PoA.1.01

Visible light cross-linking of collagen, gelatin and poly (ethylene glycol) diacrylate-based hydrogels for extrusion 3D bioprinting and their potential in tissue engineering applications.

David Rodríguez-González, M. Alejandro Fernández, Helena Herrada-Manchón

IDONIAL Tecnological Centre, Gijón, Spain

Abstract

INTRODUCTION: 3D bioprinting uses additive manufacturing technologies to build 3D structures using biological materials, biochemicals, and living cells. It creates spaces which mimic biological and mechanical environments, suitable for cell growth and function. Due to their similar properties to the natural extracellular matrix (ECM) —high water content, biocompatibility and low cytotoxicity— , hydrogels are the most prominent inks for bioprinting. However, the development of suitable hydrogel-based inks is a bottleneck as, in addition to cell requirements, they should accomplish foremost specifications. For a satisfactory printing, homogeneous and regular filament extrusion has to be ensured, as well as the stability of the final printed structure, which must retain the imposed shape.[1] In that sense, a new potential hydrogel for extrusion 3D bioprinting has been developed by following a double network hydrogel strategy. A solution of collagen and gelatin in PBS has been combined with polyethylene glycole diacrylate (PEGDA) and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), a photoinitiator whose absorbance above 400 nm enables efficient visible light polymerization [2] (Fig.1). Thus, it is possible to achieve a versatile and highly-reproducible hydrogel, suitable for producing from thin scaffolds to large-scale constructs with precision and structural fidelity, avoiding the inconvenient use of UV lamps.
RESULTS/DISCUSSION: Hydrogels showed rheological properties typical of a non-Newtonian weak gel, fitted by Herschel-Bulkley model, with a small yield stress value and thixotropic behavior. Viscosity recovery analysis revealed that, after a high shear stress, long time was needed for the hydrogel to recover its viscosity and ensure a proper shape retention (Fig.2a). In this vein, as gelatin generates thermo-reversible hydrogels, this weak point was overcome by applying a low temperature in the printing bed and inducing an in-situ gelation of the hydrogel, before the light-visible cross-linking began and provided final structural stability. The great printability of the material enabled the creation of a wide range of 3D structures with enormous possibilities in regenerative medicine: from thin self-folding scaffolds —to be potentially used to create vascularized tissues or 4D structures—, to large-scale designs with high structural fidelity (Fig.2b/2c). Moreover, been aware that the presence of collagen and gelatin enhance cell compatibility,[3] as well as the fact that its high content in water allows the exchange of substances between the medium and the hydrogel, this collagen/gelatin/PEGDA hydrogels are strong candidates for tissue regeneration and their capabilities are ready to be further explored.

REFERENCES
[1]10.1021/acs.chemrev.0c00084
[2]10.1016/j.biomaterials.2009.08.055
[3]10.1002/mabi.201900098

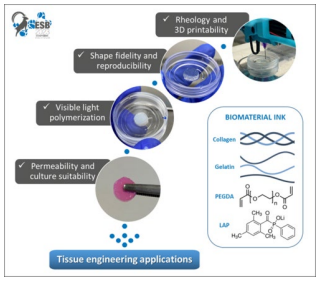


Fig.1

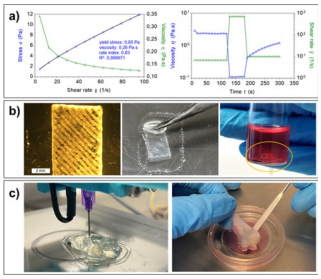


Fig.2

PoA.1.02

Fabrication of visibly degradation-monitored PCL/FNDs (Fluorescent nanodiamonds) bioscaffolds using MEW method

Xixi Wu, Thea Vedelaar, Runrun Li, Romana Schirhagl, Marleen Kamperman, Malgorzata Wlodarczyk Biegun

University of Groningen, Groningen, Netherlands

Abstract

MEW is expected as an effective method to design and manufacture thermoplastic scaffold benefited by the easy-operated and environmental-friendly features. To modify the bio-inert nature and properties like low mechanical strength and high hydrophobicity of these polymerized materials, fillers including graphene, protein and calcium phosphate, nanodiamonds were added inside.

Nanodiamond, which is a novel biocompatible material, has been gradually investigated for its distinct chemical, physical, magnetometry and quantum properties. During the exploration, multifunctional nanodiamonds have been proved that they can enhance polymeric properties as well as cell behaviour when introduced into biopolymers and also they show great potential to design sensors when properly doped.. Based on these features, beside the reinforced effect to substrates, it may broaden our horizons not only on sensoring but also non-invasive detecting and treating on biomedical fields, which will provide a promising platform to fabricate localizable and visibly degradation-monitored implants *in vivo* and discover something new in biology using treatment method that is different from the past.

Herein we fabricated polycaprolactone (PCL)/FNDs fibres at micrometre scale using melt electrowriting (MEW) method. Under the investigation of a large number of relevant literature, we find the peculiar properties of FNDs could make it practical to precisely explore how FNDs will influence the degradation process and the following bifunctionality of the composite scaffolds using our printed meshes. At the mean time, revealing of the potential of FNDs acted as a detector determinating the deterioration degree using traceable fluorescence and T1 relaxation measurements was achieved.

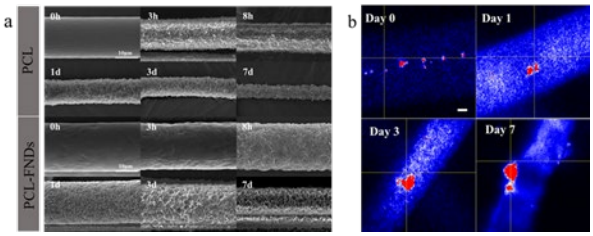


Figure 1. Degradation study. a, SEM images showing the fibres incubated in enzyme solution after 0 hour, 3 hours, 8 hours, 1 day, 3 days, 7 days; b, Use of nanodiamonds to track degradation process. Fluorescent images of nanodiamonds (0.001 wt%) in PCL fibres after 0-hour, 1-day, 3-day and 7-day enzymatic degradation. Scale bar: 3 µm.

PoA.1.03

Viscoelastic properties of gels determine the collective migration rate on PANC-1 3D models

Gema Quiñonero-López[1], Itciar González[2], Luis M Rodriguez Lorenzo[1]

[1]ICTP-CSIC, Madrid, Spain. [2]ITEFI-CSIC, Madrid, Spain

Abstract

INTRODUCTION: Cell collective migration contributes to several biological processes such as cancer spreading but the mode of migration in a 3D matrix could be different than in 2D. Thus, 3D models are paramount for translating knowledge learned on *in vitro* models to *in vivo*.
Cells rely in contractility-driven propulsion in the amoeboid migration mode. Because this mode does not require ECM degradation, amoeboid migration is faster than mesenchymal migration mode.
Alginate hydrogels do not present cell adhesion cues and therefore could promote only amoeboid migration. The purpose of this project is to test the effect of the viscoelastic properties of alginate hydrogels on the migration capacity of PANC-1 cells.
EXPERIMENTAL: Alginate based inks were rheologically characterized with a ARG2 rheometer. PANC-1 free and PANC-1-laden inks were loaded into a 3 mL cartridge, printed onto a 24-well Transwell chamber, crosslinked and incubated at 37ºC 5% CO2 for 48 h. Migrating cells were counted with a coulter Beckman and imaged after cell stained with a Live/Dead viability kit in a Paula and Leica confocal microscopes.
RESULTS/DISCUSSION: All the prepared bioinks display a shear-thinning behavior, making it possible to overcome the Barus effect so that they can be bioprinted without losing cellular viability. The recovery tests allow to predict a higher printing fidelity for the 5% S.A. bioink. After crosslinking, the 3 prepared inks yield the following viscoelastic values:

	Storage modulus (Pa)	Tan(delta)	% migrating cells (seeding)	(bioprinting)
5SA - 1.5 CaCl2	91.25 ± 8.7	2.65 ± 0.06	14+3	13+3
5SA - 3 CaCl2	257.50 ± 12.6	1.89 ± 0.02	8+1	4+1
8SA - 1.5 CaCl2	411 ± 9.3	1.50 ± 0.02	6+1	4+2

A prevailing liquid-like behaviour even after the crosslinking is observed. A higher migration is obtained for PANC-1 seeded than for bioprinted constructs, though the expected ameboid migration mode in both scenarios is appreciated. A lower cell migration rate associated to an increment in the value of the storage modulus (up to 257 Pa) but a higher migration for higher loss tangent could be observed. A better fit for a curve model could be calculated for the loss tangent (R^2= 0.9983) than for the storage modulus (R^2=0.976), suggesting the relevance of the viscous component of the bioinks in the ability of PANC-1 cells for migrating.
CONCLUSIONS: Three bioinks with different viscoelastic properties were designed. A more accurate relationship has been proposed associating the migration rate with the loss tangent.

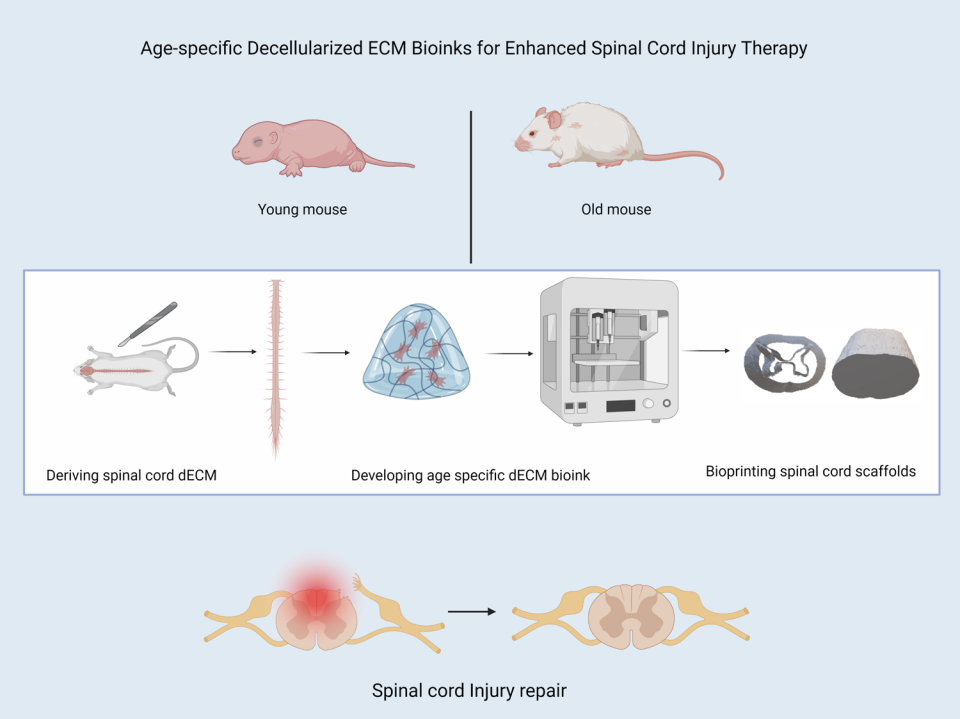
PoA.1.04

Age-specific Decellularized ECM Bioinks for Enhanced Spinal Cord Injury Therapy

Palash Chandravanshi[1], Natacha Levy[1], Zaida Álvarez Pinto[1,2]

[1]Biomaterials for Neural Regeneration Group, Institute for BioEngineering of Catalonia (IBEC), Barcelona, Spain. [2]Simpson Querrey Institute for BioNanotechnology (SQI) Northwestern University, Chicago, USA

Abstract



Traumatic spinal cord injury (SCI) affects a significant number of people worldwide, with up to 500,000 cases reported annually, resulting in a loss of motor function and reduced quality of life[1]. The development of effective therapies has proven to be challenging due to the complex and dynamic extracellular matrix (ECM) environment after the injury[2]. To tackle this problem, our study focuses on the use of age-specific decellularized ECM (dECM) bioinks, which aid in developing rationally designed 3D tissue constructs for repairing acute and chronic spinal cord lesions in a mouse model. Our preliminary results have shown that young spinal cord-derived dECM promotes advanced axonal regrowth, reduced glial scar formation, and improved functional recovery when used as a single injection after a complete transection mouse model. We are now investigating various methods to generate dECM bioinks and create bioprinted human spinal cord constructs for therapeutic use. This new therapeutic tool will elucidate novel mechanisms in both material design and injury physiopathology.

References:
1. Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance. J Spinal Cord Med 39, (2016).
2. Gaudet, A. D. & Popovich, P. G. Extracellular matrix regulation of inflammation in the healthy and injured spinal cord. Experimental Neurology vol. 258 Preprint at https://doi.org/10.1016/j.expneurol.2013.11.020 (2014).

PoA.1.05

Gelatin-based inks for the development of porous scaffolds combining 3D printing and electrospinning

Teresa Carranza[1], Jone Uranga[1], Ainhoa Irastorza[2], Ander Izeta[2], Pedro Manuel Guerrero[1,3,4], Koro de la Caba[1,3]

[1]BIOMAT research group, Donostia-San Sebastián, Spain. [2]Biodonostia Health Research Institute, Donostia-San Sebastián, Spain. [3]BCMaterials, Basque Center for Materials, Applications and Nanostructures, Leioa, Spain. [4]Proteinmat Materials SL, Donostia-San Sebastián, Spain

Abstract

Scaffold morphology is one of the limiting factors for tissue engineering. Interconnected macro- and micro-porosity are related to the scaffold success [1]. In this study, porous scaffolds were obtained by a combination of electrospinning and 3D printing. Chitin/porcine gelatin ink was formulated for 3D printing, while PVA/porcine gelatin ink was prepared for electrospinning. Both inks were stored overnight in syringe cartridges before being processed by domoBIO 2A 3D printer. Fabricated scaffolds (21 mm diameter) were composed of four 3DP ink layers with interpenetrating ES ink layers. First, rheological characterization was carried out to determine the suitability of inks to be processed by additive manufacturing. Shear thinning behaviour, with a decrease in viscosity when the shear rate increased, was observed for both inks, which is favourable for additive manufacturing processes. Additionally, good adhesion between layers was observed in the scaffolds, which showed an average porosity of 2.5 mm and 64 µm for 3D printed and electrospun layers, respectively. As can be seen in Figure 1, live/dead analysis showed cells with normal cartilage phenotype, indicating the potential of the scaffold for tissue engineering applications.

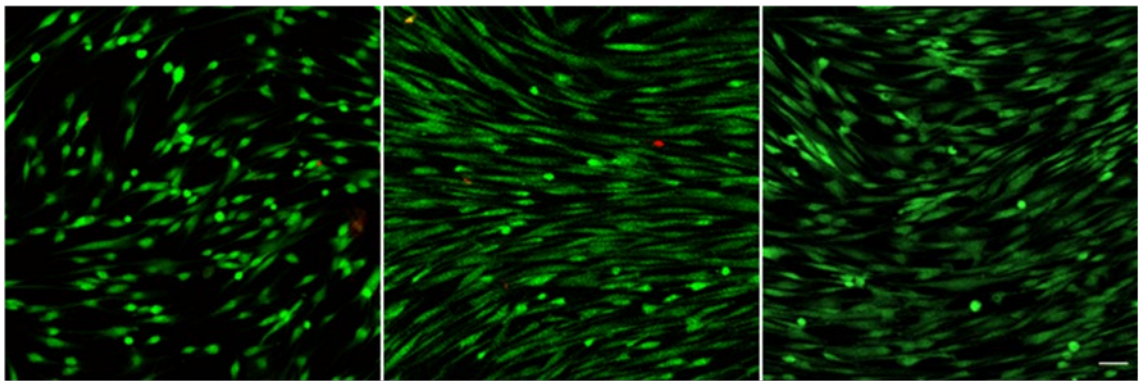


Figure 1. Long-term live/dead cell viability assay (Scale bar 50 µm).

REFERENCES

[1] Hernandez J L and Woodrow K A 2022 Medical Applications of Porous Biomaterials: Features of Porosity and Tissue-Specific Implications for Biocompatibility *Adv. Healthc. Mater.* **11** 2102087.

PoA.1.06

Biofabrication of oxidized alginate-gelatin (ADA-GEL) based structures incorporating ion releasing nanoparticles for tissue engineering applications

Hsuan-Heng Lu, Clara Froidevaux, Aldo R. Boccaccini

Institute of Biomaterials, University Erlangen-Nuremberg, Erlangen, Germany

Abstract

Bioprinting is an additive manufacturing approach to produce scaffolds with three-dimensional architectures. Oxidized alginate (ADA) attracts great interest in tissue engineering because of its biocompatibility, tunable properties, and ability to form Schiff's base with gelatin (GEL), a polypeptide enabling cell adhesion [1]. Incorporation of bioactive inorganic fillers (BIFs) into ADA-GEL can influence the properties of inks and cell behavior [2]. In this work, BIFs are SiO_2-CaO nanoparticles made by sol-gel methods [3]. BIFs were combined with ADA-GEL, and cell-laden structures were printed.

ADA solution was mixed with BIFs and further with GEL solution. ADA-GEL and ADA-GEL-BIFs incorporated C2C12 myoblasts were used as bioinks. The effect of BIFs on the printability, stability, and mechanical properties of ADA-GEL based hydrogels and the development of C2C12 cells were investigated.

ADA-GEL and ADA-GEL-BIFs inks were applied to print 3D constructs. After external crosslinking, the printed structures exhibited intact shapes and could be handled easily, as shown in Figure 1. The cytotoxicity of BIFs was examined by an indirect test, and the relative cell viability of C2C12 cells cultured with extracts from BIFs over 3 days is displayed in Figure 2. All BIFs with a concentration of 0.1% (w/v) were cytocompatible to myoblasts. Bioprinting of the chosen inks was carried out and the mitochondrial activity of myoblasts in the bioprinted structures was assessed over 14 days. ADA-GEL-BIFs showed higher cell viability after 7 days of incubation compared to ADA-GEL, indicating that the incorporation of BIFs positively affected cell proliferation. After 14 days of incubation, ADA-GEL and ADA-GEL-BIFs had similar values, which demonstrated good cytocompatibility of all inks in biofabrication.

ADA-GEL based hydrogels combined with BIFs were investigated in this work. BIFs, at low concentrations, were cytocompatible to myoblasts. The bioprinted ADA-GEL and ADA-GEL-BIFs showed shape integrity and good cytocompatibility, indicating their great potential in tissue engineering applications.

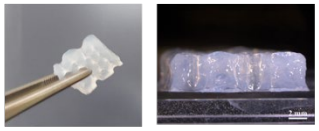


Figure 1. Digital images of a crosslinked printed structure.

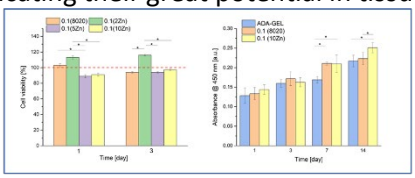


Figure 2. Cell viability of C2C12 cultured with extracts from BIFs and mitochondrial activity of bioprinted cells ($p < 0.05$).*

[1] B. Sarker et al. J. Mater. Chem. B, vol. 2, no. 11, pp. 1470–1482, 2014.
[2] S. Heid et al. Bioprinting, vol. 26, no. March 2022.
[3] K. Zheng and A. R. Boccaccini, Adv. Colloid Interface Sci., vol. 249, pp. 363–373, Nov. 2017.

The authors express appreciation to the German Research Foundation (DFG); Collaborative Research Center SFB/TRR225 (subproject B03) for the financial support.

PoA.1.07

Enzyme Bioink for the 3D Printing of Biocatalytic Materials

Luca A Altevogt, Rakib H Sheikh, Thomas G Molley, Joel Young, Kang Liang, Patrick Spicer, Kristopher A Kilian, Peter R Wich

UNSW, Sydney, Australia

Abstract

The field of 3D bioprinting still faces big challenges on the road to printing major full functional tissues and organs. One of them is adding functionality to the newly formed tissue for replicating the exact native biochemical environment.
Enzymes represent a highly attractive class of bioactive agents for applications in tissue engineering. The enzymes' specificity and selectivity enable them to react with target molecules only, and their *in vivo* biocatalytic processes makes them highly biocompatible. Hence, in contrast to their toxic and side-effect-prone drug alternatives, enzymes evolve as ideal candidates for adding functionality in biomaterials.
However, the difficulty in the application remains in delivering enzymes to the targeted site in adequate amounts for a long time. In previous studies, enzymes are rarely used as bioactive agents in the field of 3D bioprinting and are only physically entrapped or loosely injected inside the hydrogel. This results in low retention within the biomaterial scaffold and short-term effects of the enzymes.
Here, we present the development of a biocatalytic enzyme bioink for extrusion-based bioprinting by covalently attaching enzymes to the bioink scaffold in one quick crosslinking step. The enzymes become an integral part of the network and demonstrate higher stability inside the gel leading to an increased concentration and prolonged catalytic activity than solely physically entrapping the enzymes inside the hydrogel. Being able to 3D print this enzyme bioink, this opens up a novel cytocompatible biocatalytic bioink for precise and controlled applications at a targeted site within a three-dimensional structure.

PoA.1.08

Preparation and printability of a composite silk fibroin-hyaluronic acid-PLGA biomaterial ink as co-delivery carrier

Jhaleh Amirian[1,2,3], Jacek K. Wychowaniec[3], Matteo D'Este[3], Andrea Vernengo[3], Agnese Brangule[1,2], Dace Bandere[1,2]

[1]Department of Pharmaceutical Chemistry, Rīga Stradiņš University, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia. [3]AO Research Institute Davos, Davos, Switzerland

Abstract

INTRODUCTION: Composite materials with excellent biocompatibility, biodegradability, and controlled drug release properties are crucial in bone tissue engineering. Two naturally occurring components allowing formation of tuneable biomaterials are hyaluronic acid (HA)[1]and silk fibroin (SF)[2]. simvastatin (SIM)[3] stimulates bone formation by activating the BMP-2 receptor, whereas dexamethasone (DEX), which is an anti-inflammatory drug, stimulates osteogenesis *in vitro*[4]. Here, HA and SF were chemically functionalized to produce a composite hydrogel that can serve as both an extrudable bioink and dual drug delivery system.

EXPERIMENTAL PROCEDURE: Here we synthesized: i) methacrylated silk-fibroin (SFMA) from SF extracted from *Bombyx mori* and ii) methacrylated hyaluronic acid (HAMA) using aminoethyl methacrylic acid. SFMA/HAMA was combined with polylactic-co-glycolic acid (PLGA) particles carrying SIM, followed by crosslinking under UV illumination. Physio-chemical properties of HAMA, SFMA, and SFMA/HAMA composite hydrogels were assessed by FTIR, 1H NMR and oscillatory rheology as a function of components ratio. Selected formulations were extruded into a carbopol supporting bath using a 3D Discovery (RegenHU) printer. Finally, the cytotoxicity of the hydrogels was evaluated.

RESULTS: The successful syntheses of SFMA and HAMA were confirmed by FTIR and 1H NMR. The composite hydrogels reached complete gelation in 17 seconds. Addition of 1% HAMA to 5, 10, and 20% of SFMA resulted in composite hydrogels with significantly increased storage modulus and swelling in comparison to respective SFMA hydrogels without HAMA. 20% SFMA with 1 % HAMA showed shear thinning characteristics suitable for printing into circular filaments using a free form printing. DEX and PLGA-SIM were successfully incorporated into SFMA/HAMA composite hydrogels.

CONCLUSIONS: We have prepared multifunctional bioinks with dual drug delivery systems which are under investigation for bone tissue engineering.

ACKNOWLEDGEMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 857287 (BBCE).

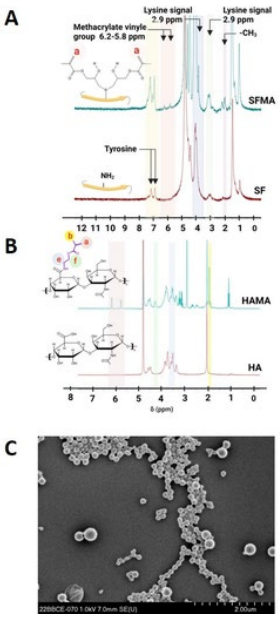


Figure 1. *A) H HNMR of SF and SFMA, B) HA and HAMA, C) SEM images of PLGA-SIM*

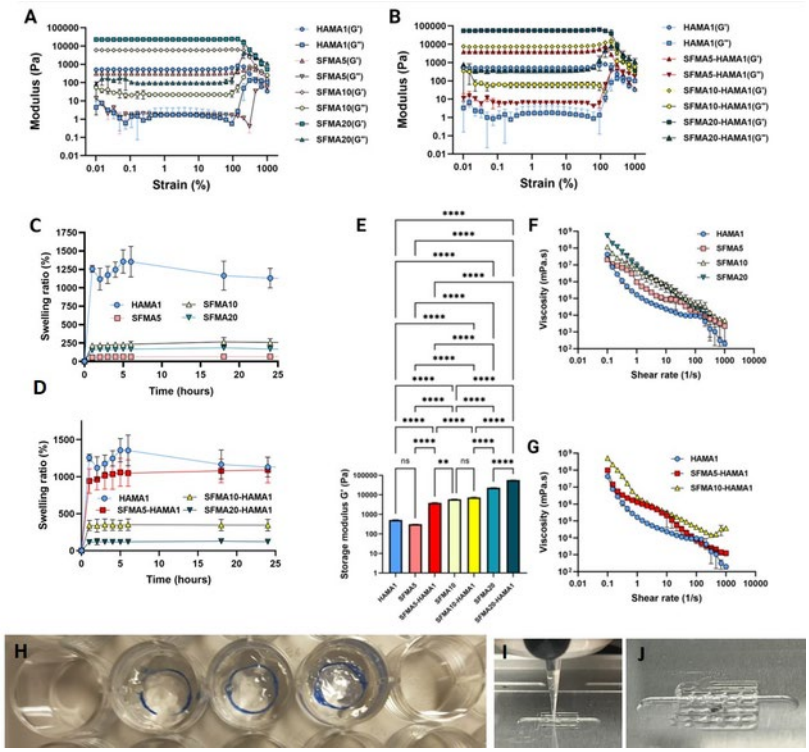


Figure 2. *Rheological properties of SFMA hydrogels A, B) Mechanical stiffness C, D) Swelling behaviour, E) Amplitude sweeps at 1 Hz and 0.2%, F, G) flow curves of HAMA, SFMA, SFMA-HAMA hydrogels, H) printing in Carbopol bath, and I, J) glass substrate.*

REFERENCES

1. Schuurmans, C. C. L. Biomaterials 2021, 268, 120602.
2. Zheng, H. Journal of Materials Chemistry B 2021, 9, 1238.
3. Gupta, S. Int J Implant Dent 2019, 5, 17.
4. James, C. G. BMC Genomics 2007, 8, 205.

PoA.1.10

Composite 3D printing inks based on natural biomaterials for hard tissue regeneration and repair

Andrada Serafim[1], Filis Curti[1,2], Elena Olaret[1], Sorina Dinescu[3,4], Iuliana Samoila[3], Adriana Lungu[1], Izabela C Stancu[1,5]

[1]Advanced Polymer Materials Group, University Politehnica of Bucharest, Bucharest, Romania. [2]Zentiva SA, Bucharest, Romania. [3]Department of Biochemistry and Molecular Biology, University of Bucharest, Bucharest, Romania. [4]Research Institute of the University of Bucharest, Bucharest, Romania. [5]Faculty of Medical Engineering, University Politehnica of Bucharest, Bucharest, Romania

Abstract

One of the main concerns of modern research in the field of tissue engineering is the fabrication of scaffolds that concomitantly mimic the micro-architectural features and biological function of the natural tissues. Natural materials are increasingly investigated as building blocks for new and improved formulations to be used in tissue regeneration and repair.

The present work describes the fabrication of 3D printed scaffolds of thick paste-like inks based entirely on natural materials [1]. Sodium alginate (SA), fish gelatin (FG) and cuttlebone (CB) were rationally combined to produce 3D scaffolds that closely match the bone architecture. The microarchitecture of the scaffolds under mechanical loading was investigated through micro-computed tomography (μ-CT) and cell-responsiveness was assessed through *in vitro* tests using MC3T3-E1 murine preosteoblasts.

Several 3D printing experiments were performed to identify the adequate processing conditions (table 1). Subsequently, the samples were submitted to a post-processing crosslinking treatment through incubation in glutaraldehyde and calcium chloride baths. All scaffolds had a biphasic structure, with composite filaments consisting of numerous CB particles homogeneously embedded within an amorphous hydrogel phase.

μ-CT imaging performed under mechanical load revealed that the addition of SA ensures better integrity and stability: while in the case of the SA-richest scaffold (GA1.5CB) a displacement of 1.5 mm has almost no impact on the microarchitectural features of the sample, the GCB control is completely crushed (figure 1,). This work also revealed that the addition of small amounts of SA also resulted in improved cell–scaffold interactions (figure 1) and favoured mineralization during MC3T3-E1 murine preosteoblast-containing CM incubation.

Sample	Mass ratio in the Final Mixture			Printing parameters	
	FG	SA	CB	Pressure (kPa)	Feed rate (mm/s)
GCB	27.78	-	72.22	550 ± 50	3.3 – 3.5
GA0.5CB	27.74	0.14	72.12		2.9 – 3.1
GA1CB	27.7	0.28	72.02		2.5 – 2.7
GA1.5CB	27.66	0.42	71.92		1.8 – 2.2

Table 1. Details of the composition and printing parameters of the biocomposite inks

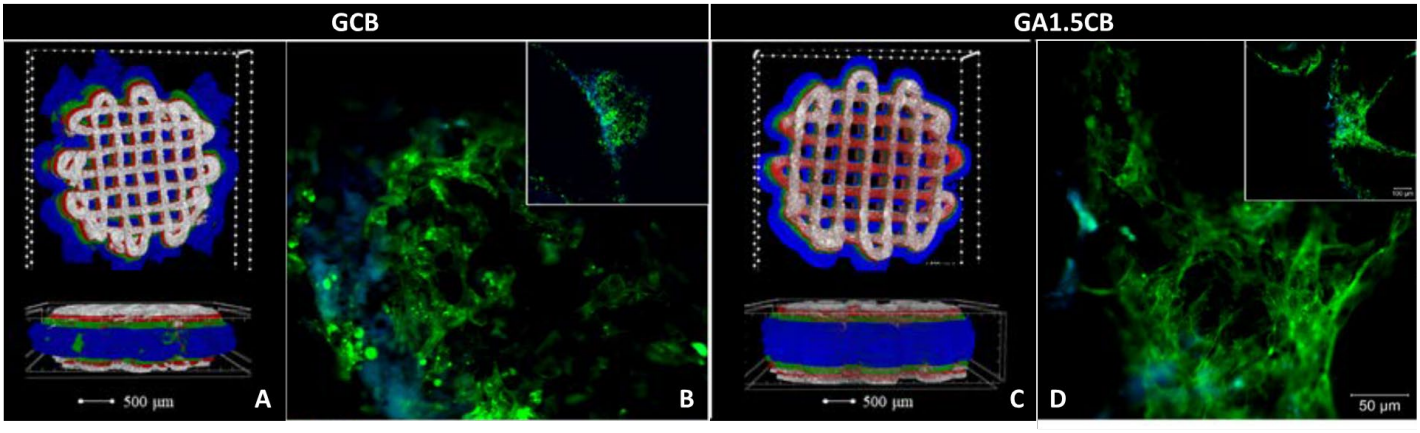


Figure 1: µ-CT images (A and C) and cell adhesion assay (48 h) (B and D) for GCB and GA1.5CB respectively

Acknowledgments: This research was supported through project PN-III-P4-PCE-2021-1240, PCE88/2022. 3D printing and µ-CT were available due to the project P_36_611, MySMIS code 107066 (INOVABIOMED).

References:

[1] Curti, F.; Serafim, A.; Olaret, E.; Dinescu, S.; Samoila, I.; Vasile, B.S.; Iovu, H.; Lungu, A.; Stancu, I.C.; Marinescu, R. Development of Biocomposite Alginate-Cuttlebone-Gelatin 3D Printing Inks Designed for Scaffolds with Bone Regeneration Potential. Mar. Drugs 2022, 20, 670.

PoA.1.11

Patient-specific meniscus prototype based on 3D bioprinting of high concentrated collagen bioink

Alfredo Ronca[1], Ugo D'Amora[1], Carla Zihlmann[2], Niklaus Stiefel[2], Girish Pattappa[3], Peter Angele[3,4], Luigi Ambrosio[1]

[1]Institute of Polymers, Composites and Biomaterials – National Research Council of Italy, Naples, Italy. [2]Geistlich Pharma AG (Geistlich), Wolhusen, Switzerland. [3]Experimental Trauma Surgery, Department of Trauma Surgery, University Regensburg Medical Centre, Regensburg, Germany. [4]Sporthopaedicum Regensburg, Regensburg, Germany

Abstract

Current approaches for meniscus repair focus on the combination of scaffolds acting as a carrier for relevant cell populations involved in meniscal healing that can also be supplemented with growth factors to increase the healing potential induced by the cells. A function of the meniscus is distributing complex loading within the knee between the femur and tibia, thus any replacement should withstand significant these stresses with minimal distortion. Collagen is the most abundant extracellular matrix (ECM) protein in the meniscus, helping to maintain the biological and structural integrity of the ECM and regulating cell activity. To create stable and high-precision 3D printed collagen scaffolds, ink formulations must possess good printability and cytocompatibility. Material printability depends upon its rheology coupled with printing parameters that allow the deposition of materials in filament/strand form. Specifically, the shape fidelity and the mechanical properties of the final construct after lyophilization depend on the ink composition and concentration. However, one of the main limitations of collagen bioinks is in its poor mechanical properties. In the case of pure collagen, it has been demonstrated that its use as an ink in low concentrations (usually between 10-15 mg/ml) does not exhibit good rheological properties for creating 3D scaffolds, greater than 1-2 mm thick, due to structural collapse. This study sought to develop a high concentration collagen bioink (125 mg/ml) for the creation of a 3D printed meniscus-like scaffold. Specifically, we attempted to create an ink, derived entirely from collagen that would potentially result in an inherently porous printed structure. The bioink was specifically designed to have load-bearing capacity upon printing. Once the ink was characterized and validated, a 3D model medial meniscus tissue was created, based on MRI scans to obtain an STL model of the structure. Following printing, a series of post-processing steps, including freeze-drying and crosslinking, were performed, and optimized to maintain the stability of the engineered construct. 3D printed structures were characterized in terms of mechanical and morphological properties and an *in vitro* biological characterization, using human meniscus derived cells with gene expression and histology performed. Moreover, a dynamic mechanical analysis (DMA) was performed on cell-seeded scaffolds and cell-free scaffolds after 28 days culture to evaluate the effect of cell presence on scaffold mechanical properties.

ACKNOWLEDGMENTS
The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 814444 (MEFISTO).

PoA.1.12

Biopolymer-based hybrid hydrogel inks serving liver tissue engineering

Nathan Carpentier[1], Ye Shicheng[2], Kerstin Schneeberger[2], Bart Spee[2], Peter Dubruel[1], Sandra Van Vlierberghe[1]

[1]Ghent University, Ghent, Belgium. [2]Utrecht University, Utrecht, Netherlands

Abstract

Introduction: Annually, 2 million people die because of liver failure. The current time on the waiting list is 12 months, while 20% never receives a new liver. We developed novel biomaterial-inks serving liver tissue engineering(LTE) to contribute to the development of donor alternatives. Hydrogel formulations consisting of thiolated gelatin(GelSH)[1] in combination with a norbornene(NB)-modified polysaccharide were selected and optimized towards long-term constant viscosity inks (i.e.preventing autogelation) enabling reliable bioprinting by the addition of tris-(2-carboxyethyl)fosfine(TCEP). Benchmarking was performed against materials reported before in LTE.[2,3]
Methods: NB-modified dextran(DexNB) was developed by coupling the hydroxyls of dextran with the carboxylic acids of 5-norbornene-2-carboxylic acid using DMAP and DCC.
NB-modified CS(CSNB) was developed(CSNB(12) and CSNB(34)) by coupling of the hydroxyls of CS with NB carboxylic acid using DMAP and Boc_2O.
The materials were physico-chemically characterized using proton nuclear magnetic resonance(^{1}HNMR), gel permeation chromatography(GPC), swelling and gel fraction experiments. The mechanical properties were assessed using atomic force microscopy(AFM) and rheological experiments assessing the crosslinking kinetics, storage modulus(G') and viscosity.
The biocompatiblity of the novel formulations was investigated using fibroblast, and the potential of the hydrogels to support proliferation and differentiation of intrahepatic cholangiocyte organoids(ICOs) was researched.
Results and discussion: The modifications of GelSH, DexNB and CSNB were successfull as evidenced by HNMR, while the molecular weight did not significantly decrease. A constant viscosity of the ink formulation was obtained for at least 24 hours with the addition of 0.25eq of TCEP according to the amount of thiols present in GelSH, while retaining a biocompatible hydrogel formulation as assessed using fibroblasts. This resulted in superior printing performance(Fig.1). DexNB(15)-GelSH and CSNB(12)-GelSH exhibited physico-chemical properties highly mimicking natural liver tissue resulting in a hydrogel that promoted the proliferation of ICOs in the same extent as Matrigel and PIC-LEC. CSNB(34)-GelSH exhibited higher mechanical properties, however outperformed the other materials in supporting the differentation of ICO's towards hepatocyte-like(HLC) cells(Fig.2).
Conclusion: The novel optimized formulations are promosing in the field of LTE. They exhibit superior printing performance, while retaining biocompatiblity. DexNB-GelSH supported the proliferation of cultured ICOs, while CSNB(34)-GelSH promoted the differentation towards HLC.

[1]VanVlierberghe,Eur.Polym.J.47:1039–1047,2011
[2]VanDenBulcke,Biomacromolecules.1:31-38,2000
[3]Ye,Adv.Funct.Mater.30,2000893,2020

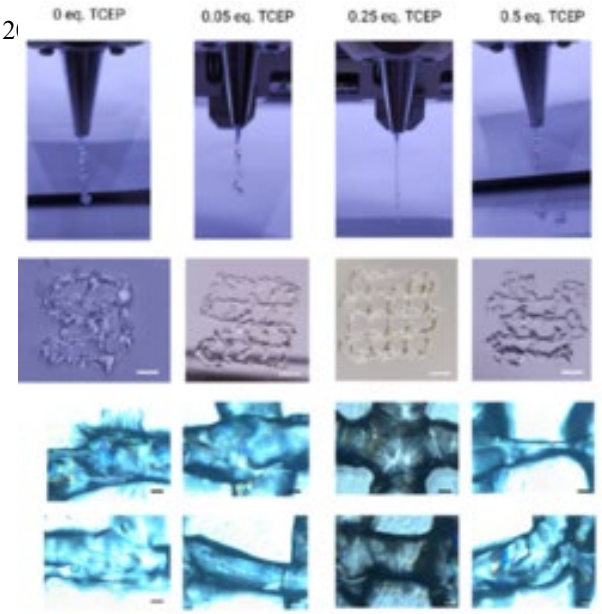


Figure 1: Evidence of superior printing.

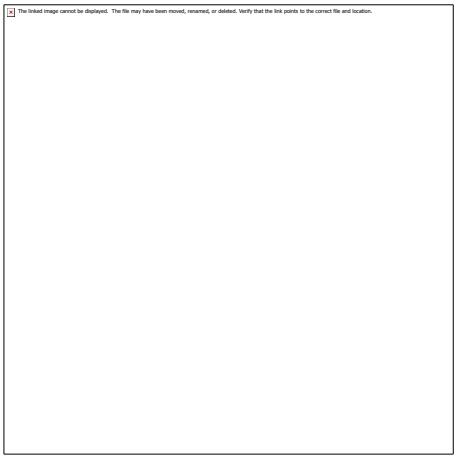


Figure 2: qPCR results of ICOs differentiated in the different materials.

PoA.1.13

Visible light 3D bioprinting: Synthesis and characterization of biodegradable poly(α-amino acid)s based bio-inks

Jana Dvorakova, Anna Golunova, Elena Moran, Vladimir Proks

Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic

Abstract

Many scientists are focusing on printing 3D objects similar to biological tissue, but constructing complex and functional structures remains a challenge. Hydrogels show promise in this field, but the synthesis of biodegradable hydrogels, the bioprinting process, and the biocompatible crosslinking of bio-inks also present various challenges.

Photocrosslinking of bio-inks to form hydrogels is a promising strategy due to its non-invasiveness and easy control of light intensity and exposure. Nowadays, photocrosslinking is frequently initiated by ultraviolet (UV) or visible light. However, UV light may cause cell mutation and affect cell fate, so visible light is considered more biocompatible for bioprinting [1].

This work proposes fully synthetic and biodegradable bio-inks based on poly(α-amino acid)s (PolyAA), which offer an alternative to commercial bio-inks based on gelatin, collagen, or alginate. The modular chemical structure of polyAA, adjustable physical parameters, along with enzymatic degradation [2], make them a highly potential toolbox for 3D bioprinting that remains undeveloped.

For that, we synthesized water soluble, biodegradable poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) based precursors that undergo photocrosslinking at 450 nm using two photoinitiation systems: a) tris(bipyridine) ruthenium (II) chloride and ammonium persulfate; b) riboflavin and L-arginine. Tyramine or methacrylate functional groups allowing the photocrosslinking of the polymer were introduced via postpolymerization modification of the initial poly(γ-benzyl-L-glutamate). The polymers were analyzed with several physicochemical methods (NMR, UV-Vis, GPC) to confirm the structure and showed the shear-thinning behaviour required for bio-inks. The printability of PHEG-based bio-inks was confirmed with the extrusion-based Cellink Bio XTM bioprinter, using a temperature control printhead in combination with a photocuring tool head. Printed hydrogels were characterized by gel yields, swelling capacity and mechanical properties were tested.

Keywords: 3D bioprinting, bio-inks, bioprinter, photoinitiation, hydrogels, poly (α-amino acid)s, blue light

Acknowledgements: Financial support from the Czech Science Foundation (No. 21-06524S)

References:

[1] Zheng, Z., Eglin, D., Alini, M., Richards, G. R., Qin, L. & Lai, X. (2021). Visible Light-Induced 3D Bioprinting Technologies and Corresponding Bioink Materials for Tissue Engineering: A Review. Engineering, 7 (7), 966-978.

[2] Sedlacík, T., Studenovská, H. & Rypáček, F. (2011) Enzymatic degradation of the hydrogels based on synthetic poly(α-amino acid)s. J. Mater. Sci. Mater. Med., 22, 781–788.

PoA.1.15

Controlling renal epithelial cell behavior by improving the viscoelastic and biofunctional characteristics of a kidney ECM derived bioink for 3D bioprinting

Isabelle Schmidt[1], Alexandra Meisner[1], Elin Pernevik[2], Philipp Linke[3], Volodymyr Kuzmenko[2], Ellena Fuhrmann[1], Itedale Namro Redwan[2], Zeno Guttenberg[3], Hanna Hartmann[1]

[1]NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany. [2]CELLINK Bioprinting, Gothenburg, Sweden. [3]ibidi GmbH, Munich, Germany

Abstract

Bioprinting is an advancing technology, having the potential to build-up functional test systems, such as an *in vitro* model of the proximal tubule to study nephrotoxicity of new drugs. However, suitable biomaterial inks are still limited, that fulfil all necessary prerequisites: good printability and biofunctionality, allowing e.g., cell adhesion and self-organisation by migration. We therefore tested two commonly used biomaterials, namely alginate and gelatine methacryloyl (GelMA), which were modified by the addition of nanofibrillated cellulose (NFC) as a thickener and/or decellularized extracellular matrix from the kidney (dECM) to provide attachment sites and achieve the desired features.

Rheology and printability were investigated, both inks exhibited good printability and showed only a slight tendency to over-gelation for the GelMA based inks, whereas the alginate biomaterial ink was not stackable. Cell attachment and monolayer formation of renal proximal tubule epithelial cells (RPTEC) on GelMA/NFC could be successfully increased by adding dECM. Compared to the alginate ink containing dECM, the biocompatibility of GelMA/NFC/dECM was superior. Also, 3D cell migration was successfully achieved in GelMA based biomaterial inks. In both, alginate and GelMA based biomaterial inks, RPTEC showed a rounded cell morphology, but larger cell clusters were observed in GelMA based bioinks. GelMA/NFC/dECM was used to print hollow channels which were lined by tubule epithelial cells and cultivated for 7 days. The viability and protein expression of the cells was confirmed by fluorescent markers. This confirms that the biocompatibility was preserved after all steps of bioink processing, including mixing, casting, or 3D bioprinting.

The here developed GelMA/NFC/dECM is therefore a suitable bioink for 3D bioprinting of proximal tubulus epithelial cells and might be a valuable tool for 3D bioprinting of kidney models and functional drug testing.

Acknowledgments: This work is funded by EUROSTARS grant project PTubeChip 113681. This work also received financial support from the State Ministry of Baden-Wuerttemberg for Economic Affairs, Labour and Tourism and the Federal Ministry of Education and Research.

PoA.1.16

Development of Hybrid 3D-Printed Scaffolds with Aligned Drug-Loaded Fibers Using In-Situ Custom Designed Templates

Kirsty Muldoon

Ulster University, Belfast, United Kingdom

Abstract

Electrohydrodynamic atomization technology employs an electric field to create a Taylor cone at the needle orifice using a polymer-solvent-based solution, resulting in the production of micro- to nanoscale particles or fibers on a collector. Although fiber alignment is crucial in various emerging applications, such as drug delivery systems[1], tissue engineering[2], structural reinforcement of materials[3], and energy storage devices[4], conventional mechanical methods of fiber alignment, such as rotating mandrels or disks, have limitations in terms of material combination and geometry arrangement due to the possibility of brittle fiber breakage caused by the required rotating speeds.

This research aims to use custom-designed templates made with conductive ink to control the alignment of drug-loaded polymer fibers on a 3D printed scaffold produced using 3D microscale printing[5] (fig 1). Three different geometries were analyzed in the electrospinning process, each constructed using defined spacing to determine the impact of template distance on fiber diameter and pattern. The hybrid structure was characterized using SEM, EDX, FTIR, TGA, DSC, mechanical properties, and drug release behavior. The results are expected to demonstrate successful control of aligned fibers on printed structures using grounded conductive ink geometric electrodes. The results obtained from the hybrid structures are expected to provide a novel mechanism for integrating electrospun fibers and 3D printed scaffolds through the combination of 3D printing and electrospinning technologies.

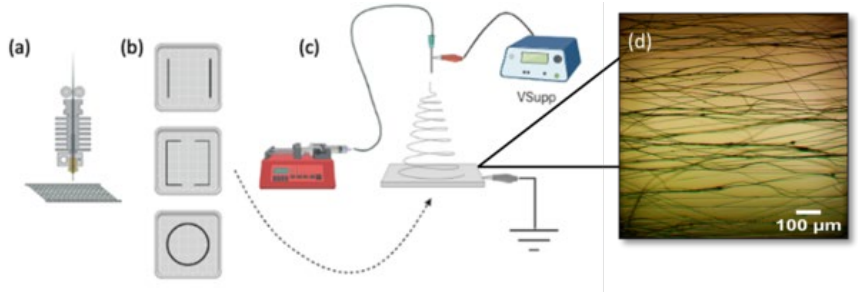


Figure 1. Schematic of (a) hot melt extrusion printing system, (b) 3D printed scaffolds with various geometries of conductive ink, (c) electrospinning system (d) optical microscopy of aligned fibres.

References

1. Cui, C. et al. Optimizing the chitosan-PCL based membranes with random/aligned fiber structure for controlled ciprofloxacin delivery and wound healing. Int. J. Biol. Macromol. 205, 500–510 (2022).
2. Mancino, C. et al. Electrospun electroconductive constructs of aligned fibers for cardiac tissue engineering. Nanomedicine Nanotechnology, Biol. Med. 44, 102567 (2022).
3. Bas, O. et al. Enhancing structural integrity of hydrogels by using highly organised melt electrospun fibre constructs. Eur. Polym. J. 72, 451–463 (2015).
4. Wang, N. et al. Structural design of electrospun nanofibers for electrochemical energy storage and conversion. J. Alloys Compd. 935, 167920 (2023).
5. Muldoon, K. et al. A Refined Hot Melt Printing Technique with Real-Time CT Imaging Capability. Micromachines 13, (2022).

PoA.1.17

The design and rheological analysis of printable ink formulations

Rency Geevarghese[1], Malgorzata Wlodarczyk Biegun[1,2], Daniele Parisi[2]

[1]Silesian University of Technology, Gliwice, Poland. [2]University of Groningen, Groningen, Netherlands

Abstract

Introduction: Bioink is a formulation comprising biomaterials, cells, and biochemical cues possessing distinct properties of visco-elasticity, printability, and biocompatibility making it an appropriate fit for 3D bioprinting applications. However, the experimental bioink optimization is challenging and time consuming. Detailed rheological analysis can be used as an effective tool to predict and assess material printability. The current work focuses on design and development of a well-printable bioink based on common biopolymers, such as sodium alginate (Alg), carboxy-methyl cellulose (CMC) and methacrylated gelatin (GelMA). Alg improves viscosity and shear-thinning property of formulation, CMC serves as an emulsifier and photocrosslinkable GelMA enhances mechanical strength and support cellular growth. Bioink compounds are mixed at different ratios and link between rheology and 3D-printing is studied with aim of detecting the optimized conditions for printability, shape fidelity and stability of 3D-printed specimen. **Methods:** Inks composed of 4% Alg-(4,8,10 & 12) %CMC, 4%Alg-10% CMC and 4% Alg-10%CMC-GelMA (4,8 &12)% (w/v) were prepared in PBS. The rheological experiments were performed in TA rotational rheometer (US), including flow sweep, amplitude sweep, frequency sweep, thixotropic test, time sweep under UV-curing and temperature ramp. GeSiM bioprinter was used for printing. For experiments involving UVcuring 0.25% (w/v) photoiniator (Lithium phenyl trimethylbenzoyl phosphinate) was added to formula. Printing with included fibroblasts was performed to assess cell viability in the optimized formulation. **Results and Discussion:** The rheological testing of 4%Alg-CMC conc. revealed that the addition of CMC improved the G', G'' and η*(Fig 1 A) and optimal printing of 4%Alg-10%CMC (Fig 1. B) was associated with these rheological properties. Further incorporation of GelMA (4,8 &12)% to 4%Alg-10%CMC led to increase in G', G'', η* (Fig 2. D&E) yet all the solutions were well-printable. 4%Alg-10%CMC-12%GelMA (with highest GelMA content) showed best printability defined as the possibility of obtaining the thinnest printed strands (Fig 2. A&C). We concluded that GelMA can moderately influence printability and addition of GelMA also influenced cell performance.
Conclusions: Characterization of rheological properties of tested materials gave a strong indication of shape fidelity, stability and printability, and is a powerful tool in the development of optimal printable materials.

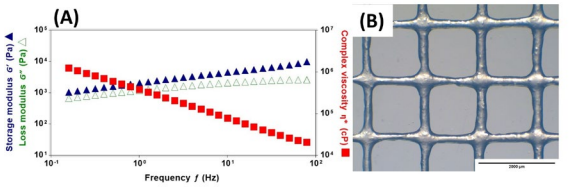


Fig 1. (A) Frequency sweep and (B) printability test of 4%Alg-10%CMC depicting best printability among 4%Alg-(4,8,10 and12)%CMC bioinks.

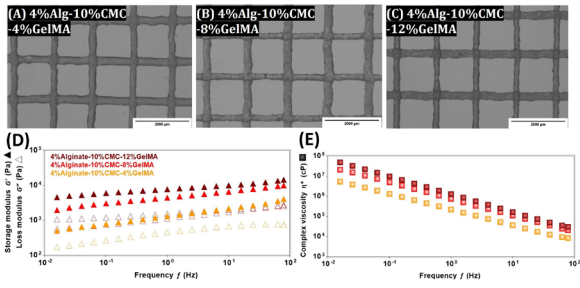


Fig 2. Printability test of the proposed inks formulations. (A) 4%Alg-10%CMC-4%GelMA, (B) 4%Alg-10%CMC-8%GelMA, (C) 4%Alg-10%CMC-12%GelMA. (D,E) Frequency sweep of 4%Alg-10%CMC-GelMA conc. proving 4%Alg-10%CMC-12%GelMA to be the best printable bioink owing to high modulus, viscosity and thinnest fiber deposition in comparison to other formulations.

PoA.1.18

3D bioprinted tubular constructs functionalized with platelet-rich plasma promote endothelial tissue regeneration

Varvara Platania[1], Nikoleta Natalia Tavernaraki[1], Ioanna Gontika[2], Eirini Fragiadaki[3], Helen A Papadaki[4,5], Kalliopi Alpantaki[6], Marina Vidaki[7,8], Maria Chatzinikolaidou[1,9]

[1]Department of Materials Science and Technology, University of Crete, Heraklion, Greece. [2]Haemopoiesis Research Laboratory, Faculty of Medicine, University of Crete & Public Umbilical Cord Blood Bank of Crete, University Hospital of Heraklion, Heraklion, Greece. [3]Haemopoiesis Research Laboratory, Faculty of Medicine, University of Crete, & Public Umbilical Cord Blood Bank of Crete, University Hospital of Heraklion, Heraklion, Greece. [4]Haemopoiesis Research Laboratory, Faculty of Medicine, University of Crete, Heraklion, Greece. [5]Public Umbilical Cord Blood Bank of Crete, University Hospital of Heraklion, Heraklion, Greece. [6]Department of Orthopaedics and Trauma, Venizeleion General Hospital of Heraklion, Heraklion, Greece. [7]Department of Basic Science, Faculty of Medicine, University of Crete, Heraklion, Greece. [8]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece. [9]Institute of Electronic Structure and Laser, Foundation for Research and Technology Hellas, Heraklion, Greece

Abstract

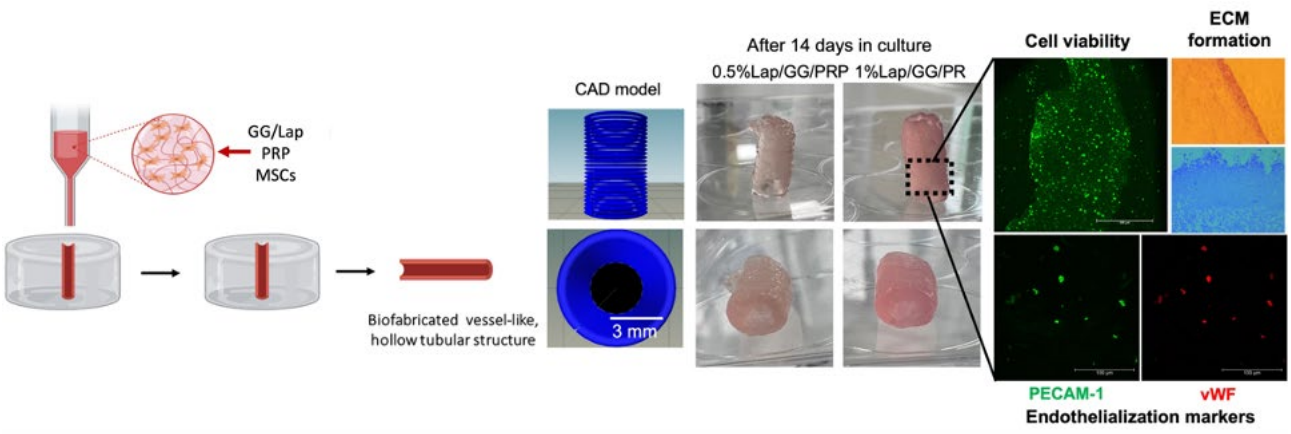
One of the most popular biofabrication techniques is 3D bioprinting which can be used to produce personalized constructs for tissue engineering (TE). Platelet-rich plasma (PRP) is a source of patient-specific growth factors exhibiting tissue repair capacity and angiogenesis promotion. Gellan gum (GG) is a natural biocompatible polysaccharide with angiogenic properties. The addition of laponite (Lap) aims to improve printability and increase pore size favoring the formation of vascular networks.

Four different blends 0.5%Lap-GG-PRP, 0.5%Lap-GG, 1%Lap-GG-PRP and 1%Lap-GG were combined with Wharton jelly mesenchymal stem cells (WJ-MSCs), bioprinted using a CAD model of hollow tubular structures and crosslinked with 2.5%w/v KCl. The swelling and mechanical properties have been determined. Biological evaluation of the constructs includes the live/dead staining, the evaluation of ECM formation by visualization of the produced collagen and glycosaminoglycans, and the endothelialization markers PECAM-1 and vWf by immunohistochemistry. *In vivo* immunological response of the bioinks was examined in C57BL6 mice subcutaneously for 14 days.

Biofabricated constructs were designed with an inner diameter of 3 mm and a wall thickness of 1 mm. Stress/strain analysis revealed the elastomeric properties of the hydrogels with Young modulus of 10 MPa. Increasing the Lap concentration led to a non-significant decrease of swelling ratio. Live/dead assay revealed cell viability of at least 76% from days 3 to 21 for all compositions. On day 3 the 0.5%Lap-GG and the 1%Lap-GG presented 85% and 88% viability respectively, followed by an increase over 99% on day 7 for both bioinks. After 21 days the 0.5%Lap-GG maintained viability level up to 99%, while a slight decrease was observed for 1%Lap-GG. Gradual increase of GAGs accumulation and collagen production through days 7, 14 and 21 indicate the promotion of ECM formation. *In vitro* functional evaluation depicts the expression and membranous localization of PECAM-1 from day 7. Granular intracellular localization of vWF was detected after 2 weeks. The results revealed the expression of both vWF and PECAM-1 by WJ-MSCs entrapped in all four construct compositions with significantly higher expression of vWF in the presence of PRP.

Cell-laden Lap-GG bioinks were used to fabricate vessel-like structures of high shape fidelity, high viability levels, high elasticity and swelling properties. The biological effect of PRP/GG/Lap bioinks has been validated towards endothelial regeneration. The integration of PRP improved the cellular behavior and

promoted endothelial differentiation. No adverse reactions of the implanted constructs in mice have been observed.



PoA.1.19

Development and Characterization of Graphene Derivative-GelMA Hybrid Bioinks for the Generation of Bioartificial Tissue Substitutes via 3D Bioprinting

María del Prado Lavin-López[1], Oscar Darío García-García[2,3], Fernanda Condi de Godoi[4], Bart Thewissen[4], Mercedes Griera-Merino[5], Sergio de Frutos[5,6], José Manuel Baena[7,8], Víctor Carriel[2], Diego María Rodríguez-Puyol[5,9], Noelia Campillo[7,8]

[1]Graphenano SL, Murcia, Spain. [2]Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain. [3]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain. [4]Tessenderlo Innovation Center and PB Leiner, Tessenderlo Group nv, Brussels, Belgium. [5]Graphenano Medical Care SL, Madrid, Spain. [6]Unit of Physiology, Department of Systems Biology, School of Medicine, University of Alcalá, Madrid, Spain. [7]REGEMAT 3D SL, Granada, Spain. [8]BRECA Health Care SL, Granada, Spain. [9]Department of Medicine and Specialized Medicine, School of Medicine, University of Alcalá, Madrid, Spain

Abstract

Introduction: 3D bioprinting (3DBP) is a promising alternative based on the automated deposition of cell-laden biomaterials (bioinks) for the production of allograft tissues to alleviate the global demand. Gelatin methacrylate (GelMA) hydrogels have shown great potential due to their high biocompatibility, biodegradability and tunable mechanical properties. Nonetheless, the supplementation of GelMA with additives may confer superior mechanical and biological performance for 3DBP tissue engineering (TE) applications. The aim of this work was to develop and characterize novel graphene derivative-GelMA hybrid hydrogels tailored for extrusion-based 3DBP. Methods: ClaroTM BG800 GelMA (PB Leiner) was blended with BioGraph® (Graphenano Medical Care), a highly-purified graphene-derivative. BG800 prepolymer concentration in the hydrogel was maintained either at 5% (BG800-5) or 10% (BG800-10)(w/v), while growing concentrations of BioGraph (up to 0.3% w/v) were tested. All formulations contained 0.1% (w/v) LAP photoinitiator. Rheological characterization was conducted in a MCR302 rheometer (Anton Paar) equipped with a light source providing an irradiance of 60 mW/cm2 at 405 nm. Printability tests were performed in a REG4Life bioprinter (REGEMAT 3D) equipped with a refrigerated syringe and a 405 nm photocuring module. The biocompatibility of BG800 and BioGraph®-BG800 was investigated in 3D constructs containing rat adipose-derived mesenchymal stem cells. Cell viability and proliferation were assessed via Live/Dead and WST-1assays.
Results: Hybrid hydrogels containing BioGraph® concentrations up to 0.1% are compatible with extrusion-based 3DBP systems, showing good extrudability with reduced clogging at the printing temperatures, effective photocrosslinking at the irradiances tested, high shape-fidelity and high resolution of the printed scaffold. In situ photocrosslinking tests revealed that GelMA concentration had a positive effect on both the speed of photocrosslinking and the stiffness of the cured matrix, while high concentrations of BioGraph® caused significant reduction in the storage modulus (G'). The sample BG800-10 depicted the highest storage modulus (G'=72±5 kPa), while BG800-5+0.3% BioGraph® showed the lowest (G'=13±4 kPa). *In vitro* studies indicated that BioGraph® content ≤0.1% did not have an adverse impact on cell viability and proliferation, and high proliferation rates were observed with BioGraph®. Conclusion: The Biograph®-BG800 hydrogels developed in this work show superior biological and rheological properties, tailored for extrusion-based 3DBP. Their tunable mechanical properties make them ideal for a wide range of applications, including TE of soft and hard tissues.
Funding: Study financed by Grant CPP2021-009070 and FIS P20-0318. N.Campillo is granted by PTQ2019-010731.

PoA.1.20

Chemically Crosslinked Shear-thinning and Self-healing Collagen Hydrogel for Biomedical Applications

Mahsa Jamadi[1], Sareh Soroushzadeh[2], Ardeshir Talebi[3], Ayan Samanta[1]

[1]Macromolecular Chemistry, Department of Chemistry – Ångström, Uppsala University, Uppsala, Sweden. [2]Pathology Department, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, Islamic Republic of. [3]Pathology Department, School of Medicine, Isfahan University of Medical Sciences,, Isfahan, Iran, Islamic Republic of

Abstract

Introduction. The development of injectable hydrogels with self-healing properties as scaffolds for delivering cells and bioactive molecules is highly desirable. The Diels-Alder (DA) reaction between furan and maleimide has been proposed as an in situ forming cross-linking strategy for developing biomolecule or cell-laden hydrogels. The covalent bonds formed by the DA reaction have the unique property of being dynamic under physiological conditions, which can give the resulting hydrogels self-healing properties. In this research, for the first time, we have developed an injectable, self-healing collagen hydrogel that could be crosslinked in situ using DA reaction.

Method. Collagen was modified with furfuryl to install a diene. The furan-modified collagen was combined with 8-arm PEG-maleimide, acting as dienophiles, to form stable hydrogels through DA reaction. To evaluate the self-healing properties of hydrogels, precursor components were first mixed in a syringe mixing system and kept for curing in the syringe for 48 h at room temperature. Afterwards, the crosslinked hydrogel in the syringe was extruded and cast between two glass slides that were separated by a 1 mm spacer (Figure 1A). To evaluate the hydrogels' shear-thinning properties, all hydrogel components were mixed, and the crosslinking reaction was allowed to proceed in the syringe, followed by extrusion onto the rheometer plate. Then after, viscosity under continuous flow was measured by increasing the shear rate.

Results. An essential feature of DA cycloadditions is the DA adduct's ability to undergo mechanically induced cycloreversion when placed under force. We believe the shear force during injection acts as a force-actuator, resulting in a mechanochemical decoupling of the furan-maleimide DA adduct and concomitant release of the polymer chains. As such, the slow crosslinking via DA reaction and the force-induced retro DA reaction allow the hydrogels to be injectable at least up to 48 hours post-mixing of the hydrogel components (Figure 2A). Extruded samples could recover to form a homogeneous hydrogel (Figure 1B). As shown in figure 2B, the storage modulus and loss modulus is promisingly recovered after healing. Physical crosslinks, including entanglements, hydrogen bonding, and hydrophobic interactions, also play a role in the self-healing properties of developed hydrogels.

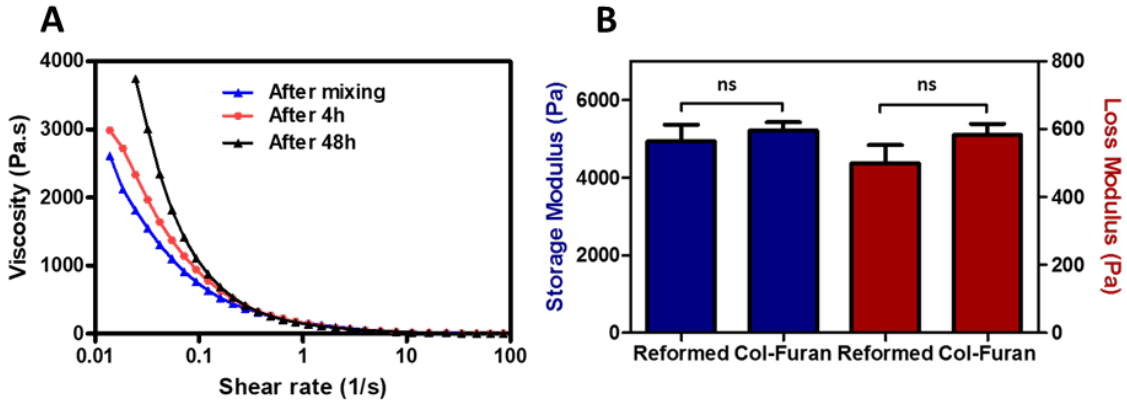


Figure 1. A. Schematic of the self-healing experiment; B. Photograph of the reformed hydrogel.

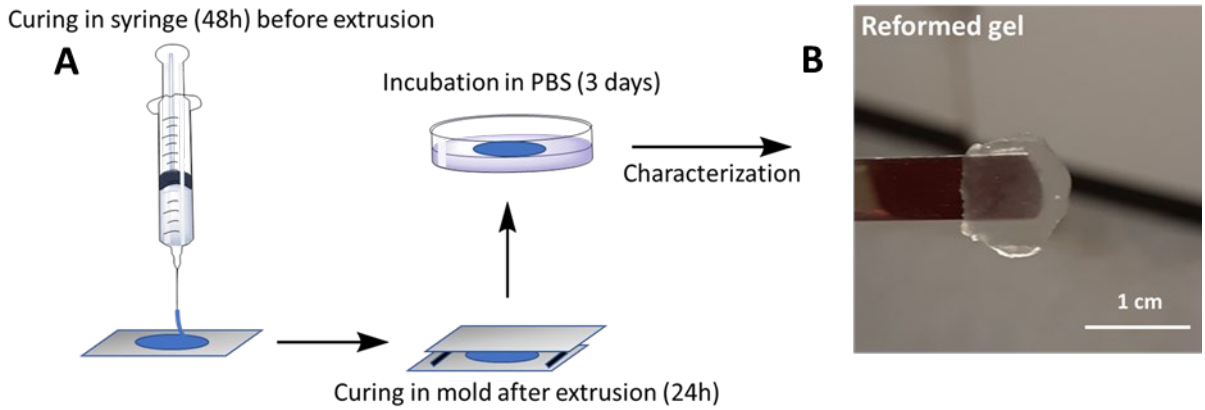


Figure 2. A. shear-thinning properties of developed hydrogels; B. The storage and loss moduli at 1 % strain of reformed samples compared to original samples.

PoA.1.21

3D bioprinted gellan gum/kappa-carrageenan scaffolds for enhanced angiogenesis

Konstantinos Loukelis[1], Varvara Platania[1], Nikos Koutsomarkos[1], Maria Chatzinikolaidou[1,2]

[1]Department of Materials Science and Technology, University of Crete, Heraklion, Greece. [2]Foundation for Research and Technology Hellas (FORTH)-Institute of Electronic Structure and Laser (IESL), Heraklion, Greece

Abstract

A variety of different research studies have emerged focusing on the construction of bioinks, which are 3D printable structures comprising cells combined with biocompatible materials. Kappa-carrageenan (KC) is a natural linear polysaccharide derived from red seaweed with remarkable biocompatible properties. Gellan gum (GG) is another biomaterial with strong gelling capabilities that promotes cell adhesion. This study focuses on the fabrication of bioinks containing kappa-carrageenan and gellan gum by high precision extrusion 3D bioprinting for the production of constructs with specific geometry that facilitate a prime environment for endothelial tissue growth. Two different blend compositions, 4% w/v GG with 1.5% w/v KC (GG_1.5KC), and 4% w/v GG with 2% w/v KC (GG_2KC) were prepared in ultrapure water and allowed to stir at 90 ^{o}C for 4 h to efficiently homogenize. Subsequently, the blends were mixed at room temperature with cell suspensions of 5x106 cells/ml. Live/dead assay has been conducted with L929 fibroblasts for 7 and 14 days to determine the cell viability and proliferation status inside the bioinks, while also the biodegradation rate of the scaffolds was assessed oved a period of 21 days. Collagen deposition as a marker of ECM formation was validated after 7 and 14 days. Additionally, rheological analysis was performed to deduce the printability and the mechanical properties of the scaffolds, including dynamic strain sweep (DSS), dynamic frequency sweep (DFS), viscometry, as well as recovery rate of viscosity after the application of 200% strain. Cell viability in GG-1.5KC bioinks was over 90% at days 7 and 14. At day 14, both bioinks had formed an extracellular matrix as validated by the collagen production. The biodegradation rate ranged between 19 and 37% for the GG_1.5KC composition and 21 and 26% for the GG_2KC. Both blends indicated shear-thinning behaviour, a characteristic that can enhance extrusion bioprinting and reduce cell death during the printing process. The DSS experiments revealed that the GG_1.5KC composition had a yield point at 18 kPa and the GG_2KC samples at 28 kPa. Both concentrations had comparable loss tangent factor values (tanδ) in the area of 0.08-0.12. Moreover, the recovery percent of the viscosity after the application of the 200% strain was found to be at 97% and 94% for the GG_1.5KC and GG_2KC, respectively. These results indicate the suitability of these novel constructs as potential medical devices for endothelium tissue engineering.

PoA.1.22

Hemocompatibility assessment of biomaterials commonly used in 3D bioprinting of organs and tissue models with a flow system.

Marta Klak[1,2], Oliwia Janowska[1], Katarzyna Woźniak[1], Natalia Wisińska[1], Sylwester Domański[1], Andrzej Berman[1,2,3], Michał Wszoła[1,2,3]

[1]Polbionica Sp. z o.o., Warsaw, Poland. [2]Foundation of Research and Science Development, Warsaw, Poland. [3]Medispace Sp. z o.o., Warsaw, Poland

Abstract

Introduction: Blood-biomaterial interactions can activate the coagulation cascade. Therefore, it is crucial for medical devices, such as vascular prostheses, stents, catheters, valves, as well as bioinks used for 3D bioprinting of bionic organs, have hemocompatible properties. Biomaterials should allow direct contact with blood without causing side effects such as thrombosis, and platelet activation. Understanding the interaction of blood components and material surfaces is essential for the development of a new hemocompatible biomaterial for medical applications.
Material and methods: The tests were carried out on pig whole blood taken without anticoagulant. Hemocompatibility studies were performed on the following biomaterials: methacrylated gelatin (GELMA), methacrylic hyaluronic acid (HAMA); methacrylated alginate (ALGMA), methacrylated chitosan (CHIMA), biomaterials based on extracellular matrix (dECM), methacrylic extracellular matrix (ECMMA), pluronic (PLU), alginate (ALG), hyaluronic acid (HA) and PEGDA. A positive control (maximal hemolysis) was also performed. The blood was added to the biomaterials. The samples were incubated at 10 time intervals: 1,3,5,8,10,12,15,20,25 and 30 minutes. After this time, 1ml of water was added. The absorbance reading was taken at 540nm. The higher the absorbance value, the higher the concentration of hemoglobin, which means less blood clotting on the surface of the biomaterial.
Results: It has been shown that the tested materials differ significantly in their hemocompatibility properties. The lowest absorbance was shown for PLU-coagulation proceeded immediately. Thus, it was shown that this material, despite very good support properties when printing bionic organs, is not suitable for constructs that intended to be implanted into a living organism. At the 10th minute of the experiment, the degree of hemolysis was significantly lower in the case of: ALG and PEGDA- 2 biomaterials commonly used in 3D bioprinting. The highest degree of hemolysis was found in dECM-based bioinks. The difference from the control samples was in the range of 10-15% with no visible clots on macroscopic examination. In the case of application of HAMA, the degree of hemolysis was 2-fold greater than in the case of non-methacrylated hyaluronic acid. It seems interesting that the methacrylation process may have a significant impact on the hemocompatible properties of biomaterials.
Conclusion: The conducted research has shown that not every material, despite preferential physicochemical properties and a beneficial effect on the viability and functionality of cells in *in vitro* tests, will be suitable for bioprinting of bionic organs having direct contact with blood after implantation.

PoA.1.23

Biomaterials Based on the Extracellular Matrix as the Future of Tissue Engineering. Is There an Upper Limit on the dECM Content in Bioprinting Tissue?

Marta Klak[1,2], Dominika Ujazdowska[2], Anna Filip[2], Tomasz Dobrzański[1], Sylwester Domański[1], Oliwia Janowska[1], Katarzyna Woźniak[1], Kamila Jankowska[2], Daria Nitarska[1], Joanna Rogalewicz[1], Andrzej Berman[1,2,3], Michał Wszoła[1,2,3]

[1]Polbionica Sp. z o.o., Warsaw, Poland. [2]Foundation of Research and Science Development, Warsaw, Poland. [3]Medispace Sp. z o.o., Warsaw, Poland

Abstract

Introduction:The most popular biomaterials in tissue engineering are those based on the extracellular matrix (dECM). They are characterized by a natural composition and complex biochemical properties. However, what is the most optimal dECM concentration in the bioink? Does more mean better? The aim of this study was to find answers to these questions.
Methodology:Two types of biomaterials were prepared - bioink precursors, made on the basis of ECM derived from decellularization process of pancreas. They were prepared using enzymatic methods. The final bioink contained 2%-16% dECM. The variants with 16%,14%,13%,11%,10%,8%,5% and 2% of dECM were analyzed. The assessment of cell viability, functionality was performed using a glucose stimulation test (GSIS test conducted for 21 days), cytotoxicity (MTT test) and histological evaluation. Tests were performed on β-cells (INS-1E).
Results:With all 8 variants it was possible to bioprint homogeneous, stable constructs. In case of increasing dECM content, the necessity to use higher pressures and temperature. Pressure was 6-48kPa and temperature was 16-25°C. Also, to obtain a better print resolution, the printing speed was 5-20 mm/s. The next step was to evaluate the cytotoxicity of the analyzed bioinks. The least favourable results were obtained for variants above 13% dECM content. The most positive results for viability were shown with a dECM content up to 11%. The results showed a significantly higher percentage of viability depending on the tested variant, the difference was even 5% lower survival of β-cells. The addition of dECM in the range of 8-11% showed even 25% higher viability than in the group of untreated cells.
The functionality of β-cells was the highest in the case of the bioink containing up to 11% dECM. Histological analysis confirmed the advantage of bioinks up to 11%, showing a 3-fold increase in insulin signal. In these analyzes, the 5% and 8% variants also showed a stronger signal for insulin secretion.
The results confirm that optimal content of dECM in bioprinted constructs should vary from 5-10%. These results are confirmed by a cascade of biological research.
Conclusions:The experiments carried out in this study prove that the appropriate concentration of dECM must be properly validated in case of tissue engineering. In case of β-cells, it shouldn't exceed a total of 10%. It should be remembered that dECM serves not only as physical protection for cells in the native environment, but also helps in various biological functions.

PoA.1.24

Fabrication of multi-scale biomimetic vascular networks via microfluidic bioprinting

Nehar Celikkin[1], Maria Celeste Tirelli[1], Marco Costantini[2]

[1]Institute of Physical Chemistry, Polish Academy of Sciences,, Warsaw, Poland. [2]Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

Abstract

Organ and tissue loss due to disease and injury is inevitable, and the current medical solutions are only based on organ transplantation and artificial prosthetic organs. Yet there is no perfect solution for patients who suffer organ and tissue loss; tissue engineering, an interdisciplinary field that applies engineering and life science principles to promote regeneration, was proposed to restore diseased/injured tissues with engineered tissues potentially. Despite the clinical success in several studies with engineered tissue substitutes, the success of the clinical trials in the field is mainly precluded due to the lack of vascularization. Unfortunately, without a functional vascular network, the nutrient and gasses' diffusion is limited to a couple of hundred micrometers. Thus, apart from the avascular tissues, the design of the built-in vascular network should also be considered for each engineered tissue. Moreover, when designing the vascular network for relevant vascular maturation and endothelization, creating a biomimetic architecture, designing the biomaterial, and specifying the manufacturing strategy should be considered as the key players of this task.

Here, we present a microfluidic enhanced 3D printing technique for biomimetic decellularized matrix-based (dECM) biomaterials inks to fabricate omnidirectional multi-scaled vascular networks. During the printing, the low-viscosity, thermoresponsive dECM hydrogels were extruded through the presented microfluidic extrusion nozzle in an agarose-based support bath to ensure spatial resolution. The thermally crosslinked scaffolds exhibited suitable mechanical stability and an ideal microenvironment for endothelial cell proliferation, spreading, and neo-vascular tissue formation. Additionally, particular attention was paid to the assembly of perfusable vascular networks, which was an essential feature for the future integration of the proposed strategy into more complex *in vitro* tissue models.

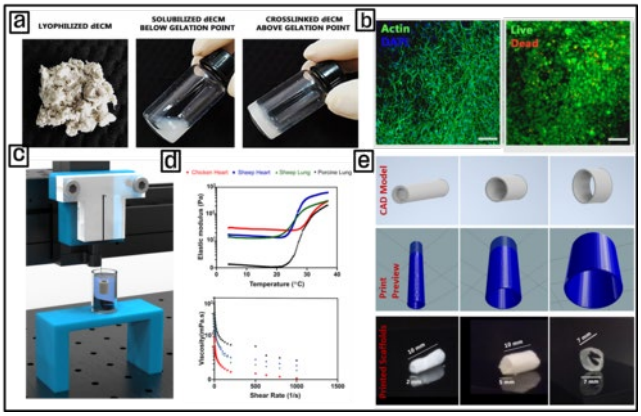


Figure 1. decellularization of highly vascularized tissues (a), cell elongation and cell viability (b), representation of 3d printer and sacrificial agarose gel (c), rheological properties of solubilized dECM biomaterial inks (d), 3d printed tubular constructs (e)

Acknowledgements

This study is also supported by the National Science Centre Poland (NCN) within PRELUDIUM 19 Project No. 2020/37/N/ST5/03272 to NC.

PoA.1.25

Design of nanofiber-reinforced bioinks for the engineering of hierarchical anisotropic tissues

Gerardina Ruocco, Elena Marcello, Irene Carmagnola, Valeria Chiono

Politecnico di Torino, Turin, Italy

Abstract

Anisotropy is a key architectural feature of many tissues such as cardiac, smooth muscle and nerve tissue, giving them directional-specific properties. Structural anisotropy emerges from the oriented disposition of ECM filaments and/or cells and has been proven to guide cell differentiation, proliferation and functional organization [1]. The ability to provide cells with topographical stimuli in 3D engineered structures is a key goal for the *in vitro* modeling of anisotropic tissues.
The work aimed at the design of bioprinted scaffolds with topographical cues able to induce cell alignment. An Alginate/modified Gelatin (Alg/Gel) bioink was exploited, filled with polycaprolactone nanofibers, longitudinally aligned along bioink filaments through shear stresses.
Aligned NFs were fabricated through electrospinning, then they were mechanically pre-fragmented by homogenization and chemically reduced into short and separated fibers through hydrolysis. Fragmented NFs (f-NFs) were surface modified by type A Gelatin (f-NFs-G) in order to increase wettability and biomimicry. f-NFs-G dimensions were investigated by SEM and confocal microscopy while the effectiveness of G grafting was analyzed by FTIR analysis and BCA assay. f-NFs-G were added to Alg/Gel solution and then mixed through a double-syringe system (Alg/Gel/f-NPs-G). The resulting bioink was printed by R-GEN 200 Bio-Plotter and double crosslinked by Ca2+ and Genipin. The effect of different f-NFs-G content on Alg/Gel/f-NPs-G hydrogel rheological properties and stability was studied. Alignment degree of embedded f-NFs-G along micro-extruded filaments was demonstrated by confocal microscopy. Finally, Alg/Gel/f-NPs-G bioink embedding C2C12 cells was bioprinted into grid-shaped scaffolds and cell organization and maturation were investigated.
SEM analysis demonstrated the production of homogenous f-NFs with an average diameter of 200 nm and a length lower than 50 µm. f-NFs were successfully integrated in the Alg/Gel/f-NPs-G bioink showing good printability. The presence of f-NFs, increased hydrogel viscosity and improved bioink shear thinning properties. 3D grid-shaped scaffolds with good shape fidelity were successfully bioprinted (Figure 1) with embedded C2C12 with aligned distribution following nanofiber topographical cues.
In conclusion, we successfully designed Alg/Gel/f-NPs-G bioinks for prospective *in vitro* engineering of anisotropic tissues. The use of such innovative bio-inks may provide the cells with topological cues in an engineered 3D microenvironment, promoting specific cell responses.

[1] Datta et al, Journal of Bionic Engineering, 16, 842–868 (2019).

ACKNOWLEDGEMENTS
BIORECAR project founded by European Research Council, (Horizon 2020, No 772168).

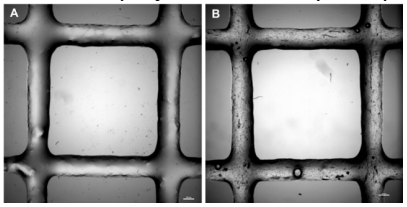


Figure 1 Printed bio-inks without (A) and with (B) f- NFs.

PoA.1.26

s-Hep3Gel: an ECM-based ink for the biofabrication of *in vitro* models of hepatic steatosis

Giuseppe Guagliano[1], Cristina Volpini[2], Francesco Briatico-Vangosa[1], Livia Visai[2], Paola Petrini[1]

[1]Department of Chemistry, Materials, and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy. [2]Molecular Medicine Department (DMM), Centre for Health Technologies (CHT), UdR INSTM, University of Pavia, Pavia, Italy

Abstract

Introduction: Aiming to lean the drug development pipeline, numerous 3D models of the liver have been proposed in recent years, but most of them mimic the physiological conditions of the organ. Conversely, pathological-like complex *in vitro* models are less diffused and are still in their infancy. [1] Here we propose an ECM-based bioink, specifically engineered to model severe stages of hepatic steatosis.
Materials and methods: s-Hep3Gel is produced through internal gelation of alginate, by sequentially mixing 4 parts of hydrogel precursor solution, 1 part of 1.5% (w/v) CaCO3 suspension, 1 part of HepG2 suspension (3 x 106 cells/ml), and 1 part of 7% (w/v) of GDL solution. All solutions were prepared in HepG2 medium. The hydrogel precursor solution was prepared dissolving 7.5% (w/v) alginate and 1 mM Na-oleate into a 1.4% (w/v) suspension of porcine liver ECM powder. [2]
The concentration of Na-oleate was optimized combining viability analyses of 2D HepG2 cultures exposed to different oleate concentrations, with qualitative microscopies to observe the formation of intracellular lipid vesicles. Crosslinking kinetics of s-Hep3Gel were characterized through time-sweeps rheological measurements. Its viscoelastic properties were characterized as a function of the time through discretized frequency-sweeps analyses. Printability of s-Hep3Gel was optimized as a function of the time both *a priori*, through the combination of two custom-made rheological tests, and *a posteriori*, through the analyses of different shape-fidelity parameters. The viability of HepG2 cells within 3D-bioprinted constructs and the formation of intracellular lipid vesicles were characterized up to 5 days in culture.
Results and discussion: The final viscoelastic properties of s-Hep3Gel mimic the ones characterizing the pathological tissue. The presence of Na-oleate in the bioink causes the formation of intracellular lipid vesicles, comparable to those featuring the hepatic steatosis *in vivo*, and causes a decrease of cell viability that is comparable with pathological scenarios.
s-Hep3Gel displays optimal printability when the crosslinking is not complete. The printability was therefore optimized following a reactive bioprinting approach [3] to fabricate cell-laden constructs.
Conclusions: s-Hep3Gel allows to fabricate 3D models of hepatic steatosis that are chemomechanically relevant. Further studies are needed to explore its effects on more metabolically relevant cell types (*e.g.,* HepaRG, iPSC-derived hepatocytes), as well as in the case of coculture with other hepatic cell types (*e.g.,* stellate cells).

References

[1] *Macromol. Biosci. 22 2200264*
[2] *ACS Biomater. Sci. Eng. 9 211–29*
[3] *Soft Matter 17 8105–17*

PoA.1.28

3D-printed biomimetic hydroxyapatite scaffolds with triply periodic minimal surface geometries by direct ink writing

Laura del-Mazo-Barbara[1,2], Anna Diez-Escudero[1], Irene Lodoso-Torrecilla[1], Morteza Aramesh[3,4], Cecilia Persson[3,4], Maria-Pau Ginebra[1,5]

[1]Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Engineering and Research Centre for Biomedical Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain. [2]Additive Manufacturing for the Life Sciences Competence Center (AM4Life), Uppsala, Spain. [3]Department of Materials Science and Engineering, Division of Biomedical Engineering, Uppsala University, Uppsala, Sweden. [4]Additive Manufacturing for the Life Sciences Competence Center (AM4Life), Uppsala, Sweden. [5]Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology, Barcelona, Spain

Abstract

Triply periodic minimal surfaces (TPMS) have attracted great interest in the recent years as nature-inspired models. Specially, they allow designing biomorphic scaffolds, with pore architectures close to the structure of trabecular bone, with interconnectivities and surface curvatures that are favourable for cell growth, migration, and vascularization. This project explores the feasibility of 3D printing three types of TMPS structures by Direct Ink Writing, using a self-setting calcium phosphate ink, and analyses the effect of pore architecture on the physicochemical properties and *in vitro* response of pre-osteoblastic cells (OB).

Cylindrical TPMS models of Gyroid (G), Diamond (D) and Schwarz (S) structures were created by Matlab codes, with different porosities. The ink consisted of a 30 wt.% Pluronic hydrogel loaded with 70 wt. % of α-tricalcium phosphate powder. After printing, the scaffolds were immersed in water at 37ºC to harden them by conversion to hydroxyapatite. After optimising the printing, the scaffolds were analysed by microcomputed tomography and tested mechanically in uniaxial compression. The microstructure and composition were assessed by X-ray diffraction and scanning electron microscopy respectively. Their effect on blood permeability was assessed as well as their influence on Saos-2 OB cell adhesion, morphology, proliferation, differentiation and mineralization. Orthogonal-patterned (OP) scaffolds with the same porosity were used as control.

The three TPMS geometries were successfully printed with a porosity around 20 %, resulting in interconnected, more concave and relatively larger pores compared to the control, therefore, more attractive for cells. A reduction of the compressive strength was observed for the TPMS scaffolds compared to the OP, although the fracture mechanism evolved from brittle column splitting for the OP to progressive compaction, especially for S (Fig. 1A). The interconnected pores in the TPMS geometries resulted in an outstanding improvement in blood permeability, 5-, 22- and 65-fold for the D, G and S respectively (Fig. 1B). The pronounced increase for the S is associated to the large and vertical pores present in this structure. Pore architecture also affected the in-vitro response of OB cells. Higher adhesion was found for G, D (Fig. 2), and S, resulting in 2 to 5-fold increase compared to OP. After 21 days of OB proliferation (Fig. 2), D samples depicted the highest proliferation rate and ALP differentiation. These findings correlate well to the blood permeability trends observed, and which could be the reason for the similarity in OB proliferation and differentiation rates.

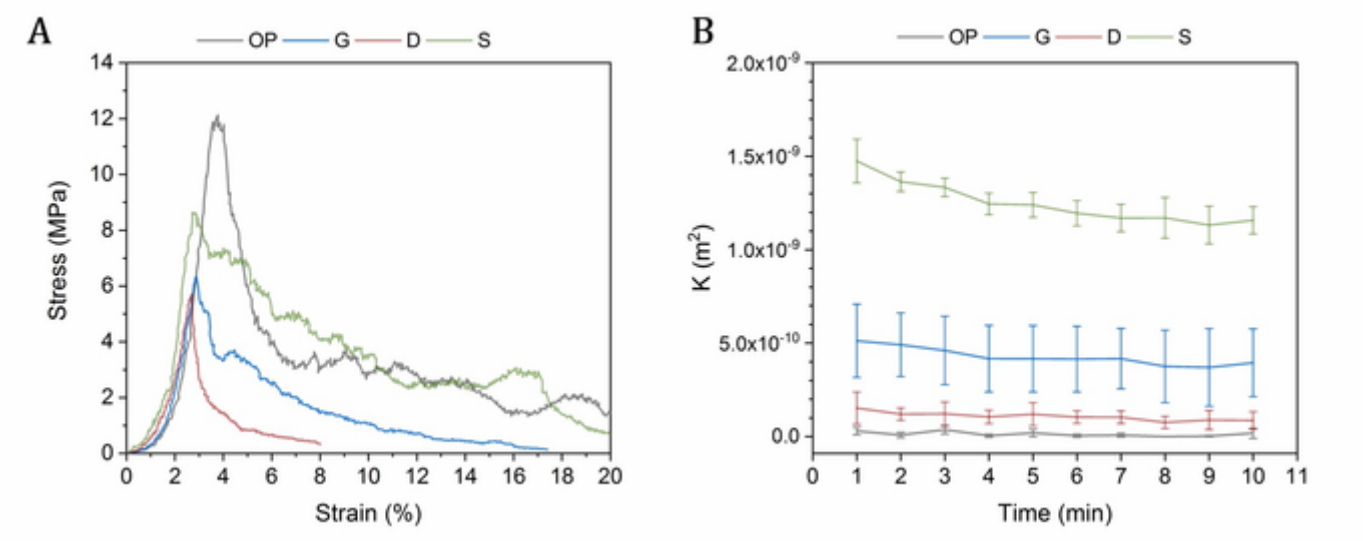


Figure1: A) compression and B) permeability.

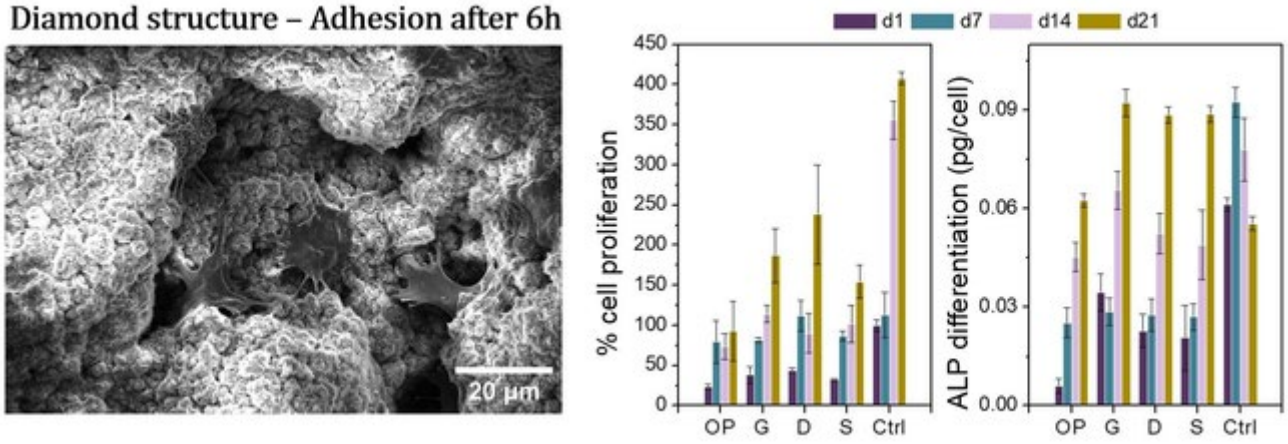


Figure2: In-vitro response.

PoA.1.29

Bioprinting models of early organ morphogenesis using cell-responsive hydrogels

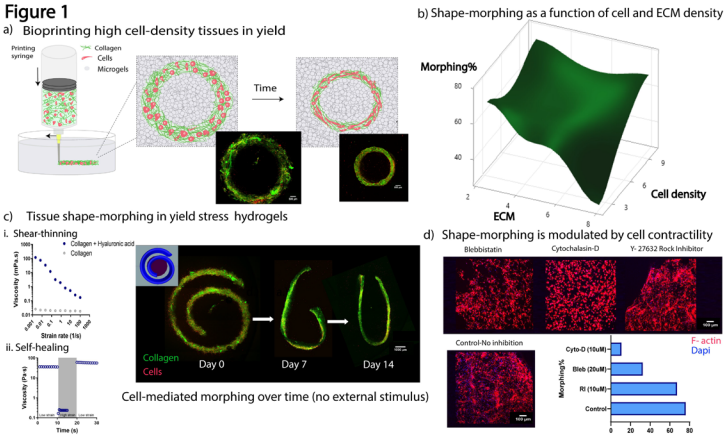
Ankita Pramanick[1], Abhay Pandit[1], Andrew Daly[1,2]

[1]CÚRAM - Science Foundation Ireland Research Centre for Medical Devices, Galway, Ireland. [2], Biomedical Engineering, University of Galway, Ireland, Galway, Ireland

Abstract

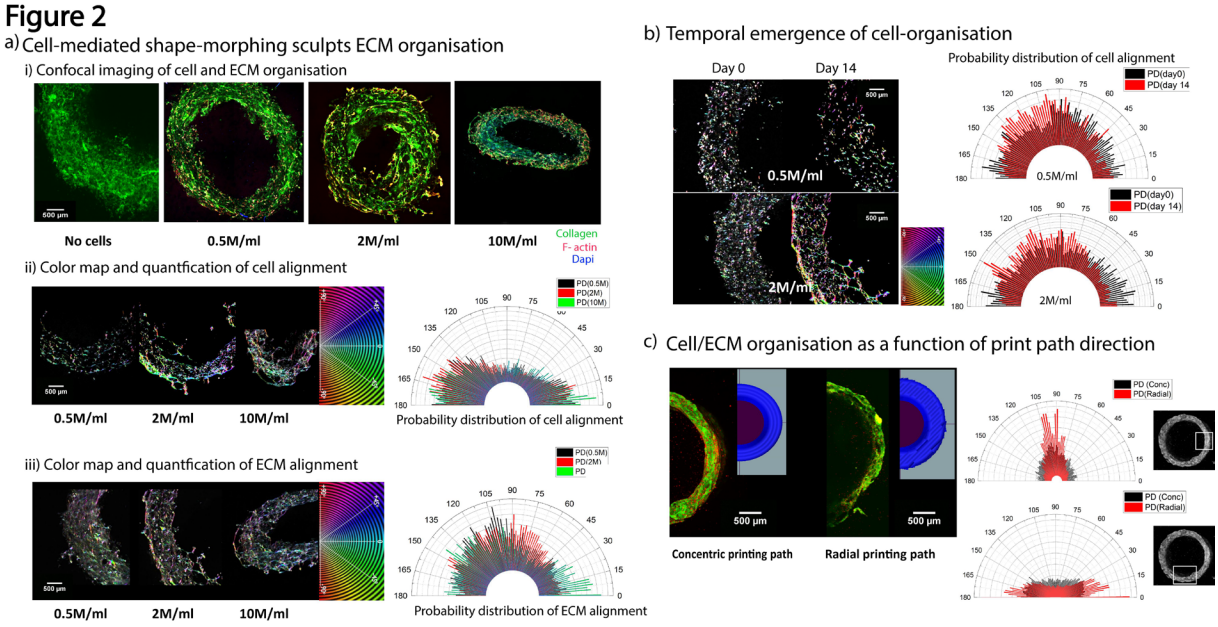
INTRODUCTION: During embryogenesis, organs undergo shape-transformations that sculpt their final composition and function[1]. Organ shape-morphing behaviours are driven by spatiotemporal variations in cellular behaviours such as proliferation and active contractile forces[2]. Despite this, existing bioprinting technologies typically employ static bioinks that restrict the cellular behaviours that drive morphogenesis. To overcome this limitation, the objective of this work was to develop a bioprinting platform for fabricating tissues that undergo programmable shape-morphing driven by cell-generated forces. To do this, we used embedded bioprinting to deposit ECM-based bioinks in yield-stress support hydrogels that can locally fluidize to accommodate shape-morphing. The platform was used to explore how cellular contractile-forces influence shape-morphing across tissue geometries ubiquitous in early organ development. METHODS: ECM bioinks (collagen and hyaluronic acid) were deposited into granular support hydrogels using embedded bioprinting to create a range of developmentally inspired tissue geometries (e.g., sheets, tubes). Tissue shape-morphing was studied across varied cell (2-10M/ml) and ECM (2.4-8mg/ml collagen) concentrations, and cell types (fibroblast/endothelial/epithelial/iPSC cells), based on full-factorial design. To explore shape-morphing mechanisms, tissues were treated with inhibitors that disrupted actomyosin machinery. Confocal microscopy was used to map shape-morphing patterns at the cell/ECM/tissue scales. RESULTS: Following deposition into the yield-stress support hydrogel, bioprinted constructs underwent significant shape-morphogenesis during culture, with distinct variations in morphing patterns depending on the initial print geometry. Importantly, the extent of shape-morphing behaviour was tunable, with increased shape-change observed at low collagen concentrations and high cell densities. Treatment with inhibitors of actomyosin contractility inhibited shape-morphing (Fig 1).

Figure 1



Interestingly, shape-morphing patterns influenced internal cell-ECM organization, with circumferential alignment emerging in shrinking tubes. We confirmed that morphing patterns were responsible for sculpting cell/ECM alignment, with circumferential reorganisation emerging irrespective of the print path employed (Fig 2).

Figure 2



DISCUSSION/CONCLUSION: By combining ECM bioinks with yield-stress support hydrogels we developed a bioprinting platform for exploring how cell-generated forces drive shape-morphogenesis in developing tissues. Tissue-scale shape-morphing behaviour depended on the relative cell/ECM density, along with the initial geometrical features. Importantly, shape-morphing patterns drove changes in internal cell and ECM alignment, which provides a new tool for programming tissue and organoid maturation.

ACKNOWLEDGEMENTS: The project was funded by
1) SFI (co-funded under the European Regional Development Fund 13/RC/2073_P2)
2) ERC Starting Grant (Project 101077900),
3) Irish Research Council GOIPG.

REFERENCES:

1. Xiong et al. Cell (2014)
2. Stooke-Vaughan et al. Review Curr Opin Genet Dev.(2018)

PoA.1.31

Cell-laden biomimetic tissue-engineered blood vessels with Human Induced Pluripotent Stem Cells-derivatives

Jagoda Litowczenko-Cybulska[1,2], Martyna Michalska[3], Karine Tadevosyan[1,2], Laura Casado[1,2], Léa Pourchet[1,2], Ioannis Lazis[1,2], Jose Carlos Rodriguez-Cabello[4], Ioannis Papakonstantinou[5], Angel Raya[1,2,6]

[1]Regenerative Medicine Program, Bellvitge Institute for Biomedical Research (IDIBELL) and Program for Clinical Translation of Regenerative Medicine in Catalonia (P-CMRC), L'Hospitalet del Llobregat, Spain. [2]Centro de Investigación Biomédica en Red—Bioingeniería, Biomedicina y Nanomedicina (CIBER-BBN), Madrid, Spain. [3]Department of Mechanical Engineering, University College London, London, United Kingdom. [4]BIOFORGE (Group for Advanced Materials and Nanobiotechnology), University of Valladolid, Valladolid, Spain. [5]Photonic Innovations Lab, Department of Electronic & Electrical Engineering, University College London, London, United Kingdom. [6]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Abstract

Cardiovascular diseases, responsible for 17.9 million fatalities annually, are the leading cause of death worldwide. While they affect people of all ages, those aged 60 years or older are particularly vulnerable, with one-third of deaths occurring prematurely. Vascular diseases caused by thrombosis, atherosclerosis, or aneurysms are a leading cause of death and require timely treatment to prevent life-threatening conditions. The standard approach is graft transplantation using autologous or synthetic grafts to bypass and replace diseased vascular segments [1]. Synthetic/allogeneic graft replacements have disadvantages such as lack of ability to grow, repair/remodel, potential thrombogenicity, furthermore some patients cannot receive grafts due to the small size of the target vessels or a lack of suitable autologous tissue [2]. Vascular tissue engineering offers a promising approach for constructing blood vessels but requires a large amount of human endothelial cells. Induced Pluripotent Stem Cells (iPSCs) can solve this challenge as they can be derived from a person's own cells and differentiated into blood vessel cell types. 3D bioprinting – a versatile tool of tissue engineering capable of generating biocompatible vessel grafts with growth potential – is particularly powerful in developing anatomically structured grafts for a range of patients including those with arterial tortuosity. However, a successful blood vessel mimicry with 3D cell-laden vascular grafts remains a challenge that could be possibly addressed by the development of a bioink consisting of extracellular matrix proteins that can be tuned to support the development, elasticity and organization of the target tissue [3].

Here, we present a unique approach that combines the hierarchical architecture of elastin-collagen-based composites with nano-/micro-patterned cues for luminal surface patterning designed to enhance endothelial cell adhesion and migration [Fig 1]. Remarkably, we achieved nanopatterned tubes with critical dimensions of 300 nm – a persisting challenge in fabrication community – by a combination of nanoimprint and soft lithography. We then tested the ability of two different approaches to utilize iPSC-derived cell-laden hydrogels for the fabrication of tissue-engineered blood vessel conduits. We discuss the accuracy of 3D bioprinting, cell-material interactions, and compare the effects of materials with various Young's moduli and pattern on endothelial cell adhesion [Fig 2].

Acknowledgement

This project received funding from the European Union's Horizon 2020 Research&Innovation program under the Marie Skłodowska-Curie Grant Agreement (nr101025242).

References

1-Ruan Y, et al. BMC Public Health; 2018;

doi:10.1186/s12889-018-5653-9.
2-Gui L, et al. Biomater. 2016; doi:10.1016/j.biomaterials.2016.06.010.
3-Litowczenko J, et al. Bioact Mater. 2021; doi:10.1016/j.bioactmat.2021.01.007.

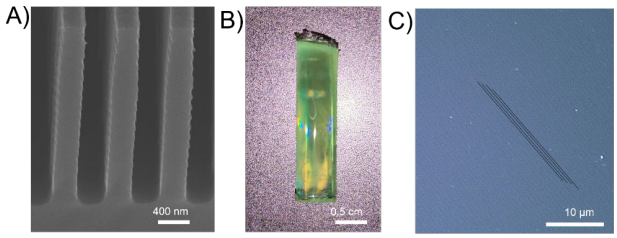


Figure 1. Nanopattern fabrication. A) Scanning electron microscope (SEM) image of silicon nanopatterned master obtained by laser interference lithography and deep reactive ion etching. B) A digital photograph of developed 3D nanopatterned tube stamp replicated from the silicon pattern in (A). C) Optical microscope image of elastin-collagen-based hydrogel stamp, the black lines indicate the direction of topographic paths

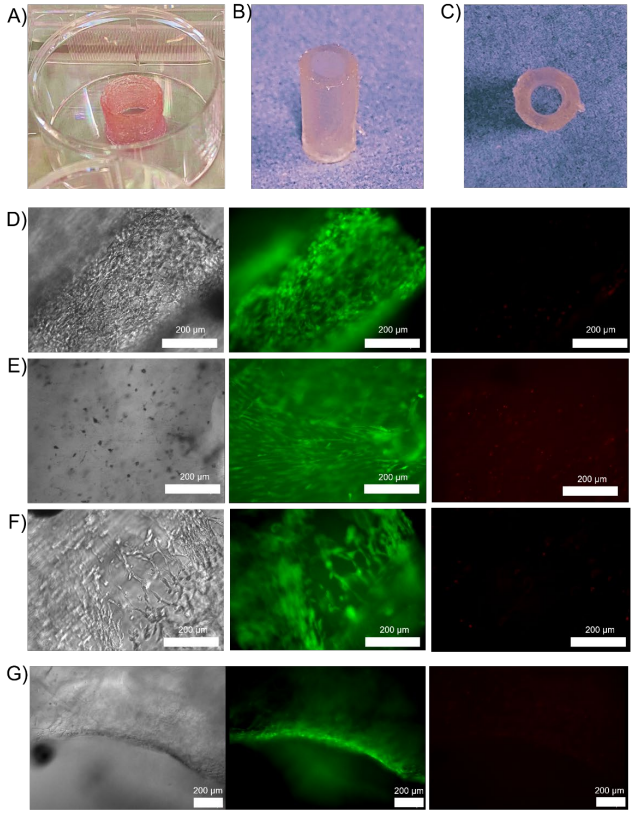


Figure 2. Cell-laden constructs and cells visualisation. Digital photographs of A) 3D printed construct with iPSC-derived fibroblasts, B) tube with the internal pattern, and C) the same tube showcasing the lumen. iPSC-derived cells D) E) fibroblasts in 3D hydrogel constructs obtained by different 3D approaches. iPSC-derived F) endothelial cells cultured on patterned hydrogels, G) endothelial cells cultured on the lumen of the patterned tube visualized by fluorescent microscope with images of stained cells with fluorescein diacetate (FDA, green) and propidium iodide (PI, red).

PoA.1.32

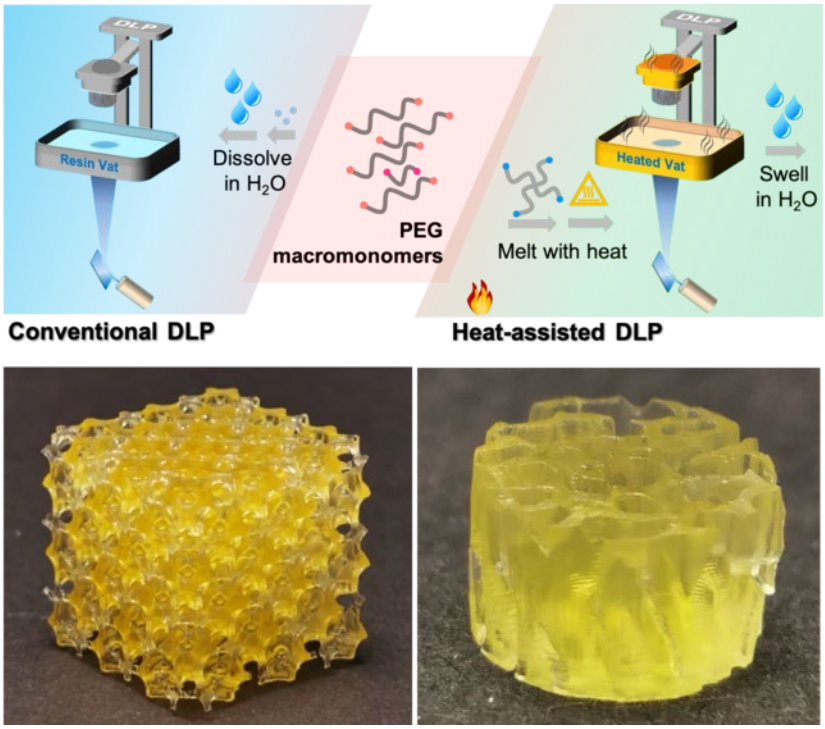
Bioactive Complex PEG Hydrogels via Heat-assisted Digital Light Processing

Safira Noor Anindita, Riccardo Conti, Doris Zauchner, Nevena Paunović, Wanwan Qiu, Marina Green Buzhor, Adva Krivitsky, Zhi Luo, Ralph Müller, Hansjörg Grützmacher, Xiao-Hua Qin, Jean-Christophe Leroux, Yinyin Bao

ETH Zurich, Zurich, Switzerland

Abstract

Digital light processing (DLP) of structurally complex poly(ethylene glycol) (PEG) hydrogels with high mechanical toughness represents a long-standing challenge in the field of 3D printing. Here, we report a 3D printing approach for the high-resolution manufacturing of structurally complex and mechanically strong PEG hydrogels via heat-assisted DLP. Instead of using aqueous solutions of photo-crosslinkable monomers, PEG macromonomer melts were first printed in the absence of water, resulting in bulk PEG networks. Then, post-printing swelling of the printed networks was achieved in water, producing high-fidelity 3D hydrogels with complex structures. By employing a dual macromonomer resin containing a PEG-based four-arm macrophotoinitiator, “all-PEG” hydrogel constructs were produced with compressive toughness up to 1.3 MJ m^{-3}. By this approach, porous 3D hydrogel scaffolds with trabecular-like architecture were fabricated, and the scaffold surface supported cell attachment and the formation of a monolayer mimicking bone-lining cells. This study highlights the promises of heat-assisted DLP of PEG photopolymers for hydrogel fabrication, which may accelerate the development of 3D tissue-like constructs for regenerative medicine.



PoA.1.33

Development of cellulose nanofiber-based 3D printing materials

Sungchul Shin

Seoul National University, Seoul, Korea, Republic of

Abstract

The optimization of the ink material's rheological characteristics is crucial for achieving precise 3D bioprinting, allowing the creation of cell structures using hydrogel materials in any desired shape. Here, we outline the fundamental rheological factors observed during the printing process of cellulose nanofiber (CNF) hydrogels, which include shear thinning, yield stress, and self-healing properties. The CNFs exhibited exceptional shear thinning behavior, and the CNF bioink displayed high viscosity at zero shear and maintained structural accuracy even at low dispensing pressure. Moreover, incorporating CNFs improved the mechanical stability of the bioink, indicating their vital role in enhancing the structural properties of the composite hydrogels. We anticipate that the CNF-based bioink holds promise for future biomedical applications, such as constructing artificial blood vessels and engineered vascular tissue scaffolds.

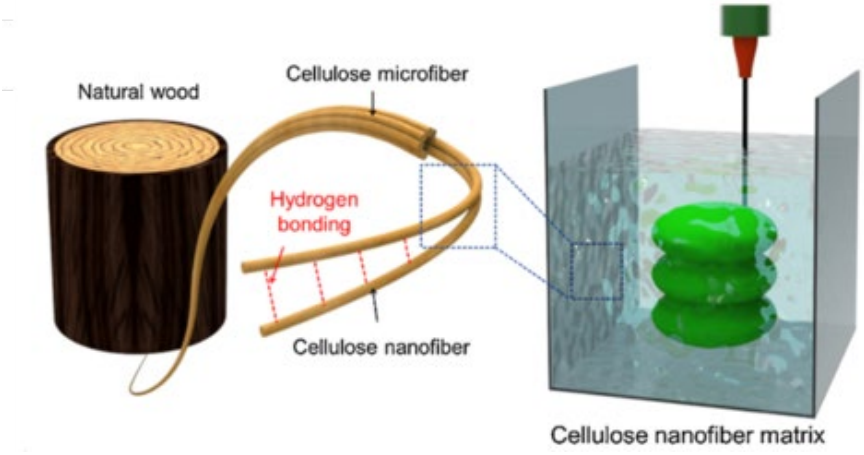


Figure. Schematic of transforming the natural wood pulp into a CNF matrix for 3D printing.

PoA.1.34

3D bioprinted gelatin/hyaluronic acid scaffolds for engineered cartilage

Kyoung Choi, Jun Shik Choi, Chun-Ho Kim, Sang Jun Park

Korea Institute of Radiological and Medical Sciences, Seoul, Korea, Republic of

Abstract

3D bioprinting technology show great potential in tissue engineering as a therapeutic tool. Biocompatible and biodegradable materials like gelatin and hyaluronic acid (HyA) are ideal for use as biomaterial inks in 3D bioprinting. However, non-crosslinked gelatin/HyA (GH) 3D structures lack mechanical strength and are highly soluble in aqueous solutions. To overcome these limitations, the stability and printability of the GH scaffold were improved through cross-linking processes using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride n-hydrate (DMTMM) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS). The size and remaining mass of the crosslinked 3D scaffold were evaluated, and the surface morphology was examined using scanning electron microscopy. Cytotoxicity of DMTMM was evaluated by culturing human mesenchymal stem cells (hMSCs) on the cross-linked GH scaffold. Following a 21-day culture period, various analyses such as gene expression, glycosaminoglycans (GAG) assay, and confocal imaging confirmed the successful chondrogenesis of hMSCs on the GH scaffold. The study revealed that the incorporation of HyA into the gelatin-based scaffold had a positive impact on its rheological properties and printability. Moreover, the utilization of DMTMM as a cross-linker effectively improved scaffold stability without inducing cytotoxic effects. As a result, the GH scaffolds demonstrated the ability to support chondrogenic differentiation, indicating their suitability for tissue engineering applications.

PoA.1.35

Facile transdermal delivery of NAD+ precursor molecule using 3D printed solid microneedle arrays

Masood Ali[1], Sarika Namjoshi[1], Heather Benson[2], Tushar Kumeria[3], Yousuf Mohammed[1]

[1]Frazer Institute, The University of Queensland, Brisbane, Australia. [2]Curtin University, Perth, Australia. [3]University of New South Wales, Sydney, Australia

Abstract

Purpose: A major challenge of transdermal microneedle patch technology is to design and manufacture micron-scale modifications on its surfaces with higher resolution and reduced manufacture time when using low-cost 3D printers. Making microneedles using a 3D printer to date have displayed low aspect ratios and poor tip sharpness. Herein, we present a simple and customisable 3D printed microneedle fabrication technique by optimising design parameters such as modifying resin, print angle, aspect ratio and print curing time. **Methods and results:** Methacrylate resin for 3D printing was diluted with methanol to attain high resolution microneedles, FTIR confirmed no chemical change between original and modified resin. 3D print layer height of 25 µm yielded smoother surface. The print angle at 90° yielded 84.28 ± 2.158 % (n = 5) of the input height (1.2mm), with tip diameter as 48.52 ± 10.43 µm (n = 5). The print aspect-ratio at 1:1.7 (output height: base ratio) yielded 88.37 ± 2.047 % (n = 5) of the input height (1.0 mm), an improvement on the output. Fluorescein (with surface tension reductant: Sucrose and tween-20) was then used to coat the surface of the microneedles and its deposition amount was determined as a function of coating and microneedle parameters (using fluorescence spectroscopy and wetting contact angle). An array of 4 X 4 microneedles with a height of 800 µm contained 823 ± 57 µg (n = 5) of coated Fluorescein. The dye-coated microneedle arrays were inserted into human skin (in-vitro), showing fluorescence signal at a depth of 150 µm (n = 3) into the skin. To study the drug diffusion kinetics in the skin, NAD+ precursor molecule was coated onto the 3D microneedle surface and its diffusion kinetics was studied in full thickness human skin in-vitro using the Franz diffusion setup. Approximately 261 ± 43 µg (34% of nominal coat) of NAD+ precursor molecule was delivered through the full thickness skin, while 121 ± 23 µg (14% of nominal coat) was left in the skin. **Conclusion:** Our study presents a low-cost method of fabricating high-fidelity microneedles. Secondly, coating formulations can be designed to address the hydrophobic nature of the microneedle surface, and lastly, transdermal delivery of NAD+ precursor molecule using microneedle arrays as an additional means of delivering drugs for the treatment of NAD+ deficient related disorders.

PoA.1.36

Glass-ceramic based 3D printed tissue analogues and its bioassay towards affordable healthcare.

Samir Das, Santanu Dhara

IIT Kharagpur, Kharagpur, India

Abstract

Tissue damage and degeneration are common pathological phenomena in higher organisms specifically in mammalians. The traditional method of organ transplantation is a gold standard treatment option for live saving, which is dependent upon the availability of a donor. In case of Scarcity of organ donors, risk of graft rejection are mainly associated with adverse immune response. Evolution of 3D printing involves fabrication of biological tissue analogues and has widespread applications in healthcare such as anatomical models, surgical guides, and customized implants. During implantation design and biomaterials selection for scaffold played an important role for developing macroporous bioactive tissue analogues hierarchy can offer direct bone bonding and improved Osseointegration by incorporating Hullbert's tissue ingrowth concept.

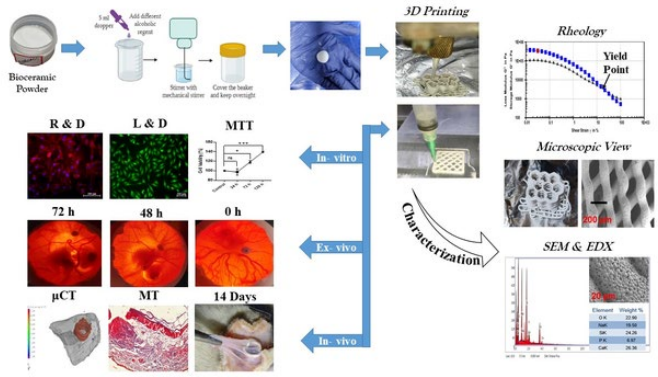


Figure 1. Schematic of fabrication, characterization of 3D printed glass scaffold and its bioassay.

Here we have studied slurry based techniques to develop ink loaded glass ceramic powders with required proportions of binder and lubricant followed by mechanical mixing with the different solvent. Rheological characterization was carried to determine the gelation kinetics and gel strength of the formulated ink. The respective storage and loss moduli were recorded to determine the linear viscoelastic zone and confirms the shear-thinning behavior of developed ink. Various tissue analogues hierarchy was modelled in stereolithography (STL) format using SOLIDWORK with different infill percentage based on strength, anatomical location and application followed by slicing and splitting of the scaffold using CURA. Subsequently, scaffold was printed using optimized printing parameters by an extrusion based 3D printer and fired for sintering at amorphous glassy phase. Physicomechanical characterizations of the printed scaffold were performed to determine the osteogenesis during scaffold and tissue interaction. Finally, the cyto and tissue compatibility of the scaffold templates was confirmed by various biological assays in terms of enhanced host to material response. This finding not only encourages surgical planning, opens up opportunities for enhanced recovery during tissue regeneration that stimulate exciting innovations towards the healthcare sector.

PoA.1.37

Magnetoactive 3D printed scaffolds from a PCL/PLLA co-polymer and anisotropic Fe_3O_4 ferromagnetic nanoparticles for enhanced osteochondral tissue regeneration

Maria Kalogeropoulou[1], Milad Takhsha[2], Franca Albertini[2], Pierpaolo Fucile[1], Lorenzo Moroni[1]

[1]Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands. [2]Institute of Materials for Electronics and Magnetism (IMEM), National Research Council (CNR), Parco Area delle Scienze 37/A, Parma, Italy

Abstract

The use of magnetoactive materials in regenerative medicine is gaining significant momentum, due to their potential to stimulate tissue formation, under both static and dynamic culture conditions. However, the influence of magnetic nanocomposites on osteochondral tissue regeneration is yet to be fully understood. In this study, we embark on investigating the effect of magnetic 3D printed scaffolds from a polycarpolactone/poly-L-lactic acid (PCL/PLLA) random co-polymer and ellipsoidal magnetite (Fe_3O_4) nanoparticles (MNPs), on osteo- and chondrogenesis. Anisotropic MNPs were synthesized by reduction of hematite nanoellipsoids by hydrogen, which was adsorbed during the thermal decomposition of TiH_2. The MNPs were characterized by transmission electron microscopy (TEM) and vibrating-sample magnetometry (VSM) and were found to maintain the anisotropic shape of the hematite while exhibiting a ferromagnetic behaviour (M_r ~ 20.18 Am^2/kg, H_c ~29 mT). Three magnetic nanocomposites were prepared by twin-screw extrusion, with 5wt%, 10wt% and 20wt% MNPs, respectively. The magnetite content of the composites was detected in both X-ray diffraction (XRD) and Energy Dispersive X-ray Spectroscopy (EDS) and the recorded signal was observed to increase proportionally to the increasing MNPs concentrations. The three magnetic nanocomposites were used for the fabrication of 3D printed scaffolds using an FDM printer, with a simultaneous pneumatic and screw-driven extrusion. The selected scaffold design was a typical woodpile pattern (0^o-90^o), which guaranteed a minimum effect of the scaffold geometry on stem cell behavior. Axial compression tests of the 3D printed constructs showed that the incorporation of MNPs in the copolymer resulted in a stiffness increase. The potential use of the magnetic scaffolds for in situ magnetomechanical compression was also demonstrated by cyclic magnetic actuation using a displacement-measuring laser sensor. *In vitro* experiments were conducted by seeding and culturing human mesenchymal stromal cells (hMSCs) on magnetic and non-magnetic scaffolds (controls) for 14 days. The cytotoxicity of the scaffolds was assessed using the LDH assay, and no significant difference was detected between the magnetic scaffolds and the controls, indicating the excellent biocompatibility of the nanocomposites. Cell adhesion, spreading and pore bridging was observed for all conditions. Thus, our preliminary results indicate that the magnetic nanocomposites are strong candidates for advanced osteochondral tissue engineering, providing a platform for further exploration of remotely actuated 3D constructs.

Acknowledgements
This publication is part of the project 3D-MENTOR (project number: 18647) of the VICI research programme, which is financed by the Dutch Research Council (NWO).

PoA.2.02

Novel Polymer Coatings to Reduce Infection on Blood Contacting Devices

Claire F Saxby[1], Jeni Luckett[2], Derek Irvine[3], Paul Williams[2], Morgan Alexander[1]

[1]School of Pharmacy, University of Nottingham, Nottingham, United Kingdom. [2]School of Life Science, University of Nottingham, Nottingham, United Kingdom. [3]Faculty of Engineering; University of Nottingham, Nottingham, United Kingdom

Abstract

Following insertion of medical devices, those in blood contacting environments undergo rapid adsorption of blood proteins to their surfaces, leading to suitable conditions for bacteria and fungi to adhere and develop into biofilms [1]. The biofilm environment increases tolerance to both antimicrobial agents and the host immune system. Once a biofilm has formed on the device surface, removal is often the only effective treatment [2], causing pain and discomfort to the patient, as well as increasing health care costs and hospital stay durations. We aim to prevent biofilm formation using polymers that are unsupportive of biofilms, rather than being biocidal. This strategy aims to increase the effectiveness of drug treatments, while minimising the development of antimicrobial resistance. Non-bactericidal polymer coatings have been shown to reduce bacterial biofilm formation on urinary catheters, *in vitro* and *in vivo* [3], as well as in the clinic [4]. However, the addition of blood proteins presents an additional challenge to the coatings' performance [5]. Protein adsorption can cause activation of various cascades within the blood, making haemocompatibility an important consideration in the development of new coatings. In this work, we create *in silico* models of biofilm resistance profiles. In addition, we synthesise polymer coatings and analyse their *in vitro* ability to prevent biofilm formation in a non-bactericidal way after conditioning in whole human blood, with the final goal of identifying a candidate coating for *in vivo* testing.

[1] R. Murga, J. M. Miller, and R. M. Donlan, "Biofilm formation by gram-negative bacteria on central venous catheter connectors: Effect of conditioning films in a laboratory model," J. Clin. Microbiol., vol. 39, no. 6, pp. 2294–2297, 2001.
[2] J. Guenezan, B. Drugeon, N. Marjanovic, and O. Mimoz, "Treatment of central line-associated bloodstream infections," Crit. Care, vol. 22, no. 303, 2018.
[3] A. L. Hook et al., "Combinatorial discovery of polymers resistant to bacterial attachment," Nat. Biotechnol., vol. 30, no. 9, pp. 868–875, 2012.
[4] N. Jeffery et al., "A new bacterial resistant polymer catheter coating to reduce catheter associated urinary tract infection (CAUTI): A first-in-man pilot study," Eur. Urol. Suppl., vol. 18, no. 1, p. e377, 2019.
[5] T. Singh et al., "Discovery of hemocompatible bacterial biofilm-resistant copolymers," Biomaterials, vol. 260, p. 120312, 2020.

PoA.2.03

Weak organic acid loaded hydrogel coatings for the prevention of catheter-associated urinary tract infections.

Jane Burns, Colin P McCoy, Matthew P Wylie, Nicola J Irwin

Queen's University, Belfast, United Kingdom

Abstract

Introduction: The main difficulties associated with catheter-associated urinary tract infections (CAUTIs) that make them problematic to treat is device encrustation and colonisation by uropathogens. Encrustation occurs due to the presence of urease-producing bacteria, which can lead to catheter lumen blockage (1). Weak organic acids (WOAs) have been used as naturally occurring preservatives for centuries and are reported to exert their antimicrobial activity by the flow of unionised molecules through bacterial cell membranes (1,2). Subsequent reduction in intracellular pH due to accumulation of acidic anions and hydrogen ions causes damage to enzymes, increase in turgor pressure and oxidative stress, thereby inhibiting metabolic processes (1). Previously, WOAs have shown promising antimicrobial activity against uropathogens and anti-encrustation (3). Herein, the antibacterial activity of WOA-loaded hydrogel catheter coatings against uropathogens for the prevention of CAUTI is investigated.

Methods: Coating formulations of ethylene glycol dimethacrylate (EGDMA), hydroxyethyl methacrylate (2-HEMA), polymethyl methacrylate (PMMA), polyvinylpyrrolidone (PVP), Irgacure® 2959 and citric/propionic acids were dip coated onto silicone sheets and all-silicone catheters at 200 mm min^{-1} before dried and cured in a UVA light chamber. The antibacterial activity against *Proteus mirabilis* (*P. mirabilis*) ATCC 51286 was investigated according to the CLSI agar diffusion protocol. Catheter bridge assays assessed *P. mirabilis* swarming inhibition. Adherence and *in vitro* bladder model assays were performed for determination of bacterial viability, urinary pH and time to catheter blockage.

Results: WOA-loaded hydrogel coatings produced zones of inhibition and were observed to reduce viable *P. mirabilis* adherence of >99%, relative to uncoated silicone controls. However, hydrogel-coated catheter sections (with and without WOA), were found to equally permit *P. mirabilis* swarming. In bladder models, the lumens of control catheters blocked within 46.38 h ± 3.16. Catheters with hydrogel coatings containing the citric/propionic acids combination displayed a small extension in time to blockage (53.51 h ± 13.12) indicating promising synergistic activity against uropathogens and encrustation.

Conclusion: The infection-resistant WOA-loaded hydrogel coatings identified present a promising new approach for the prevention of CAUTI and catheter lumen blockages. Future work focused on extending the localised delivery of WOAs in the catheterised urinary tract is imperative.

References: (1) Piper P *et al. Micro.* 2001:2635-42. (2) Ricke SC. *Poultry Sci.* 2003:632-9. (3) Burns J *et al. J. Hosp. Infection.* 111(3):69-77.

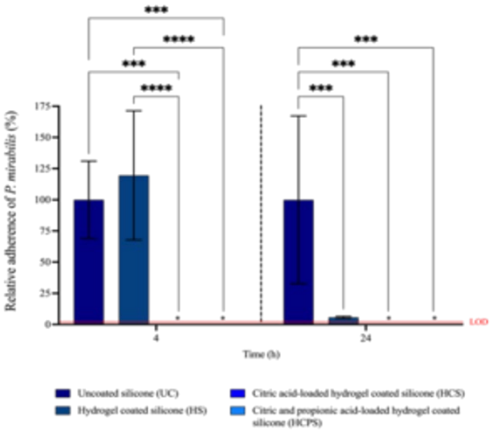


Figure 1: Adherence (%) of P. mirabilis to WOA-loaded hydrogel coated silicone, relative to uncoated silicone controls, after 4 h and 24 h. Error bars represent S.D. of mean values, (n=5).

PoA.2.04

Zinc doped hydroxyapatite nanoparticles for the treatment of intracellular bone infections.

Lizzy A.B. Cuypers, Rong Wang, Pascal Bertsch, Fang Yang, X. Frank Walboomers, Sander C.G. Leeuwenburgh

Radboud university medical center, Nijmegen, Netherlands

Abstract

Bone infections pose a significant concern to healthcare. These infections are often difficult to treat since bacteria, such as Staphylococcus aureus (*S. aureus*), can hide intracellularly in several cell types including osteocytes and macrophages. Commonly used antibiotics cannot penetrate cell membranes to kill these intracellularly hiding bacteria, and thus remain therapeutically ineffective. Hence, new treatment strategies that deliver therapeutics in a targeted manner to effectively treat intracellular infections are urgently required. In this study the use of hydroxyapatite nanoparticles (HA NPs) is proposed as potential solution due to their ability to be internalized by cells. Moreover, HA NPs can be doped with therapeutic ions like zinc (ZnHA NPs) to enhance their antibacterial properties. Herein, ZnHA NPs were fabricated and their antibacterial properties, cytocompatibility and cellular uptake were studied in detail.

ZnHA NPs with different zinc content (0, 10, 15 and 20 mol%) were synthesized through a precipitation reaction and characterized by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) to determine their crystal and molecular structure, respectively. The antibacterial properties of the NPs were tested against *S. aureus*, and cytocompatibility was assessed with pre-osteoblast MC3T3 cells. Additionally, the internalization of ZnHA NPs was evaluated using confocal microscopy after culturing fluorescently labeled THP-1 macrophages with fluorescently labeled ZnHA NPs for 24h.

The XRD diffractograms and FTIR spectra confirmed the presence of characteristic peaks of HA in all samples, indicating that zinc doping did not impede crystallization of the nanoparticles. The addition of ZnHA NPs to *S. aureus* culture resulted in a dose-dependent delay of the log-phase growth compared to the non-treatment control group, confirming the bacteriostatic effect of the NPs. ZnHA NPs did not have a significant impact on the metabolic activity of pre-osteoblast MC3T3 cells after 24h, which was also confirmed by LIVE/DEAD assays. Furthermore, confocal microscopy demonstrated that the ZnHA NPs were internalized by macrophages corresponding to lysosomal uptake of the NPs (Figure 1).

In conclusion, our results confirm the potential of ZnHA NPs for treatment of intracellular bone infections and warrant further preclinical studies of their antibacterial efficacy.

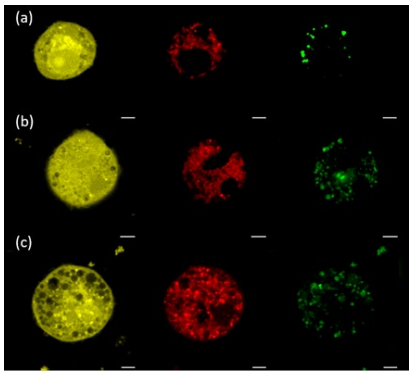


Figure 1: Internalization of (a) 10 mol%, (b) 15 mol%, (c) 20 mol% ZnHA NP in THP-1 macrophage cells. Cell cytoplasm visualized in yellow, lysosomes in red, and ZnHA NPs in green. Scale bar 5µm.

PoA.2.05

Determination of cyclic di-nucleotide second messengers and their degradation products and precursors in biomaterial-colonizing bacteria using LC–MS/MS

Silvio Uhlig[1], Kun Cai[2], Krystyna A. Liskiewicz[2], Maria Pain[1], Lene A. Grutle[1], Hanne Røberg-Larsen[3], Roger Simm[2]

[1]Nordic Institute of Dental Materials, Oslo, Norway. [2]Institute of Oral Biology, University of Oslo, Oslo, Norway. [3]Section for Chemical Life Sciences - Biomolecules, Bio-inspired Materials and Bioanalytics, University of Oslo, Oslo, Norway

Abstract

Bacterial biofilms on medical implants such as dental prostheses are a serious problem. The cyclic di-nucleotides (CDNs) 3',5'-c-di-guanosyl monophosphate (c-di-GMP), 3',5'-c-di-adenosyl monophosphate (c-di-AMP) and 3',3'-c-AMP–GMP (cGAMP) are bacterial second messenger molecules that play key roles for the survival and adaptation of bacteria. Cyclic-di-GMP has been shown to regulate processes such as biofilm formation, motility and virulence, while c-di-AMP in addition appears to be involved in e.g. stress response, cell wall homeostasis and cell division. The roles of cGAMP have not been investigated much in bacteria, but isomers of 3',3'-cGAMP in metazoan species are well-known initiators of immune signaling pathways. The regulation of CDN levels appears to be critical for diverse cellular processes, and could give information on the ability of pathogenic bacteria to colonize biomaterials. Thus, the production of c-di-AMP is directly linked to biofilm formation in some bacteria. On the other hand, CDNs may modulate cellular activities of host cells. Their expression might thus contribute to the pathogenicity of bacteria on the tissues that surround e.g. dental prostheses.

The concurrent liquid chromatographic (LC) analysis of CDNs and the related linear nucleotides (5'-phosphadenylyl-adenosine (pApA), 5'-phosphate-guanylyl-guanosine (pGpG), adenosine monophosphate and triphosphate (AMP and ATP), guanosine monophosphate and triphosphate (GMP and GTP)) is challenging because of phosphate-metal interactions on instrumental surfaces and multiple pKa values of the nucleotides. We aimed to develop a LC method for the analysis of the nucleotides in connection with selective tandem mass spectrometric detection (MS/MS). The simultaneous LC–MS/MS analysis of both CDNs and linear nucleotides was not satisfactory. Thus, CDNs were separated in reverse-phase mode using a sorbent that is especially designed for the analysis of basic and highly ionizable compounds (Zorbax Bonus-RP), and linear nucleotides were separated using hydrophilic interaction chromatography using a zwitterionic stationary phase (SeQuant ZIC-pHILIC). The limits of quantification were in the lower nanomolar range. We also explored different sample preparation strategies, i.e., dispersive solid-phase extraction as well as strong and weak anion-exchange, to reduce background noise during instrumental analysis, but found that bacterial extracts were best analyzed without any additional treatment.

PoA.2.07

Antibiofilm Properties of Linoleic Acid-loaded Bone Cement with and without Gentamicin

Linglu Hong[1,2], Karin Hjort[2], Cecilia Persson[1], Dan I. Andersson[2]

[1]Department of Materials Science and Engineering, Division of Biomedical Engineering, Uppsala University, Uppsala, Sweden. [2]Department of Medical Biochemistry and Microbiology, Uppsala University, Upppsala, Sweden

Abstract

Introduction: Polymethyl methacrylate (PMMA) bone cement is widely used in e.g., vertebroplasty to treat osteoporosis-induced vertebral compression fractures. Treatment-related complications include adjacent vertebral fractures (AVFs) and infection. Linoleic acid (LA) has been investigated as a promising additive to reduce the mechanically-related AVFs, and it has also shown antibacterial effects. This study aimed to investigate the antibiofilm properties of LA-loaded bone cement as well as the interaction between LA and gentamicin and the feasibility of using them together.

Experimental Methods: To evaluate biofilm formation on different bone cements with different bacterial strains, bone cement with and without LA (12 vol% of the liquid part of commercially available V-Steady bone cement) was molded into peg shapes using the FlexiPeg biofilm device for biofilm evaluation. Bone cement pegs were incubated with bacteria for 24h and 48h and then biofilm formation was quantified by viable bacteria count and visualized by scanning electron microscopy (SEM).

To investigate any influence of residual monomer release on bacterial growth, the minimum inhibitory concentration (MIC) of MMA and the released MMA concentration were determined. The MIC of MMA was determined by the broth microdilution method. Extracts from PMMA at 1h, 24h, 72h, and 7 days after preparation were used to measure the released MMA. To evaluate combinations of LA and gentamicin against the bacterial strains, a checkerboard assay was conducted in 96-well plates. The effect on bacterial growth of each individual component and the combination was determined.

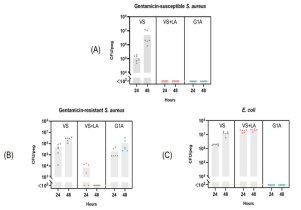


Figure 1 Viable bacteria count of (A) Gentamicin-susceptible S. aureus, (B) Gentamicin-resistant, and (C) Gentamicin-susceptible E. coli after 24h and 48h of biofilm growth on the VS, VS+LA, and G1A bone cement pegs. The grey bar indicates the mean and the dash line (<10^2 CFU per peg) indicates the detection limit, i.e., no colonies were observed on agar plates after plating the lowest dilution. (VS: V-steady commercial-available bone cement. VS+LA: V-Steady loaded with linoleic acid. G1A: commercial-available gentamicin-loaded bone cement.)

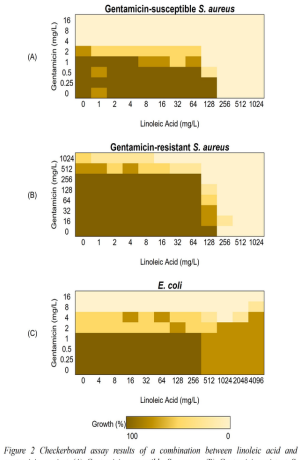


Figure 2 Checkerboard assay results of a combination between linoleic acid and gentamicin against (A) Gentamicin-susceptible S. aureus, (B) Gentamicin-resistant S. aureus, and (C) E. coli. From the lightest yellow to the darkest yellow indicates 0-20%, 20%-40%, 40%-60%, 60%-80%, and 80%-100% bacterial growth rate.

Results and Discussion: Viable bacteria count (Figure 1) and SEM images showed that LA-loaded bone cement could significantly inhibit *Staphylococcus aureus* (*S. aureus*) biofilm formation, including gentamicin-resistant strains, but had a limited effect on *Escherichia coli*. The MIC of the MMA against S. aureus was 40 g/L and the released MMA from LA-loaded cement reached only around 1 g/L after 7-day accumulation, i.e., the released MMA did not have a significant influence on bacterial growth. The checkerboard assay (Figure 2) showed that the LA and gentamicin combination could broaden the antibacterial spectrum and increase the gentamicin efficacy.

Conclusions: LA, incorporated in small amounts into PMMA bone cement, could effectively inhibit S. aureus biofilm formation without contribution from residual MMA. LA was also found suitable to use together with gentamicin to broaden the antibacterial spectrum. LA merits further investigation as an antibacterial agent in bone cement, alone or in combination with antibiotics.

PoA.2.08

The Potential of Nanoscale Patterning and Microalloying for Antibacterial Metallic Glass Design

Juan J Londono[1,2], Paula M Giraldo[3], Adam B Turner[3], Florian Spieckermann[1], Silke Prades-Rodel[2], Alexandra Levesque[2], Jan Schroers[4], Margarita Trobos[3], Anders Palmquist[3], Andreas Blatter[2], Jürgen Eckert[1,5]

[1]Department of Materials Science, Montanuniversität Leoben, Leoben, Austria. [2]Research and Development Department, PX Services, La chaux-de-Fonds, Switzerland. [3]Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. [4]Mechanical Engineering and Materials Science, Yale University, New Haven, USA. [5]Erich Schmid Institute of Materials Science, Austrian Academy of Sciences, Leoben, Austria

Abstract

Metallic glasses are promising materials for use in hard tissue implants due to their superior strength, elasticity, and corrosion resistance compared to traditional crystalline alloys. The adoption of metallic glasses in medical device manufacturing could be transformative, enabling the realization of functional designs previously unattainable due to material limitations.

To be suitable for medical implant applications, next-generation alloys should not only improve manufacturability, mechanical strength, and corrosion resistance, but also be biocompatible, and promote wound healing and osseointegration, while inhibiting biofilm formation on the material's surface. However, there has been limited research on the intentional design of antibacterial metallic glasses, mainly relying on high quantities and release of well-known antibacterial elements like Ag or Cu, which compromise biocompatibility. This work aimed to design metallic glasses with antibacterial properties that meet the requirements of hard tissue implant applications, following two strategies: microalloying and nanopatterning. We demonstrate the effectiveness and potential for innovative medical devices by implementing these strategies in the model material $Pd_{43}Cu_{27}Ni_{10}P_{20}$ (Pd-MG).

First, microalloying excellent glass-forming compositions with multiple metallic species can maintain superior mechanical and corrosion properties. This approach can reduce the total release of cytotoxic and pro-inflammatory metallic ions, while improving the material's antibacterial efficiency through a synergistic effect between the various ion species. We showcase this strategy by adding small amounts of Ag and Ga to Pd-MG.

Second, the unique formability of metallic glasses allows for fast thermoplastic patterning of surfaces with well-controlled geometry and sizes. We create nanopillars with aspect ratio ~10 and diameters of 40, 80, 160, and 250 nm on Pd-MG to implement this strategy. We evaluate the mechanobactericidal effect of the nanopillars and how they influence the adhesion and polarization of macrophages.

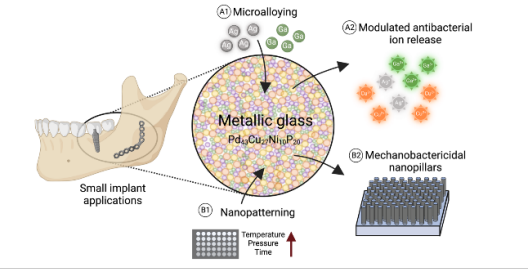


Figure 1 Microalloying and thermoplastic nanopatterning as viable strategies to create antibacterial metallic glasses for implant applications.

In summary, our research presents two innovative strategies to design metallic glasses with antibacterial properties. We show the application of this strategy on Pd-MG, the effects on the physical properties of the glass, and evaluate cytocompatibility, antibacterial efficiency, cell adhesion, and biofilm formation.

PoA.2.09

Playing with self-assembly of biopolymers: from antibacterial to antiviral materials

Philippe Lavalle[1,2], Varvara Gribova[1], Eloïse Lebaudy[1], Lorène Tallet[1], Cynthia Calligaro[2], Viola Mazzoleni[1], Leyla Koçgözlü[1], Hadda Zebiri[1], Engin Nihal Vrana[1]
[1]Inserm / University of Strasbourg, Biomaterials and bioengineering, Strasbourg, France. [2]SPARTHA Medical, Strasbourg, France

Abstract

All implantable biomedical systems face risks of infections leading to bacterial biofilms hard to eradicate. To prevent such infections, a multifunctional surface coating that can address this issue would significantly improve clinical outcomes[1].
Polyarginine (PAR), polylysine (PLL), or polyornithine (POR) are synthetic highly cationic homopolypeptides that can act as antimicrobial agents due to their positive charges. We developed a family of new supramolecular coatings based on these homopolypeptides assembled with hyaluronic acid (HA)[2,3] in a layer-by-layer process. We demonstrate that exclusively coatings constructed with homopolypeptide chains of 30 residues in length provide a strong antimicrobial activity. These coatings have an inhibitory effect on all pathogenic bacteria associated with infections of medical devices, including antibiotic resistant bacteria.
This system can also be fabricated in the form of hydrogel, useful to provide antibacterial properties to porous implants like surgical meshes[4,5]. In this case, similar components are used, HA to produce the hydrogel, and PAR as the active ingredient. A precise controlled release of PAR with adequate MW can be achieved with strong efficiency over multi-infections.
Finally, during the pandemic, theses hydrogels based only on biopolymers were tuned to combine an antiviral property to the antibacterial ones. Strong efficacy against SARS-CoV-2 was demonstrated. Applications are numerous in the field of bandages, sexual protection ophthalmology or daily use disinfectants.

References

1. Tallet L, Gribova V, Ploux L, Vrana NE, Lavalle P., “New smart antimicrobial hydrogels, nanomaterials, and coatings: earlier action, more specific, better dosing?”, Adv. Healthc. Mater., 2021,10, e2001199.
2. Mutschler A., Betscha C., Ball V., Senger B., Vrana N. E., Boulmedais F., Schroder A., Schaaf P., Lavalle P. "Nature of the polyanion governs the antimicrobial properties of poly(arginine)/polyanion multilayer films", Chem. Mater., 2017, 29, 3195.
3. Mutschler A., Tallet L., Rabineau M., Dollinger C., Metz-Boutigue M.-H., Schneider F., Senger B., Vrana N. E., Schaaf P., Lavalle P., "Unexpected bactericidal activity of poly(arginine)/hyaluronan nanolayered coatings", Chem. Mater., 2016, 28, 8700.
4. Gribova V., Petit L., Seguin C., Fournel S., Kichler A., Vrana N. E., Lavalle P., “Polyarginine as a simultaneous antimicrobial, immunomodulatory and miRNA delivery agent within polyanionic hydrogels”, Macromol. Biosci., 2022, 22:e2200043.
5. Gribova V., Boulmedais F., Dupret-Bories A., Calligaro C. Senger B., Vrana N. E., Lavalle P., “Polyanionic Hydrogels as Reservoirs for Polycationic Antibiotic Substitutes Providing Prolonged Antibacterial Activity”, ACS Appl. Mat. Int., 2020, 12, 19258-19267.

PoA.2.12

Carboxymethylcellulose loaded with evolved bacteriophages and vancomycin for treatment of *Staphylococcus aureus* fracture-related infection

Marco Chitto[1], Baixing Chen[2,1], Virginia Post[1], Pamela Nylund[1], Stephan Zeiter[1], Jeroen Wagemans[2], Mercedes Gonzales Moreno[3], Andrej Trampuz[3], Rob Lavigne[2], Matteo D' Este[1], Fintan Thomas Moriarty[1]

[1]AO Foundation, Davos, Switzerland. [2]KU Leuven, Leuven, Belgium. [3]Charité-Universitätsmedizin Berlin, Berlin, Germany

Abstract

Fracture fixation devices allow for management of complex musculoskeletal injuries, however, the risk of developing fracture-related infection (FRI) remains. Most infections are caused by *Staphylococcus aureus* whose ability to form antibiotic-tolerant biofilms and evade host defenses is the major challenge in preventing and treating infections. Alternative therapies, such as a combination of antibiotics and/or bacteriophages, are currently being considered for FRI. Biodegradable biomaterials, such as hydrogels, may be employed for local delivery of both the antibiotic and bacteriophage in combination.

In this work, two evolved bacteriophages with increased antibiofilm activity (ϕR14 and ϕR23), were used as monophages, or as a 1:1 cocktail of both. Phage ϕR14 suspension (10^{10}PFU/ml), phage ϕR23 suspension (10^{10}PFU/ml), or vancomycin solution (37.5 mg/ml) were mixed with 3% carboxymethylcellulose (CMC) powder to form a homogeneous hydrogel (P+V Gel). Stability of both antimicrobials in the hydrogel was evaluated at day 0, 1, 4, and day 8 at 37°C. *In vivo*, the same composition was tested in the treatment of a 5-day-old *S. aureus* infection of a tibial plate osteotomy in mice. Treatment involved debridement and 5 days of systemic vancomycin (0.1 mg/kg b.i.d.) therapy in *i*-Systemic antibiotic only; *ii*- Systemic antibiotic with P+V Gel; *iii*- P+V Gel without systemic antibiotic treatment; and *iv*- untreated group (n=7 mice/group). The bacterial load was monitored at euthanasia by colony forming unit (CFU) and plaque forming unit (PFU) quantification.

In vitro, the number of viable phages and the stability of vancomycin in P+V Gel remained constant over a period of 8 days. *In vivo*, the reduction of bacterial load was most significant in the in soft tissue when treated with systemic antibiotic and P+V Gel, with a tenfold CFU reduction compared to Systemic antibiotic alone or P+V Gel alone. At euthanasia, twofold more phages particles were detected in soft tissue, bone and implant in the animals that received P+V Gel only compared to P+V Gel combined with Systemic antibiotics.

In summary, the CMC hydrogel allows for phages and vancomycin to be retained and released without affecting antimicrobial activity. Additionally, these results demonstrate that phages in combination with systemic antibiotics can improve soft tissue burden of MRSA *in vivo*.

PoA.2.13

Copper-electroplated Ti6Al4V is bactericidal against *S. aureus* and enhances macrophage phagocytosis

Adam B Turner[1], Paula M Giraldo-Osorno[1], Sebastião M Barros[2,3], Simone Guttau[2], Robin Büscher[2], Anders Palmquist[1], Margarita Trobos[1]

Abstract

Implanted biomaterials are used to restore or prevent the loss of function associated with ageing and physical trauma. However, infection and ineffective immune responses to these devices result in a significant proportion of implant failure. As of yet, no prosthetic or fixation device is completely resistant to bacterial colonisation and as such, it is vital that new biomaterials are investigated. In this project, we aimed to characterise the microbiological and host cell responses to a novel biomaterial, in which metallic copper was electroplated onto an orthopaedic Ti6Al4V-ELI surface, to endow it with functional bactericidal and immunomodulatory properties. In this investigation, bacterial viability counting and scanning electron microscopy were performed to quantify and visualise the direct and indirect bactericidal effects of the Cu-electroplated titanium (Cu-Ep Ti) against a lab strain (*Staphylococcus aureus* ATCC 25923), and a clinical *S. aureus* periprosthetic joint infection (PJI) isolate in tryptic soy broth (TSB) and Roswell Park Memorial Institute (RPMI) at early (4 h) and extended (24, 48 h) time points. The effect of the copper electroplating on human THP-1 macrophage adhesion and viability was analysed by nucleocounting. Further, characterisation on how interactions with the copper surface modulated macrophage phagocytosis of pHrodo S. aureus bioparticles was evaluated. Results showed a potent antimicrobial activity against *S. aureus* ATCC 25923 and clinical PJI isolate, alongside promising host-immunomodulatory properties. Direct and indirect exposure to Cu-Ep Ti surfaces produced potent bactericidal effects resulting in significant reductions in bacterial viability at 24 h in TSB (94-98 %) and RPMI (99-100 %), with complete eradication of *S. aureus* in some cases. As expected, cytotoxicity was observed in THP-1 macrophages without media exchange, though when media was exchanged at 8, 24 and 48 h THP-1 cell viability was equivalent to that of the Ti control. Interestingly macrophages adhered to the copper material or grown in the presence of copper ions from the material showed a significant increase in phagocytosis of *S. aureus* bioparticles compared to control Ti (7-fold increase), suggesting a dual bactericidal and host immunomodulatory mechanism. In conclusion, we have shown that this novel Cu-electroplated Ti biomaterial can limit bacterial contamination on the implant surface, whilst simultaneously promoting a beneficial antimicrobial immune response.

PoA.2.14

BIOCOMPATIBLE AND ANTIMICROBIAL COATING FOR MULTI-MATERIAL INVASIVE MEDICAL DEVICE

Cynthia Calligaro[1], Lucie Lieu[1], Aghilas Akkache[1], Lauriane Petit[2], Yijie Li[1], Chloé Guilbaud-Chéreau[1], Philippe Lavalle[2], Nihal E Vrana[1]

[1]SPARTHA Medical, Strasbourg, France. [2]INSERM UMR-S 1121, Strasbourg, France

Abstract

Background & Objectives: Healthcare-associated infection is a significant problem in orthopaedic implant surgeries. An antimicrobial coating process on implants can be a solution but might be complexified by the geometry and the variety of the implant composition. To solve this, we developed multimodal bioactive coatings made of polypeptides and polysaccharides, which are easy to build and show reliable antimicrobial properties [1-3].
Here, we designed poly(arginine) and hyaluronic acid (HA) layer-by-layer coating that applies simultaneously to all the components of a single implant and is effective against relevant bacteria without cytotoxicity or adverse effects on the implant's mechanical properties.
Methods: Four materials found in orthopaedic implants were investigated simultaneously: titanium (Ti), cobalt-chrome (CoCr), polyetheretherketone (PEEK), and ultra-high molecular weight polyethylene (UHMWPE). To construct a coating on these materials, an anchorage layer made of polydopamine (p(DOPA)) was first deposited, and then the (PAR/HA) multilayer film was constructed by alternate dipping in PAR and HA solutions until 24 layers. A full study was conducted on the coated materials, with i) cytotoxicity evaluation, ii) antimicrobial activity assays, iii) efficacy after sterilization or storage, and iv) measurement of osteoblast attachment.
Results: p(DOPA)+(PAR/HA)24 coating was successfully constructed on all materials and showed no cytotoxicity on the Balb-3T3 cell line according to the ISO10993-5 standard.
The coating showed strong bactericidal activity against Staphylococcus aureus on both surrounding planktonic bacteria and on the implant sample surface. Planktonic bacteria growth of Escherichia coli was not fully inhibited in the tested conditions, but no adherent bacteria were found at the surface of the coated materials.
The coating still sustained high antimicrobial efficacy after steam sterilization (121°C, 20 minutes) or after accelerated aging equivalent to 1 year of storage at room temperature.
The Saos-2 osteoblast cell line was used for attachment assays, and cell attachment was low after 4 or 24 hours of contact. However, a better cell attachment was found when cell seeding was done after incubating coated materials in the cell culture medium. This shows the coating allows cell attachment after interacting with the biological environment.
p(DOPA)+(PAR/HA)24 coating is, therefore, biocompatible, antimicrobial, and can be deposited on different substrates. This coating appears to be a versatile and powerful antimicrobial system, especially for implantable medical devices.

References:
1.(Özçelik H. Adv Healthc Mater. 2015;4:2026-2036)
2.(Mutschler A. Chem Mater. 2017;29:3195–3201)
3.(Gribova V. Macromol Biosci 2022,2200043)

PoA.2.15

Vancomycin and Bupivacaine loaded UHMWPE for the development of therapeutic implant materials

Nicoletta Inverardi[1,2], Anthony Marzouca[1], Keita Fujino[1], Amita Sekar[1,2], Mehmet D Asik[1,2], Jean Yuh[1], Eileen Walsh-Rock[1], Orhun K Muratoglu[1,2], Ebru Oral[1,2]

[1]Massachusetts General Hospital, Boston, USA. [2]Harvard Medical School, Boston, USA

Abstract

Eluting therapeutics from total joint replacement implants is a promising strategy to obtain local delivery to address both pain management and peri-prosthetic joint infections, which are a major patient and healthcare burden.[1,2] In this work, we investigated the synergistic activity of the antibiotic vancomycin hydrochloride and the analgesic bupivacaine hydrochloride loaded into ultrahigh molecular weight polyethylene (UHMWPE).

METHODS: UHMWPE was blended with bupivacaine hydrochloride (BP) and vancomycin hydrochloride (VC) at different total drug loading (1, 5 and 7 % w/w). The blends were dehydrated at 90 °C under vacuum for 18-24h and they were compression molded at a temperature of 170 °C for 10 min under 20 MPa, followed by cooling under the applied pressure. The composition of the materials was confirmed by thermogravimetric analysis (sample: 10mg, heating ramp: 30 - 600 °C under nitrogen flow). Tensile testing was performed after die-cutting samples (ASTM D638-10) (2000N load cell; crosshead speed of 10 mm/min). The antibacterial performance of samples (3×5×10 mm^3) was investigated against *Staphylococcus aureus* (ATCC 12600) by incubating with 1.35ml of 10^5 CFU/ml *S. aureus* in Mueller Hinton broth at 37 °C under shaking (n=3, virgin UHMWPE as control). At given timepoints, the bacterial viability in real-time was determined using an ATP-based chemiluminescent assay. The spent media was discarded after centrifugation and bacteria were resuspended in fresh broth.[3]

RESULTS AND DISCUSSION: The antibacterial efficacy of VC-loaded UHMWPE was highly dependent on the drug loading: 1% did not suggest any eradication, whereas 7% led to bacteria eradication after 3 days. The tensile properties decreased as the drug loading increased (Table 1). By keeping fixed the weight percent of VC to 1% and supplementing with BP, the antibacterial properties significantly improved. The composition with the lowest drug loading, which was effective in eradicating bacteria was 1%VC+4%BP loaded UHMWPE; fixing the total drug loading to 5% allowed also to limit the decrease in the tensile properties (Table 1). CONCLUSION: Dual-therapeutic loaded UHMWPE can provide a synergistic antibacterial effect while also releasing analgesic that can help in pain management.

Formulation	Tensile properties		Antibacterial performance
	Elongation at break (%)	Ultimate tensile strength (MPa)	Index "Day at eradication"
Virgin UHMWPE	448 ± 34	50.81 ± 2.52	(10^8 CFU/ml at all points)
1 % Vancomycin	372 ± 22	38.81 ± 1.54	No full eradication
5% Vancomycin	337 ± 17	32.02 ± 0.73	Not tested
7% Vancomycin	337 ± 12	29.83 ± 0.55	3
1% Vancomycin + 4% Bupivacaine	336 ± 17	32.54 ± 0.54	2
1% Vancomycin + 6% Bupivacaine	249 ± 17	22.35 ± 0.59	2

Table 1 Tensile and antibacterial results

1. V. Suhardi et al., Nature Biomedical Engineering. 2017; 1:80.
2. S. Grindy et al., Acta Biomaterialia. 2019; 93:63-73.
3. A. Sekar et al., Journal of Visual Experimentation (Accepted)

FUNDING: National Institutes of Health Grant R01AR077023.

PoA.2.16

Development of antibacterial surfaces based on a titanium alloy functionalised with antimicrobial peptides

Diana S. Gomes, Artemis Stamboulis

School of Metallurgy and Materials, University of Birmingham, Edgbaston, B15 2TT, Birmingham, United Kingdom

Abstract

Introduction: When the surface of orthopaedic implants is colonised by unwanted microorganisms following surgical procedures, the resulting infection can lead to severe complications and ultimately to implant failure. Antibiotics have limited activity in these situations, especially if biofilm-forming or antibiotic-resistant microorganisms are present. Coatings using immobilised molecules can improve the antimicrobial properties of implants. Antimicrobial peptides (AMPs) are promising candidates because of their broad-spectrum antimicrobial activity, low toxicity, and less likelihood to induce resistance. Focused on titanium alloy Ti-6Al-4V, this work will use small peptide sequences with an electrostatic affinity for titanium and add them to the AMP sequence to create a solid-binding antimicrobial peptide with an affinity for implant surfaces. Metal surfaces will also be submitted to a combination of treatments to favour electrostatic attachment by increasing oxide species on the surface and creating a morphology more suitable for bioactivity.
Experimental Methods: Ti-6Al-4V samples were first polished to a mirror-like finish, then submitted to a combination of mild chemical treatment (CT, immersion in 10 mL of H2O2 8.8 M HCl 0.1 M, 80 ºC, 30 minutes) and heat treatment (HT, thermal oxidation at 450, 550, 650 and 750 ºC for 60 minutes). The peptide construct containing KR-12 (smallest fragment of human cathelicidin LL-37) and the titanium-binding peptide was produced by solid-phase peptide synthesis. Coating of samples was performed at room temperature to electrostatically immobilise the peptide construct on the modified surfaces. Treated surfaces will be tested against Gram-negative and Gram-positive bacteria to determine antibacterial capacity and evaluated for cytotoxicity.
Results: Physicochemical characterisation of modified surfaces using Raman spectroscopy, XRD, AFM, WCA, SEM, EDX were performed. Combined CT and HT at higher temperatures increased the surface oxide layer (contributions of TiO_2 crystalline phases were found, and a higher percentage of surface oxygen was detected). Also, it induced higher surface roughness values and lower WCA compared to polished surfaces and treatments at lower temperatures, with surface roughness dependent on specific treatment combinations.
Conclusions: Prevention of pathogenic bacteria proliferation on surfaces coated with AMPs can provide alternatives to antibiotics. This study aims to produce new and simple functionalisation procedures to prevent infection related complications on materials used in orthopaedic applications.

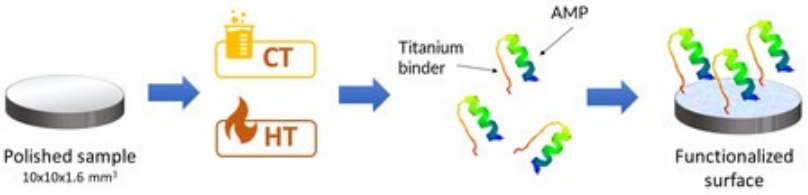


Figure 1 - Overall view of functionalisation procedure of titanium surfaces.

PoA.2.17

Anti-bacterial properties of a calcium hydroxide-based coating for implant surfaces

Harald Holeczek[1], Olivier Braissant[2], Michael de Wild[3], Jasmine Rüegg[4]

[1]Medicoat AG, Mägenwil, Switzerland. [2]University of Basel, Alschwil, Switzerland. [3]University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, Switzerland. [4]Medartis, Brisbane, Australia

Abstract

INTRODUCTION: Peri-implantitis is of growing concern today with increasing numbers of implants used. To prevent inflammation after surgery it would be ideal to inactivate any bacteria that might reach the implant surface in the operating theatre or during introduction of the implant into the body. Such a bactericidal effect should not affect the body of the patient with possible long-term effects. One approach is to rise the pH directly on the implant surface to a level that inactivates most bacteria until the body immune system can kill them.

METHODS: Titanium disks and screws have been coated first with a rough titanium layer by thermal plasma spraying and afterwards with a layer of $Ca(OH)_2$. The surface of the coated samples was moistened with LB medium containing S. epidermidis and S. aureus with a concentration of 105 CFU ml^{-1}. Samples were then placed in a micro-calorimeter. The activity of the bacteria was measured indirectly via the produced metabolic heat. RESULTS: The diagram resulting from Gompertz curve fitting (Fig. 1) shows the reduced metabolic heat for the $Ca(OH)_2$ coated sample. The growth rates of the different populations (Fig. 2) were calculated from the measurement data and show a considerably reduced growth on the samples with anti-bacterial $Ca(OH)_2$-coating, compared to those with hydroxy apatite (HA) or pure titanium (Ti20).

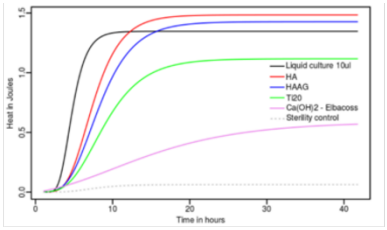


Figure 1: Modelled data from Gompertz curve fitting of micro-calorimeter measurements.

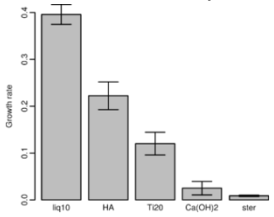


Figure 2: Growth rates computed from calorimeter data.

CONCLUSIONS: Samples coated with Ca(OH)2 show a significant reduction of bacterial metabolism activity which points to substantially reduced growth. Thus, the anti-bacterial effect of such a coating has been shown. As the coating consists only of Ca and hydroxyl ions which are both completely resorbed by the body, the active coating is removed after some time. The contact-killing effect is only active directly on the surface of the coated specimen. No long-term effects are expected when the coating is used in the body. Our in-vivo study showed good osseointegration of coated implants in rats [1].

ACKNOWLEDGEMENTS: The authors thank the Forschungsfonds Aargau for supporting the project 20140331_07_Z_ELBACOSS.

References:

[1] N. Harrasser, M. de Wild, J. Gorkotte, A. Obermaier, S. Feihl, M. Straub, R. von Eisenhart-Rothe, H. Gollwitzer, J. Rüegg, W. Moser, Ph. Gruner, R. Burgkart, Evaluation of calcium dihydroxide- and silver-coated implants in the rat tibia, J Appl Biomater Funct Mater 14(4): e441-e448 (2016).

PoA.2.18

Unveiling the antibacterial properties of irradiated graphene oxide surfaces

Ana Brites[1,2], Margarida Ferreira[3], Catarina Silva Monteiro[3,4], Maria Miguel Morim[1,2,5], Orlando Frazão[3], Ana Patrícia Henriques[1,2], Inês de Castro Gonçalves[1,2]

[1]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [2]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [3]INESC-TEC - Instituto de Engenharia de Sistemas e Computadores, Tecnologia e Ciência, Porto, Portugal. [4]FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, Portugal. [5]FCUP - Faculdade de Ciências da Universidade do Porto, Porto, Portugal

Abstract

Antibiotic resistance is increasing, despite the development of new antibiotics lagging behind. Graphene-based materials (GBMs) have great potential as antimicrobial agents, especially in light-based therapies, and considering their low potential of causing bacterial resistance. GBMs films have been shown to eliminate ~99% of planktonic bacteria under low-power near-infrared (NIR) light exposure through photodynamic and mild-photothermal effects [1].

This work aimed to assess antibacterial effectiveness of irradiated graphene oxide (GO) using different light sources with varying wavelengths and optical powers. Envisaging the fighting of healthcare infections, bacteria's ability to recover and regrow after GO single or repeated exposure to the light was evaluated (reusability tests), as well as the influence of the presence of plasma proteins on GO's antibacterial activity.

Vacuum filtration was used to produce GO films from a commercial GO suspension. GO films were single- or multiple-irradiated with different light sources (lasers and LEDs, the latter with different viewing angles ($2\theta_{50\%}$.)) within the NIR range (730 and 850nm), at different optical powers (45–150mW; safe low-power for biomedical applications). Antibacterial performance (colony forming units assay) was conducted from 45min to 24h against *Staphylococcus epidermidis* incubated in TSB medium, with/without plasma proteins.

Planktonic bacteria required 45min to 4h to be fully eliminated, depending on light source and optical power. Decreasing power reduced antibacterial efficacy of the light source. NIR laser@850nm required 4h irradiation at 150mW (highest optical power) to eradicate bacteria (100% death), similar to LEDs@850nm. However, the same death percentage could be obtained at 74mW (smaller $2\theta_{50\%}$ LED) and at 110mW (higher $2\theta_{50\%}$ LED), demonstrating that smaller viewing angles are more effective, requiring less optical power to kill bacteria. With LED@730nm, bacteria growth was delayed by 88% after 4h at 133mW. Only laser @850nm was able to eradicate both planktonic and adherent bacteria, since after irradiation followed by 24h of incubation, no regrowth was observed.

Reusability tests showed a slight decrease (100% to 98%) in GO's antibacterial performance when exposed to the laser @850nm twice, with a 24h gap, but no performance reduction was observed with the smaller $2\theta_{50\%}$ LED @850nm, suggesting once again the impact of the light source.

Interaction between GO and safe low-power NIR irradiation is a promising technology to fight infections and antibiotic resistance, suggesting this is a safe approach for different applications, including the biomedical field.

References: [1] Henriques, P.C., et al., Carbon, 2021.

Acknowledgements: Wellcome Trust [WT223940/Z/21/Z]; La Caixa Foundation [LCF/PR/CI21/00256].

PoA.2.19

Towards pH-sensitive chitosan nanoparticles for treating biofilms on orthopaedic implants

Pardis Keikhosravani, Tim Bollen, Azin Khodaei, Saber Amin Yavari, Harrie Weinans

Utrecht medical center, Utrecht, Netherlands

Abstract

Introduction: Implant-associated infection (IAI) results from bacteria that initially colonize on the surface of the implants, then proceed to form a biofilm. Since biofilms are encased in an extracellular matrix and are tolerant to antibiotics and immune cell clearance. One promising strategy for delivering drug inside biofilm is using nanoparticles with smart pH-responsive properties to target distinct pH environments and improve the efficacy of drugs. Antibacterial peptides (AMP) are a class of compounds with the potential to combat bacterial infections, including those caused by Staphylococcus aureus (S. aureus) biofilms. LFchimera is a synthetic peptide designed by combining the functional domains of lactoferrin and melittin, while IDR1018 is an innate defense regulator peptide. In this study, the pH-sensitive smart chitosan nanoparticles (CSNPs) loaded with either LFchimera or IDR peptide were optimized to achieve maximum encapsulation efficiency, as well as improve its ability to penetrate the biofilm matrix and reduce drug toxicity, allowing increasing the chances of successful treatment.
Materials and methods: To prepare the CSNPs, the ion-gelation method using sodium tripolyphosphate (TPP) was utilized. In short, a solution of chitosan (5 mg/mL in 1% (v/v) acetic acid) was prepared and mixed with TPP in double distilled water. To load the LFchimera and IDR drug, they were mixed separately into the CSNPs at various weight ratios and any remaining chemicals or by-products were eliminated through centrifugation at 14,000 rpm for 30 min. Nanoparticle penetration toward biofilm, *in vitro* biomass reduction of biofilm, and cytotoxicity of the samples were investigated by confocal microscope visualization, crystal violet method, and Alamar blue activity assay, respectively.
Results and Discussion: The electrostatic interaction between chitosan and TPP allowed for the formation of CSNPs. These CSNPs facilitated the encapsulation of the LFchimera and IDR1018 drugs with 80 and 85% drug loading efficacy, respectively. Our materials characterization and biological assays verified that CSNPs could effectively penetrate and disrupt the biofilm matrix to be degraded under acidic conditions. Both LFchimera and IDR1018-loaded CSNPs reduced biofilm biomass. AMPs chitosan nanoparticles are more effective at reducing biofilm biomass than the peptides alone, indicating that the chitosan nanoparticle enhances the peptide's antimicrobial activity without cytotoxicity. In conclusion, both developed LFchimera and IDR1018-loaded CSNPs could potentially be applied to biofilms in the post-antibiotic era.

PoA.2.20

Using Low Temperature Plasma (LTP) to Develop Scaffolds with Antimicrobial Properties for Tissue Regeneration

KOMAL VIG

ALABAMA STATE UNIVERSITY, MONTGOMERY, USA

Abstract

Bypass surgery, using the autologous vein has been one of the most effective treatments for cardiovascular diseases (CVD). More recently tissue engineering including engineered vascular grafts to synthesize blood vessels is gaining usage. Dacron and ePTFE has been employed for vascular grafts, however, these does not work well for small diameter grafts (<6 mm) due to intimal hyperplasia and thrombosis. In the present study PTFE was treated with LTP to improve the endothelialization of intimal surface of graft. Scaffolds were also modified with polyvinylpyrrolidone coated silver nanoparticles (Ag-PVP) and the antimicrobial peptides, p753 and p359. Human umbilical vein endothelial cells (HUVEC) were plated on the developed scaffolds and cell proliferation was determined by the MTT assay. Cells attachment on scaffolds was visualized by microscopy. mRNA expressions levels of different cell markers were investigated using quantitative real-time PCR (qPCR). X ray photoelectron spectroscopic confirmed the introduction of oxygenated functionalities from LTP air plasma. Microscopic and MTT assays indicated increase in cell viability in LTP treated scaffolds. Gene expression studies shows enhanced expression of cell adhesion marker Integrin- α 5 gene after LTP treatment. The KB test displayed a zone of inhibition for Ag-PVP, p753 and p359 of 19mm, 14mm, and 12mm respectively. To determine toxicity of antimicrobial agents to cells, MTT Assay was performed using HEK293 cells. MTT Assay exhibited that Ag-PVP and the peptides were non-toxic to cells at 100µg/mL and 50µg/mL, respectively. Live/dead analysis and plate count of treated bacteria exhibited bacterial inhibition on develop scaffold compared to non-treated scaffold. SEM was performed to analyze the structural changes of bacteria after treatment with antimicrobial agents. Gene expression studies were conducted on RNA from bacteria treated with Ag-PVP and peptides using qRT-PCR. Based on our initial results, more scaffolds alternatives will be developed and investigated for cell growth and vascularization studies.

Acknowledgements: This work is supported by the NSF EPSCoR RII-Track-1 Cooperative Agreement OIA-2128653 and by NSF EIR award 1831282.

PoA.2.21

Role of the flagellar hook in the structural development and antibiotic tolerance of *Pseudomonas aeruginosa* biofilms

Jules D. P. Valentin[1,2], Hervé Straub[1], Qun Ren[1]

[1]Empa, St. Gallen, Switzerland. [2]University of Fribourg, Fribourg, Switzerland

Abstract

Pseudomonas aeruginosa biofilms exhibit an intrinsic resistance to antibiotics and constitute a considerable clinical threat. In cystic fibrosis, a common feature of biofilms formed by *P. aeruginosa* in the airway is the occurrence of mutants deficient in flagellar motility. This study aimed to investigate the role of the flagellum in adaptation and cell survival during biofilm development. Mutations in the flagellar hook protein FlgE influenced greatly *P. aeruginosa* biofilm structuring and antibiotic tolerance. Phenotypic analysis of *flgE* knockout mutants compared to the wild type (WT) revealed elevated fitness under planktonic conditions, and dramatically increased formation of microcolony aggregates in a microfluidic environment. Biofilm cells of *flgE* knock-out mutants displayed enhanced tolerance towards multiple antibiotics, whereas their planktonic cells showed similar resistance to the WT. Confocal microscopy study demonstrated that gentamicin does not affect the viability of cells located in the inner part of *flgE* knock-out mutant biofilms due to reduced penetration. These findings suggest that deficiency in flagellar proteins like FlgE in biofilms and in cystic fibrosis infections represent phenotypic and evolutionary adaptations that alter the structure of *P. aeruginosa* biofilms conferring increased antibiotic tolerance.

PoA.2.22

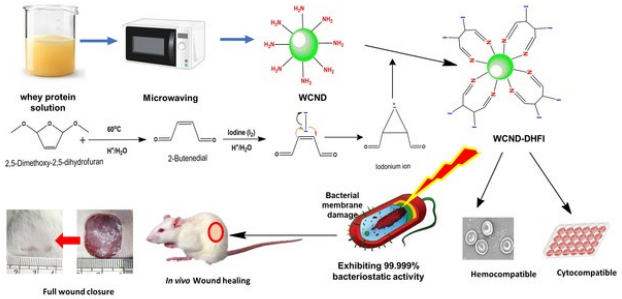
Iodine functionalized 2,5-dimethoxy-2,5-dihydrofuran (DHFI) crosslinked carbon nanodots for antibacterial and wound healing application

Sayan Mukherjee[1], Anurag Kumar Pandey[2,1], Nantu Dogra[1], Santanu Dhara[1]

[1]School of Medical Science and Technology, Indian Institute of Technology Kharagpur, Kharagpur, India. [2]School of Nano Science and Technology, Indian Institute of Technology Kharagpur, Kharagpur, India

Abstract

Antibiotic-resistant bacterial infections have become a severe biomedical challenge causing increased death rates and morbidity. There is a contentious urge to develop alternative bacteriostatic strategies and bactericidal agents to prevent and treat infections to counter antimicrobial-resistant organisms. Treatment for severely infected cutaneous wounds would need additional administration of antibacterial wound dressing solutions to protect the wound from bacterial infection and inflammation. In the current research, carbon nanodots (CND) have come to the fore as fascinating antibacterial nanomaterials due to their inherent bacteriostatic activity, low toxicity, and easy surface functionalization compared to metal-based nanoparticles and semiconductor quantum dots. In this regard, whey protein-derived carbon nanodots (WCND) were synthesized using the microwave irradiation method, and its amine-rich surface functionality was crosslinked with covalently bound Iodine functionalized 2,5-dimethoxy-2,5-dihydrofuran (DHFI) to produce WCND-DHFI. The physicochemical characterization of both WCND and WCND-DHFI were performed and compared to comprehend the consequence of iodination on the characteristics of WCND. The suitability of the sample in biological environments was evaluated through *in vitro* cytocompatibility as well as a hemocompatibility study. Cellular regeneration and propagation have been observed in dermal fibroblast cells isolated from rat skin. WCND-DHFI has shown improved cell viability and cellular propagation. Further, the antibacterial properties of WCND-DHFI were studied against both gram-positive and gram-negative bacterial strains. The WCND-DHFI has depicted a stable and prominent bacteriostatic activity for up to 6h for both strains of bacteria. The sample also has denoted a 99.996% and 99.999% loss of bacterial viability for gram-positive and negative strains, respectively. Further, the sample was investigated for *In vivo* wound healing efficacy with wound margins created in rat models and accelerates healing in comparison to standard market available dressing solutions. The wound healing capability was assessed by hematoxylin & eosin staining, as well as collagen deposition and tissue regeneration were confirmed by Masson's trichrome staining. WCND-DHFI can be used as a promising cytocompatible material for antibacterial and wound healing applications.



PoA.2.23

Antimicrobial Conjugated Oligoelectrolytes Containing Triphenylphosphonium Solubilizing Groups

Samuel Chan, Guillermo C. Bazan

National University of Singapore, Singapore, Singapore

Abstract

Conjugated oligoelectrolytes (COEs) are a class of amphiphilic antimicrobial anti-infective agents with a modular molecular framework that enables rapid synthetic derivatization. The molecular topology and spatial distribution of ionic groups is analogous to that of a phospholipid bilayer, and with appropriate molecular dimensions, these compounds readily interact with and permeabilize the bacterial cell membrane. The modular nature of this molecular platform enables facile tuning of the physicochemical properties of each molecule to achieve a desired activity profile. However, previous generations of COEs have been focused on improvements to activity against *Escherichia coli*. Here we designed a series of COEs derived from a stilbene conjugated segment and featuring triphenylphosphonium (TPP) groups and compared their activity relative to their quaternary ammonium counterparts. We show that substitution of the traditional quaternary alkylammonium moieties with a more lipophilic TPP cation enables broad-spectrum antimicrobial activity, while retaining the necessary aqueous solubility for drug development. The therapeutic scope was expanded relative to previous analogues to include drug resistant ESKAPE pathogens and mycobacteria. The lead compound was shown to perturb the bacterial membrane through various membrane-based assays and transmission electron microscopy. In addition, we have achieved successful performance of these membrane active molecules in an *in vivo* model, with lower treatment doses compared to clinically relevant antibiotics. This work thus highlights how simple structural modifications focused on tuning the hydrophobic-hydrophilic balance in a molecule can serve to improve its antimicrobial activity.

PoA.4.01

A polysaccharide-based platform for the production, growth, and chemotherapy evaluation of tumoroids

Elliot Lopez[1,2], Claire Wilhelm[2], Teresa Simon Yarza[1]

[1] Université Paris Cité, Université Sorbonne Paris Nord, LVTS Inserm U1148, 75018, Paris, France. [2] Physico-Chemistry Curie (PCC), CNRS UMR168, Institut Curie, Paris, France

Abstract

To answer the need for more accurate and personalized cancer treatments, two parallel trends are at stake among tumor models: increase of the throughput, and refining. Thanks to the recent development of microfluidics and microfabrication, in vitro 3D models represent a serious candidate as they conciliate a biomimetic design with high throughput and possibility to integrate further biological and biomechanical cues. In this perspective, we aim at developing a platform for the growth of tumoroids within a novel porous support made of polysaccharides.

Polysaccharides hydrogels are molded into 3D-printed guides to produce microwells networks that can either be used after crosslinking, or freeze-dried and rehydrated, which makes them porous. This platform offers a standardized support with tunable stiffness and porosity on which tumoroids are grown in a reproducible manner for further in vitro assays (Fig. 1).

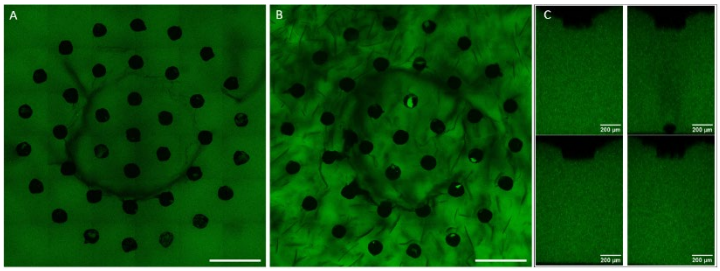


Fig. 1: (A) Microwells obtained with sterile neutralization. (B) Similar network obtained after freeze-drying and rehydration. Scale bar: 1 mm. (C) Lateral imaging of microwells within non-porous hydrogels. Scale bar: 200 µm. 0.025% FITC hydrogels.

The gels are seeded with cancer cells that rapidly aggregate into tumoroids in a reproducible and high-throughput manner. Once formed, survival assays were conducted after doxorubicin administration (Fig. 2). IC50 value were 5 to 10-fold higher for tumoroids as compared to monolayers, which highlights the resistance of cancer cells when grown in a 3D conformation. The presence of a plateau at 30% viability for high drug doses in 3D condition can pinpoint a laboring penetration of doxorubicin within the core, that will be assessed by microscopy.

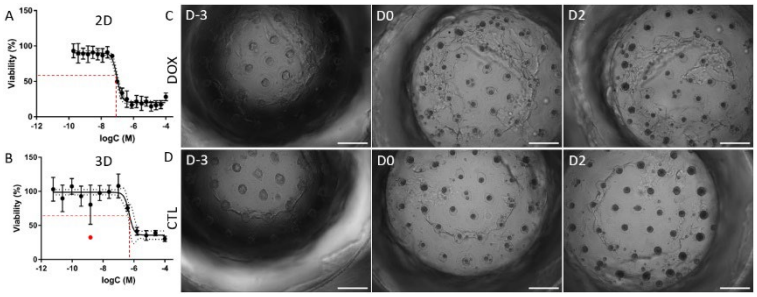


Fig. 2: Viability assay of A673 cells treated with doxorubicin for 48h in 2D (A) and in non-porous gels (B). Pictures of A673 cells in non-porous gels just after seeding, after 3 days of culture, and after 2 more days with treatment of 100 µM doxorubicin (C) or DMEM (D). Scale bar: 1 mm.

The versatility of the platform also allows deepening the role of the microenvironment on the cellular response. In this case, cancer cells are grown within porous gels doped with cancer-associated fibroblasts, and their invasion profile can be monitored by fluorescence microscopy with or without treatment. Finally, the polysaccharides hydrogels have been tested for the growth of tubular endothelial structures. Thus, the next step will be to combine tumoroids with endothelialized constructs towards a biomimetic platform of vascularized tumoroids production, growth, and integration within a microfluidic chip to include flow.

PoA.4.02

Development of 3D biocompatible hydrogels using a novel application of continuous liquid interface production (CLIP) for the sustained delivery of therapeutic neural stem cells against glioblastoma

Lauren E Kass, Cathleen DeVane, Addis Tessema, Julia Logan, Jillian Perry, Shawn Hingtgen

University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract

Background: Glioblastoma (GBM) is an aggressive brain cancer associated with a median survival of 12-15 months. A novel platform for treating GBM involves using engineered neural stem cells (NSCs) as targeted drug carriers. However, NSCs implanted into the GBM resection cavities of mice only survive there for 3-5 days. We hypothesized we could significantly improve therapeutic outcomes by increasing NSC persistence in the resection cavity using biomaterials. However, NSC migration from the biomaterial system should be maintained to enable therapeutic activity. We proposed using continuous liquid interface production (CLIP) to 3D-print biocompatible hydrogels on which NSCs could be seeded, with the 3D structure acting as a physical barrier to clearance without blocking cell migration.
Methods: A biocompatible resin made with gelatin methacryloyl (GelMA) and poly(ethylene glycol) diacrylate (PEGDA) was used to print a 3D lattice using CLIP (Figure 1). NSC loading onto the hydrogel surfaces was achieved via a static-centrifugation seeding strategy. NSC growth and morphology on the scaffolds was assessed via bioluminescence imaging and SEM imaging, respectively. Cell migration from the hydrogels was assessed *in vitro* using fluorescence microscopy. *In vivo* NSC persistence on the scaffolds was compared to NSCs directly injected in PBS using a model of GBM resection in mice with serial bioluminescence imaging.

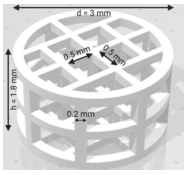


Figure 1. Rendering of CLIP Scaffold Design.

Results: NSCs on CLIP hydrogels proliferated to a density over 30-fold higher than the initial seeding density after 14 days in culture. SEM images revealed that NSC morphology on the hydrogels changes over time from a spherical shape to a flattened morphology once the cells attached to the scaffold material. *In vitro* migration experiments confirmed that NSCs cultured on CLIP hydrogels can escape the scaffolds and migrate to nearby GBM cells. *In vivo* persistence studies performed in non-tumor bearing mice showed that NSCs on CLIP hydrogels proliferated to 20% higher than the seeded dose by day 6, followed by a slow decline in persistence. In contrast, NSCs directly injected into the surgical cavity in saline showed a more immediate and rapid decrease in persistence, with 30% of the initial NSC signal lost at day 6.
Future Directions: We will next assess the therapeutic efficacy of NSC-loaded CLIP hydrogels versus directly injected NSCs in our model of GBM resection in mice. We also plan to expand our portfolio of biocompatible resins and hydrogel structures to determine which scaffold features most significantly influence NSC migration and persistence.

PoA.4.03

Tailored synthesis of dual-color silica nanosystems and its analysis by super resolution microscopy as potential tool in cancer therapy.

Maria A Ramirez Morales[1,2], Elisa M De Luca[3], Giuseppe A Mele[2], Pier P Pompa[4], Maria A Malvindi[1]

[1]HiQNano srl, Lecce, Italy. [2]Universitá del Salento, Lecce, Italy. [3]CNR NANOTEC, Institute of Nanotechnology, Lecce, Italy. [4]Istituto Italiano di Tecnologia, Nanobiointeractions&Nanodiagnostics, Genova, Italy

Abstract

Fluorescent nanoparticles are widely used in biomedicine, where their dispersion, stability, and biocompatibility are fundamental features for their potential. Silica nanoparticles are widely used due to its biocompatibility and generally synthesized by the hydrolysis and condensation of tetraethyl orthosilicate (TEOS) obtaining a wide range from 5-2000 nm. Multiple-colored nanoparticles can optimize diagnostic and therapeutic techniques by providing control and monitoring of the interaction inside living cells between the organelles and the used nanomaterial.
In this work, we presented for the first time, a two-cycle Stöber method for the tailored synthesis of dual-color fluorescent core-shell silicon dioxide (SiO2) nanoparticles using two commercially available dyes trapped each in a different silica matrix. The two-cycle Stöber method is based on the sol-gel process creating two loaded silica matrices with a fluorescent dye in each. First, a silica core with a green fluorophore covalently trapped inside was created. Subsequently, the second layer of fluorescent silica (shell) is formed through a second process of silica growing and far-red dye loading. Those nanosystems can be imaged using different excitation wavelengths precisely to get a better understanding of the behaviour of nanoparticles such as internalization, release, degradation, distribution, etc. Taking the affinity of some types of cancer towards biotin for future studies in diagnosis and treatment, we optimized the functionalization of the nanosystems surface with this molecule by two approaches: adsorption and covalent linking. All the nanosystems were characterized by Transmission Electron Microscopy (TEM), Fourier transform infrared (FT-IR) spectroscopy, Dynamic Light Scattering (DLS), UV-vis and confocal microscopy. Moreover, by using advanced super-resolution fluorescence imaging with Lattice SIM2, we demonstrated fluorescent NPs can be visualized at nanometric resolution, showing great promise for the high-resolution multicolour imaging within the complex and dynamic intracellular microenvironment in cancer studies.

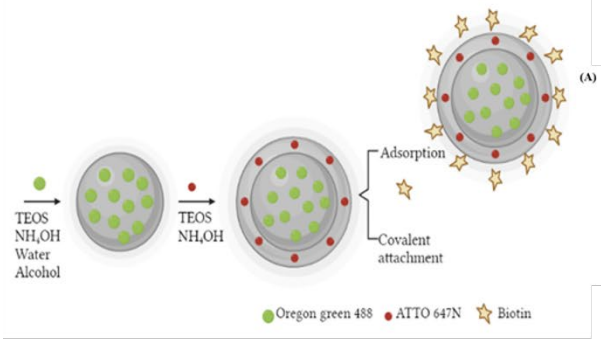


Figure 1. Schematic representation of two-cycle Stöber method.

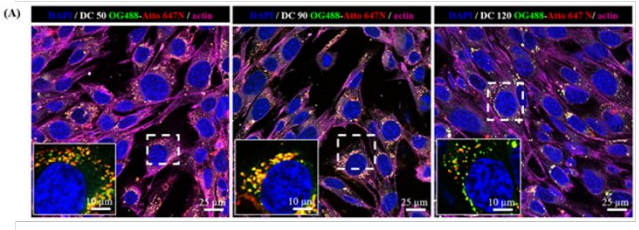


Figure 2. Nanoparticle internalization in primary human dermal fibroblasts. Representative confocal images of cells incubated for 24h with silica nanoparticles of different sizes.

PoA.4.04

Cancer Immunotherapy with Chemically Enhanced Anerobic Bacteria in Combination with Photothermal Therapy

Sheethal Reghu, Eijiro Miyako

Japan Advanced Institute of Science and Technology, Nomi, Japan

Abstract

While conventional cancer therapies such as radiation therapy, chemotherapy and surgery are extensively used and beneficial, they have a multitude of drawbacks such as resistance, side effects, inefficient targeting, costliness, which has hindered their mainstream adoption. Hence, it is crucial to update the treatment techniques to meet the growing demand for alternative medicines. Bacteria have emerged as a promising contender for cancer treatment with their unique and diverse mechanisms that cannot be achieved with traditional methods [1,2]. They can controllably induce cytotoxicity, high targeting efficiency, active tissue penetration, and are easily detectable, making them highly effective for cancer therapy [2].

The objective of this study was to augment the anaerobic bacteria *Bifidobacterium bifidum* by chemically modifying them with a photothermal agent, enabling them to possess photothermal properties to use in cancer immunotheranostics[3]. Cremophor EL (a formulation vehicle) encapsulated indocyanine green (a cyanine dye and a photothermal agent) nanocomplexes were prepared followed by their overnight incubation with *B.bifidum*. The nanoparticles penetrated through the bacterial membrane, thereby forming the modified bacteria exhibiting excellent NIR absorbance and low cytotoxicity. To explicate the modification process, we conducted fluorescence spectroscopy, UV spectroscopy, confocal microscopy, and transmission electron microscopy to identify morphological and structural distinctions between the modified bacteria and the unaltered ones. *In-vitro* studies conducted with these modified bacteria showed high-temperature elevation upon laser irradiation and low toxicity without laser irradiation. Tumor targeting efficiency was tested in *in-vivo* studies by injecting the modified bacteria intratumorally in colon26 implanted tumor mice models. Bacteria accumulations were detected exclusively in the hypoxic tumor environment using an NIR fluorescence bioimager, followed by the photothermal treatment at 0.7 W on solid tumors after 24 hours of injection using an 808nm NIR laser (Fig.1). Consequently, the tumor surface experienced a significant temperature increase, resulting in a complete tumor regression within a few days.

In conclusion, we demonstrated a simple bacteria-modification process to impart a therapeutic property by maintaining their function, structure, and morphology, without any genetic engineering. Once perfected, bacteria therapy could potentially outperform all other traditional cancer treatment methods and prove to be a valuable weapon to combat cancer.

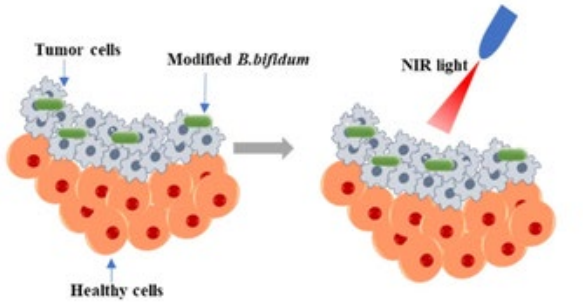


Fig. 1 Schematic illustration of localization of the modified bacteria in the tumor microenvironment.

References

1. Nair, N., et al. Anticancer Res. 2014, 34, 6289-6296.
2. Forbes, N. S. Nat. Rev. Cancer, 2010,10, 785−794.
3. Reghu, S. and Miyako, E. Nano Lett. 2022, 22, 1880-1888.

PoA.4.05

A microfluidic model of human vascularized breast cancer metastasis to bone for the study of immune-cancer cell interactions

Simone Bersini[1,2], Martina Crippa[1], Giuseppe Talò[3], Chiara Arrigoni[1,2], Matteo Moretti[1,2,3]

[1]Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Bellinzona, Switzerland. [2]Euler Institute, Università della Svizzera italiana, Lugano, Switzerland. [3]Cell and Tissue Engineering Lab, IRCCS Istituto Ortopedico Galeazzi, Milano, Italy

Abstract

The organ-specific metastatization of breast cancer to bone is driven by specific interactions between the host microenvironment and cancer cells. In this context, it is still unclear the role that circulating immune cells, including neutrophils, play during the early steps of bone metastatic colonization (i.e. pro-tumoral vs. anti-tumoral) [1]. Here we aimed at analyzing the contribution of neutrophils during the extravasation of circulating tumor cells and the growth of breast cancer micrometastases.

Based on our previous bone metastasis models [2], we microfabricated through 3D printing a microfluidic system that allows to: 1) track the flow and extravasation of breast cancer cells (BCCs) and other immune cells including neutrophils [3]; 2) independently seed human vascularized breast cancer metastatic seeds within a bone-mimicking microenvironment containing osteoblasts and endothelial cells (ECs) in a fibrin matrix [4]. ECs formed perfusable microvascular networks after 4 days (Fig.1A) and their architecture and permeability were quantified w/ and w/o BCCs. Circulating BCCs or neutrophils isolated from human buffy coats were injected into biofabricated blood vessels. Samples were live imaged using confocal microscopy.

Compared to control conditions without BCCs, the metastatic seeds compromised the architecture of microvascular networks resulting in lower number of junctions (5.7±1.2 vs. 18.8±4.5) and shorter total network length (10.5±1.0 vs. 13.4±0.8 [mm]). Vascular permeability was higher when measured in presence of BCCs ($3.8\pm6.8\times10^{-8}$ vs. $5.3\pm4.4\times10^{-9}$ [cm/s]). Neutrophil extravasation was also higher when BCCs were present compared to controls (27.9±13.7 vs. 14.7±12.4 [%]) (Fig.1B-C). However, neutrophil migration was driven by micrometastasis secreted factors promoting neutrophil interaction with BCCs rather than by the presence of leakier blood vessels. Strikingly, we observed that the percentage of dying BCCs increased in presence of neutrophils, as confirmed by flow cytometry on isolated cells. Finally, treatment with the drug eptifibatide reduced the extravasation of circulating BCCs by tackling the expression of invasive markers and improving inter-endothelial junctions.

We demonstrated that BCCs alter the bone microenvironment by modulating the architecture and permeability of blood vessels. We also showed that neutrophils have an anti-tumor phenotype by migrating towards BCCs and inducing cell death. Finally, we demonstrated that the biofabricated microenvironment can be modulated with pharmacological treatment to impair the metastatic process.

[1]Janssen et al. (2017) J. Immunother. Cancer, 5,1–14.
[2]Jeon et al. (2015) PNAS, 112(1),214-219.
[3]Crippa et al. (2021) Lab Chip, 21,1061-1072
[4]Crippa et al. (2022) Mat Today Bio, 17,100460

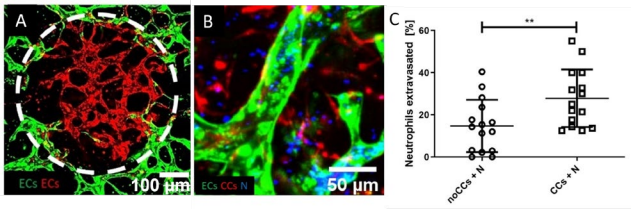


Figure 1: A) Representative image of connection between two independently seeded microvascular networks (GFP and RFP endothelial cells (ECs)) within the microfluidic device. B) Neutrophils (N, blue) flow within microvascular networks (green) and extravasate towards cancer cells (CCs, red). C) Quantification of neutrophil migration w/ and w/o cancer cells (t-test, p<0.01).

PoA.4.06

Screening for Copy Number Changes in Hepatocellular Carcinoma Using High-Resolution Multiplex PRT

Nouf Khalid Alsultan

University of Nottingham, Nottingham, United Kingdom. King Saud University, Riyadh, Saudi Arabia

Abstract

The paralogue ratio test (PRT) has been evaluated as a rapid system to detect copy number variations in several diseases. Hepatocellular carcinoma (HCC) is essentially cancer-associated in early stages with copy number variations including duplications or deletions of particular genes. The early detection of HCC is very important for a successful treatment regime. In this study, we used multiplex PRT to analyse copy number variations. As the primary aim, the copy number of desired gene loci from different chromosomes was estimated using a series of primer sets. The designed primers amplified fragments of approximately 100 bp to 200 bp. The PRT amplified fragments results clearly show the amplification of expected fragments with different primer sets, although minor variations of +/-2 bp were observed in the amplified products by capillary electrophoresis in comparison with the expected fragment lengths as estimated relative to the (ROX) size markers. The results of PRT amplification from the extracted genomic DNA from lymphoblastoid cell lines were used as a negative control for future analysis of samples from HCC patients. The calculations of standard deviation (SD) for the systems were in the range of (0.02 -0.27) and that gave reliability and credibility to the measurements.

PoA.4.07

Bone metastatic spheroid model for preclinical evaluation of novel anticancer biomaterials and therapeutics

Ceri-Anne E. Suurmond[1], Rong Wang[1], Michele Iafisco[2], Nicola Margiotta[3], Jeroen J.J.P. van den Beucken[1], Sander C.G. Leeuwenburgh[1]

[1]Radboud University Medical Center, department of Dentistry, Regenerative Biomaterials, Nijmegen, Netherlands. [2]Institute of Science, Technology and Sustainability for Ceramics (ISSMC, ex ISTEC), National Research Council (CNR), Faenza, Italy. [3]Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Bari, Italy

Abstract

Prostate and breast cancer are primary cancer types that metastasize frequently to bones. Direct treatment of bone metastases comprises surgical resection, which creates a bone defect that requires filling with a suitable graft material. (1) Ideally, such bone graft material would have dual functionality by exhibiting both bone regenerative and anticancer efficacy. Recently, hydroxyapatite nanoparticles (HAnps) loaded with cisplatin have been proposed to achieve this dual functionality. (2) However, biomaterials with anticancer efficacy are currently evaluated using relatively simple 2D cell culture models that do not resemble the physiological bone tumor conditions. (3) Therefore, we previously developed an easy-to-use but clinically relevant 3D humanized bone metastatic spheroid model that showed abundant cancer cell manifestation. Here, we aim to use this model to preclinically assess therapeutic efficacy of cisplatin and cellular uptake of HAnps in 3D.

Primary human bone marrow stromal cells (hBMSC; stained with CellTrace CFSE green) were combined with either 10% cancer cells (prostate PC3 or breast cancer MDA-MB-231; stained with CellTrace Far Red) and seeded in ultra-low attachment plates with 30 µg/mL of collagen type 1A to generate metastatic spheroids. Cisplatin was added at 10, 50, and 100 µM for a 24h exposure period. Subsequently, spheroids were imaged using confocal microscopy. HAnps were prepared by mixing 83.5 mM Ca(CH3COO2) with 50 mM Na3PO4, followed by aging for 24h at 60 °C under continuous stirring. HAnps were then collected by centrifugation, washed and diluted to 1 mg/mL. HAnp were stained with IRDye 680RD overnight and subsequently supplemented to the media of the stained spheroids at 25 µg/mL.

Spheroids show a dose-dependent response to cisplatin administration. Both types of cancer cells were more susceptible to cisplatin than hBMSCs. In addition, HAnps were taken up by spheroids after a 24h incubation period, suggesting that our bone metastatic spheroids can be used for 3D preclinical evaluation of nanobiomaterials. These data justify further validation of bone metastatic spheroids for screening of chemotherapeutics and and nanobiomaterials for treatment of bone cancer.

1. Cooper DM, et al. Cortical Bone Porosity: What Is It, Why Is It Important, and How Can We Detect It? Curr Osteoporos Rep. 2016;14(5):187-98.
2. Nadar RA, et al. Preclinical evaluation of platinum-loaded hydroxyapatite nanoparticles in an embryonic zebrafish xenograft model. Nanoscale. 2020;12(25):13582-94.
3. Li F, et al. A bifunctional MXene-modified scaffold for photothermal therapy and maxillofacial tissue regeneration. Regenerative Biomaterials. 2021;8(6).

PoA.4.09

Engineering a vascularized aerogel-based platform using biofunctionalized alginate

Mariana V. Magalhães[1,2,3], Inês Coutinho[1,2], Cristina C. Barrias[1,2,4], Sílvia J. Bidarra[1,2]

[1]INEB, Porto, Portugal. [2]i3S, Porto, Portugal. [3]FEUP, Porto, Portugal. [4]ICBAS, Porto, Portugal

Abstract

Breast Cancer is one of the most common, and is currently the primary cause of cancer-related deaths in women. Moreover, it is not the primary tumor that is usually the culprit. It is the "untreatable" nature of metastatic breast cancer that is responsible for the majority of deaths related to breast cancer. A key step in tumor progression and metastasis is angiogenesis. The development of vasculature within the breast tumor microenvironment is crucial not only for nutrient attainment but also for disposing of metabolic waste and carbon dioxide to maintain homeostasis and the erratic proliferation of cells. Overwhelming efforts have been put into designing innovative pre-clinical models that can truthfully replicate the disease *in vitro*[1]. 3D models that can incorporate multiple types of cells[2] and also replicate the vascular compartment[3], will undoubtedly help further to comprehend the complexity behind tumor vascularization and metastasis and improve success rates in drug development and screening. To address this challenge, we develop a hybrid alginate-based 3D system, combining hydrogel-embedded tumoroids (parenchymal compartment) with a macroporous alginate-based aerogel co-seeded with fibroblasts and endothelial cells (vascularized stromal compartment). For the stromal compartment, we used macroporous RGD/YIGSR alginate-based aerogels co-seeded with endothelial cells and fibroblasts. For the parenchymal compartment, a protease-sensitive alginate hydrogel with tumor cells was added to the pores of pre-vascularized scaffolds, forming a hydrogel *in situ* by peptidic crosslinking. The 3D hybrid system supports epithelial morphogenesis in tumoroids and endothelial tubulogenesis, allowing heterotypic cell-cell and cell-ECM interactions. It thus provides a versatile and powerful tool to study the role of angiogenesis during tumor progression. In the future, it can be further optimized to receive patient-derived cells for applications such as drug screening, which paves the way to bringing us closer to a world where personalized medicine is the standard of care.

References

1. Bidarra, S.J., *et al.*, A 3D *in vitro* model to explore the inter-conversion between epithelial and mesenchymal states during EMT and its reversion. Sci Rep, 2016.
2. Barros da Silva, P., *et al.*, Reshaping *in vitro* Models of Breast Tissue: Integration of Stromal and Parenchymal Compartments in 3D Printed Hydrogels. Front Bioeng Biotechnol, 2020.
3. Teixeira, F.C., *et al.*, Engineering a Vascularized 3D Hybrid System to Model Tumor-Stroma Interactions in Breast Cancer. Front Bioeng Biotechnol, 2021.

Acknowledgments

Portuguese Foundation for Science and Technology for Project EndoSWITCH (PTDC/BTM-ORG/5154/2020), MVM's scholarship (Grant No: SFRH/BD/10184/2022), and SJB's research contract DL 57/2016/CP1360/CT0006.

PoA.4.10

Quantitative spatial distribution maps of hyaluronic acid in healthy skin and melanoma using a novel methodology.

Jorge González-Rico[1], Cristina Quílez[2,3], Marisa López-Donaire[1], Enrique Cerdeira[2], Nuria Gago-López[4], Arrate Muñoz-Barrutia[2,5], Diego Velasco[2,3,5]

[1]Department of Continuum Mechanics and Structural Analysis, Universidad Carlos III de Madrid, Leganés, Spain. [2]Department of Bioengineering, Universidad Carlos III de Madrid, Leganés, Spain. [3]Fundación Instituto de Investigación Sanitaria de la Fundación Jiménez Díaz, Madrid, Spain. [4]Melanoma group. Molecular Onoclogy Programm. Spanish National Cancer Research Center (CNIO), Madrid, Spain. [5]Instituto De Investigacion Sanitaria Gregorio Marañon, Madrid, Spain

Abstract

Hyaluronic Acid (HA) is an essential component of the extracellular matrix of most tissues, particularly in the skin, where it performs both filler and specific functional roles depending on its molecular weight. However, in cases of tumor progression, also the concentration of HA is related to both prognosis and cancer type (1). Particularly in melanoma, the most lethal skin cancer, the concentration of HA has been proposed as a prognostic biomarker. However, it is still unknown whether modifications in the molecular weight of HA and architecture could be associated to melanoma progression and there are not accurate systems and protocols that localize and quantify HA in the tumor architecture and environment. Our protocol is based in the direct relationship between fluorescent signal of HA and bulk tissue HA quantification from tissue digestion followed by ELISA. The fluorescent signal is obtained from the specific interaction of tissue HA with a biotinylated HA binding protein from Versican V1 domain. Both stained and digested samples were obtained from the same sample, alternating different thickness of sectioning. Finally, from the extensive analysis from a varied range of samples (both from human and animal), a calibration curve was obtained for determining the concentration of HA in a stained sample, through image analysis (Figure 1). To validate the method, we analyzed both healthy and melanoma skin samples. Our results showed that the HA concentration in melanoma was lower than in surrounding tissue and healthy skin samples (Figure 2), consistent with previous findings (2). However, our novel method provided actual concentration values, making it a potentially useful tool for delimiting tumors and improve evaluating the prognosis in melanoma. In summary, our study offers: 1) a robust protocol for spatial quantification and mapping of HA in biological tissues, 2) highlights the importance of considering spatial distribution when assessing the role of HA in cancer development and progression.

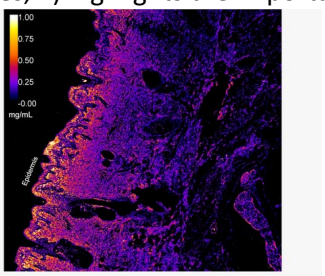


Figure 1: Quantitative spatial distribution of HA in human healthy skin

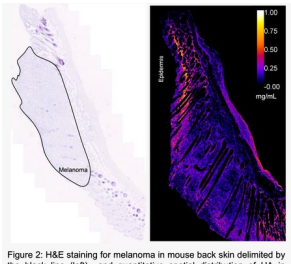


Figure 2: H&E staining for melanoma in mouse back skin delimited by the black line (left) and quantitative spatial distribution of HA in mouse back skin melanoma (right)

References

1. S. Aaltomaa et al., Urol. Int. 69, 266–272 (2002).
2. J. M. Karjalainen et al., Am. J. Pathol. 157, 957–965 (2000).

Acknowledgments

This work was supported by I+D+i PID2021-126523OB-I00, funded by AEI 10.13039/501100011033 and Programa Autonómico CAM S2018/BAA-4480.This work was partially funded by Ministerio de Ciencia, Innovación y Universidades, Agencia Estatal de Investigación, under Grants FPU19/02854 and PID2019-109820RB-I00, MCIN/AEI/10.13039/501100011033/, co-financed by European Regional Development Fund (ERDF), "A way of making Europe".

PoA.4.12

Integrating Spectroscopic and Imaging Approaches for Improved Biological Fluids and Tissues Biopsy Analysis in Colorectal Cancer Detection

Lavinia Raluca Șaitiș[1], David Andras[2,3], Alexandru Stefan Farcasanu[1], Cătălin Șaitiș[4], Ramona Crainic[1], Radu Fechete[4]

[1]Babeş-Bolyai University, Faculty of Physics, Cluj-Napoca, Romania. [2]County Emergency Hospital, Surgical Department, Cluj-Napoca, Romania. [3]Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania. [4]Technical University of Cluj-Napoca, Cluj-Napoca, Romania

Abstract

Colorectal cancer (CRC) is one of the most common and deadly types of cancer worldwide. Therefore, the early detection of CRC is critical for improving patient outcomes. Compared with the time consuming classical histopathological approach, this study discusses the application of various spectroscopic and imagistic techniques for the fast detection and diagnosis of CRC from blood plasma analysis, tissue biopsy analysis, and enzyme-based assays.

Native and deproteinized blood plasma and proteins from plasma collected from 40 patients with confirmed CRC and 20 healthy volunteers were analysis using FT-IR spectroscopy, 1D 1H NMR relaxometry and diffusometry and 2D 1H NMR T2-T2 EXSY as well as single voxel spectroscopy. Plasma samples collected from CRC patients' present higher lipid and protein content compared to healthy controls, as was detected by FT-IR and 1H NMR spectroscopy. Moreover, 1H NMR relaxometry and diffusometry can be used to measure the proton mobility and water diffusion which reflect changes in plasma composition and structure associated with CRC. Tissue biopsy analysis using FT-IR and MRI imaging (native and 2D 1H NMR T2-T2 EXSY maps as well as single voxel spectroscopy) can provide detailed information on the molecular and structural changes (presence of specific metabolites) that occur in CRC tissue. Therefore, various types of acquired MRI images can detect changes in tissue morphology and function (tissue perfusion and cellularity). The use of enzyme-based assays, such as Alkaline Phosphatase (ALP) and the conversion of MnO2 to Mn2+ in the presence of 2-phospho-L-ascorbic acid trisodium salt, in the evaluation of accuracy of CRC detection by 1D and 2D NMR relaxometry methods was investigated. ALP is an enzyme that is elevated in CRC patients, and its detection in serum can be used as a marker for CRC. The mechanism is based on the conversion of MnO2 to Mn2+ in the presence of 2-phospho-L-ascorbic acid trisodium salt (leading to a significantly decay of T2-relaxation time) and can be used to detect ALP activity in serum samples, shown to be highly sensitive and specific for CRC detection. Finally, the most relevant parameters were included into a Principal Component Analysis (PCA) and the results are systematically classified using a trained Artificial Neuronal Network (ANN) based on machine learning (ml5) library. In conclusion, the application of various techniques for the detection and diagnosis of CRC, including blood plasma analysis, tissue biopsy analysis, and enzyme-based assays, can provide valuable information for clinicians and improve patient healthy state.

PoA.4.13

3D Bioprinting for creating osteosarcoma constructs as a model for Boron Neutron Capture Therapy (BNCT) studies

Elena Delgrosso[1], Franca Scocozza[2], Laura Cansolino[1,3], Federica Riva[4], Michele Conti[2], Ian Postuma[3], Silva Bortolussi[5,3], Cinzia Ferrari[1,3,6]

[1]Dept. of Clinical Surgical Sciences; integrated unit of experimental surgery, advanced microsurgery and regenerative medicine, University of Pavia, Pavia, Italy. [2]Dept. Civil Engineering and Architecture; University of Pavia, Pavia, Italy. [3]National Institute of Nuclear Physics (INFN), Pavia, Italy. [4]Dept. Public Health, Experimental and Forensic Medicine, Histology and Embryology Unit, University of Pavia, Pavia, Italy. [5]Department of Physics, University of Pavia, Pavia, Italy. [6]Animal Care and Radiobiology Centre, University of Pavia, Pavia, Italy

Abstract

Osteosarcoma is a primary malignant bone tumor that occurs mainly in children, adolescents, and young adults. Its infiltrative growth makes it difficult to treat with current options, which involve multiagent chemotherapy and extensive surgical resection. Nevertheless, its infiltrative growth leads to a high incidence of local and distant recurrences. Boron Neutron Capture Therapy (BNCT) is an experimental alternative radiotherapy treatment that could allow a less aggressive surgery by killing infiltrative tumor cells in the surrounding healthy tissues. BNCT studies are performed on 2D *in vitro* models that are not able to reproduce pathological tumor tissue or on *in vivo* animal models that are expensive, time-consuming and must follow the 3R's principles. The aim of this project is to develop a 3D *in vitro* model of osteosarcoma using bioprinting technology, which can better reproduce the pathological tumor tissue organization and limit the use of animals.

A rat osteosarcoma cell line (UMR-106) was encapsulated into a sodium alginate-SA 8% hydrogel based, and 3D bioprinted with the Cellink INKREDIBLE+® (Cellink AB). The printing protocol, biomaterial selection, cell density, and crosslinking process were optimized to develop the 3D *in vitro* tumor model. We also try to develop a method to quantify the intracellular levels of boron uptake by the osteosarcoma cells encapsulated into the bioink. Two different treatments were tested: constructs printed with cells previously exposed to Boronophenylanine (BPA) (pre-printing treatment) to evaluate the gel interference with the 10B quantification and constructs printed with cells not previously enriched with BPA but exposed after printing to BPA (post-printing treatment) to verify the gel influence on the intracellular boron uptake and to define a method suitable to remove the residual BPA trapped in the gel matrix. We successfully obtained a fully colonized 3D living cell construct by rat osteosarcoma cell line UMR-106. The intracellular boron evaluation is a crucial point for BNCT studies. However, the method for boron quantification in cells within constructs needs to be further refined because the gel matrix interferes with the measurements. Our proposed model provides an alternative or parallel approach to 2D culture and *in vivo* animal models for BNCT experimental studies. The optimized printing protocol, biomaterial selection, and cell density offer a valuable basis for the development of further 3D osteosarcoma models for BNCT studies.

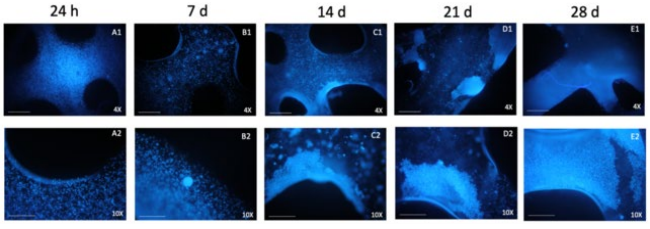


Figure1. *Printed constructs images obtained by fluorescence microscope (Olympus CX41). Nuclei cells stained using HO 33258 (blue fluorescence) highlighting cell distribution and clone formation.*

PoA.4.14

Development of mesoporous silica nanoparticles coated with pH-responsive polymers and encapsulated with MAPK inhibitor for the treatment of Colorectal carcinoma

Kornrawee Srichan, Chatchawan Srisawat, Vorapan Sirivatanauksorn, Nathachit Limjunyawong, Primana Punnakitikashem

Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol university, Bangkok, Thailand

Abstract

Colorectal carcinoma (CRC) is a significant cause of cancer-related deaths worldwide. Dysregulated epidermal growth factor receptor (EGFR) which is related to MAPK signaling pathway was overexpressed in CRC patients. Current treatments for CRC, including surgery, radiotherapy, and chemotherapy, have limited efficacy and many side effects. Therefore, targeted therapy is a new option for CRC treatment. Encorafenib is a small molecule drug that can inhibit the MAPK signaling pathway, has demonstrated potential as a targeted therapy for CRC. However, its clinical efficacy has been limited in terms of drug dosage and drug degradation. So, mesoporous silica nanoparticles (MSNs) which are nanocarriers for drug delivery have many properties such as large pore volume, tunable pore size, and ordered arrangement of pores. Additionally, the use of pH-responsive polymers, carboxymethyl chitosan (CMC), allows for controlled drug release in response to the acidic tumor microenvironment, improving therapeutic efficacy. Thus, this study aims to develop the drug delivery system for CRC treatment using encorafenib encapsulated in MSNs coated with carboxymethyl chitosan (En@MSNs-CMC). The purpose of this system is expected to increase drug accumulation at the tumor site and enhance the therapeutic efficiency of the drug, leading to improved clinical outcomes for CRC patients. MSNs were synthesized by Stober's method and demonstrated uniform spherical morphology, as confirmed by field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM) (Figure 1). TEM analysis revealed that the size of MSNs was 69.50 ± 4.82 nm. Dynamic light scattering (DLS) measurement showed hydrodynamic sizes of 114.40 ± 2.69 nm for MSNs and 271.33 ± 12.54 nm for MSNs-CMC. Moreover, the encapsulation efficiency of encorafenib encapsulated in nanocarriers revealed 92.55 ± 4.17 %. *In vitro* cell studies, the cellular uptake of coumarin-6 encapsulated in MSNs-CMC (Cou@MSNs-CMC) showed that Cou@MSNs-CMC have cellular internalization by the CRC cells (Figure 2). In addition, En@MSNs-CMC demonstrated enhanced efficiency in inhibiting CRC cells compared to control groups observed by the CellTiter®-Blue cell viability assay. These findings showed the potential of En@MSNs-CMC as an effective suppressor of CRC cells growth. These results suggested that mesoporous silica nanoparticles coated with pH-responsive polymers can improve drug delivery performance and provide a drug delivery system for efficient CRC therapy.

Keywords- Colorectal cancer, MAPK inhibitor, mesoporous silica nanoparticles, pH-responsive polymers.

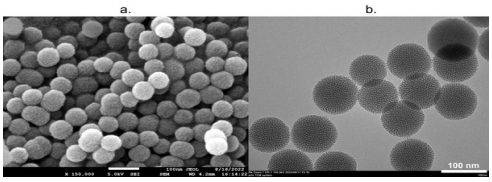


Figure 1 FE-SEM (a.) and TEM (b.) image of mesoporous silica nanoparticles (MSNs).

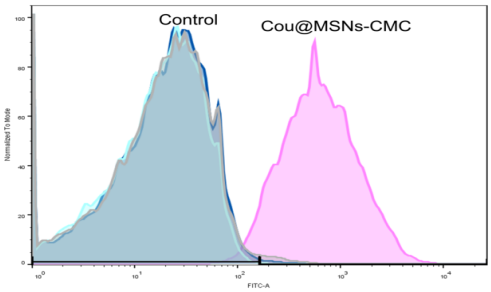


Figure 2 Flow cytometry analysis for determining cellular internalization of Cou@MSNs-CMC in CRC cells.

PoA.4.16

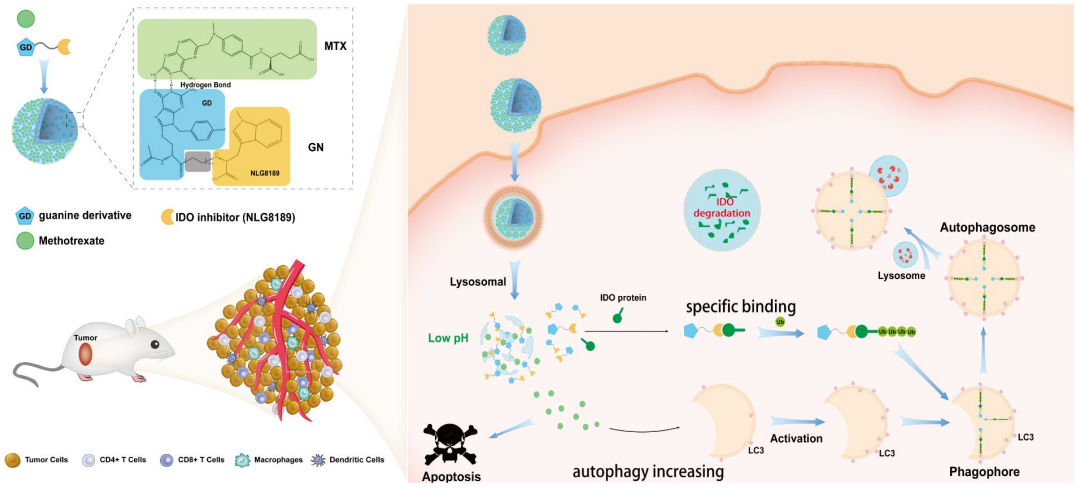
AUTAC-based supramolecular self-assembled nanostructures for immunometabolic intervention and reprogramming macrophage to M1 polarization in tumor immunotherapy

Yazhen Wang, Huile Gao, Jun Cao

Sichuan University, Chengdu, China

Abstract

Immunometabolic intervention via inhibiting the activity of associated proteins shows promising prospects in reinvigorating antitumor immunity. However, simply blocking these proteins' activities does not result in a sufficient therapeutic effect. The advent of autophagy-targeting chimera (AUTAC) allows this translation of the pharmacological inhibition paradigm into the degradation paradigm, providing a more marked therapeutic effect. In this study, we first designed and synthesized an AUTAC molecular (GN) for achieving intracellular indoleamine 2,3-dioxygenase (IDO) protein targeted degradation via an auto-lysosome system. Then, inspired by the Watson-Crick base pairing theory, GN (the guanine derivative structure) could self-assembled with a nucleoside analogue (i.e., methotrexate) by hydrogen bonding interactions to form supramolecular nanostructures (GMNPs) with chemotherapeutic and protein degradation abilities for immunometabolic cancer therapy. After accumulating at the tumor sites by enhanced permeation and retention (EPR) effects, GMNPs could be endocytosed by tumor cells and realize drug release in response to intracellular acidic lysosome environments. On the one hand, released methotrexate promoted apoptosis of tumor cells and induced autophagy, on the other hand, released GN bound with IDO protein and induced its K63-linked polyubiquitination, leading to persistent IDO degradation. Further, such a combination of immunotherapy and chemotherapy modulated the crosstalk between tumor cells and immune cells, initiating TAM reprogramming to M1-like macrophage, boosting the antitumor T-cell immunity, and effectively inhibiting tumor growth and metastasis. Thus, our study provides a supramolecular self-assembled nanostructure platform to advance AUTAC in tumor immunotherapy.



PoA.4.17

Innovative Approach for Treating Glioblastoma After Surgery: Thermo-Sensitive PEG-PCL-PEG Hydrogel Loaded with Branched Poly(amidoamine) siRNA Complexes

Nurcan Gumus[1,2], Keith Spriggs[1], Pratik Gurnani[3], Ruman Rahman[2], Cameron Alexander[1]

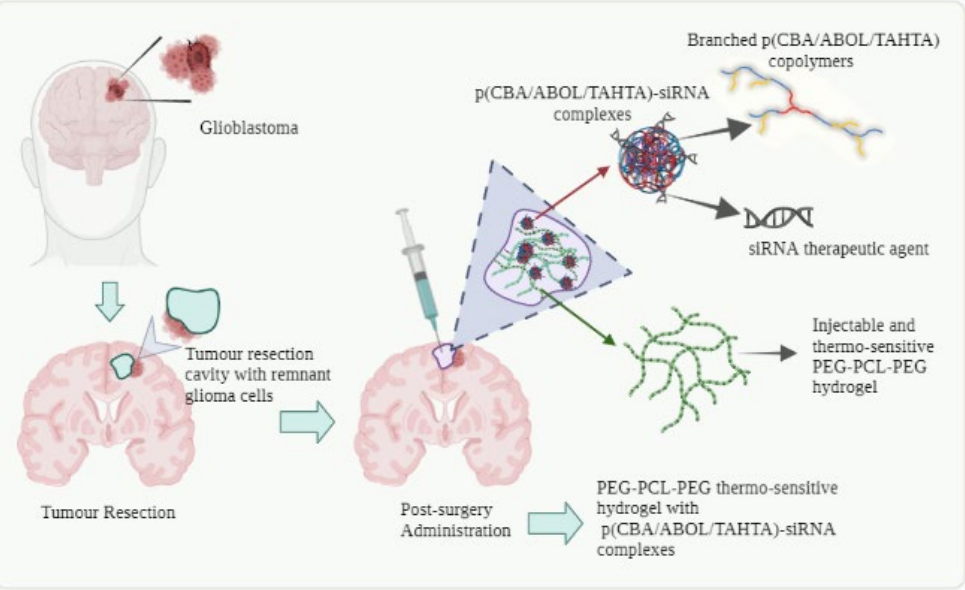
[1]Division of Molecular Therapeutics and Formulation, School of Pharmacy, University of Nottingham, NG7 2RD, UK., Nottingham, United Kingdom. [2]Children's Brain Tumour Research Centre, School of Medicine, University of Nottingham, Nottingham NG7 2UH, U.K., Nottingham, United Kingdom. [3]UCL School of Pharmacy, University College London, 29-39 Brunswick Square, Bloomsbury, London, WC1N 1AX, UK, London, United Kingdom

Abstract

Glioblastoma is the most aggressive and prevalent type of brain cancer in adults. Although immense efforts and research have been dedicated to developing glioblastoma treatments, the median survival of patients remains less than 15 months from diagnosis and has not improved in decades. One of the major contributors to poor prognosis in glioblastoma disease is tumor recurrence following surgical resection at the glioblastoma invasive margin. Small interfering RNAs (siRNAs) are promising therapeutic agents to suppress genes that contribute to glioblastoma pathogenesis by modulating protein expression of classically undruggable disease targets involved in cell migration, invasion, and proliferation. However, the delivery of siRNA to the brain is one of the issues impeding the clinical applications of siRNA-based therapy owing to short half-life in circulation and its poor permeability across blood–brain barrier (BBB). The state-of-the-art research indicates the potential for implantation of controlled release formulations directly within the tumour cavity following surgical resection to provide sustained treatment at the glioblastoma invasive margin, directly bypassing the BBB. Here, we report the development of a poly(ethylene glycol)-poly(ε-caprolactone)-poly(ethylene glycol) based hydrogel system for controlled release of siRNA formulations at the glioblastoma invasive margin.

For our siRNA formulation, we developed a novel branched poly(amidoamine) bio-reducible non-viral vector classified as p(CBA/ABOL/TAHTA) copolymers showing high transfection efficiency. The formulations exhibited 85% siRNA encapsulation efficiency and average diameters less than 200 nm. Importantly the formulations showed around 60% silencing effects *in vitro* (around 60% reduction in the luciferase activity in GIN31fluc (patient-derived glioblastoma cell lines), 25% higher than the lipofectamine positive control.

To assess the potential for post-surgical administration, siRNA polyplexes were immobilised in the PEG-PCL-PEG hydrogel. Sustainable release of the polyplexes was observed up to 5 days in phosphate buffered saline but importantly were found to be biologically active post-release with more than 30% gene silencing effect was observed. Furthermore, utilising Cy5 labelled polyplexes we observed promising penetration ability after applying the loaded hydrogel to a pseudo-resection in cadaver mouse brains. Consequently, p(CBA/ABOL/TAHTA)-copolymer is a wide promising carrier for siRNA delivery and an injectable thermos-sensitive hydrogel to advance local and sustained release of siRNA.



Scheme 1. Illustration of the thermos-sensitive hydrogel siRNA delivery system with branched p(CBA/ABOL/TAHTA) copolymers. p(CBA/ABOL/TAHTA)-siRNA complexes were loaded into the thermos-sensitive hydrogel.

PoA.6.04

Thiolate poly(lactic-co-glycolic acid) nanofibers loaded with dexamethasone and ropivacaine show enhanced sustained release in the treatment of neuropathic pain through a local therapy technique

Yoon Jin Choi

National Cancer Center, Gyeonggido, Korea, Republic of

Abstract

To treat neuropathic pain, dexamethasone (DEX, an anti-inflammatory agent) and ropivacaine (RVC, a local anesthetic) are injected into the epidural space of the patient's spine in clinics. This combinatorial drug treatment is short-acting and subjected to uncontrolled drug flow to the motor nerve. To overcome these limitations, we developed thiolate poly(lactic-co-glycolic acid) (PLGA-SH) nanofibers conjugated with mono-(6-mercapto-6- deoxy)-β-cyclodextrin (SH-β-CD) containing DEX and RVC. The anti-inflammatory effect in the PGLA-CD-DEXRVC nanofibers was assessed using bone marrow-derived macrophages (BMMs) *in vitro*. We injured the sciatic nerve in Sprague Dawley (SD) rats to create a Chronic constriction injury (CCI) model for an *in vivo* assessment. Neuropathic pain was evaluated by testing the cold allodynia response, and by Immunofluorescence (IF) staining of transient receptor potential vanilloid 1 (TRPV1, a nociceptor marker) and ionized calciumbinding adaptor molecule 1 (iba1, a microglia marker). In this study, the synthesized PLGA-CD-DEX-RVC nanofibers sustainably released RVC and DEX for more than 48 h. The PLGA-CD-DEX-RVC nanofibers suppressed polarized M1 macrophages and increased polarized M2 macrophages. The allodynia cold sensitivity was consistently relieved in the PLGA-CD-DEX-RVC group for 14 days. Moreover, the expressions of the TRPV1 and iba1 were remarkably reduced in the PLGA-CD-DEX-RVC group on day 14. PLGA-CD-DEX-RVC nanofibers can restrict drugs flow to motor nerves and of relieving pain for extended periods. We suggest that PLGA-CD-DEXRVC nanofibers can serve as a useful treatment for neuropathic pain in clinics.

PoA.6.06

Development of polymeric reinforcements for collagen-based vascular wall models

Nele Pien[1,2,3], Dalila Di Francesco[1,4], Francesco Copes[1], Michael Bartolf-Kopp[5], Victor Chausse[6], Marta Pegueroles[6], Tomasz Jüngst[5], Francesca Boccafoschi[4], Catharina De Schauwer[3], Sandra Van Vlierberghe[2], Peter Dubruel[2], Diego Mantovani[1]

[1]Laboratory for Biomaterials and Bioengineering, Canada Research Chair Tier I for the Innovation in Surgery, Department of Min-Met-Materials Engineering & Regenerative Medicine, CHU de Quebec Research Center, Laval University, Quebec City, Canada. [2]Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Department of Organic and Macromolecular Chemistry, Ghent, Belgium. [3]Faculty of Veterinary Medicine, Department of Translational Physiology, Infectiology and Public Health, Ghent University, Ghent, Belgium. [4]Laboratory of Human Anatomy, Department of Health Sciences, University of Piemonte Orientale "A. Avogadro", Novarra, Italy. [5]Department of Functional Materials in Medicine and Dentistry, Institute of Biofabrication and Functional Materials, University of Würzburg and KeyLab Polymers for Medicine of the Bavarian Polymer Institute (BPI), Würzburg, Germany. [6]Biomaterials, Biomechanics and Tissue Engineering group, Department of Materials Science and Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain

Abstract

A previously developed collagen-based vascular wall model showed promising results in mimicking the biological properties of a native vessel but lacked appropriate mechanical properties. In this work, we aim to improve this collagen-based model by reinforcing it using a tubular polymeric (reinforcement) scaffold. The polymeric reinforcements were fabricated in commercial, poly(ε-caprolactone (PCL), a polymer already used to fabricate other FDA-approved and commercially available devices for medical applications, by (i) solution electrospinning (SES), (ii) 3D printing (3DP) and (iii) melt electrowriting (MEW). The non-reinforced collagen-based model was used as a reference (COL). The effect of the processing technique and the corresponding scaffold architecture on the resulting mechanical and biological properties of the reinforced collagen-based model were evaluated. SEM imaging showed the influence of the processing technique on the scaffold's architecture at both the micro- and the macrolevel (Figure 1). The polymeric scaffold led to significantly improved mechanical properties of the reinforced collagen-based model (initial elastic moduli of 382.05 ± 132.01 kPa, 100.59 ± 31.15 kPa and 245.78 ± 33.54 kPa, respectively for SES, 3DP and MEW at day 7 of maturation) compared to the non-reinforced collagen-based model (16.63 ± 5.69 kPa). In addition to the effect on the resulting mechanical properties, the different processing techniques and polymeric tubes' architecture influenced cell behavior, in terms of proliferation and attachment, along with collagen gel compaction and ECM protein expression. The MEW reinforcement resulted in a collagen gel compaction like the COL reference, whereas 3DP and SES lead to thinner and longer collagen gels. Overall, it can be concluded that (i) the selected processing technique strongly influences the resulting mechanical and biological properties, and (ii) the incorporation of a polymeric reinforcement leads to mechanical properties closely resembling those of native arteries.

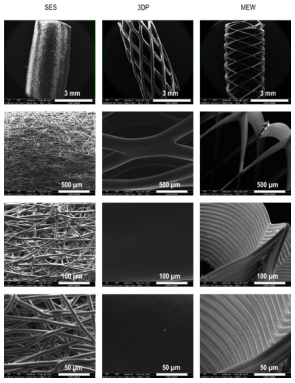


Figure 1. Visualization of the reinforcement tubes using SEM imaging, processed by SES, 3DP and MEW. Fiber diameters of SES, 3DP and MEW amounted 6.58 ± 0.30 µm, 237.04 ± 12.51 µm and 13.16 ± 0.67 µm, respectively.

PoA.6.07

Degradation of polycaprolactone dental filament composites for use as drug delivery system

Benjamin Kruppke, Therese Schüler, Celine Guder

Technische Universität Dresden, Dresden, Germany

Abstract

As one of the most significant diseases of the periodontium, periodontitis is a major economic and patient-specific health problem. Therefore, sustainable treatment of the inflammation of the gums and the fight against the bacteria that lead to the formation of pathological tooth pockets is essential. Various approaches are available for this purpose, ranging from antibacterial, bactericidal, and anti-inflammatory hydrogels, with comparatively short durability, to drug-loaded plastic chips for temporary insertion into the gingival pocket and (in the past) drug-loaded sutures, the removal of which was very time-consuming. For periodontal pockets that develop during the course of the disease, the removal of the thread after the release of the active ingredients can be avoided. For this purpose, a resorbable biopolymer should be used, which is degraded in the intended release period. The degradable biopolymer polycaprolactone (PCL) is particularly suitable for this purpose.
In a new approach, we are researching a drug-delivery system consisting of a melt-extruded PCL. The usual acidic degradation of the biocompatible degradable biopolymers should be compensated by processing it into a composite with a buffer substance to protect enamel, dentin, and dental cement from demineralization. The results of the comparison of different mineral and organic buffer substances with mass fractions between 20 and 30 % showed that the degradation rate of the polymers and the entire composite can be significantly influenced. The pH can only be kept constant to a limited extent over the entire exposure time in phosphate-buffered saline (PBS), artificial saliva, and PBS with lipase for a period of up to 28 days. The optimal combination was achieved by polycaprolactone with magnesium carbonate, in which compensation of the polymer-induced pH change and thus maintenance of a physiological pH was achieved over 28 days, while the material degraded completely and thus almost twice as fast as pure PCL. Furthermore, mineral deposits could be avoided, which could delay the degradation of the composite materials and later cause irritation and inflammation in the gingival pocket.
In summary, the PCL/magnesium carbonate composite can be used as an optimal basis for further drug immobilization with antibiotics. The processing and release studies required for this are currently ongoing so that an efficacy study can subsequently be carried out with the relevant dental bacteria. This could provide a degradable thread material that can be effectively used for periodontitis treatment without the need for time-consuming removal.

PoA.6.09

POLY(ETHERETHERKETONE) / REDUCED GRAPHENE OXIDE NANOCOMPOSITE COATINGS PREPARED BY ELECTROPHORETIC DEPOSITION WITH SELF-ASSEMBLED ARCHITECTURES: RELATION BETWEEN THE MICROSTRUCTURE, PROPERTIES, AND CELLULAR PROLIFERATION.

Eduin Ivan González Castillo[1], Arturo Elías Aguilar Rabiela[1,2], Martin Kreuzer[3], Araceli Flores[4], Yadir Torres[5], Peter Samuel Shuttleworth[4], Gary James Ellis[4], Aldo Roberto Boccaccini[1]

[1]Institute of Biomaterials, University of Erlangen-Nuremberg, Erlangen, Germany. [2]Tissue Engineering Research Group, Dept. of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland. [3]ALBA synchrotron, Barcelona, Spain. [4]Institute of Polymer Science and Technology (ICTP-CSIC), Madrid, Spain. [5]Materials and Transport Science and Engineering, Sevilla University, Sevilla, Spain

Abstract

Nanostructured coatings have great potential for orthopaedic implant applications[1]. Self-assembly is a promising strategy for developing advanced nanocomposites as it provides an approach to construct complex superstructures of diverse scales by making use of noncovalent interactions[2]. Desirable morphological features and properties of self-assembly superstructures can be further enhanced by means of electrophoretic deposition where colloid particles suspended in a liquid migrate, by effect of an electric field, towards an electrode on which they form a relatively dense coating after their deposition[3]. Respecting graphene oxide (GO), its π-conjugated structures can form strong π – π stacking interactions with benzene rings such as those found in poly(etheretherketone) (PEEK)[4], and electrophoretic deposition of PEEK/GO has been described in our work as an important stage towards PEEK/reduced graphene oxide (rGO) nanocomposite coatings[5, 6]. Both PEEK and rGO have attracted enormous scientific and technological interest in the medical field because of their properties[7].

After thermal treatment, the nanocomposite coatings were found to exhibit (i) a large-scale co-continuous morphology related to rGO nanosheets whose basal planes were mainly aligned with the coating surface, (ii) a dendritic morphology of PEEK domains, and (iii) irregular domains associated with the deposition of PEEK particles wrapped by the nanosheets (Fig.1). These morphologies were highly dependent on the nanosheet-content, permitting the surface roughness of the coatings to be tuned. Besides, the nanosheets provoked changes in the melting and non-isothermal crystallization of the polymeric matrix, as well as in the crystalline microstructure characteristics of PEEK by inducing transcrystallization. The above-mentioned morphological and microstructural features influenced not only on the near-to-surface mechanical properties, scratch resistance, and scratch damage mechanism, but also on the cellular morphology and proliferation. With the aim of improving and optimising the PEEK/rGO nanocomposite coatings, diverse characterization techniques are used and correlated each other, including synchrotron radiation infrared microspectroscopy (Fig.2), to gain a considerable insight into the relation microstructure-properties-cellular proliferation.

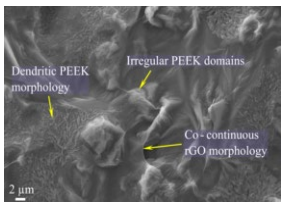


Fig. 1 SEM micrograph of a PEEK/rGO coating.

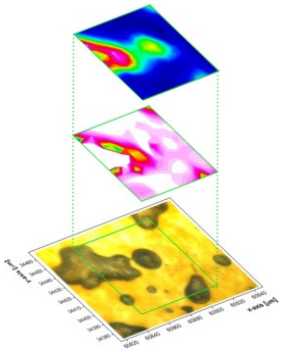


Fig. 2 Chemical imaging of PEEK/rGO microstructures using synchrotron radiation infrared microspectroscopy.

[1] Streicher RM, et al. Nanomedicine. 2007;2:861-74.
[2] Busseron E, et al. Nanoscale. 2013;5:7098-140.
[3] Louh R, Huang E. Solid State Phenomena. 2007;124-126:599-602.
[4] Shudo Y, et al. ChemElectroChem. 2018;5:238-41.
[5] González-Castillo EI, et al. Journal of Materials Science. 2020.
[6] González-Castillo EI, et al. Journal of Materials Science. 2022;57:5839-54.
[7] Kurtz SM, Devine JN. Nanomedicine (Lond). 2007:861-74.

PoA.6.10

Bioactive composite scaffolds for the regeneration of critical-sized mandibular defects

Sophia Dalfino[1,2,3,4], Martyna Nikody[1,5], Martijn van Griensven[6], Elizabeth R. Balmayor[7], Claudia Dolci[4], Gianluca Tartaglia[2,3], Lorenzo Moroni[1]

[1]Department of Complex Tissue Regeneration, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht, Netherlands. [2]Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milano, Italy. [3]IRCCS Fondazione Cà Granda IRCCS Ospedale Maggiore Policlinico Milano, Milano, Italy. [4]Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milano, Italy. [5]Department of Instructive Biomaterials Engineering, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht, Netherlands. [6]Department of cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht, Netherlands. [7]Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany

Abstract

Mandibular bone differs from long bones in its fast-remodelling kinetics and the neuroectodermal origin, therefore its regeneration has to be addressed specifically. Moreover, the mandible has complex anatomy and functions, making it challenging to design bone grafts suitable for tissue engineering.
Additive manufacturing (AM) provides high control over scaffold architecture and, in combination with osteoinductive composite materials, enables the fabrication of scaffolds with potential for the regeneration of critical-sized defects. Currently, poor bone formation, vascularization, and mechanical properties limit the clinical translation of synthetic grafts.
Herein, we aim to develop AM composite scaffolds, consisting of polycaprolactone (PCL) and a high content of different types of ceramic fillers, i.e. tricalcium phosphate (TCP) or bioactive glass (BG), to enhance the bioactivity of the constructs. The high content of TCP or BG, 40% and 20% w/w respectively, was achieved via a blending strategy. To obtain more bone-like architectures and improve cell adhesion and proliferation, scaffolds with diamond and star (0-15°)-shaped pores were printed with high shape fidelity (Figure 1A). Additionally, scanning electron microscopy (SEM) images revealed a homogenous distribution of the ceramic fillers on the surfaces and within the fibers of the scaffolds, and energy dispersive spectroscopy (EDX) analysis showed the characteristic elements of the ceramic fillers (i.e., Ca, P, Si) on the surfaces (Figure 2A). AM composite scaffolds displayed higher compressive moduli than standard PCL scaffolds. Mechanical properties were influenced by pore shapes, showing increased compressive moduli in the diamond compared to the star-shaped scaffolds, and to the standard woodpile (0-90°) grid.
The bioactivity of the composite scaffolds was assessed in culture medium (Figure 2B). The proliferation and differentiation of human mesenchymal stromal cells (hMSCs) was tested. First, the seeding efficiency using a dextran-based seeding method was investigated (Figure 1B). Preliminary data showed that characteristic osteogenic markers were upregulated in the composite constructs. Thus, composite scaffolds with high ceramic content were manufactured, enhancing their osteoinductive potential. In future studies, the angiogenic potential of bioactive glass composites will be investigated. Moreover, the scaffolds will be tested *in vivo* in pig animal models to evaluate their potential in regenerating critical-sized defects.

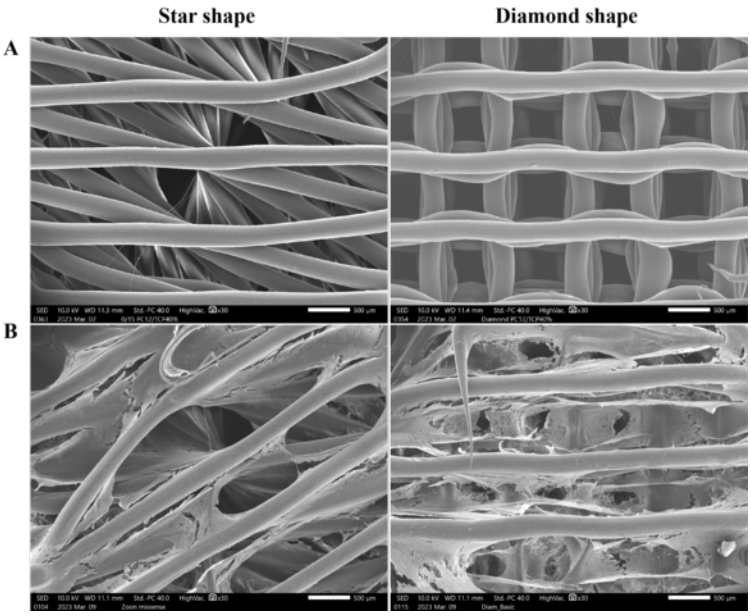


Figure 1. A – Scaffolds designs: star and diamond-shaped porous structure. B – Scaffolds after 7 days of culture with hMSCs.

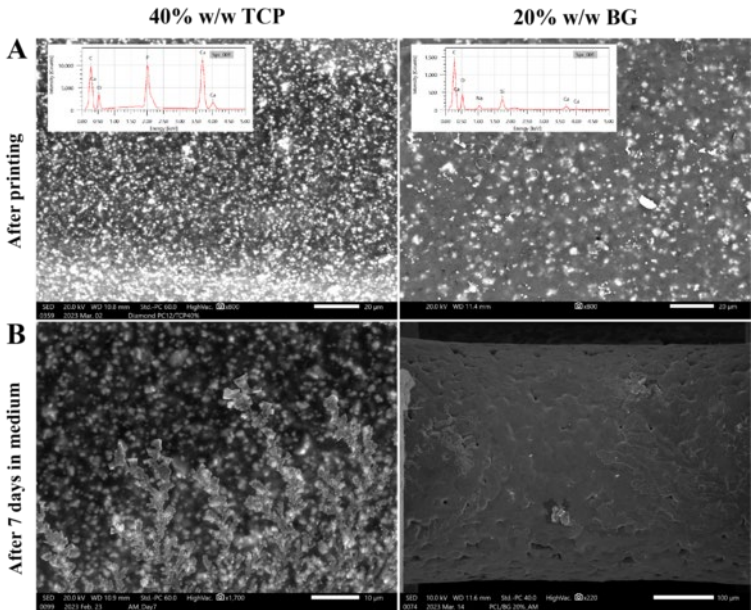


Figure 2. A – Particle distribution on the scaffolds and EDX analysis for β-TCP and BG composites. B – Acellular scaffold surfaces for β-TCP and BG composites after 7 days in culture medium.

PoA.6.11

HAP-ICG particles as a filler of biodegradable polymer-based composite for NIR fluorescence-based medical imaging

Żaneta Górecka, Joanna Idaszek, Wojciech Świeszkowski

Warsaw University of Technology, Warsaw, Poland

Abstract

Due to the fact that the image-guided procedures support the medical personnel in making immediate and more accurate decisions on treatment, the desired advantage of the implanted device is the possibility to visualise them during implantation surgery as well as during post-surgery diagnostic and treatment procedures. The need for intraoperative guidance is nowadays filled with NIR fluorescence (NIRF) imaging which enables the safe visualisation of the fluorescent object from under the tissue or blood. Thus, the development of biomaterials which are visible in NIRF-based imaging is of particular interest.

The HAP was found to be an interesting filler for biodegradable polymer-based composite (BPBC) that increase the radio-opacity. However, in this work it was applied as a carrier for indocyanine green (ICG), the only FDA- and EMA-approved NIR fluorophore. The pristine ICG has some drawbacks, such as concentration-dependent aggregation, poor photostability and rapid clearance from the body. Thus, many studies focused on improving strength and stability of fluorescent signal by ICG encapsulation in various carriers, e.g., polymeric nanoparticles, micelles, liposomes, or coatings.

In this study, the adsorption of the ICG on HAp particles was investigated with e.g., FTIR, TGA and SEM. Moreover, the properties of the BPBC with HAP-ICG particles were tested with e.g., SEM, DSC, confocal imaging, NIRF stability measurements, and cytotoxicity assay. The proposed method of preparation of the composite enabled to receive the material with homogenous microstructure together with uniform contrasting properties. Moreover, such material composition allowed thermal processing, which was impossible for materials based on ICG incorporated in, e.g., liposomes or micelles. However, the crystallisation of the matrix was slightly inhibited when the ICG was present at the HAP surface. The developed composite was characterised by the increased stability of the contrasting fluorescence-based properties compared to the materials for similar applications reported in other studies. We conclude that adsorption of ICG on HAP could force the orientation of the ICG molecule and restrict motion that inhibit the photoisomerization of the ICG. As a result of inhibited photoisomerization, the increase of quantum efficiency and fluorescence lifetime of ICG was found to occur. Moreover, the performed cytotoxicity test revealed the lack of cytotoxic effect with L929 cell line. Therefore, the proposed material could be successfully utilized for NIRF image-guided surgery.

This study was supported by the National Science Centre, Poland (grant no. 2020/37/N/ST5/04137, PRELUDIUM-19)

PoA.6.12

Evaluation of Biodegradability and Osteointegration of Carbon Fiber Reinforced Polylactic Acid Scaffolds

Mehmet Niyazi Cetin[1], Recep Ustunsoy[2], Tahsin Ertas[3], Rola Zahedah[4], Bircan Dinc[5]

[1]Bahcesehir University, Faculty of Medicine, Istanbul, Turkey. [2]Istanbul Atlas University, Faculty of Medicine, Depertment of Biophysics, Istanbul, Turkey. [3]Istanbul University, Faculty of Medicine, Depertment of Biophysics, Istanbul, Turkey. [4]Bahcesehir University, Tissue Engineering and Regenerative Medicine, Istanbul, Turkey. [5]Bahcesehir University, Faculty of Medicine, Depertment of Biophysics, Istanbul, Turkey

Abstract

Polylactic acid (PLA) is a biocompatible and one of the preferred biomaterials, especially in bone implants, tissue engineering scaffolds, and skin regeneration studies. The biodegradability of PLA is both a wanted and an unwanted feature, depending on the area of use. When PLA is reinforced with carbon fiber (CF), its biocompatibility can be increased, and durable biomaterials can be produced. In the study, PLA-CF scaffold was obtained by the crystal melting method. PLA-CF which contains 1% CF by mass material, melted up to 170 °C in the mold, cooled, and sterilized by keeping them under UV for 20 minutes. The osseointegration and biodegradability of the PLA-CF scaffold were evaluated. The outer diameter of the carbon fibers is between 190-590 nm. Its length ranges from 5-55 µm (Figure 1-A, B). Scaffolds were characterized by taking SEM images and the FTIR spectrum. In the SEM images, it is observed that the carbon fibers formed hollow and rod-like structures in the scaffold(C) . While -OH stretching mode appeared at 3300 cm^{-1} in the FTIR spectrum of PLA, the peak here disappeared in PLA-CF spectrum. In both biomaterials, -C-H stretching modes were observed at 2900 cm^{-1}. The fingerprint region of PLA appeared in both scaffolds, and characteristic peaks of carbon nanostructures was observed in CF containing scaffolds (1,2). PLA and PLA-CF scaffolds (3 x 3 x 1 mm^3) were kept in a hydrolysis solution adjusted to pH 7.4 and mass loss was evaluated by keeping at 37 °C, 120 rpm. Mass loss of PLA and PLA-CF scaffold was 25% and 0% respectively.

To investigate the integration of cells into the scaffold, $1X10^5$ osteoblast cells were seeded in each well of 6-well plates and allowed to proliferate together on both PLA and PLA-CF scaffolds for 48 hours. High cell integration was observed in wells containing PLA-CF scaffolds compared to wells containing PLA scaffolds. Cell aggregations attached to the hollow and rod structures of the PLA-CF scaffold were also observed(D). It was aimed to produce and characterize PLA scaffolds containing CF and to investigate their primary effects on cells. The scaffolds may take place in specific orthopedic implant applications (3) and are a suitable design for detailed toxicity and animal experiments.

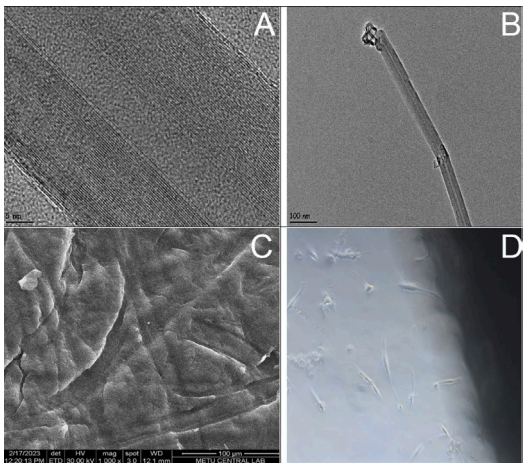


Figure 1: TEM images of CFs at 5 nm (A) and 100 nm (B). SEM image of PLA-CF scaffolds at 100 µm (C). Proliferation and integration of cells around PLA-CF scaffold (10x magnification) (D).

PoA.6.13

Tough bioactive 3D nanocellulose composites

Yeji Han, Jaehwan Kim, Junsik Choi, Jinho Hyun

Seoul National University, Seoul, Korea, Republic of

Abstract

Cellulose nanomaterials (CNMs), which can be extracted from plants or bacteria, the most abundant sustainable biomass on earth, is a nanoscale one-dimensional fiber. The unique properties of CNMs, including their sustainability, excellent mechanical characteristics, and biocompatibility, have attracted significant attention in both research and industry. However, it does not show proper bioactivity for the support of the growth and differentiation of cells. In order to compensate for the shortcomings, acrylated biomolecules can be combined with the 3D nanocellulose structure and its bioactivity is enhanced sufficiently as a biomaterial for tissue engineering. Here, a low concentration of bioactive molecules is applied in the ink and an additional freeze-thaw process is employed for the enhancement of mechanical properties. The hydrogen bonding between the hydrophilic chains increases the crystallinity in the composite hydrogel structure during the freezing process, and the dimension of the composite hydrogel is stabilized. The increase of crystallinity also improves the tensile properties and the elasticity of the hydrogel via the formation of microcrystalline domains. The composition of CNMs with acrylated biomolecules using photocrosslinking and freeze-thaw processes uniquely provides a broadly applicable tool for tough biomaterial design and fabrication.

PoA.7.01

Novel technologies for transdermal delivery of active molecules

Mehrsa RAFIE JIRDEHI[1,2], Amélie REVAUX[3], Pascale PHAM[3]

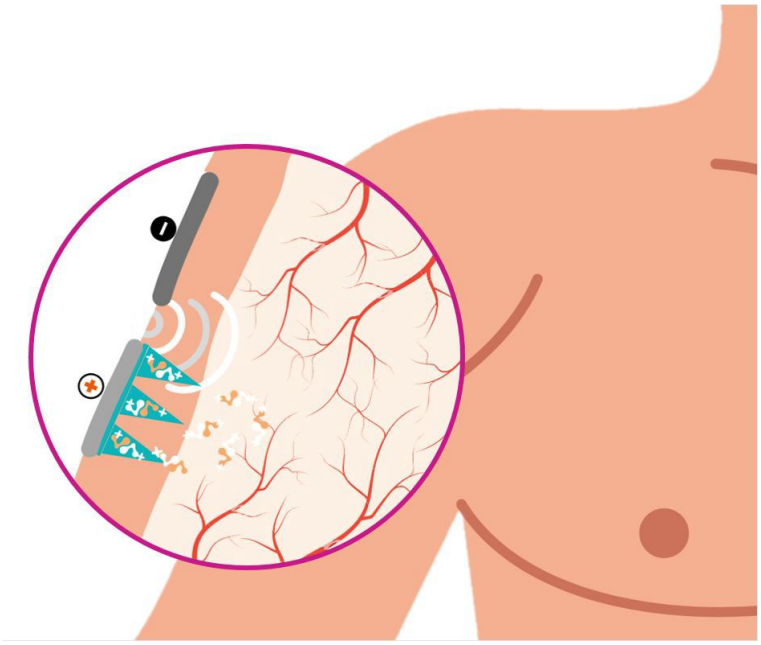
[1]CEA, Grenoble, France. [2]Université Grenoble Alpes (UGA), Grenoble, France. [3]CEA/LETI, Grenoble, France

Abstract

Transdermal drug delivery (TDD) is mostly used for topical applications such as local anaesthesia or skin diseases. However, it can also be a powerful way to deliver a wide range of molecules systemically. This route maximises bioavailability by avoiding passage through the digestive track and allows an autonomous and painless delivery, unlike enteral and parenteral routes respectively.
Microneedles (MN) and iontophoresis (ITP) are two TDD technologies used to enhance drug diffusion.

- MN perforate the outermost layer of the skin, the stratum corneum, which acts like an environmental barrier. They create a pathway to the deeper layers without reaching nerve endings.
- ITP uses a set of electrodes to apply an electric field, moving charged molecules under the effect of an electrostatic force. Finite element method is used to spatially visualise current density distribution and adapt electrode design and relative position. Electrolysis of interstitial fluids is avoided by the use of Ag/AgCl electrodes and electrochemical characterisations are performed to evaluate electrodes lifetimes.

A wearable TDD device can be designed using MN arrays on top of screen-printed electrodes, combining both technologies advantages. MN, ITP and their combination are compared to passive diffusion in a hydrogel skin model for 45 minutes.
Biomolecules such as peptides and proteins are the most difficult to deliver because of their molecular size and hydrophilicity. Fluorescein isothiocyanate-labelled bovine serum albumin (FITC-BSA) is hence used as a drug model for in-vitro comparison and quantified by UV-Visible spectroscopy. Results show the diffusion of around 30% of the drug reservoir for passive, MN, and ITP situations, while up to 50% of the reservoir diffuses with the combination of MN and ITP. Further experiments are proposed to perform a layer-by-layer quantification to estimate drug diffusion kinetics in each cases.



PoA.7.02

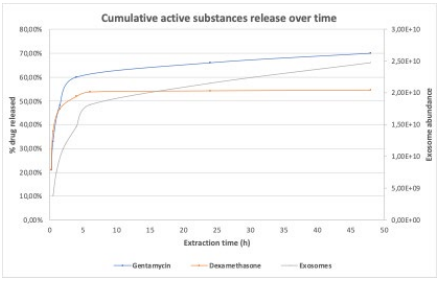
Development of a biocompatible allogenic support for gradual dispensation of active substances

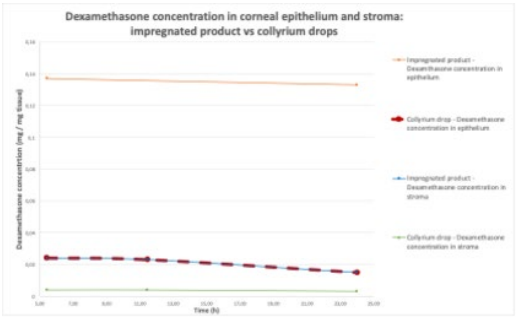
Florine Grossetête, Laurence Barnouin

TBF, Mions, France

Abstract

Introduction: TBF aims to design an allogeneic matrix that can be loaded with a large range of active substances: antibiotic, anti-inflammatory or biological components and able to provide a gradual dispensation of these substances.
Methods: TBF developed an allogenic matrix made of ground umbilical cord lining extracted from human placental tissues. The product is a molded foam, obtained from tissues that are virally inactivated, ground, freeze-dried and sterilized. Product is safe and biocompatible and consist in a small strip that can be inserted behind the lower eyelid. These products contain hyaluronic acid, growth factors and proteoglycans. They have been impregnated after gamma irradiation with a known quantity of gentamycin, dexamethasone or extracellular micro-vesicles extracted from mesenchymal stem cells cultures. Impregnated products have been placed in an extraction medium, which has been removed and replaced at defined times. Actives substances have then been quantified in the different extracts according to appropriate methods. Gentamycin has been quantified according to the Method A from the Eur. Ph., dexamethasone by an ELISA detection kit and exosomes using an ELISA detection kit based on CD63 protein binding.
A comparative study has also been performed in bioreactor. Active substances penetration in corneal tissues was evaluated for TBF product impregnated with Dexafree collyrium versus Dexafree collyrium indentical quantities. Dexamethasone quantity has been quantified by HPLC-MS/MS method in different corneal tissues.
Results: For gentamycin and dexamethasone, quantifications have shown that the products enable a progressive release during the first 6 hours of extraction of 40% to 60% of the active substance. Then a threshold is reached, and the release is stabilized over the remaining time of extraction until reaching 55% to 70% of extraction. Exosomes are constantly released in extraction medium; all observation points are similar. For the bioreactor study, the corneal tissues contained more dexamethasone in the group of the product than in collyrium group.
Conclusion: The results obtained tend to show that the product developed can be impregnated with different type of active substances and allow a gradual dispensation. It could be used in ophthalmological pathologies and replaced collyrium use, to increase contact duration between the eye and the active substance and allows a better penetration of the active substance in the tissues.





PoA.7.03

Rocket-like microneedles for long-acting hormone therapy in prostate cancer

MI-CHI TSAI[1], Kuan-Ta Chen[2], Hung-Wei Yang[1]

[1]Department of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan. [2]nstitute of Medical Science and Technology, National Sun Yat-sen University, Kaohsiung, Taiwan

Abstract

Prostate cancer (PCa) is the second common malignant tumor in men worldwide. The 5-year survival rate of non-metastatic PCa is 98.9%, however, the rate in patients with metastatic PCa (mPCa) on initial diagnoses is less than 30%.

Androgen-ablation therapy is the standard initial treatment for the patients who have been diagnosed with mPCa. The most common way to achieve ablate androgen is through chemical castration with the use of luteinizing hormone-releasing hormone (LHRH) agonists (i.e., Lupron or Zoladex) or androgen antagonists (i.e. Androcur、Casodex). To date, Zoladex is available as a subcutaneous implant (a drug delivery system that is placed just below the skin) with local anesthesia by the trained healthcare provider in clinic. Although each administration can remain active for 12 weeks, the administration is associated with pain and sharps waste generation. In order to overcome abovementioned defects, we plan to develop rocket-like microneedle patches (RMPs) comprising Zoladex-loaded self-gelation hydrogel and Bicalutamide-loaded zeolitic imidazolate frameworks (ZIFs) for on-site androgen-ablation therapy. The fluorescence images demonstrated that the Zoladex@hydrogel (green fluorescence) were evenly concentrated in the tips of the RMPs, and the Bicalutamide@ZIFs (red fluorescence) were evenly distributed in the remaining space of the needles to form obvious two-layer MNs (Fig. 1A). Once being inserted into the skin, the Zoladex@hydrogel and Bicalutamide@ZIFs are released into the epidermis within 5 min. The Zoladex can be sustainably released from the hydrogel and the Bicalutamide can be released triggered by high level of androgen to cause ZIFs disassembly (Fig. 1B). Finally, the prostate tumor xenograft mice were grouped and administered two-dose Zoladex (once per week) by subcutaneous (SC) injection or RMPs. The results showed that the tumor growth did not be inhibited while the mice received only dose of Zoladex by SC injection compared with control group (without any treatment). Conversely, the tumor growth was effectively inhibited while the mice received two-dose administration (once per week) with RMPs (Fig. 1C). The whole system permits the on-site syringe-free androgen-ablation therapy in PCa, avoiding both local anesthesia and sharps waste.

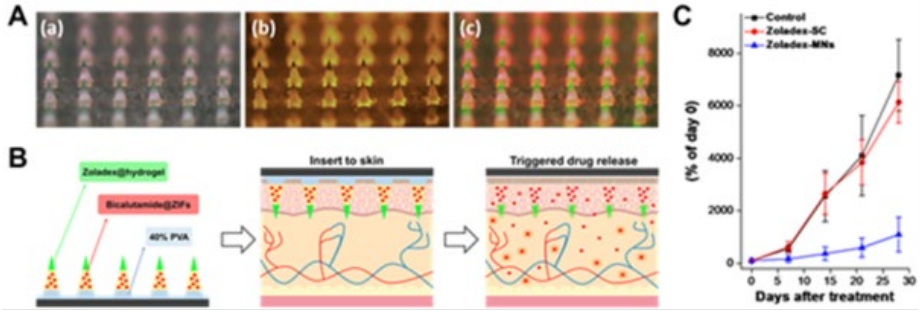


Fig. 1. (A) Representative fluorescence microscopy images of RMP with a PVA base. (a) Zoladex@hydrogel containing FITC-Zoladex were loaded at the tips of the RMP (green), (b) Bicalutamide@ZIFs were distributed in areas other than the tips of the RM (red), and (c) merged image. (B) Illustration of the fabricated RMP containing Zoladex@hydrogel and Bicalutamide@ZIFs, and schematic of the RMP for *in vivo* two-stage drug release triggered by high l evel of androgen. (C) Tumor progression observation among groups. Values are expressed as means ± SD (n = 5).

PoA.7.04

Avastin-loaded 3D-printed scaffold as an effective anti-adhesive barrier to prevent post-surgical adhesion bands formation

Fatemeh Jamshidi-Adegani, Saeid Vakilian, Fahad Al-Fahdi, Juhaina Al-Kindi, Sulaiman Al-Hashmi

Laboratory for Stem Cell & Regenerative Medicine, Natural and Medical Sciences Research Center, University of Nizwa, Nizwa, Oman

Abstract

Post-surgical adhesion bands are fibrous scar tissue bands that can cause complications such as pain, discomfort, and organ blockages, particularly in the abdominal and pelvic regions. Preventive strategies to minimize the formation of adhesion bands during surgery include careful surgical techniques, reducing tissue trauma and surgical time, using laparoscopic techniques, as well as various anti-adhesive treatments such as drugs, physical barriers, and surgical adjuvants. Vascularization plays an important role in the formation of adhesion bands by promoting the infiltration of inflammatory cells and the release of growth factors that stimulate fibroblast proliferation and collagen deposition. Avastin (Ava), a monoclonal antibody used in cancer treatment that targets VEGF, has shown potential as a preventative cue for post-surgical adhesion bands in rat models. Additionally, Alginate (Alg), a natural polysaccharide with various medical applications, has shown potential as a preventative membrane for abdominal adhesion bands. A proposed solution for preventing intra-abdominal adhesion formation involves using a 3D-printed bioactive scaffold composed of Ava, which functions as an anti-angiogenesis drug and Alg acts as a physical barrier, leading to reduced peritoneal adhesion bands by decreasing inflammation and angiogenesis at the site of injury.

In the present research, the impact of Alg and Ava alone, as well as their combination in a 3D-printed scaffold, on the strength and area of post-surgical adhesions was examined in a mouse model of abdominal surgery. Postsurgical adhesion band formation was evaluated using two scoring systems presented by Zühlke et al. and Duran et al. The main cytokines such as Interleukin 6 (IL-6), and Vascular endothelial growth factor (VEGF) levels were measured in abdomen fluid by enzyme- linked immunosorbent assay according to the manufacturer's instructions, 10 days after surgery. Histopathological of adhesion tissues were examined to evaluate the fibrosis and inflammation as two main factors causing adhesion bands formation.

Following topographical and physical characterization of the Alg and Alg/Ava 3D-printed scaffolds, the *in vivo* study showed that despite Alg and Ava groups, Alg/Ava was effective in reducing the extent and strength of adhesion bands formation, simultaneously. The histopathological evaluation of the adhesion tissues suggested that the reduction in fibrosis and inflammation is responsible for the prevention of adhesion bands by Alg/Ava scaffold. Moreover, the cytokine assessment revealed that this happen via the inhibition in the secretion of the VEGF and IL-6, suppressing inflammation and vascularization pathways.

PoA.7.05

Novel drug delivery vehicle: doxorubicin-loaded octacalcium phosphate

Ilijana Kovrlija[1,2], Oznur Demir[1,2], Marta Laizane[1,2], Janis Locs[1,2], Dagnija Loca[1,2]

[1]1 Rudolfs Cimdins Riga Biomaterials Innovation and Development Centre, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Pulka 3, Riga, LV-1007, Latvia., Riga, Latvia. [2]2 Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia., Riga, Latvia

Abstract

Introduction: In the past decades octacalcium phosphate (OCP) has been explored as an effective drug delivery system (DDS). Upsurge of malignant bone tumors and the need for localized DDS optimization, are requiring that the research on novel DDS becomes enriched with new amalgamations. With its exceptional structure, interlayered apatite and water levels, OCP provides an elegant approach to drug incorporation, hence, doxorubicin hydrochloride (DOX) has been used as a model drug. Even though the variety of OCP loaded drugs increases, the *in situ* incorporation of DOX, via α-tricalcium phosphate (α-TCP), was not assessed. Consequently, the objective of the study was to examine the possibility of DOX incorporation into OCP and DOX influence on OCP structural changes and determination of drug release kinetics.

Experimental Methods: 100 mg of α-TCP and a range of DOX (1 – 20 wt%) amounts were added into 50 mL of 0.0016M H_3PO_4. The reaction was continued for 24h at 22°C, under unremitting stirring, and pH monitoring. The products were centrifuged at 3000 rpm, and dried overnight (37°C). To corroborate the OCP phase, X-ray powder diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR-ATR) were employed. DOX release profile was determined by infusing the DOX-OCP in phosphate buffered saline (PBS), for 42 days. DOX content in PBS was verified using ultraviolet–visible spectroscopy (UV/VIS) at λ=480 nm.

Results and Discussion: Phase composition of all DOX-OCPs, was confirmed by the XRD, with characteristic maxima aligning to the theoretical structure of OCP. However, above 10wt% (theoretical loading) of DOX, OCP formation was inhibited. Chemical composition of the attained OCP phase was corroborated with FT-IR (PO_4^{3-} stretching vibration and HPO_4^{2-} stretching were present). Finally, from used 10wt% of DOX, only 2 ± 0.6wt% was incorporated into DOX-OCP, possessing the initial burst release in the first 2h (16±1%), and reaching 38±2% DOX release after 42 days.

Conclusion: DOX-OCP was successfully employed as a DDS for potential cancer studies. To test the maximum DOX loading, while preserving the OCP phase, different wt% of DOX were tested. For the maximum utilization of the OCP as a DDS, further research is needed.

Acknowledgments

This project was funded from the EU's Horizon 2020 research and innovation programme under the GA No.860462 (PREMUROSA) and GA No.952347 (RISEus2). The authors also acknowledge the access to the infrastructure and expertise of the Baltic Biomaterials Centre of Excellence (BBCE, GA No.857287).

PoA.7.06

Tailoring Liposomal Membrane Elasticity for Enhanced Biodistribution and Anti-Inflammatory Response in Systemic Rheumatoid Arthritis Treatment

Dahwun Kim[1], Suyeon Lim[1], Siyan Lyu[1], Hwira Baek[2], Jin Woong Kim[2], Jihoon Jeong[1]

[1]School of Pharmacy, Sungkyunkwan University, Suwon, Korea, Republic of. [2]School of Chemical Engineering, Sungkyunkwan University, Suwon, Korea, Republic of

Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes inflammation and destruction of the joints. Current RA treatments have limitations due to poor bioavailability, lack of specificity in targeting, and side effects. Liposomes are a promising drug delivery platform due to their biocompatibility and tunability of physiochemical properties. In addition to size, surface charge, and surface absorption, liposomal membrane elasticity is an emerging controllable parameter for drug delivery. Research shows that elastic moduli can selectively target different diseases, prolong retention in the systemic circulation, and increase drug accumulation in target cells, thereby improving therapeutic efficacy. Although the elasticity of the liposomal membrane is known to be influenced by the physical characteristics of the lipid composition, such as the length and saturation of the acyl chains and the amount of cholesterol present, lipid/polymer hybrid liposomes have brought attention to a promising drug delivery carrier for various diseases due to their unique membrane elastic properties resulting from the combination of polymers with mechanical robustness and chemical versatility. Our research goal is to adjust the elasticity of liposome membranes, which could establish a platform to create liposomes that target specific areas and improve their biological fate. In this study, we developed stem cell extract and microRNA-co-encapsulating liposomes with the addition of poly(ethylene oxide)-b-poly(ε-caprolactone)-b-poly(ethylene oxide) (PEO-PCL-PEO) (Tri-Liposome). The liposomal membrane elasticity of Tri-Liposome increased with increasing content of PEO-PCL-PEO, leading to different pharmacokinetic/pharmacodynamic profiles in a collagen-induced arthritic (CIA) murine model. We systemically injected a semi-elastic Tri-Liposome into CIA mice, which remained in circulation for an extended period. Moreover, it accumulated in inflamed joints more than its soft and rigid counterparts, resulting in the suppression of pro-inflammatory cytokines in synovial tissues, leading to a reduction in joint inflammation and alleviation of rheumatoid arthritis progression. Our Tri-Liposome platform could enhance blood circulation and anti-arthritic effects by regulating liposomal membrane elasticity. Therefore, it provides new insight into a fundamental parameter in designing drug nanocarriers for targeted RA therapy.

PoA.7.07

Functionalization of a meniscus prosthesis with polymer coatings for the sustained release of anti-inflammatory drugs.

Alfonso F. Blanco[1,2], Gustavo Lou[1,2], Inés Rubio[1,2], José Crecente-Campo[1,2], María José Alonso[1,2]

[1]Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), IDIS Research Institute, Universidade de Santiago de Compostela, Santiago de Compostela, Spain. [2]Department of Pharmacology, Pharmacy and Pharmaceutical Technology, School of Pharmacy, Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Abstract

Knee osteoarthritis (OA) is a joint disease caused by the progressive degeneration of cartilage and bone with a rising prevalence worldwide (1). One of the leading causes of this disease is the deterioration of the menisci which leads to meniscus removal. Substituting the damaged meniscus with a non-biodegradable prosthesis is an innovative solution for elder patients trying to avoid knee OA (2). However, the continued administration of anti-inflammatory drugs to relieve pain can result in off-target side effects such as renal, cardiovascular, and gastrointestinal complications. To solve these problems, local administration of these drugs by intra-articular injection is often proposed, but it leads to patient discomfort, dissatisfaction, and occasionally, further cartilage damage (3). From this perspective, the local and sustained administration of antiinflammatory drugs from a non-biodegradable prosthesis could enhance their therapeutic effect, limit their side effects, and improve patient compliance.

In this work, a functional coating for a meniscus prosthesis, based on biodegradable polymers, was designed to achieve a sustained release of two anti-inflammatory drugs (named AID1 and AID2) with two different release kinetics (1-7 weeks for AID1 and 3-9 months for AID2) that allow modulating the post-surgical and chronic inflammatory environment in the joint cavity of the knee, respectively. More precisely, the effect of polymer type and molecular weight, and drug loading on the release kinetics were evaluated and optimized during a thorough primary screening, resulting in two different double-layer polymer/drug prototypes. These prototypes have been characterized regarding their degradation and mechanically evaluated by dynamic mechanical analysis. Furthermore, the toxicity and activity of the prototypes were tested *in vitro* using human monocyte-derived macrophages. Altogether these results are promising to carry on towards *in vivo* experiments.

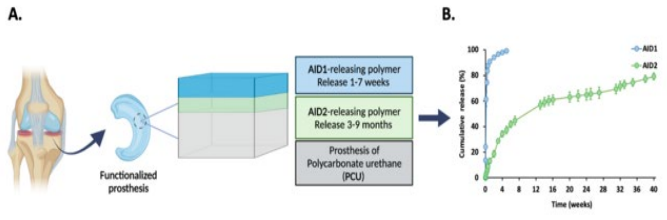


Fig. 1 A. Schematic representation of the functionalized meniscus prosthesis. B. The sequential release of AID1 and AID2 in terms of percentage (%) released from a double-layer polymer/drug coating consisting of a first polymer coating of PC/ PB with AID2 and a second polymer coating of PD with AID1.

Acknowledgments

This work was supported by funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 814444 (MEFISTO).

References:

[1]. Felson DT, Epidemiologic Reviews. 1988, vol. 10(1), p. 1–28.

[2]. Abbadessa A, Crecente-Campo J, Alonso MJ, Tissue Engineering - Part B: Reviews. 2021, vol. 27, p. 133–54.

[3]. Mora JC, Przkora R, Cruz-Almeida Y, Journal of Pain Research. 2018, vol. 11, p. 2189-96.

PoA.7.08

Copper nanoclusters as active agents for a pediatric orphan disease

Ariane Boudier[1], Audrey Malardé[2], Jean-Marc Alberto[2], Grégory Pourié[2], Benjamin Creusot[1], Alexa Courty[3], Bruno Madebène[3], Benoit Tremblay[3], Ali Ouadi[4], David Brasse[4], Igor Clarot[1], François Feillet[2,5]

[1]Université de Lorraine, CITHEFOR, Nancy, France. [2]UMR Inserm 1256 NGERE, Université de Lorraine, Nancy, France. [3]Sorbonne Université, MONARIS, CNRS-UMR 8233, Paris, France. [4]Institut Pluridisciplinaire Hubert Curien, Université de Strasbourg, CNRS, UMR 7178, Strasbourg, France. [5]Centre de Référence des Maladies Métaboliques de Nancy, Nancy, France

Abstract

Menkes disease is a genetic orphan pediatric disease with a fatal outcome in early childhood (less than 3 years) induced mainly by a severe neurodegeneration. Menkes disease is due to the deficiency of the copper transporter, ATPase7A, in the gut and the blood brain barrier which induces a severe multisystemic copper deficiency with a dramatic decrease in all cuproproteins activities [1]. Up to now, the only treatment using copper-histidine complex remains palliative with a poor biodistribution to the brain and unchanged fatal prognosis. Copper nanoclusters (CuNC) were synthetized, characterized and tested in Moblo mice (knock down model for ATPase7A transporter) [2]. Copper NC (0.7 nm) with a metal core surrounded by biodegradable ligands were obtained. They can be stored for a long time (> 1 year) under a lyophilized form. After sub-cutaneous injection in Moblo new-born mice, they increased their survival in a dose-dependent manner without sign of toxicity. They restored the activity of copper-dependent enzymes in both the systemic and the central systems. For example, the activity of tyrosinase inducing the production of melamin was demonstrated with the restoration of normal fur pigmentation in the treated mice. More interestingly, the activity of Cytochrome c oxidase (complex IV of the respiratory chain) restored in the brain indicating demonstrated that CuNC cross the blood brain barrier. Images obtained by positron emission tomography after radiolabeled 64CuNC injection suggested the biodistribution to the brain. The neurobehavioral evaluation of animals in terms of post-natal maturation of the brain using a structure-function approach is currently evaluated. Thanks to these results, an orphan drug designation is already obtained at the European Medicine Agency. The scale-up process is ongoing to produce the first batches for clinical trials, according to the good manufacturing practices. A pharmaceutical company will start the clinical trials in the next year.

[1] K. Kodama, Y. Murata, Molecular genetics and pathophysiology of Menkes disease, Pediatr. Int. 41 (1999) 430–435. https://doi.org/10.1046/j.1442-200x.1999.01091.x
[2] A Boudier, I. Clarot, F. Feillet,Patent WO2022/238656 A1

PoA.7.09

Biphasic porous structures formed by monomer/water interface stabilization with colloidal nanoparticles

Fabio Pizzetti, Valeria Vanoli, Roberta Magaletti, Giovanna Massobrio, Franca Castiglione, Filippo Rossi

Politecnico di Milano, Milan, Italy

Abstract

Introduction
Bicontinuous jammed emulsion gels (known as bijels) are Pickering emulsion where the aqueous and organic phases are present as continuous phases. These emulsions, stabilized by colloidal nanoparticles at the interface between the two phases, can be used in a variety of applications. The goal of this study is that of using a hydrophobic monomer, able to polymerize in bulk, thus forming a bicontinuous structure with polymer and water present as immiscible phases.
Material and Methods
ε-caprolactone, selected as monomer, has been inserted in the reacting cylinder, along with TBD as catalyst. The system was mounted on an orbital shaker, and a stirring velocity of 1000 rpm has been set. Once the polymerization has occurred, an aqueous solution of NPs (both organic and inorganic have been tested) has been added, and the stirring speed has been increased up to 1700 rpm for 1 minute. Then, the stirring velocity has been decreased back to 1000 rpm until the bicontinuous structure formation occured. Release tests have been performed by soaking the bicontinuous structures in 2 mL of PBS at 37 °C for mimicking the physiological conditions. After certain timepoints, 1 mL has been withdrawn and replaced with 1 mL of fresh PBS.

Results
DOSY analyses were able to confirm the bicontinuity of such structures, and their mechanical and chemical properties have been fully characterized through different analyses (GPC, NMR, ESI-MS, DSC, Fluorescent confocal microscopy). Furthermore, the results obtained for release in PBS and solid media gave encouraging results. Important topic to be highlighted is the temperature control for the production protocol, since the final material strongly depends on it.
Discussion
These materials have been demonstrated able to load both hydrophilic and hydrophobic molecules and their release properties have been intensively studied. Through HR-MAS analysis diffusional studies have been performed, for tracking changes in release properties changing the NPs used (inorganic and organic). Furthermore, the possibility of codelivery of two different molecules (hydrophilic and hydrophobic respectively) has been characterized, highlighting an interaction between the two compounds that influence the final diffusivity values.

PoA.7.10

Development of injectable HPMC-hydrogel loaded with pH and thermo-responsive poly((PLA-co-MAA)-b-(EG_2MA-co-EG_8MA)) nanoparticles

Elisa Lacroce, Francesca Cianniello, Giuseppe Nunziata, Filippo Rossi

Politecnico di Milano, Milan, Italy

Abstract

Recently the use of nanocomposite materials, such as polymer-nanoparticle hydrogels, has gained more attention in biomedical research for drug release studies. Thanks to the implementation of organic nanoparticles (NPs) inside 3D-polymeric structures, it is possible to tune the mechanical properties of the gel and develop a new type of drug delivery system at the same time [1]. The physical interaction between organic NPs and hydrogel leads to a material able to change its structure once stress is applied and recover the original form after the stress removal. In addition, these systems are characterized both by hydrophilic and hydrophobic regions having the possibility to release drugs with different hydrophilicity and dimensions. The use of pH/thermo-responsive NPs inside the hydrogel confers the possibility of modulating the drug release by exploiting a specific physiological condition or by applying an external stimulus [2]. In this work, we developed an injectable hydroxypropyl methylcellulose (HPMC) hydrogel loaded with pH and thermo-responsive NPs derived by the synthesis of poly((lactic acid-co-methacrylic acid)-b-(di(ethylene glycol) methyl ether methacrylate-co-poly(ethylene glycol) methyl ether methacrylate)) poly((PLA-co-MAA)-b-(EG_2MA-co-EG_8MA)) copolymer. Ring opening polymerization (ROP) and reversible addition-fragmentation chain transfer (RAFT) reactions were performed to synthesize the copolymers [3] and then NPs were obtained through nanoprecipitation. Average size, pH and thermo-responsivity of NPs were verified with DLS and UV analysis. Several analyses of drug release at different pH and temperatures were conducted to study the drug release kinetic. Finally, rheological characterization was performed in order to characterize the mechanical properties of the final polymer-nanoparticle hydrogel.

[1] E. A. Appel, M. W. Tibbitt, M. J. Webber, B. A. Mattix, O. Veiseh, and R. Langer, “ARTICLE Self-assembled hydrogels utilizing polymer-nanoparticle interactions,” 2015, doi: 10.1038/ncomms7295.

[2] R. S. Kalhapure and J. Renukuntla, “Thermo- and pH dual responsive polymeric micelles and nanoparticles,” Chem. Biol. Interact., vol. 295, pp. 20–37, Nov. 2018, doi: 10.1016/J.CBI.2018.07.016.

[3] M. Sponchioni, R. Ferrari, L. Morosi, and D. Moscatelli, “Influence of the polymer structure over self-assembly and thermo-responsive properties: The case of PEG-b-PCL grafted copolymers via a combination of RAFT and ROP,” J. Polym. Sci. Part A Polym. Chem., vol. 54, no. 18, pp. 2919–2931, Sep. 2016, doi: 10.1002/POLA.28177.

PoA.7.12

Electrically responsive drug delivery hydrogel for ureteral stent application

Beatriz Domingues[1,2], Eduardo Silva[1,2], Estêvão Lima[3], Alexandre A Barros[1,2], Rui L Reis[1,2]

[1]I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Braga, Portugal. [2]ICVS/3B's–PT Government Associate Laboratory,, Braga/Guimarães, Portugal. [3]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

Abstract

The increasing interest in personalized pharmacotherapy has promoted the development of sophisticated stimuli-responsive drug delivery systems. Using an external trigger, as an electrical field, enables the spatial-, temporal-, and dosage-controlled release of drugs.[1] Electrically responsive drug delivery systems represent an attractive alternative to treat ureteral stent-associated pain, a frequent side effect of stent placement.[2] A hybrid approach incorporating a conducting polymer, such as polyaniline, within a polymeric scaffold, such as gelatin, may present advantages for drug delivery application, including electrochemical activity, excellent biocompatibility, and tissue-like mechanical properties.[3] Here, we show a biodegradable electroactive hydrogel capable of programmed drug delivery for ureteral applications.

Polyaniline was chemically synthesized, and its structure was characterized by nuclear magnetic resonance and Fourier-transform infrared spectroscopy. Electroactive hydrogels, composed of gelatin, polyaniline, and ropivacaine (a local anesthetic with proven intravesical effectiveness[4]), were chemically crosslinked with genipin at 37°C. Conductivity analysis, swelling behavior, and degradation assays were performed to characterize the hydrogels. The cytotoxicity of hydrogels was assessed following ISO10993-5 and using L929 mouse fibroblast (ATCC NCTC clone 929). In an ex vivo porcine assay (Figure 1), different electrical stimuli were applied, and the amount of ropivacaine released from hydrogels was quantified by high-performance liquid chromatography.

It was confirmed that hydrogels are semiconductor materials whose conductivity varies proportionally with the amount of polyaniline in the material. The hydrogel composed of 10% gelatin, 3% polyaniline, and 0,25% genipin showed a conductivity of 4,19E-04 ± 6,30384E-05 S/cm. Due to the appropriate properties of swelling, degradation, and cytotoxicity, the beforementioned formulation was chosen to undergo ex vivo assays. Results showed that drug release from hydrogels with polyaniline was higher when electrical stimuli were applied, compared with no electrical stimulation, which can lead to a more effective therapeutic effect.

We proved that the developed hydrogel system is capable of programmed drug delivery in response to electrical stimuli. Loaded with a suitable anesthetic, these biodegradable electroactive hydrogels may represent a novel strategy for efficient ureteral stent-pain management.

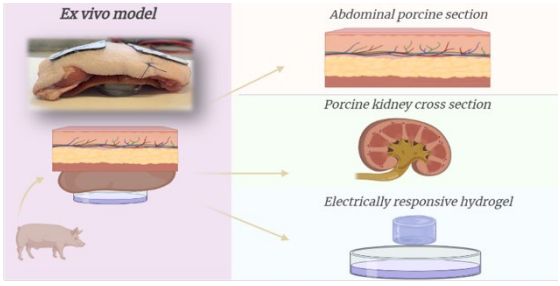


Figure 1 – Ex vivo model to study ropivacaine release upon electrical stimulation

ACKNOWLEDGMENTS

The authors would like to thank the FCT (Grant no: PD/BD/150478/2019) for financially supporting this project.

REFERENCES

1. Bansal, M. et al. J. Control. Release 328, 192–209 (2020).
2. Koprowski, C., et al. J. Endourol. 30, 744–753 (2016).
3. Guiseppi-Elie, A., et al. Am. Chem. Soc. 38, 608–609 (1997).
4. Sur, R. L. et al. 22, 473–478 (2008).

PoA.7.13

Development of an *in vitro* model to test senolytic formulations in cardiac disease

Filippo Cuni[1], Lorenza Garau Paganella[1], Giovanni Bovone[1], Celine Labouesse[1], Sara Mantero[2], Costanza Giampietro[3], Mark Tibbitt[1]

[1]Macromolecular engineering laboratory, ETH, Zurich, Switzerland. [2]Politecnico di Milano, Milan, Italy. [3]ETH, Zurich, Switzerland

Abstract

Cellular senescence is a state of permanent cell cycle arrest that has been shown to aggravate cardiovascular diseases (CVDs) by limiting the regeneration potential of the tissue [1]. Cardiac inflammation plays a key role in inducing endothelial senescence resulting in increased cytokine levels in the tissue and an inflammatory feedback loop between inflammation and senescent cells [2]. Clearance of senescent cells by senolytic drugs has been investigated as a new therapeutic strategy to restore proliferative potency of the tissue. However, while these drugs effectively kill senescent cells, they usually lack selectivity and cause adverse effects to non-senescent cells due to the high doses administered [1].
To overcome this, our work focuses on developing a local injectable senolytic loaded hydrogel, capable of selectively eliminating senescent cardiac cells *in situ*. Navitoclax, an inhibitor of the BCL-2 anti-apoptotic family, was chosen as the senolytic drug [3]. As navitoclax is highly insoluble and unstable in aqueous solvents, we formulated it by producing navitoclax nanoparticles via nanoprecipitation, resulting in stable particles with average diameter of ~115 nm.
To deliver navitoclax, we used polymer–nanoparticle (PNP) hydrogels. This platform exploits physical reversible interactions between poly(ethylene glycol) *block*-polylactide (PEG-b-PLA) nanoparticles, hydroxypropylmethylcellulose, and the embedded navitoclax nanoparticles. The PNP hydrogel is injectable due to its shear-thinning and self-healing hydrogel properties, maintains shape fidelity *in situ*, and has predictable drug release and degradation [4].
To test the efficacy of navitoclax nanoparticles and our injectable system, we used an *in vitro* model of inflammatory senescence using TNF-α treated HUVECs. Senescent phenotype was validated by protein levels (P21 and KI67) and gene expression analysis (IL6, IL8 and CDKN1A1(p21)). Compared to non-formulated navitoclax, navitoclax nanoparticles resulted in higher selectivity for senescent cells and shifted the therapeutic window to lower drug concentrations. Three days of treatment of senescent cells with navitoclax nanoparticles resulted in the elimination of senescent cells and the reversion to a proliferative cell population, comparable with healthy cells.
In the future, we will further examine the drug release profile from hydrogels and test our injectable formulation *in vitro*.

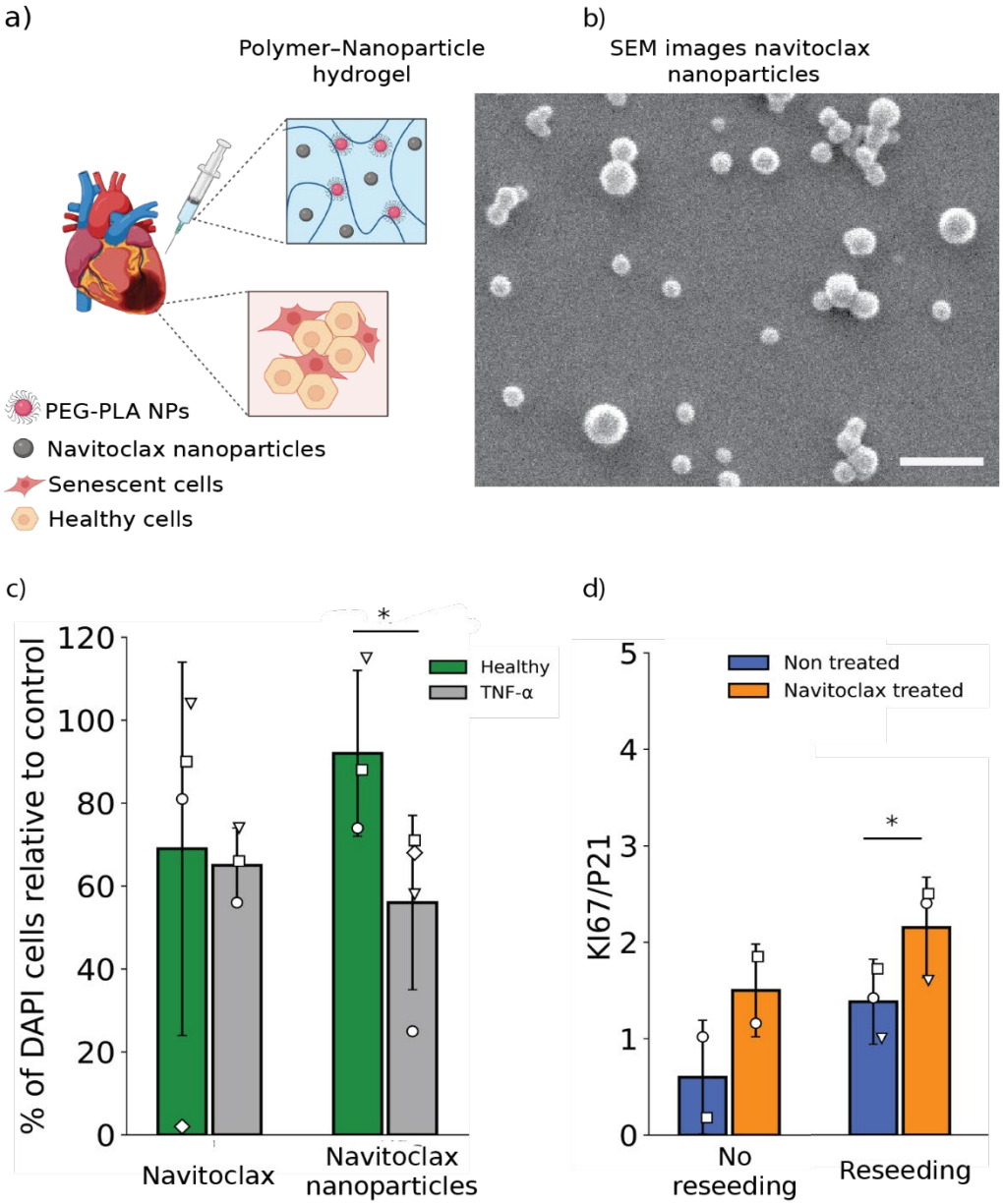


Figure 1: a) Polymer–nanoparticle hydrogel administration *b) Particle shape c) Navitoclax selectivity d) Senescent cells recovery after navitoclax*

[1] Childs BG. et al, Nat Rev Drug Discov. 2017.
[2] Jia G. et al, Biochim Biophys Acta Mol Basis Dis. 2019.
[3] Chen J. et al, Mol Cancer Ther. 2011.
[4] Guzzi EA. et al, Small. 2019.

PoA.7.14

Mold-less microneedle for transdermal drug delivery with protein protective property via dextran-based zwitterionic polymer

Kazuaki Matsumura[1], Harit Pitakjakpipop[1,2], Paisan Khanchaitit[2], Kittipong Tantisantisom[2], Robin Rajan[1]

[1]School of Materials Science, Japan Advanced Institute of Science and Technology, Nomi, Japan. [2]National Nanotechnology Center (NANOTEC), Pathum Thani, Thailand

Abstract

The microneedle technology obtains high attention in transdermal drug delivery systems (TDDS) research. This is due to the limitations in the oral and parenteral drug delivery systems. In this research, we employ a photolithography microneedle fabrication method that does not require molds. It takes less than 5 minutes for each fabrication process that includes adjusting the length and shape of microneedle by varying the time for UV irradiation and the photomask patterns. This method improves the suitability of the microneedles for industrial scale production.

Aggregation and deactivation of proteins loaded in microneedles is a problem in TDDS of proteins. Therefore, we decided to produce microneedles with a sulfobetaine (SPB) polymer with protein-protective activity that can prevent protein aggregation and maintain protein activity.

We fabricate four-point star-shape microneedles via a photolithography method and use SPB monomer as one of the components of the hydrogel network. The other component is dextran-glycidyl methacrylate/acrylic acid (Dex-GMA/AAc) (Figure 1).

The microneedles exhibit high drug loading capacity, rapid drug release, and inhibit protein aggregation. We use a transparent acrylated epoxidized soybean oil (AESO) sheet as the microneedle substrate. It does not absorb any drug during drug loading and conforms along with skin surface when the microneedle patch is applied on it, demonstrating its ease of application on any body part (Figure 2).

To enhance the stability and activity of therapeutic proteins loaded in the microneedles, we design a pre-drug loading platform that uses a Dex-GMA/SPB/AAc hydrogel. The lactate dehydrogenase (LDH) enzyme activity study shows that this hydrogel inhibits protein aggregation while loading enzyme into the microneedle. It has a higher enzyme kinetic activity than Dex-GMA/AAc hydrogel that does not have the poly-SPB side chains even under external stress. These microneedles release the proteins in their native state. The thioflavin T assay that determines the fibril formation in human insulin indicated that Dex-GMA/AAc and Dex-GMA/SPB/AAc loaded with human insulin suppressed fibril formation under dry and high-temperature conditions, with only 25% and 20% aggregation, respectively. The combination of hydrogel microneedle and poly-SPB side chains has a potential for biopharmaceutical transdermal drug delivery, especially protein-based drug that increases the efficiency bioavailability.

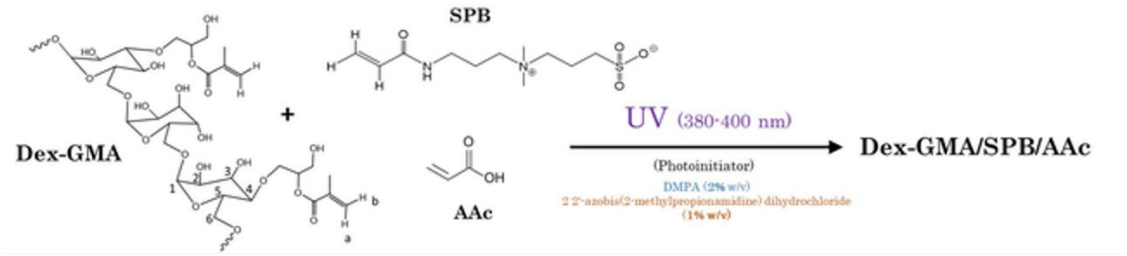


Figure1. Chemical reaction of the microneedle preparation with Dex-GMA, AAc and SPB

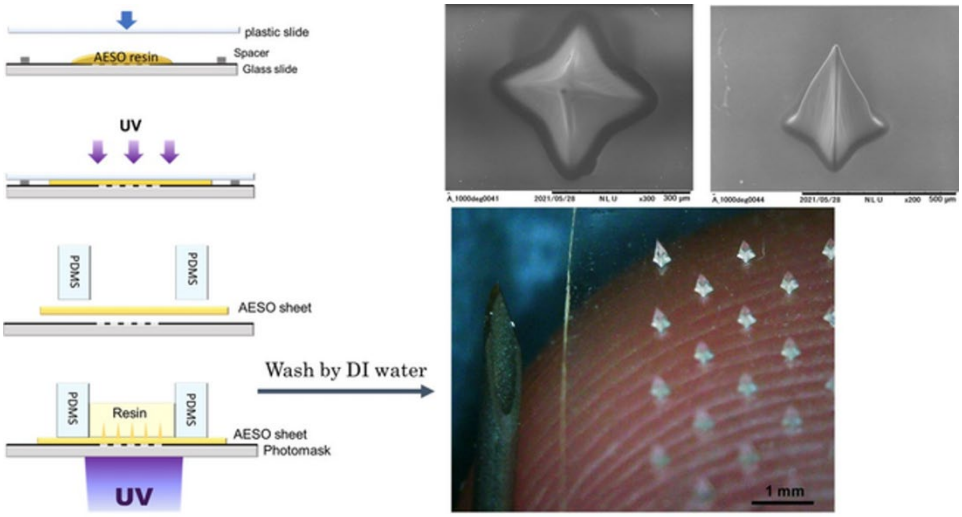


Figure 2. Schematic illustration of the fabrication of microneedle arrays via inverted light UV lithography

PoA.7.16

Redox responsive nanogels for intracellular drug delivery

Stéphane H.F. Bernhard[1], Carolina Söll[1], Giovanni Bovone[1], Emanuele Mauri[2], Mark W Tibbitt[1]

[1]ETH Zürich, Zürich, Switzerland. [2]Politecnico di Milano, Milan, Italy

Abstract

Nanogels are a class of nanoscale cross-linked polymer networks exhibiting high performances as nanocarriers for drug delivery.[1][2] Nanogels bring the favorable properties of hydrogel biomaterials, including high water content, swellability, and simple drug loading, to the nanoscale while facilitating cellular uptake and the delivery of therapeutics to the cytosol. However, diffusion of the therapeutics through the nanogel network leads to premature release before internalization of the carrier. Stimuli-responsive release of the therapeutics within the cell would reduce off target side effects. Disulfide containing chemistries can act as responsive linkers for specific cleavage in reducing environments or in the presence of glutathione (GSH),[3] which has a higher concentration in tumor cells compared with healthy tissues.

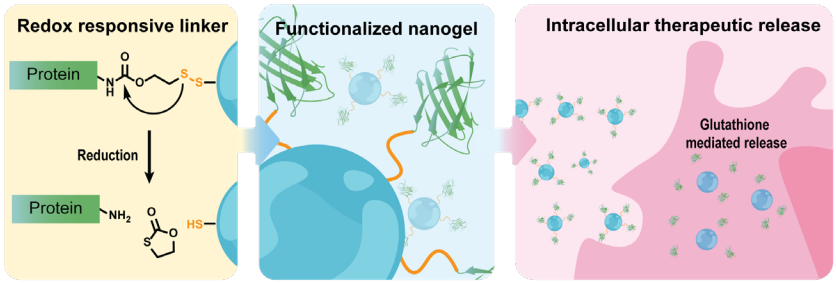


Figure1: Disulfide base linker enable, upon reduction, the release of biologics free of chemical modification. Drug functionalized nanogel were synthetized enabling responsive release of the load upon internalization by tumour cell.

In this work, a redox responsive nanogel drug delivery carrier was synthesized using a self-immolating disulfide linker. Functional nanogels were synthetized through emulsion-evaporation of azide-functionalized poly(ethylene glycol) (PEG) and poly(ethyleneimine) (PEI). A model protein was then modified with a disulfide linker and coupled to the nanogel through click chemistry. Upon reduction of the disulfide bond the linker reacts with itself, releasing the therapeutic without leaving traces of the original chemical modification. We are currently investigating the release efficiency of the model protein from the nanogel and its bioactivity upon release in various cleavage conditions.

Overall, this work aims to develop a redox responsive nanogel drug delivery carrier for intracellular release of therapeutics free of chemical modification.

[1] E. Mauri et al. (2018) ACS Appl. Nano Mater., 1, 12, 6525–6541.
[2] E. Mauri et al. (2017) Eur. Polym. J., 94, 143–151.
[3] C. F. Riber et al. (2015) Adv. Healthc. Mater., 4, 12, 1887–1890.

PoA.7.17

Xeno-free cardiac tissue repair enabled by human protein-based microparticles as cell carriers

Sara C. Santos[1], Catarina A. Custódio[1,2], João F. Mano[1]

[1]CICECO - Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal. [2]Metatissue, Ílhavo, Portugal

Abstract

Human platelet lysates (PL) have been explored for multiple applications as a source of growth factors and proteins involved in cell function [1,2]. Methacryloyl platelet lysates (PLMA, Metatissue®) were recently reported as precursor to produce matrices for cell culture. PLMA can be cured upon light exposure to form hydrogels with tuneable mechanical properties and increased stability *in vitro* [2]. The synthesized hydrogels have proven to support growth, sprouting and migration of human-derived cells. Moreover, it was reported the production of PLMA-based porous scaffolds capable of supporting cell maintenance and function in the absence of animal-derived serum supplements [3].

Microcarriers have emerged as potential delivery platforms for cell-based tissue engineering strategies as they offer the possibility of large-scale cell culture, often required for cell-based therapies. As such, herein we propose the use of PLMA-based microparticles as platforms for cell culture and cardiac tissue engineering.

PLMA microparticles were produced by microfluidics. Water-in-oil microdroplets were formed by using PLMA solution with a photoinitiator as a continuous phase and mineral oil as dispersed phase. Afterwards, microdroplets were crosslinked using light to produce PLMA microparticles. Structural properties of PLMA microparticles were assessed. PLMA microparticles were afterwards evaluated as microcarriers to culture cardiomyocytes and endothelial cells.

Monodispersed PLMA microparticles with diameters around 360µm were produced by flow-focusing microfluidics. H9C2 cell line was used to evaluate the ability of such microparticles to serve as anchor for cardiomyocyte attachment and proliferation. PLMA microparticles were seeded with H9C2 cells or H9C2 co-cultured with endothelial cells. Our results show that PLMA microparticles can support cell adhesion and promote the formation of cell and microparticles aggregates in xeno-free conditions.

PLMA-based scaffolds were already described as platforms that support cell culture even in the absence of animal-derived serum supplements. Herein, we describe the use of the same precursor material to produce microparticles to be used as microcarriers for cell proliferation and cardiac tissue engineering. Our results show great potential of such microparticles to serve not only as platforms for cell proliferation but also as potential microcarriers for cardiac tissue engineering. Such microparticles can have an autologous origin, being adequate to be used as injectable systems with no risk of cross-reactivity, immune reaction or disease transmission.

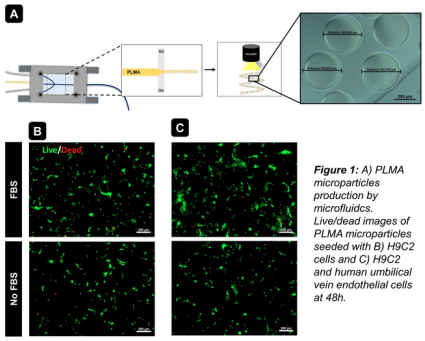


Figure 1: *A) PLMA microparticles production by microfluidcs. Live/dead images of PLMA microparticles seeded with B) H9C2 cells and C) H9C2 and human umbilical vein endothelial cells at 48h.*

[1] Santos SC et al, Tissue Eng. Part B: Reviews. 2018; 24(6):454-462
[2] Santos SC et al, Adv. Healthcare Mater. 2018;7, 1800849.
[3] Santos SC et al, Adv. Healthcare Mater. 2022; 11(12):e2102383

PoA.8.03

Improved osseointegration using novel bioceramic surface layers on titanium alloys for dental implants

Nigel Gordon Smith[1], Pavel Shashkov[2], Aleksey Yerokhin[3]

[1]BioCera Medical Ltd, Cambridge, United Kingdom. [2]Cambridge Nanolitic Ltd, Cambridge, United Kingdom. [3]Manchester University, Manchester, United Kingdom

Abstract

We present upon the characterisation and biological behaviour of the use of electro-chemical oxidation (ECO) to yield four different bioactive coatings on Grade 5 (Ti_6Al_4V) titanium alloys. By applying bipolar pulsed electrical field to the titanium alloys in an electrolyte and particulate components to yield a dense, robust bioceramic surface modified with zirconium and titanium oxides and hydroxyapatite to provide white surfaces.

Test articles were prepared using Ti_6Al_4V substrate: disks of 25 mm diameter and thickness of 5 mm were used for characterisation and *in vitro* experiments; Ti_6Al_4V wire of 0.6 mm diameter was used for *in vivo* investigation. Specimens were coated using various parameters to vary, thickness, porosity and composition. Four coatings were derived from common base electrolyte (Type 1) augmented with nano-powders of zirconium oxide (Type 2), titanium oxide (Type 3) and hydroxyapatite (Type 4).

The study reports upon the surface composition, adhesion, optical properties, corrosion resistance, in-vitro and in-vivo bioactivity. Test piece surfaces were evaluated to determine in-vitro mineralisation, bioactive response to the differentiation of soas-2 cells. Cell viability was determined by alamar blue assay after 1, 14 and 28 days of culture.

The ECO treatments have shown to provide enhanced corrosion resistance and results in white ceramic surface to the substrate. Exposure of these surfaces to simulated body fluid (SBF) shows the surfaces are biocompatible and the surface layer inhibited the release of Al^{3+}, Ti^{4+} and Zr^{4+} and minimal leakage of V^{5+}ions.

Results from in-vitro tests demonstrated the ability to form apatite, enhanced osteoblast adhesion and higher cell proliferation on the coated surfaces compared to the uncoated substrate. The implants were tested *in vivo* by implantation in mice tibia and osteointegration assessed by bone-implant contact, bone ingrowth and these parameters were significantly higher compared with uncoated titanium implant ($P<0.05$). This study demonstrates a multifunctional implant surface with good osseointegration, and corrosion resistance properties can be applied which could further improve the performance and appearance of medical grade titanium alloys used for dental implants.

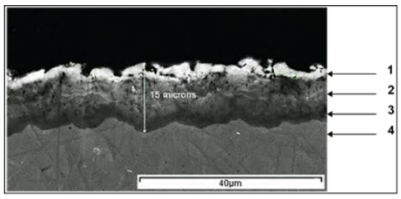


Fig.1 Cross-sectional image of the ZrO2-TiO2 surface [1. Porous layer; 2. Compact layer; 3 Barrier layer and 4. Substrate

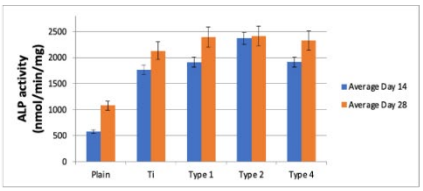


Fig 2. ALP activities of the Soas-2 cells

The proposed coatings can provide an efficient strategy to enhance osseointegration and mitigate the release of metal ions from unmodified implants. The benefits of the ECO coating are expected to improve the aesthetic appearance, limit ion release, and improve the lifetime of such dental implants in situ.

PoA.8.04

AFM ANALYSIS AND NANOINDENTATION OF THE MECHANICAL PROPERTIES OF THE SUPERFICIAL LAYERS OF TEETH AFTER BLEACHING.

Claudio Pasquale, Fabrizio Barberis, Alberto Lagazzo, Matteo Schiaffino

University of Genoa, Genoa, Italy

Abstract

Aesthetics played a huge role in the history of human society and civilization. Nowadays, maintaining good looks has become absolutely crucial and, in particular, teeth whiteness impacts the quality of life before and after the bleaching treatment. [1] Indeed, despite intrinsically stained teeth being a consequence of the etiology of the pathologic process involved, widespread attitudes such as drinking wine, coffee and tea, smoking, feeding coloured food, and abuse of antibiotics, can give rise to stains. Tannins, furfurals, carotenes, artificial colour and tetracyclines may affect the structure of dentine or enamel inducing tooth discolouration.[2] On the other hand, although studies have reported a positive self-perception in patients that underwent tooth bleaching, dental sensitivity and gingival irritation due to incorrect treatment can lead to a negative effect on people's lives.[3] Furthermore, bleaching agents may have antibacterial effects leading to oral microbiome dysbiosis. Therefore, white teeth' positive-influence on the quality of life and the tooth's sensitivity negative-influence on it are related. [4] Chemically, bleaching with products based on calcium peroxide or hydrogen peroxide are prominent opinion for in-office and over-the counter use. [5] However, the tooth is not simply inorganic material but a highly optimized and complex organic-inorganic biocomposite. [2-6] Therefore, aggressive hydrogen peroxide bleaching can also damage the organic matrix in the tooth. Additionally, tooth surface structure changes and damages [7] and microscopic pores and defects on its surface may lead to reversible pulpitis and correlated diseases. [8] In this work we conducted comparative studies between different bleaching agents, specifically taking into consideration the new product BlancOne ULTRA+ (IDS SpA) and classic market products for professional use such as Opalescence Extraboost (ULTRADENT), Zoom WhiteSpeed (Philips Research Eindhoven High Tech) and Pola Office (SDI Limited). We first analyzed the bleaching efficacy of each product using a spectrophotometer before and after the treatments, highlighting the statistical differences between them. In the second analysis we conducted detailed studies on the possible three-dimensional morphological damage of the enamel structures following the treatments. Using an atomic force microscope (AFM) we were able to analyze the treated surfaces before and after bleaching, thus obtaining images and data capable of quantifying the differences in surface changes. Finally, through a nano-indentation process we were able to verify whether, like a modification of the enamel surface, there could also be a change in the mechanical resistance of the dental enamel structure.

PoA.8.05

Palatal expansion screws: analysis of the force expressed by 7 different configurations

MATTEO SCHIAFFINO[1], MARCO MIGLIORATI[2], ALBERTO LAGAZZO[1], FABRIZIO BARBERIS[2], CLAUDIO PASQUALE[1]

[1]Università degli studi di Genova, Genova, Italy. [2]Università degli studi di Genova, GENOVA, Italy

Abstract

The treatment of young adults and adults with transverse skeletal deficits of the upper jaw, in which skeletal maturation is too advanced to use tooth-borne solutions, is represented by miniscrew assisted rapid palatal expansion (MARPE). However, since this is an innovation of recent development, the scientific evidence to support it is poor and there are many configurations and variables to be taken into account.
The purpose of this study is to analyze some of these variables by measuring the force expressed by seven different configurations of orthodontic expansion screws, produced by Leone®, HDC® and Tiger Dental®, through an *in vitro* investigation.
The study used an experimental model reproducing the maxillary dental arch with the palate to fit all screws and standardize the position.
All screws tested had 1.5 mm diameter arms laser-welded to the body. To estimate the stiffness of the screws, a Zwick® testing machine with a 0.5 kN load cell was used to record the forces generated by the expander. The expander was placed in the Zwick® machine by gripping the arms with the upper and lower clamps of the machine, trying to keep the expander as aligned as possible in the vertical plane. The screw was activated a quarter turn (0.2 mm of expansion for the Leone® and HDC® screws) or a sixth turn (0.17 mm of expansion for the Tiger Dental® screw) and the resulting compressive force was recorded. Activations were performed by a stainless steel Leone® wrench of 1.2 mm of diameter. The test was repeated 3 times for each configuration. The results shows maximum developed force values of 184.2 N, obtained by the 11 mm Leone® TAD screw, and minimum force of 91.83 N, developed by the 12 mm Leone® standard screw.
The values obtained are lower than those of the study conducted by Camporesi et al. in 2013, which reached almost 230N with Leone® A2620 screw and just over 200N with Hyrax® screw.
The best-performing devices were found to be those with connection arms that had less distance between parallel arms on the same side and no divergent bends. The best performing device had no bends on the connection arms at the weld on the expansion screw. The new Leone® screws for TAD develop higher forces than conventional expansion screws. The device shows greater effectiveness the stiffer the structure is and consequently sees the bends in the connection arms as its weakest point.

PoA.8.07

Precision Remineralising Technologies to Advance Dental Enamel Health

Palwinder Kaur[1], Helen Chappell[1], Mohammed Al-Mosawi[1], Maisoon Al-Jawad[1], Ursula McDonnell[2], Christabel Fowler[2]

[1]University of Leeds, Leeds, United Kingdom. [2]Haleon, Surrey, United Kingdom

Abstract

INTRODUCTION: Erosion of dental enamel is becoming a significant problem, affecting many European children and adults. Several studies have been conducted to understand the underlying mechanism behind enamel erosion, and efforts have been made to develop therapeutic techniques capable of remineralising the eroded enamel. However, experimental data from these studies are limited in providing detailed elemental information about the demineralised and remineralised enamel. For this reason, theoretical methods, including first-principles modelling, have become essential to mineral studies to comprehend and predict their properties. Combining computational modelling with Synchrotron X-Ray Diffraction (S-XRD) will allow us to understand and predict the crystallographic orientation and the chemistry of dental enamel as a function of acid attack and subsequent remineralisation processes.

METHODS: We employed the first principles-based code, CASTEP, to perform enamel bulk models' structural optimisations. Elemental substitutions with strontium (Sr^{2+}), magnesium (Mg^{2+}) and tin (Sn^{2+}) ions were performed with bulk models, and their formation energies (*Ef*) were calculated. Human tooth specimens were divided into three groups for the laboratory experiments: healthy enamel (control), artificially demineralised and remineralised enamel using a remineralising solution containing: i) Ca^{2+} and PO_4^{3-} ii) plus Sr^{2+} ions. Samples were analysed using a scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDS). For S-XRD measurements, these samples were sectioned and polished to ~50 µm. Regions of interest near the enamel surface were mapped using XRD with an incident energy of 15 keV and a beam spot size of ~4 µm. The 002 reflection azimuthal curves from each diffraction pattern were fitted using the Gaussian function with an in-house built software. The crystallites' alignment was determined using the peak width analysis.

RESULTS AND DISCUSSION: Serial replacements of Ca^{2+} ions in enamel models were performed, and their *Ef* values indicated that substitutions of Mg^{2+} and Sn^{2+} are thermodynamically unstable, while the Sr^{2+} substitution is favourable. Following the positive developments in computational modelling, Sr^{2+} was used as a remineralising ingredient. SEM images showed that, compared to the control, the crystallites formed were much broader following the treatment with a remineralisation (Sr^{2+} ions) solution. EDS analysis of these crystallites exhibited an Sr^{2+} peak, indicating the incorporation of Sr^{2+} in the remineralised enamel surface.

CONCLUSION**:** In this study, we employed first-principles methods and advanced laboratory techniques to study the effect of substituting different ions in the hydroxyapatite lattice. The best candidate for enamel repair is Sr^{2+} due to the negative formation energy and formation of acid-resistant crystallites.

PoA.8.08

The flowability of various root canal sealers in room temperature and human body temperature: preliminary study.

SEOKWOO CHANG

Kyung Hee University, Dental School, Seoul, Korea, Republic of

Abstract

Aim: The aim of this study was to compare the viscoelastic properties of four dental root canal sealers, (AH Plus, CeraSeal, Guttaflow 2, and Well Root St.) at room temperature (25°C) and human body temperature (37°C)
Materials and Methods: AH Plus (Dentsply, USA), CeraSeal (Metabiomed, South Korea), Guttaflow 2(Coltene, Switzerland), and Well Root St (Vericom, South Korea) were used in this experiment. Strain-controlled rheometer (ARES, Rheometric Scientific, London, UK) was used. Time sweep tests were carried out both in 25°C and 37°C and the results were compared (n=5).
Results: In Room temperature (25°C) the complex viscosity was the highest in Guttaflow 2 (86.89 ± 18.84) and decreased in the order of AH Plus (60.52 ± 11.10), CeraSeal (45.23 ±12.93), and Well root st (12.06 ± 1.74). In human body temperature (37°C) the complex viscosity was the highest in Guttaflow 2 (35132.52 ± 11015.66) and decreased in the order of CeraSeal (29.53 ± 11.52), AH Plus (25.77 ± 6.26) and Well Root st (15.10 ± 4.02).
Conclusion: In Room temperature and human body temperature, the flowability of Well Root st was the best in materials tested.

PoA.8.09

Novel dental implants materials based on the β-type titanium and zirconium alloys produced with the application of mechanical alloying and field-assisted sintering technique

Mateusz Marczewski[1], Piotr Siwak[2], Flávia Pires Rodrigues[3], Kamil Kowalski[4], Mieczysław Jurczyk[5], Dariusz Garbiec[1]

[1]Łukasiewicz Research Network - Poznań Institute of Technology, Poznań, Poland. [2]Institute of Mechanical Technology, Poznań University of Technology, Poznań, Poland. [3]School of Dentistry, Paulista University—UNIP, São Paulo, Brazil. [4]Institute of Materials Science and Engineering, Poznań University of Technology, Poznań, Poland. [5]Department of Biomedical Engineering, Institute of Materials and Biomedical Engineering, Faculty of Mechanical Engineering, University of Zielona Góra, Zielona Góra, Poland

Abstract

Titanium-based alloys are used widely as dental implant materials. That is because of their low Young modulus of about 100 GPa, high corrosion resistance in the body fluids, and high specific strength. In order to improve the mechanical properties of Ti-based alloys a new generation of β-type titanium alloys was discovered. Their advantages are lower Young modulus than the older generation of alloys and higher biocompatibility because of the limitation of the use of toxic elements. Recently, the need for a limitation of titanium use is also needed cause of its classification as a critical raw material. It is possible by developing materials based on zirconium. The formation of β-type zirconium alloys allows improvement of these alloys' properties, similarly as in the case of titanium.

In the course of recent studies of our group, the Ti and Zr-based alloys in the ternary Zr-Ti-Nb system produced with the application of mechanical alloying and field-assisted sintering techniques were studied. Mechanical alloying is the process conducted in the high-energy ball mill allowing mechanochemical synthesis based on the competitive cold welding and fracturing caused by the high density of dislocations. On the other hand, spark plasma sintering is a novel technique of powder densification based on the Joule heating of powder particles caused by the high current flow through the specimen. It allows conduct processed with lower temperatures and times in comparison to the more conventional hot pressing techniques. Within the work, the influence on processing parameters (milling and sintering) as well as the materials composition were studied. The conducted work proved the possibilities of the formation of alloys with more than 90% content of Ti(β) and Zr(β) phase and the nanoindentation modulus of 89 GPa (about 40% than commercially pure titanium). Moreover, other prior research within the development of these groups of alloys and their relation to the recent findings will be presented: surface and composite functionalization within the application of bioceramics, antibacterial additives, as well as electrochemically developed biocompatible coatings such as anodized coatings or calcium phosphates coatings. The properties of developed materials such as hardness, Young modulus, corrosion resistance and biological properties will be presented.

The presented work will aim to prove, that the developed materials might be used for the novel generation of dental implants to improve the properties of commercially used products and also their accessibility to society.

PoA.8.10

Development of a 3D *in vitro* mineralized bone model to reproduce the osseointegration process of dental implants

Ghannaa Shayya[1], Olivier Chassande[1], Marie Laure Stachowicz[1], Hugo De Oliveira[1], Anne Lise Chopard Lallier[2], Robin Siadous[1], Sylvain Catros[1,3]

[1]Inserm BioTis, Laboratory for the Bioengineering of Tissues, University of Bordeaux, Bordeaux, France. [2]Anthogyr SAS, Sallanches, France. [3]Department of Oral Surgery, University Hospital of Bordeaux, Bordeaux, France

Abstract

Background: Dental implants have been clinically used for five decades with high success rates. The major clinical issue observed with this technique is peri-implantitis, an infectious/inflammatory disease resulting in peri-implant bone resorption and implant loss: there is thus a need for the development of novel implants biomaterials and surfaces that would reduce this risk. Current dental implant research is extensively based on animal models which are associated with high cost and ethical issues. Additionally, *in vitro* research models are limited into the 2D- experiments, which do not reproduce the complexity of clinical settings. This makes the development of a novel *in vitro* dental implant model for implant screening of critical importance.

Aim: The aim of our study was to fabricate, using biofabrication methods, the first *in vitro* model combining osseointegration model and soft tissue integration model around a titanium implant to study the implant integration process.

Materials and Methods: The osseointegration model consisted of a solid scaffold to hold the different components together, titanium implant and a cell-laden hydrogel. The first part of this study aimed at designing each component of the system. The scaffold was obtained by the 3D printing of polylactic acid using fused deposition modeling (FDM) and characterized. The gel candidates tested were methacrylated gelatin, methacrylated collagen, or methacrylated collagen with hyaluronic acid. Various cell candidates (SAOS2, MC3T3-E1, and immortalized MSCs) were cultured in the different gels in 3D to examine their survival, metabolic activity, proliferation and differentiation, using live-dead assay, alamar blue, and fluorescence detection of red fluorescent protein, respectively. We have used long-term culture (35 days) with basal and osteodifferentiation medium to assess mineralization within gels by alizarin red staining and o-cresolphthalein complexone assay to quantify calcium content.

Results: We have chosen methacrylated collagen with hyaluronic acid as a hydrogel component and MC3T3-E1 or immortalized MSCs as cellular component of the model. This system allowed attachment, proliferation, and differentiation of cells with optimal production of mineralized matrix in the hydrogel.

Conclusions and Future experiments: We optimized appropriate 3D culture conditions to select the hydrogel and an osteoblastic cell source to obtain a mineralized tissue *in vitro*. Ongoing experiments include insertion of the titanium implants to evaluate the mineralized matrix / dental implant connection by measuring the strength of the gel-implant interaction (pull-out tests). Future experiments will combine this osseointegration model with gingival soft tissue model to obtain a representative *in vitro* model of dental implant.

PoA.8.12

Mechanically loaded Zirconia and Titanium implants in artificially induced peri-implant inflammation.

Christoph Martin Sprecher[1], Susi Wolf[2], Stefan Milz[3], Henriette Engelhardt-Woelfler[4], Michael Gahlert[2], Simon Janner[5], Boo Meng[6], David L Cochran[6], Stefan Röhling[2]

[1]AO Research Institute Davos,, Davos, Switzerland. [2]University of Basel, Basel, Switzerland. [3]University of Munich, Munich, Germany. [4]University of Bamberg, Bamberg, Germany. [5]University of Berne, Bern, Switzerland. [6]University of Texas, Austin, USA

Abstract

INTRODUCTION: The current study compares the mineralized tissue reaction on artificially induced peri-implant inflammation and infection around the upper part of mechanically loaded dental implants made of Zirconia or Titanium.
METHODS: Five male American Foxhound dogs received 4 implants, made of Titanium or Zirconia with micro-rough surface topography. Twelve weeks after teeth removal dental implants were placed. After insertion of the prosthetic supra construction, ligatures around the implant neck were installed to induce local inflammation. The ligatures were removed 8 weeks later. After additional 16 weeks, the animals were euthanized. Tissue blocks with implant blocks were obtained for further investigation (Fig. 1). Giemsa/Eosin-stained resin sections (Fig. 2) were prepared and peri-implant bone density, peri-implant contact ratio and bone loss of the alveolar crest were histomorphometrically determined in a buco-lingual plane.
RESULTS: Around Titanium implants the bone resorption was significantly increased compared to Zirconia implants (Fig. 2). The effect was most prominent on the lingual side of the implants. No further significant differences were found. From a clinical point of view, all implants appeared stable.
DISCUSSION & CONCLUSIONS: In the present inflammation and infection study Zirconia implants exhibited significantly reduced peri-implant bone loss when compared to Titanium implants with a comparable surface topography and roughness.
ACKNOWLEDGEMENTS: This study was supported by ITI-Grant Number 920_2018.

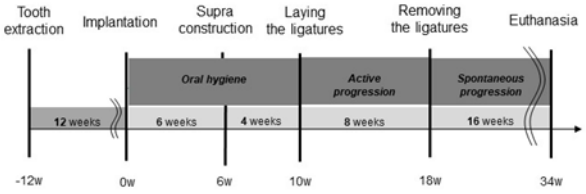


Fig. 1: The different phases of the study.

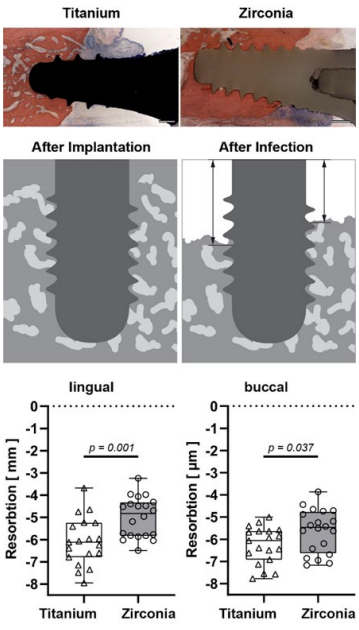


Fig. 2: Histological appearance and histo-morphometrical results of Titanium (left) and Zirconia (right) implants. Scale bars: 1000 µm.

PoA.8.13

Fluoride ion release from PMMA/PEO/NaF composite for dental application

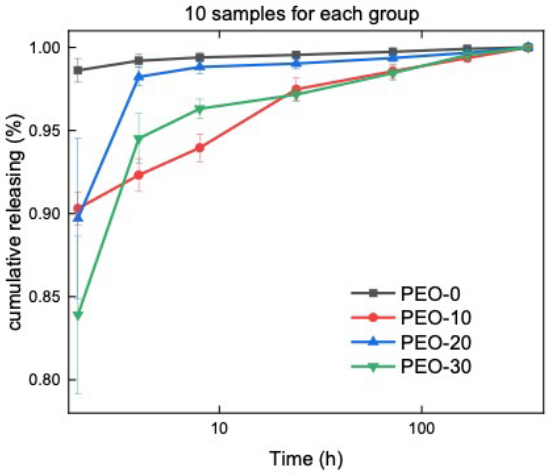
Jia Wang[1], Wen Kou[2]

[1]Department of Physics, Faculty of Science and technology, Umeå University, Umeå, Sweden. [2]Dental Material Sciences, Institution of Odontology, Umeå University, Umeå, Sweden

Abstract

Objective: The prevalence of temporary mandibular joint disorder (TMD) is around 10% of the adult population worldwide. One of the most common ways to manage symptomatic TMD is the provision of oral splint. Oral splint is often made with thermoset polymethylmethacrylate (PMMA). The main functions of oral splint are to enhance the muscle relaxation in the facial area and to provide protection against tooth wear. Splints are typically worn during sleep for 7-8 hours, providing an optimal platform for the controlled release of fluoride ions. To improve the effectiveness of PMMA oral splints in reducing dental caries, we have developed a novel polymer composite strategy involving the introduction of amphiphilic polyethylene oxide (PEO) into the PMMA matrix. This composite creates a pathway for ion release within the PMMA, and the addition of an appropriate amount of sodium fluoride enables controlled release of fluoride ions during the wearing period, reducing the incidence of dental caries. In the present conceptual study, PMMA/ PEO/ sodium fluoride (NaF)/ composite films were made, and the fluoride ion release was studied. **Material and methods:** Sodium fluoride salt was mixed with PEO with four various concentrations, later the mixture was incorporated into polymethylmethacrylate based material. Thin films were made by solution-casting method and following specimens were conducted: PEO-0 (No PEO), PEO-10 (10 wt% PEO), PEO-20 (20wt% PEO) and PEO-30 (30wt% PEO) (n=10/group). The films were then soaked in milli-Q water separately, and the fluoride ion release were detected with a selective fluoride electrode. The cumulative fluoride ion releasing rate were measured after 2 hours, 4 hours, 8 hours, 24 hours, 72 hours, 168 hours and 336 hours. The top, bottom and cross-sectional surfaces were studied with optical microscopy and also SEM. **Results:** PEO-0 released more than 98% of fluoride after 2 hours. PEO-20 and PEO-30 showed a similar releasing trend, while PEO-30 released 5% less fluoride. At 2 hours, PEO-10 released about the same amount of fluoride ions as PEO-20. PEO-10 samples showed the slowest releasing rate up to 8 hours. PEO-10 and PEO-30 show similar releasing after 72 hours (Fig 1).

Significance: All PMMA/PEO/NaF composite samples showed a slower fluoride release compared to PMMA/NaF samples. This result indicates that by PEO incorporation slower fluoride ion release would be achieved.



PoA.8.14

Mucoadhesive patches for topical application of steroids on the oral mucosa

Elena Maria Varoni[1], Andrea Cochis[2], Lorenzo Bonetti[3], Alessandro Scalia[2], Marcello Manfredi[2], Luigi De Nardo[3], Lia Rimondini[2], Lina Altomare[3]

[1]University of Milan, Milan, Italy. [2]University of Piemonte Orientale, Novara, Italy. [3]Politecnico of Milan, Milan, Italy

Abstract

Introduction: Clobetasol-17-propionate (CP) is the most potent corticosteroid, produced only for skin application because of high lipophilicity. CP is effective for the local management of oral immune-mediated diseases, despite a gold standard formulation for the oral mucosa is lacking[1,2]. We developed an innovative bilayer chitosan (CS)-based muco-adhesive patch via electrophoretic deposition (EPD), loaded with CP. Preclinical analyses assessed: physico-chemical characteristics; patch mucoadhesion and CP absorption throughout epithelial layers; patch cytocompatibility and CP bioactivity via proteomics.
Materials and methods: *EPD of bilayer CS-CP patches:* CP (1g/L) was mixed with CS (1g/L) in a 30% water + 70% ethanol bath (pH = 4.8); EPD conditions: titanium square (2cmx2cm) cathode; square waves (100-75 V / Dc= 0.17 t= 5 min); double step deposition for bilayer patches: a first deposition containing only CH and, after drying, a second deposition using a CS solution with or without CP, respectively CS-CP and CS.
Morphological analysis: SEM and optical microscopy.
In vitro CP release from patches: Liquid chromatography mass spectrometry (LC-MS).
CP release (ex vivo mucosa model): patches were adjusted onto mucosa at the air-liquid interface and allowed to spontaneously release CP for 0.5, 3 and 6 hours. CP amount determined by LC-MS.
Patch mucoadhesion (ex vivo porcine oral mucosa): A tack –test, between the porcine oral mucosa and the patch, was performed using rheometer.
Patch cytotoxicity and CP bioactivity (human oral mucosa 3D model): metabolic activity of cells was evaluated by Alamar Blue assay. Proteomics was performed with UHPLC Vanquish system coupled with Orbitrap Q-Exactive Plus. *Statistical analysis:* one-way ANOVA (Tukey's test a post-hoc analysis); significance $p < 0.05$. Results: Patches showed a homogeneous and defined porous structure, an excellent swelling rate after re-hydration. CP was successfully loaded in the patches (loading capacity: 3.4 ± 0.9 µg/mL). No significant differences were observed between CS and CS-CP in term of mucoadhesion. The release profile was assessed (Fig. 1).
Conclusion: The mucoadhesive CS-based patches represent a promising CP drug delivery system for oral mucosa.

References
1. Oren-Shabtai et al. Drugs Aging. 2021;38:125-136.
2. Lodi et al. Cochrane Database Syst Rev. 2020;2:CD001168.

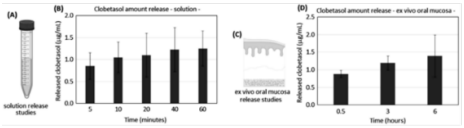


Figure 1. (A-B) CP release in physiological solution and (C-D) ex-vivo mucosal tissue. Using the 3D model of human mucosa, patch cytocompatibility was confirmed (Fig. 2); proteomics showed 7 peptides down-regulated and 10 up-regulated, which correlated with the anti-inflammatory CP activity.

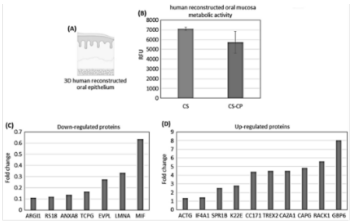


Figure 2. Cytocompatibility (A-B) and proteomics of the human 3D reconstructed oral epithelium in contact with patches.

PoA.8.15

Light-responsive and multifunctional antimicrobial coatings to prevent peri-implantitis

Teresa Gagliardi[1], Paulo Palma[2], Chiara Tonda-Turo[3], Akhilesh Rai[2,1]

[1]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal. [2]Faculty of Medicine, University of Coimbra, Coimbra, Portugal. [3]POLITECNICO DI TORINO, Torino, Italy

Abstract

Introduction: The placement of dental implants is widely known as reconstructive treatment to replace missing or damaged teeth. The bacterial infection leads to inflammation in the gingiva followed by bone loss around the dental implants, which is called peri-implant disease. Implant failure due to peri-implantitis has been increasing at a significant rate of 8%, which corresponds to more than 1 million failures globally. Here, we demonstrate the coating of near-infrared (NIR) light-responsive polydopamine (PDA) on Ti surfaces followed by the covalent conjugation of LL37 peptides. LL37-PDA-Ti surfaces have an antimicrobial property and are biocompatible with human gingiva fibroblasts (HGFs) and human oral keratinocytes (HOKs) cells.

Experimental details: PDA-coated Ti surfaces were functionalized with LL37-SH peptides. Zeta potential, XPS, QCM-D and FTIR measurements were done to characterize the coatings. To evaluate antimicrobial activity, LL37-PDA-Ti surfaces were tested against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* in 10% human serum (HS) upon exposure to 808 nm NIR light. Biocompatibility of LL37-PDA-Ti surfaces was evaluated to HOFs and HOKs using ATP and LDH assays. Unpaired T-test and 1-way ANOVA were performed to do statistical analysis of the data.

Results and discussion: A thin layer (5 ±2 nm) of PDA is coated on Ti surfaces. The photothermal measurement indicates that the exposure of NIR light for 5 min increases the temperature of Ti surfaces to 15 ±3°C. Zeta potential measurement shows that LL37-PDA-Ti surfaces are positively charged compared to negatively charged PDA-Ti surfaces. FTIR and XPS studies also indicated the conjugation of LL37 peptides on PDA-Ti surfaces. Importantly, there is no leaching of LL37 peptides from the surfaces after incubation in PBS for 24h. LL37-PDA-Ti surfaces are potent to kill *S. aureus*, *E. faecalis* and *E. coli* bacteria in 10% HS upon exposure to NIR light for 2 min. Moreover, LL37-PDA-Ti surfaces destroy *E. faecalis* biofilm after exposure to NIR light (Figure). There is no cytotoxicity induced by LL37-PDA-Ti surfaces to HOKs and HGFs after exposure to NIR light compared to the control. LL37-PDA-Ti surfaces promote the adhesion and proliferation of HOKs. Immunofluorescence analysis shows that LL37-PDA-Ti surfaces favour the formation of hemidesmosomes of HOKs.

Conclusion: We show that LL37 peptides can be conjugated on PDA-Ti surfaces. LL37-PDA-Ti surfaces kill Gram-positive and gram-negative bacteria without inducing major cytotoxicity to human cells. Overall, Ll37-PDA-Ti surfaces could be used to prevent peri-implantitis.

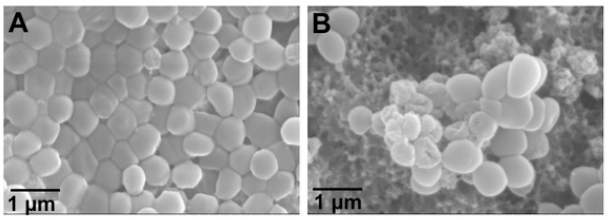


Figure: SEM images of *E. faecalis* without treatment (control, **A**) and after treatment with LL37-PDA-Ti surface + NIR light exposure (**B**).

Acknowledgements: AR would like to thank FCT for the LightImplant project (PTDC/CTM-CTM/1719/2021).

PoA.8.16

Hemocompatibility, biocompatibility, and oxidative stress indicators in human periodontal ligaments cells: Interactions with cerium/calcium doped mesoporous nanoparticles

Ioannis Tsamesidis[1], Georgia K. Pouroutzidou[1,2], Anastasia Beketova[1], Maria Bousnaki[1], Anna Theocharidou[1], Eleana Kontonasaki[1]

[1]School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece;, Greece.
[2]School of Physics, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece;, Greece

Abstract

INTRODUCTION: Periodontitis, is a common oral disease, affecting in its most severe form, approximately 20% of the population. Various biomaterials have been developed and used to treat periodontal defects clinically; however, suboptimal results have been observed along with a lot of toxicity issues[1]. There is a critical need for the development of new treatment strategies. Cerium and calcium-based nanomaterials have drawn a lot of interest from scientists all around the world[2]. In this work, human periodontal ligament cells (hPDLCs) were exposed to calcium or/and cerium-doped MSNs and their viability, hemocompatibility, ROS, and TAC levels were evaluated.

Sample	SiO_2	CaO	CeO	Zeta Potential (eV)	Ce^{+3}/Ce^{+4}
MSi	100			-31.5	-
MSiCe	95		5	-26.3	57.7
MSiCa	95	5		-22.3	-
MSiCaCe	60	35	5	-17.0	53.4

Table 1. Nominal composition (in % mol) and physicochemical characteristics of the obtained Mesoporous nanoparticles.

EXPERIMENTAL METHODS: Mesoporous silica-based nanoparticles (MSNs) were synthesized via a modified sol-gel method in a basic environment. The obtained MSNs underwent calcination at 600°C for 5 hours after drying at 60°C overnight. The biocompatibility, red blood cells (RBCs) activity, and ROS levels were assessed as previously described [3] following ISO standard 10993-4:2017.

RESULTS AND DISCUSSION: The composition and physicochemical properties of the synthesized MSNs are

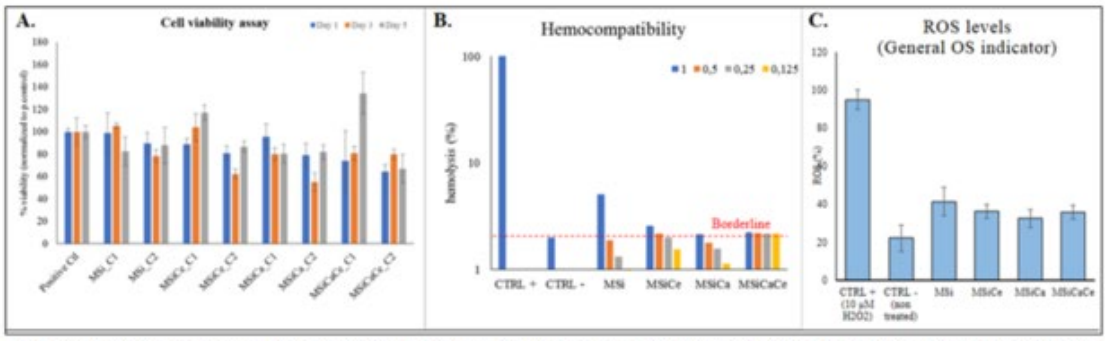


Fig.1: (A) Cell viability assay of hPDLCs after 1,3 and 5 days of incubation with the tested MSNs, (B) hemocompatibility of the tested MSNs after 24 hours of incubation and (C) ROS levels of hPDLs after 5 days of incubation

presented in table 1. The viability assay of hPDLCs with the tested MSNs revealed a biocompatible profile at the lowest tested concentration for all the materials after 1,3 and 5 days of incubation. Moreover, MSNs doped both with cerium and calcium (lowest zeta potential compared with all MSNs) presented an increase in cell proliferation status of the cells after 5 days of incubation. Also, all the doped MSNs appeared to be hemocompatible, while undoped MSNs with the highest zeta potential created hemolysis (8%). On the other hand, ROS levels of hPDLCs were higher after incubation with the MSNs compared with control cells. No significant differences of ROS levels among the MSNs were observed.

CONCLUSION: In conclusion, low zeta potential of MSNs appeared to play a significant role in their biocompatibility for both tested human primary cell lines (hPDLCs and RBCs). Also, a synergistic combination of Ca and Ce in silica-based MSNs was observed and by monitoring the Ce and Ca amount, suitable MSNs for periodontal tissue regeneration strategies can be developed.

REFERENCES

1. Tassi, S. A. et al. Periodontal Res. 52, 793–812, 2017
2. Kurtulu,F. et al. Mater. Sci. Eng. 124, 2021
3. Tsamesidis I., et al. Pharmaceutics, 15, 2023

ACKNOWLEDGMENTS

This work is supported by European Union's Horizon 2020 research and innovation program under grant agreement No 953128, project: Smart, Multifunctional Dental Implants: A solution for peri-implantitis and bone loss.

PoA.8.17

Modelling and in-vitro study of ultrashort-pulsed laser irradiation on human enamel for tooth restoration: A hybrid technique

Sarathkumar Loganathan, Eric Kumi Barimah, Neelam Iqbal, Simon Strafford, Brian Nattress, Sue Pavitt, Anna Nielsen, Animesh Jha

University of Leeds, Leeds, United Kingdom

Abstract

Ultrashort pulsed laser is an emerging non-invasive tool for performing high-precision surgery in restorative dentistry. The present study demonstrated the employability of ultrashort-pulsed laser as a hybrid tool for both tooth preparation and filling damaged tooth enamels. For the restoration of enamel, the laser irradiation experiments were carried out at two different pulse energy regimes (500 µJ and 60 µJ) using 800 nm, Ti:Sapphire femtosecond laser (100 fs, 1 kHz, 5 mm/s and 5 µm scanning line overlap) for tooth surface preparation and filling damaged surface using laser sintering. The finite element (FE) model was developed for optimizing the laser operating conditions for optimizing the removal damaged tissue and minimizing the collateral damage to healthy dentine underneath. (Fig. a). The rectangular cavity (1 x 3 mm2) with a depth of 20 µm was prepared on the flat enamel surface by operating the laser at a high pulse energy regime (500 µJ, Fig. b). Subsequently, the cavity was filled with the slurry containing 1:4 ratio of iron-oxide doped hydroxyapatite and chitosan. The laser sintering experiment was carried out at the low pulse energy regime (60 µJ) using different scanning strategies (single and dual scan) to ensure uniformity of sintering across the depth (Fig. c). The study was also extended for the in situ evaluation of restored enamel slabs positioned onto a dental appliance in the volunteers mouth for ascertaining the statistical efficaciousness of the procedure and for comparing the acid-resistant properties with natural enamel as control (Fig. d). The surface morphology, chemical compositions, mechanical and interfacial properties of the restored enamel surface were evaluated, and its adhesive mechanisms were analysed. The in-situ evaluation study confirmed that the enamel restored with the dual scan strategy was stable in the oral environment. The proposed method of restoring damaged enamel with a single-source ultrashort-pulsed laser as a hybrid tool is feasible and has a promising future in dental applications.

Keywords: Tooth preparation, Cavity filling, FE model, Human enamel, Femtosecond laser, Tooth restoration, Laser sintering

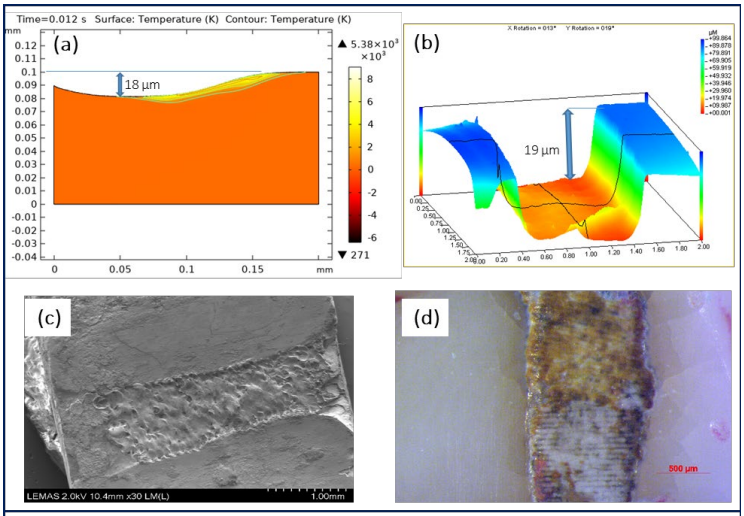


Fig. (a) FE Model to optimize the laser processing conditions for cavity preparation, (b) Optical profilometry image of the cavity, (c) SEM image of restored enamel surface after laser sintering, (d) Microscopic image of the sintered surface adopted dual laser scan strategy after In-situ trail.

PoA.9.02

Phytotherapeutic agent functionalized cotton wool-like bioactive glass-based fibers for wound healing applications

Irem Unalan, Lea Schoeppach, Aldo Roberto Boccaccini

Institute of Biomaterials, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Abstract

In recent years, the combination of electrospinning and sol-gel methods has been of growing interest to fabricating three-dimensional bioactive glass (BG)-based fibers for biomedical applications [1,2]. However, a bacterial infection in wound healing is still a critical health concern. Lately, natural antibiotic-free agents such as phytotherapeutics have been of renewed interest in suppressing bacterial infection [3]. In particular, essential oils such as cinnamon oil (CO) are the most promising phytotherapeutics aiming to promote wound healing and decrease bacterial infection [3]. This study aims to fabricate novel phytotherapeutic cotton wool-like BG-based fibers. In this regard, cotton wool -like BG-based fibers were produced by electrospinning using inorganic sol-gel solutions [1] and then coated with soy protein (SP) or CO-incorporated SP solution by dip-coating method. The morphology of the CO cotton wool-like BG-based fibers was investigated by Scanning Electron Microscopy (SEM). The results revealed that the cotton wool-like structures comprised long and entangled fibers with interconnected pores, and coating with CO did not affect the scaffolds' morphology (3D porosity). The presence of CO and the total phenolic content of CO-coated cotton wool-like BG-based fibers was confirmed by ATR-FTIR and UV-Vis spectroscopy analysis. Additionally, antioxidant activity in terms of the DPPH free radical scavenging activity assay indicated that coating CO enhanced the antioxidant property of cotton wool-like BG-based fibers; thereby, it could be a potential biomaterial in the treatment of oxidative stress during the wound healing process. Furthermore, the CO-coated cotton wool-like BG-based fibers exhibited antibacterial activity against Staphylococcus aureus and Escherichia coli. Moreover, the *in vitro* cytotoxicity results showed that CO-coated cotton wool-like BG-based fibers did not have cytotoxic effects on normal human dermal fibroblast (NHDF) cells. Consequently, our findings revealed that phytotherapeutic cotton wool-like BG-based fibers could be an alternative approach to avoid bacterial infection and protect against oxidative stress damage in wound healing applications.

Keywords: Electrospinning, Bioactive glass, Sol-gel, Essential oils, Antibacterial activity, Antioxidant activity, Wound healing

References

[1] L. Liverani, T. Reiter, K. Zheng, Z. Neščáková, A.R. Boccaccini, Mater. Lett. X. 14 (2022) 100133. https://doi.org/10.1016/J.MLBLUX.2022.100133.

[2] H.H. Lu, K. Zheng, A.R. Boccaccini, L. Liverani, Mater. Lett. 334 (2023) 133712. https://doi.org/10.1016/J.MATLET.2022.133712.

[3] I. Unalan, T. Fuggerer, B. Slavik, A. Buettner, A.R. Boccaccini, Mater. Sci. Eng. C. (2021). https://doi.org/10.1016/j.msec.2021.112320.

PoA.9.03

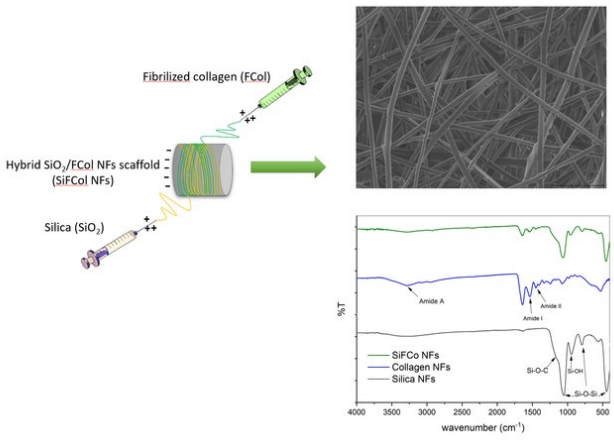
Exploring the Potential of Silica/Collagen Hybrid Nanofibers Scaffolds in Bone Tissue Engineering

Sara Jalali, Benjamin Kruppke

Technische Universität Dresden, Dresden, Germany

Abstract

Replacing hard tissues is always a major challenge for orthopedic surgeries. However, bone substitute biomaterials can be utilized to fill the gap caused by missing bone tissue and stimulate the formation of new bone tissue. Silica and collagen are among the materials that are particularly promising for bone tissue engineering owing to their unique biocompatibility, degradability and osteogenic properties. Furthermore, silica-based biomaterials are known for their strong mechanical strength and could facilitate bone mineralization or calcification, aiding in the process of periosteal ossification. Collagen, which is the most abundant protein in mammals and forms the structural framework of most tissues, provides high tensile strength, high water affinity, low antigenicity, hemostatic properties, low inflammatory and cytotoxic properties, and the ability to promote cellular attachment, growth, and differentiation.
The aim of this study is to use silica and collagen for creating a new scaffold material for bone tissue engineering. The proposed method involves producing hybrid fiber mats of collagen and silica nanofibers of almost identical fiber dimensions. This was achieved through a dual electrospinning process by using two syringes, one containing a TEOS solution as a silica precursor and the other filled with a suspension of fibrilized collagen. The morphology of the scaffolds was investigated using SEM, while their structure was analyzed by FTIR. The stability of the resulting scaffolds has been improved by chemical crosslinking and investigating the parameters of different glutaraldehyde vapor reaction times. The biological properties of the hybrid nanofibers have been also evaluated for their ability to promote cell attachment and proliferation with human osteoblasts *in vitro*.
In conclusion, the mechanical properties and biocompatibility of these hybrid nanofibers have demonstrated their potential as a scaffold material for bone tissue engineering. This study presents the first successful synthesis of hybrid electrospun nanofibers without heat treatment composed of collagen and silica and provides a basis for further research on their use in bone regeneration.



PoA.9.04

MORPHOLOGICAL COMPARISON OF PCL AND PCL/GEL ELECTROSPUN NANOFIBERS AND *IN VITRO* TOXICITY EVALUATION WITH PIG TENDON-DERIVED TENOCYTES AND STEM CELLS

Seyma Sereflioglu[1,2], Matt Shephard[1], Nicholas Forsyth[1], Aldo Boccaccini[2]

[1]School of Pharmacy and Bioengineering, Keele University, Staffordshire, United Kingdom. [2]Institute of Biomaterials, University of Erlangen-Nuremberg, Erlangen, Germany

Abstract

To create tissue scaffolds that meet requirements like biocompatibility, biomimetic interface, mechanical properties close to native tissue, and promotion of cell attachment and differentiation, synthetic and natural polymer composites are investigated as tissue engineering approaches. When compared to natural polymers, synthetic polymers are chosen for tissue engineering applications because they may be nontoxic, degrade gradually, and are readily shaped into numerous forms. However due to the unfavourable effects of the degradation products *in vivo*, such as inflammatory responses and their relatively poor integration with host tissues, the use of synthetic polymers is often limited. Natural polymers, on the other hand, have the advantage of being recognisable by cells, similar to the extracellular matrix found in natural tendon, bioactive, and compatible with biological processes, which promote cell adhesion, migration, proliferation, and differentiation. Their limited mechanical strength and rapid degradation, however, prevent them from being widely used in tissue engineering.

In this work, we used polycaprolactone (PCL), and a blend with gelatin (PCL/Gel) to create nanofibrous tissue scaffolds in random and aligned orientations. Through combination of synthetic polymer PCL and the natural polymer Gel, we sought to boost hydrophilicity and improve biocompatibility while maintaining the advantages of PCL's mechanical properties and slow degradation rate. As another strategy to increase hydrophilicity, we also applied surface oxygen plasma treatment to enhance the cell-material interaction. The surface wettability of nanofibrous scaffolds were characterized followed by morphological assessment by SEM to generate insight about the effect of plasma treatment and orientation. Their cellular toxicity as a scaffold for tendon tissue regeneration was determined.

We routinely generated uniform and beadles random nanofibers with sub 200 nm diameter with a rotating drum collector at 1000 rpm. Optimised aligned nanofibers were 200-400 nm diameter at 3000 rpm. PCL hydrophobicity was decreased by the addition of Gel from 130 ± 8 to 73.3 ± 0.7, and further decreased with plasma surface treatment to 19.9 ± 2.8 in random-oriented nanofibers. The alignment orientation which mimics the native tendon structure also decreased the water contact angle compared to random oriented scaffolds. *In vitro* cytotoxicity testing of scaffolds with tenocytes and tendon stem cells demonstrated that scaffolds are nontoxic and suitable for further biological exploration.

PoA.9.05

Mechanical and biological characterization of electrospun scaffolds as biomimetic dural substitutes

Nathalia Oderich Muniz, Jean-Philippe Tosiani, Timothee Baudequin

Universite de technologie de Compiegne, CNRS, Biomechanics and Bioengineering, Compiegne, France

Abstract

Motivations. Dura mater is the most external meninge, a half-rigid membrane located between the central nervous system and bone tissues. When injured or removed, artificial substitutes are commonly used but complications are still frequent (up to 40% of cerebrospinal fluid leakage with bovine pericardium grafts). Due to the morphological similarities between the native dural extracellular matrix and polymer fibres produced by electrospinning, we aim at developing a novel multiphasic electrospun dural substitute mimicking the transition from skull to meningeal dura. We expect it to be more bioactive (thanks to mechanical and architectural resemblance) and more functional (thanks to specific layers providing advanced properties such as watertightness), leading to fewer postoperative complications.
Methods. Electrospun fibres were produced from 10% and 12%w/v polycaprolactone (PCL) solution, pure or combined with bioactive molecules to promote bone or dural cells development (hydroxyapatite nanoparticles, silk fibroin). Random and aligned fibres were compared (Figure 1AB). PCL scaffolds were also coated with aqueous polyurethane solution to increase watertightness (Figure 1C). The mechanical properties of each fibre network (elastic modulus, ultimate tensile strength, elongation at break) were evaluated by tensile tests. To assess biocompatibility and bioactivity, primary human dural fibroblasts or immortalized adipose-derived stem cells were cultured on the scaffolds (1 week) before measuring metabolic activity (Alamar Blue, MTS) and viability (Live/Dead).
Results. PCL scaffolds showed lower mechanical properties than native dura according to literature (for instance 8.61 (PCL) VS 68.1 MPa (dura) for elastic modulus), but all modifications performed on the scaffolds led to increased mechanical properties mimicking better the actual tissue (Figure 2). Polyurethane coating increased slightly the elongation at break of PCL fibres without altering elasticity, ultimate tensile strength nor cell viability. From the biological point of view indeed, both cell types were able to attach, spread and proliferate on all scaffolds with high viability.
Outlooks. Multiphasic structures will therefore be investigated in future studies to approach the optimal mechanical behaviour. Biological functionality (matrix composition, stem cell differentiation) will also be evaluated in the longer term before moving on to *in vivo* implantations of the most promising scaffolds.

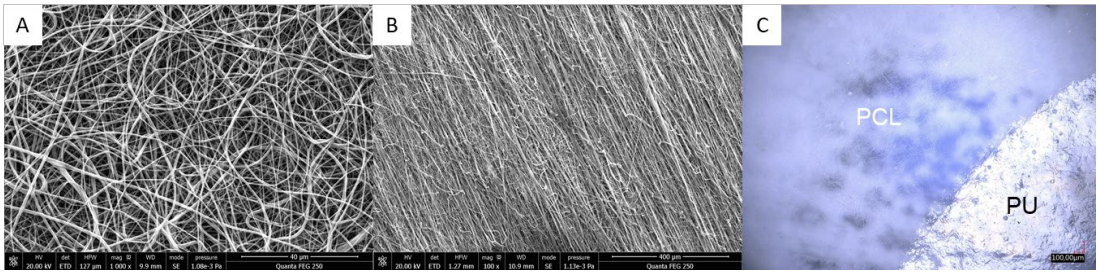


Figure 1: (A, B) Scanning electron microscopy observations of random (A) and aligned (B) pure PCL fibre networks. (C) Keyence HR optic microscopy of a PCL scaffold partially coated with polyurethane (PU) solution.

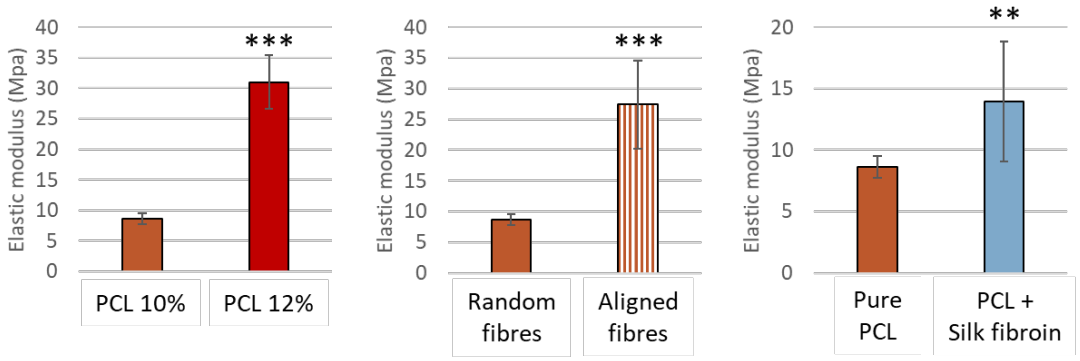


*Figure 2: Evolution of the elastic modulus of PCL scaffolds following changes in concentration (left), alignment (middle) or composition (right). Mean±SD, ***: p<0,001, **: p<0,01.*

PoA.9.06

Advanced experimental and numeric simulation method used for the characterization of nanofibers biomaterials produced by electrospinning

Ramona - Crainic[1], Alexandru Stefan Farcasanu[1], Petru - Pascuta[2], Lavinia Raluca Saitis[1], Radu - Fechete[2]

[1]Babeş-Bolyai University, Faculty of Physics, Cluj-Napoca, Romania. [2]Technical University of Cluj-Napoca, Faculty of Material and Environmental Engineering, Cluj-Napoca, Romania

Abstract

Electrospinning is a versatile technique used for producing nanofibers from various types of biopolymers. These fibers have a high surface area to volume ratio, which makes them suitable for modern applications in tissue engineering, drug delivery, and wound healing. In this study, we focused on four different biomaterials produced from raw marine collagen, chitosan, polyvinyl alcohol (PVA), and polyethylene glycol (PEG).

Various collagen/chitosan, PVA/chitosan, PEG/collagen and collagen/chitosan/PVA in different ratios were dissolved in acetic acid and formic acid to form solutions with concentration from 2 - 90 % (g/ml) to study the effect of different molecular format (e.g. native and denatured collagen). These solutions were used in the electrospinning experiments in the same day at room temperature. The bio-polymeric solutions were placed into a 50 ml syringe with a needle of 0.8 mm inner diameter. The high voltage power supplier (12 kV) was connected to the needle via a conductive clamp. A piece of aluminum foil was fixed with Tessa film onto the rotary collector acted as ground was placed at 100 mm distance at the same height as the needle. The feed rates of the solution were optimized between 0.8 and 3.2 ml/h.

We characterized the raw materials, bio-polymeric solutions and finite nanofibers biomaterials using several techniques: FT-IR spectroscopy, scanning electron microscopy (SEM), low-field NMR relaxometry and high field ^{1}H and ^{13}C NMR as well as NMR imaging and single voxel ^{1}H NMR spectroscopy. Low field 2D T_1-T_2 COSY and T_2-T_2 EXSY and high field COSY were employed. For each nanofiber the FT-IR, ^{1}H and ^{13}C NMR spectra and as well as 2D T_1-T_2 COSY and T_2-T_2 EXSY revealed characteristic changes on specific peaks, as a result of new interaction between functional groups indicating the formation by electrospinning of a new morphology with a new molecular dynamics highlighting the relevant parameters. The degree of crystallinity was established from X-ray diffraction measurements. To analyze the SEM images, we employed artificial neural network (ANN), based on machine learning library (ml5), to correlate the collector speed with the alignment degree of nanofibers. In this sense the ANN was trained to identify patterns and structures. The analysis was proved to be helpful for a better understanding of the produced nanofibers properties. This study demonstrates the potential of electrospinning for producing nanofibers and highlights the importance of using multiple characterization techniques for a comprehensive understanding of the fibers properties.

PoA.9.08

Development and characterization of nanofibrous membrane for the treatment of congenital diaphragmatic hernia

Rodolphe Migneret[1,2], Isabelle Talon[3,4], Emeline Lobry[1], Guy Schlatter[1], Hamdi Jmal[2], Amane-Allah Lachkar[3,4], Nadia Bahlouli[2], Anne Hébraud[1]

[1]ICPEES UMR 7515, Strasbourg, France. [2]ICube UMR 7357, Strasbourg, France. [3]INSERM U 1121, Strasbourg, France. [4]Pediatric Surgery Departement-Hôpital de Hautepierre, Strasbourg, France

Abstract

Congenital Diaphragmatic Hernia (CDH) is a rare malformation, a hole in the diaphragm is formed early in the embryogenesis. For the most severe cases of CDH, a prosthesis is necessary to fill the hole in the diaphragm. The major issue is that the currently used prosthesis cannot follow the growth of the child. Their mechanical properties are not suitable in terms of stretchability and rigidity, leading to the break of the stitches and recidivism of the hernia.

We aim to change the paradigm by designing a biocompatible prosthesis adapted to the disease with two distinct faces: one smooth side to impair the intestine adherence on the abdominal side and one fibrous side to enhance the cellular colonization to improve the integration of the prosthesis to the diaphragm. To follow the growth of the child, the mechanical specifications are the following: a 300 % minimum stretchability and a rigidity similar to the muscle.

A medical grade elastomeric thermoplastic polyurethane (TPU) was processed using electrospinning. A semi-diluted entangled polymer solution is stretched in a jet-like form, under a strong electric field, leading to the formation of a nano-fibrous membrane on a grounded collector. Electrospinning formulation and process parameters, using a 4-needles set-up, were studied to obtain fibrous membranes. Then, the preparation of bilayered membrane with a smooth film layer and a rough fibrous layer was investigated. To avoid delamination between the two layers, the membranes were fabricated in a continuous process by varying the flow rate of the solution during electrospinning. A 4-needles emitter was used to increase the production yield and its translation was optimized to ensure the thickness homogeneity of the membrane (Figure 1).

It was shown that mechanical properties are impacted by the ratio of film to total thickness. A panel of membranes was thus designed varying this ratio from 0 (only fibers) to 1 (only film) allowing to modulate the mechanical properties of the membrane (tensile strength, elongation at break, Young modulus, Figure 2). The membrane exhibiting the best mechanical properties was compared with the current prosthesis. The specifications in terms of stretchability were successfully doubled (600 %) and the rigidity was considerably lowered (2.5 MPa). Further biological and mechanical tests, such as uniaxial tensile tests on the stitched membrane or bi-axial tensile tests, are currently ongoing to complete the study.

PoA.9.09

The morphology and topography of Chitosan-Zn complex fiber mats influence the viability and attachment of stromal cells and mouse fibroblasts

Nurshen Mutlu[1,2], Fatih Kurtuldu[1,2], Aleksandra Ewa Nowicka[1], Liliana Liverani[2], Dušan Galusek[1,3], Aldo R. Boccaccini[2]

[1]FunGlass, Alexander Dubček University of Trenčín, 911 50, Trenčín, Slovakia. [2]Institute of Biomaterials, Department of Material Science and Engineering, University of Erlangen-Nuremberg, 91058, Erlangen, Germany. [3]Joint Glass Centre of the IIC SAS, TnUAD and FChFT STU, FunGlass, 911 50, Trenčín, Slovakia

Abstract

Electrospinning is a promising technique for fabricating fibrous materials that mimic the structure of the extracellular matrix. Electrospun fiber mats with therapeutic potential hold great promise as an effective wound healing biomaterial. So far, however, limited attention has been paid to the role of the fiber topography on cell attachment and its potential synergistic effect with biochemical cues. This study aims to compare biophysical properties and biocompatibility of four different biodegradable fiber mats: chitosan and chitosan-zinc complex (ChiZn) fibers with diameters either in nano or micron-sized range. ChiZn was synthesized by the in-situ precipitation method. The influence of zinc chelation on the structure and morphology of fibers was assessed by XRD, FTIR, SEM and EDX: complexation and homogeneous distribution of zinc were documented. Subsequently, ChiZn was blended with polyethylene oxide (PEO) to enable electrospinning in a benign solvent system and crosslinked with glutaraldehyde vapor. The morphology was examined by SEM: fibers with nanosized (~200 nm) and micron-sized (~1000 nm) diameters were observed. The roughness of the fiber mats was measured by AFM. The effect of topography and composition of the fiber mats on the viability, adhesion, and proliferation of stromal cells and of mouse fibroblasts was investigated. Higher cell viability was observed on the mats composed of nanosized fibers. Early attachment and spread of the cells were evaluated by SEM after 1- and 7-days incubation. On the nanosized fibers, the cells only spread on the surface of the mats, while infiltration of the cells into the mat was observed for micron sized fibers. The results confirm that topographical features and surface chemistry play an important role in material-cell interactions. Synergistic effects of topographical and biochemical cues on cell behavior in contact with ChiZn fibers remain a topic for future research.

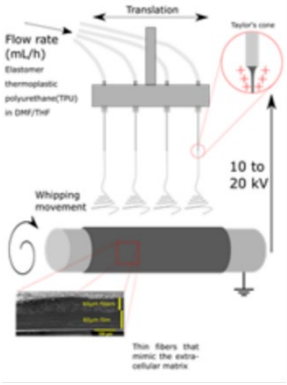


Figure 1

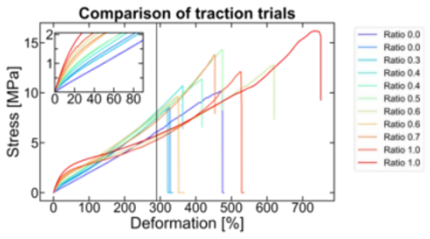


Figure 2

PoA.9.10

Poly(Beta-amino)ester electrospun wound dressing with modulated degradation kinetics for Chronic wound treatment.

Liam AYADEN[1], Mickael MATON[2], Feng CHAI[2], Nicolas BLANCHEMAIN[2], Stéphanie DEGOUTIN[1]

[1]University of Lille, UMET, Lille, France. [2]University of Lille, U1008, Lille, France

Abstract

Chronic wounds show no healing tendency after a period up to 4 to 6 weeks despite any local treatment1. This wound presents a persistence of the inflammatory stimulus and a high sensitivity to bacterial infection. Engineering innovative wound dressing is a key to facilitate the wound treatment, reduce healing time and prevent recurrent infections. In this context, this project focused on the design of electrospun wound dressing based on Poly(Beta-amino)esters polymers (PBAE) and loaded with antibiotic (ciprofloxacin, CFX). The release kinetics of CFX was studied by varying the structure of PBAE polymers and therefore their biodegradability rates.

PBAE macromers were synthesized through bulk Michael addition of Poly(ethylene glycol) diacrylate (Mn= 575 and Mn= 250) and Isobutylamine at 90°C for 72H and characterized by NMR and SEC2. The electrospinning of PBAE macromer, Poly(ethylene oxide) (900 kDa, PEO), 2-dimethoxy-2-phenylacetophenone (DMPA) (mass ratio of 73:27 PBAE:PEO) and CFX solutions in N,N-Dimethylformamide (DMF) was optimized and followed by UV-crosslinking for 120 min. Fiber diameters were quantified using ImageJ (v1.42q, NIH) on SEM micrograph (FLEXSEM1000). Degradation (n=6) of fibrous samples was performed in phosphate-buffered saline (PBS) at 37°C for up to 96H. The CFX release from fibrous mats was studied under dynamic conditions using a USP apparatus 4 (Sotax®). The *in vitro* cytotoxicity of fibers was assessed with a NIH3T3 cell line, according to ISO 10993-5 standard, with extraction method by AlamarBlue® Assay.

We successfully synthesized viscous liquid PBAE macromer with two different PEGDA leading to two different molecular weights (av. 1900 and 2300 Da) and hydrophilicities. SEM images of electrospun PBAE membranes confirmed the formation of homogeneous and defect-free fibers with micron scale diameters (Figure 1). UV post-treatment was successfully monitored using FTIR analysis and led to reduced degradation rates in PBS (~70% mass loss after 48h). The cytocompatibility of membranes was proved by cell viability (>70% with regard to control) of NIH3T3 cells. Release assays confirmed the different kinetics relative to the PEGDA used.

A promising biocompatible fibrous membrane with suitable degradation and release kinetics was developed. This PBAE/PEO membrane could be used as bilayered wound dressings for the release of two different drugs with distinct time scale release kinetics.

1. Han G, Ceilley R.. Adv Ther. 2017;34(3):599-610. doi:10.1007/s12325-017-0478-y

2. Metter RB, Ifkovits JL et al.. Acta Biomater. 2010;6(4):1219-1226. doi:10.1016/j.actbio.2009.10.027

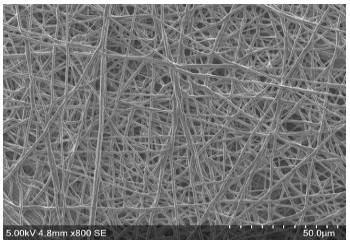


Figure 1 - SEM Images of electrospun

PoA.9.11

Fabrication of fish gelatin-PiPOx and GelMA- PiPOx fibrous scaffolds

Elena Olăreț[1], Florica A Jerca[2], Valentin V Jerca[2], Raluca E Ginghină[3], Izabela C Stancu[1,4]

[1]Advanced Polymer Materials Group, University Politehnica of Bucharest, Bucharest, Romania. [2]Centre for Organic Chemistry "Costin D. Nenitescu", Romanian Academy, Bucharest, Romania. [3]Research and Innovation Center for CBRN Defense and Ecology, Bucharest, Romania. [4]Professor, Faculty of Medical Engineering, University Politehnica of Bucharest, Bucharest, Romania

Abstract

The present work aimed the development of biohybrid microfibrous scaffolds based on poly(2-isopropenyl-2-oxazoline) (PiPOx) and gelatin, for wound repair. PiPOx emerged as a versatile platform to develop advanced functional materials, reported recently in literature by Jerca's group, showing high potential for biomaterials [1]. The versatility of PiPOx polymer consists in its hydrophilic and biocompatible character, and in the fact that it can be modified to simultaneously introduce multiple and challenging functional handles, by ring opening addition reactions in the presence of various reactive groups. This advantage further allows the combination of the synthetic PiPOx with the biopolymer, to tackle the problems related to the mechanical and hydrolytic stability of the resulted biohybrid microfibers. In this study, first step was to optimize the fabrication of novel hybrid fibrous scaffolds based on fish gelatin (FG) or its methacryloyl derivative (fGelMA) and PiPOx for. FG was chosen for its high biocompatibility and spinnability while fGelMA for its potential to be crosslinked through UV photopolymerization. The fabrication of the FG-PiPOx and fGelMa-PiPOx fibrous scaffolds was performed using a controlled environment (25°C and 40% relative humidity) electrospinning equipment. Electrospinning precursors were prepared as aqueous solutions of the synthetic polymer and protein, in various ratios ranging from 1:10 to 10:1 w/w respectively. Morphological and structural characteristics were investigated through scanning electron microscopy and Fourier-transform infrared spectroscopy. In addition, aqueous media affinity and biodegradation under physiological simulated conditions were investigated. This work reports the optimal composition and fabrication parameters for obtaining hybrid fibrous scaffolds with potential in biomedical applications.

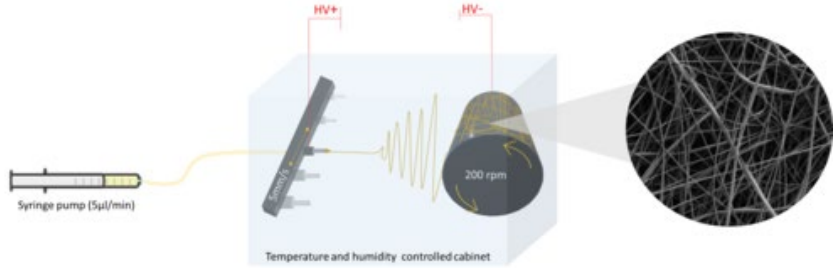


Figure 1. *Schematic representation of fabrication process*

Acknowledgements

This research was founded through PN-III-P2-2.1-PED-2021-1776, no. PED 588/20) grant. The fabrication through electrospinning technique was possible due to European Regional Development Fund through Competitiveness Operational Program 2014-2020, Priority axis 1, ID P_36_611, MySMIS code 107066, INOVABIOMED.

References

[1] Jerca, F. A., Jerca, V. V., & Hoogenboom, R. (2021). *In vitro* Assessment of the Hydrolytic Stability of Poly (2-isopropenyl-2-oxazoline). Biomacromolecules, 22(12), 5020-5032.

PoA.9.12

Medical Lace Embroidery – A promising technique for customised and malleable orbital floor implants

Cindy Elschner[1], Karla Günther[1,2], Judith Hahn[1], Laura Meinig[1], Axel Spickenheuer[1], Markus Stommel[1,2], Paula Korn[3]

[1]Leibniz-Institut für Polymerforschung Dresden e. V., Dept. Materials Engineering, Dresden, Germany. [2]Technische Universität Dresden, Faculty of Mechanical Science and Engineering, Dresden, Germany. [3]Technische Universität Dresden, University Hospital Carl Gustav Carus, Dept. Oral and Maxillofacial Surgery, Dresden, Germany

Abstract

Injuries of the bony orbit are common and result from a blunt frontal impact to the eyeball or socket. The purpose of surgical treatment after fractures of the orbital floor or wall is to restore function and aesthetic appearance by repairing the traumatic defect and relocating the ocular globe to its correct position. In addition to the timing and surgical approach, the choice of implant material is a third crucial factor contributing to the success of orbital trauma surgery. There is no universal therapy concept for the materials selection. Rather, a variety of different materials and combinations thereof are used to stabilise the fracture. The conclusion to be drawn from this and from recurrence rates of up to 30 % is that the ideal implant has not yet been found. The scientific community would like these implants to be able to adapt to the anatomical shape, to be stable at least until the injured bone is fully restored, and to be radiopaque.

To get closer to these goals, a new concept for creating malleable, porous and customised textiles based on titanium wires and surgical sutures is presented. Lace embroidery was chosen for the manufacture of these biomedical textiles because of the flexibility of pattern design, ability to influence mechanical properties and suitability for small sizes and quantities. Moreover, embroidery is a simple and inexpensive way to produce customised biomedical textiles.

Based on computed tomographic (CT) patient records, a workflow was developed that enables generating patient-specific textiles using embroidery techniques. Data processing up to computer-aided design (CAD) format was done with free open-source software (3S Slicer (The Slicer Community); Blender (Blender Foundation)). The embroidery designs were created with commercial punch software (Pulse DG16 (Tajima Software Solutions Inc.); EDOpath (Complex Fiber Structures GmbH)). In addition, the textile processability of different titanium-based wires (variation of alloy and diameter) was investigated and analysed to identify whether they are suitable for mouldable textiles.

Using embroidered, tape-like test specimens, the mechanical properties of the titanium-based textiles were determined via uniaxial tensile testing. It will be demonstrated that the mechanical properties of the textiles can be adjusted flexibly by the embroidery pattern. In this way, stiffnesses more similar to natural bone can be achieved than with pure titanium, or ceramics as well as implants made of polymers.

PoA.9.13

Electrospun bacterial cellulose mesh electrosprayed with chitin nanofibrils as a regenerative and sustainable patch for tympanic membrane perforation

Bahareh Azimi[1], Atefeh Rasti[2], Claudio Ricci[1], Alessandra Fusco[3], Giovanna Donnarumma[3], Roohollah Bagherzadeh[4], Ipsita Roy[5], Andrea Lazzeri[1], Serena Danti[1]

[1]University of Pisa, Pisa, Italy. [2]Amirkabir University of Technology, Tehran, Iran, Islamic Republic of. [3]University of Campania "Luigi Vanvitelli",, Napoly, Italy. [4]Institute for Advanced Textile Materials and Technologies, Amirkabir University of Technology, Tehran, Iran, Islamic Republic of. [5]University of Sheffield, Sheffield, United Kingdom

Abstract

Bacterial cellulose (BC) is a biopolymer synthesized by microorganisms that holds promise for tissue engineering and wound healing applications due to its high purity and mechanical properties, as well as ability to promote cellular growth and differentiation [1]. Chitin nanofibrils (CNs) are bio-based nanomaterials with potent anti-inflammatory activity [2]. In this study, we investigated the potential benefits of combining BC ultrafine fibers and CNs to develop a wound dressing mesh for tympanic membrane (TM) perforations, which occur due to underlying infectious pathologies creating a highly inflamed environment. We aimed to develop a bio-based and bioactive mesh for TM perforation healing. To achieve this, BC was used to produce electrospun fiber meshes that were surface-modified via electrospray of CNs to achieve a uniform distribution. A 3 w% BC/solvent solution was prepared using a stepwise procedure of adding BC to an ionic liquid, i.e., 1-Butyl 3-methylimidazolium acetate ([Bmim]OAc), at 75°C and dimethyl sulfoxide (DMSO) was added as a co-solvent ([Bmim]OAc: DMSO, 1:3 w/w). Electrospinning was performed using a flow rate of 0.5 mL/h and a 23 kV voltage on a 9 cm needle-distant collector, rotating inside a water coagulation bath at 50 rpm with a ground charge. BC nanofibers with size of 227.65 ± 77.50 nm were successfully produced (Figure 1 (a)), which were uniformly functionalized with CNs with average size of 180 nm ± 47 nm (Figure 1 (b)).

The samples and were cultured *in vitro* with human dermal keratinocytes (HaCaT) and real time RT-PCR was performed to assess pro- and anti-inflammatory cytokine expression. Results showed that the presence of CNs improved the indirect antimicrobial and anti-inflammatory activity of the electrospun fiber meshes. Specifically, the downregulation of essential pro-inflammatory cytokines and upregulation of human beta-defensin 2 expressions were observed.

By combining the advantageous features of BC and CNs, we have developed a natural and eco-sustainable mesh with the potential to address the unique challenges posed by TM perforations and their associated inflammatory response.

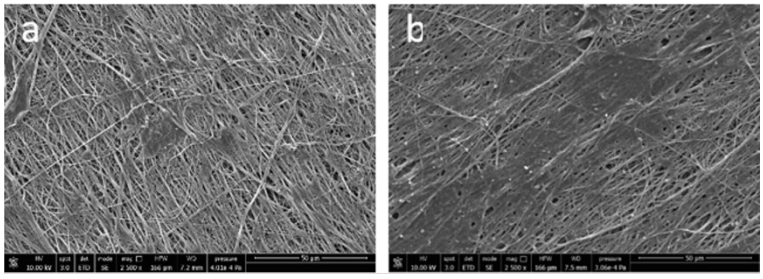


Figure 1. SEM images of a) BC electrospun nanofibers and b) CN-coated-BC electrospun nanofibers.

References:

1. Azimi, B. et al. Cellulose-Based Fibrous Materials from Bacteria to Repair Tympanic Membrane Perforations, Frontiers in Bioengineering and Biotechnology, 2021, 9, 669863
2. Danti, S. et al. Chitin nanofibril application in tympanic membrane scaffolds to modulate inflammatory and immune response." Pharmaceutics, 2021, 13(9): 1440.

PoA.9.14

Characterization of Polycaprolactone Antibacterial Electrospun Membranes For Drug Delivery

Mickaël Maton[1], William Aulard-Dorche[1,2], Maxime Lebaz-Dubosq[1,2], Kim Vanden Broeck[1], Feng Chai[1], Christel Neut[3], Jonathan Sobocinski[1,2], Bernard Martel[4], Nicolas Blanchemain[1]

[1]Univ. Lille, INSERM, CHU Lille, U1008, Advance Drug Delivery System, Lille, France. [2]Institut Cœur Poumon, Lille University Hospital (CHU Lille), Lille, France. [3]Univ. Lille, INSERM, CHU Lille, U1286, Institute for Translational Research in Inflammation, Lille, France. [4]Univ. Lille, CNRS UMR8207, UMET – Unité Matériaux et transformations, Villeneuve d'Ascq, France

Abstract

INTRODUCTION: Polycaprolactone (PCL) is a biocompatible and biodegradable polymer widely used in medical device [1]. Electrospinning is a process to obtain loaded non-woven membranes with drugs for drug delivery application. The aim of this work is to optimized and characterized electrospun membranes with ciprofloxacin to prevent infections.

EXPERIMENTAL METHODS: Experimental solutions were prepared by solubilization of 12% PCL (80 KDa) in formic /acetic acid mixture (50/50, v/v) [2], without or with 1%, 3% or 5% of ciprofloxacin. The electrospinning of these solutions allows to obtain different membranes, respectively PCL/CFX-0%, PCL/CFX-1%, PCL/CFX-3%, PCL/CFX-5%. A morphological analysis was determined by scanning electron microscopy to determine the impact of CFX concentration on fibers morphology. This analysis was completed by EDX to show the CFX distribution. The total amount of CFX was determined by HPLC-DAD at 278 nm, after solubilization of 11mm diameter samples in 1mL of chloroform and liquid-liquid extraction with 8mL of phosphate buffer at pH 10.4. Release profiles were determined for each membrane under static and dynamic conditions. Briefly, the static release process was realized with 11mm diameter samples in 1mL of PBS. At 0.5h, 1h, 2h, 3h, 4h, 6h, and 24h, PBS was collected and replaced by fresh media, and analyzed by HPLC-DAD. Antibacterial activity of each solution was determined by Kirby-Bauer method, by deposing 50µL on incubated Muller Hinton agar plate with Escherichia coli K12 or Staphylococcus aureus ATCC6538. The dynamic release was realized with SOTAX CE7-smart apparatus with 5x5cm samples in 80mL of PBS and analyzed by HPLC-DAD.

RESULTS AND DISCUSSION: The SEM analysis shows homogenous nanofibers for all membranes with diameter of 146,53nm; 152,33nm; 145,71nm; 116,26nm respectively for PCL/CFX-0%; PCL/CFX-1%; PCL/CFX-3%; PCL/CFX-5%. The release profile determined under static conditions presents a more important burst for the PCL/CFX-5%. This burst increases with the CFX quantity in electrospun solutions, but the released quantity seems not to be proportional to the initial quantity of active substance. After 24h, CFX concentrations in release media are around 0,078mg/mm², 0,157mg/mm², 0,1769mg/mm² respectively for PCL/CFX-1%, PCL/CFX-3% and PCL/CFX-5[n1] %. The antibacterial activity study shows an activity against E.coli until 6h for PCL/CFX-1% and 24h for PCL/CFX-3% and PCL/CFX-5%.

CONCLUSION: The morphological analysis shows a good morphology of fibers for all membranes. It seems that the CFX hasn't an impact on electrospinning, with a homogenous repartition of the CFX on the surface. Kinetic releases showed a sustained release of our membranes.

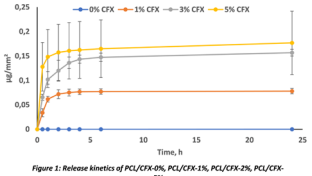


Figure 1: Release kinetics of PCL/CFX-0%, PCL/CFX-1%, PCL/CFX-2%, PCL/CFX-5%

PoA.10.01

Injectable and in situ nitric oxide-generating hydrogel as dynamic matrices for tissue regenerative applications

Dieu Linh Tran, Phuong Le Thi, Donghwan Oh, Ki Dong Park

Department of Molecular Science and Technology, Ajou University, Suwon, Korea, Republic of

Abstract

In situ forming hydrogels have been widely used as bioactive materials for tissue regeneration, owing to their extracellular matrix mimicking properties and minimally invasive surgical procedure. Recently, nitric oxide (NO), an endogenous gas molecule, have been indicated as critical modulators for various therapeutic applications, such as treatment of vascular disorders, wound healing, and cancer treatment. However, the short half-life of NO in living systems are great challenges to its clinical applications. Herein, copper (Cu) ions (a NO-generating catalyst) were incorporated in the phenol-rich gelatin-based hydrogel (GH/Cu) for in situ generations of NO in the presence of endogenous S-nitrosothiol. The NO-releasing GH/Cu hydrogels were prepared by mixing GH solutions containing horseradish peroxidase (HRP)/tyrosinase (Tyr) and hydrogen peroxide (H2O2)/copper sulfate (CuSO4), respectively. The physico-chemical properties of GH/Cu hydrogels such as gelation time, mechanical strength and degradation rate were well controlled with varying the concentrations of HRP and H2O2. Most importantly, *in vitro* release profile of NO from hydrogel matrices was precisely controlled in a wide range, over 2 weeks. The effect of released NO on cell viability and tube formation was carried out using human umbilical vein endothelial cells (HUVECs). Interestingly, the optimal NO release concentrations from hydrogels could stimulate the migration and tube formation activities of HUVECs. Besides, the ex ovo chick chorioallantoic membrane assay and *in vivo* subcutaneous injection assay showed that the GH/Cu hydrogels promoted the neovascularization and host tissue infiltration via material–tissue interactions. As a result, we are successful to develop NO-releasing gelatin-based hydrogels as injectable and dynamic matrices for tissue regenerative applications. The concentration of NO release was accurately controlled by simply varying the CuSO4 concentration. By optimizing the NO release amount from hydrogels, we can modulate the cellular functions for various biomedical applications, including wound healing, vascular disorder, anti-infection and stem cell based therapeutic products.

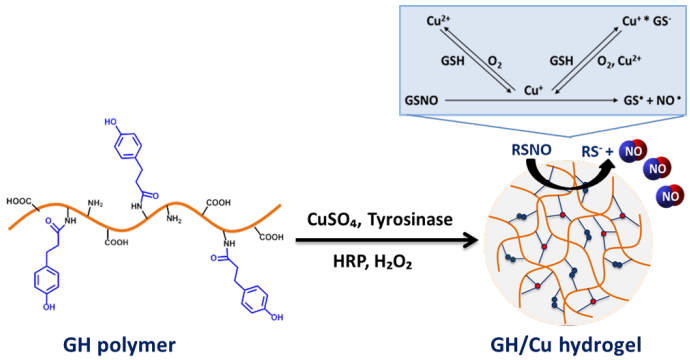


Figure 1. Schematic illustration for preparation of injectable and NO-releasing gelatin hydrogel

REFERENCES

[1] Nouf Alharbi. Materials Today Communications. March 2023.

[2] Malo Dufay. Materials Today Communications. January 2021.

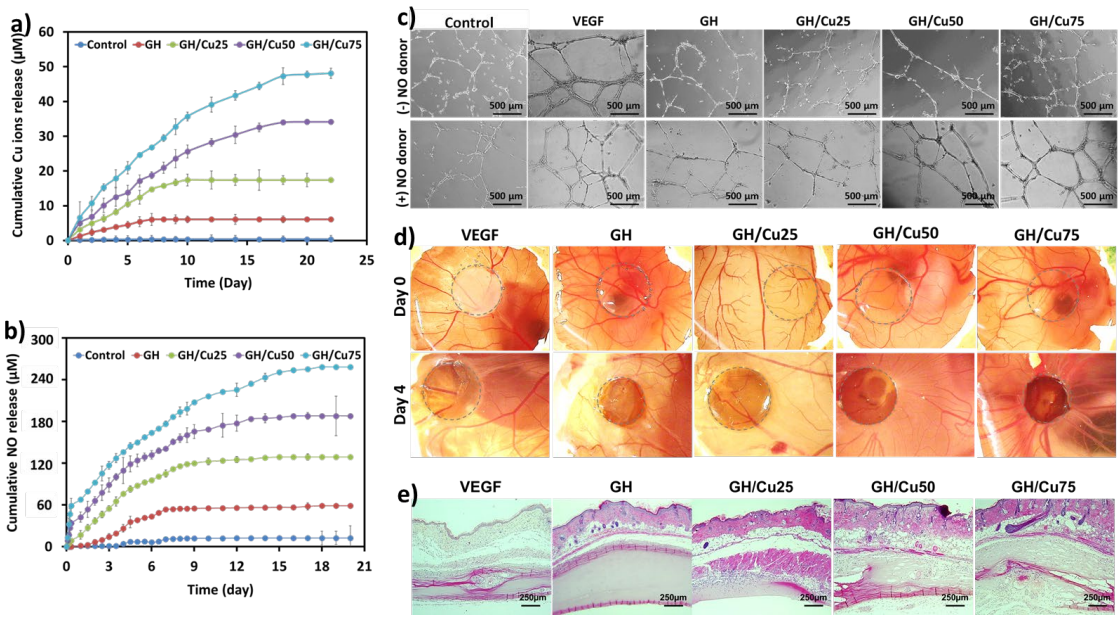


Figure 2. Cumulative release of Cu ions (a) and NO (b). Effect of NO release from hydrogels on (c) in vitro tube formation, (d) ex ovo CAM assay and (e) in vivo subcutaneous injection in rat model

Acknowledgements: RS-2020-KD000033, NRF-2019R1A6A1A11051471, and KEIT 20018560/NTIS 1415180625

PoA.10.02

Exploring new hydrogel based on chondroitin sulphate and kefiran exopolysaccharide for TERM application

Hajer Radhouani[1,2], Cristiana Gonçalves[1,2], F. Raquel Maia[1,2], Eduarda Pinheiro Oliveira[1,2], Rui L. Reis[1,2], J. Miguel Oliveira[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, Guimarães, Portugal. [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal

Abstract

Natural polysaccharides are a renewable resource that has been attracting considerable attention in tissue engineering and regenerative medicine fields, especially in creating new biomaterials. Chondroitin sulphate is a glycosaminoglycan that has been used alone or in combination with glucosamine, as a dietary supplement, for example, to treat arthritis disease (1). Moreover, kefiran, an exopolysaccharide produced by the microflora of kefir grains, has recently received particular interest for its biomedical applicability (2). However, since natural polymers are characterized by weak mechanical properties, fragility properties, and low impact strength, numerous cross-linking approaches are currently performed to overcome these shortcomings. Thus, it is important to highlight that chemical modification is the most extensively used method to reinforce the polymer's matrix and to enhance polymer's properties by grafting these polymers (3). In this current research, we successfully synthesized a new hydrogel based on reacting kefiran exopolysaccharide with chondroitin sulphate (CS) based on the diaminopropane modification. The developed hydrogel was fully characterized, and its properties were evaluated using several methodologies, including 1H nuclear magnetic resonance spectroscopy (^{1}H-NMR), fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), Gel permeation chromatography−Size exclusion chromatography (GPC-SEC), scanning electron microscopy (SEM), micro-computed tomography (Micro-CT), and rheology. Furthermore, its cytocompatibility was also evaluated by AlamarBlue® assay.

Therefore, the grafting of chondroitin sulphate to kefiran was successfully confirmed by 1H-NMR and FTIR spectra. The average molecular weight of the new developed CS/kefiran material, estimated by GPC, was about 1649 ± 4.52 kDa. The DSC spectra revealed a degradation temperature of 230ºC, showing that CS/kefiran material has high thermal stability. The CS/kefiran scaffold showed an elastic behaviour with a phase angle of 11.17 ±3.23. The scaffold demonstrated a highly porous and homogeneous structure with an open, fully interconnected geometry as determined by micro-CT and SEM analysis. Furthermore, the *in vitro* studies revealed that the metabolic activity of human adipose derives stem cells, when exposed to the developed CS/kefiran scaffold, was maintained over 72h. These results suggested that the newly developed CS/kefiran material has attractive and interesting properties for a wide range of TERM applications.

Acknowledgements: H. Radhouani and C. Gonçalves were supported by FCT-Portugal, with references CEECIND/00111/2017 and SFRH/BPD/94277/2013, respectively.

References:
(1) Sood A, et al. (2021) Carbohydr. Polym. Technol. Appl. 2:100067.
(2) Radhouani H, et al. (2018) J Bioact Compat Polym. 33 (5) 461-478.
(3) Ferreira P, et al. (2015) J Coatings Technol Res 12:463–475.

PoA.10.05

Hybrid Glyco-Biomaterials mimicking the ECM microenvironment for 3D bioprinted *in vitro* models

Federica Barbugian[1], Laura Russo[1,2], Francesco Nicotra[1]

[1]UNIMIB, Milan, Italy. [2]Curam, Galway, Ireland

Abstract

3D bioprinted personalized tissue models for regenerative medicine and animal-free drug screening require morphological and biomolecular features mimicking at best the natural ones. Complex glycan imprinting at cellular and extracellular levels is emerging as a potent regulator of cell fate in physiological and pathological events. Generation of glycoconjugate biopolymers to mimic ECM assembly is therefore an interesting approach to obtaining custom *in vitro* systems replicating a personalized cellular microenvironment.

In this work, we generated a library of glycosylated biomaterials based on hyaluronic acid (HA) in combination with various ECM proteins. The diverse application prospects are based on the possibility to fine-tune the physicochemical properties of HA-based hydrogels by adjusting the concentration and degree of functionalization, resulting in tailored gel behavior, viscosity, elasticity, mechanical strength, and bioresponsive biological systems. The final goal of the project is to understand and test the biochemical and physical behavior of the different hydrogels in order to obtain bioinks that resemble ECMs as much as possible. The selected formulations were then tested with three different cell lines to obtain *in vitro* 3D bioprinted GBM models suitable for high-performance predictive screening and research of the tumor microenvironment. Furthermore, a flow rate was used to exploit the difference between static and dynamic conditions.

Since the lack of vascularization remains a major limitation in tissue engineering, we also created a vascularized tissue model by combining template leaching additive manufacturing with hydrogels to recreate a complex and physiologically relevant vascularised tissue. In addition, the tissue model was characterized by the success of vascularisation of the channels constructed through template leaching, as well as its functional properties.

PoA.10.06

Application of injectable glycol chitosan thermogel for inner ear drug delivery

Thi Phuc Le[1], Kang Moo Hur[1], Yong-Ho Park[2]

[1]Department of Polymer Science and Engineering, Chungnam National University, Daejeon, Korea, Republic of.
[2]Department of Otolaryngology, College of Medicine, Chungnam National University, Daejeon, Korea, Republic of

Abstract

We prepared a new injectable thermogel to enhance the efficiency of inner ear delivery of dexamethasone (DEX). Hexanoyl glycol chitosan (HGC) was synthesized and evaluated as an amphiphilic thermogel (Tgel ~ 32 °C) for use as a solubilizing agent as well as an injectable carrier for intratympanic delivery of the hydrophilic and hydrophobic forms of DEX. Various thermogel formulations with different drug types and concentrations were prepared, and their physicochemical and thermogelling properties were characterized by 1H NMR, ATR-FTIR, and rheometer. They exhibited versatile release kinetics from several hours to more than 2 weeks, depending on drug type and concentration. Our formulations further showed good residual stability for more than 21 days without any cytotoxicity or inflammation in the middle and inner ear and could deliver a considerably high drug concentration into the inner ear. Therefore, HGC thermogel has great potential as an effective and safe formulation for inner ear drug delivery.

PoA.10.07

Improved swelling property of tissue adhesive hydrogels based on alpha-cyclodextrin/decyl group-modified Alaska pollock gelatin inclusion complex

Hiyori Komatsu[1,2], Shiharu Watanabe[2], Shima Ito[1,2], Kazuhiro Nagasaka[1,2], Akihiro Nishiguchi[2], Tetsushi Taguchi[1,2]

[1]University of Tsukuba, Tsukuba, Japan. [2]National Institute for Materials Science, Tsukuba, Japan

Abstract

Introduction: Closure of the cerebral dura mater is one of the fundamental procedures after brain surgery. Suture or staple have been used for closing dure mater, adhesives/sealant have been subsequently used, for the prevention of cerebrospinal fluid leakage from the anastomotic site. Commercial adhesives enable to close dura mater, however, they have still disadvantages on adhesiveness, biocompatibility and swelling property in physiological condition. We have developed a surgical adhesive consisting of Alaska pollock-derived gelatin (ApGltn) with various hydrophobic groups and a polyethylene glycol crosslinker (4S-PEG). Adhesion strength was effectively improved by introducing hydrophobic groups in ApGltn [1]. On the other hand, decreased adhesive strength caused by self-assembly between hydrophobic groups was also observed at high substitution degree of hydrophobic group [2].

In this study, we focused on the molecular inclusion property of α-cyclodextrin (αCD) to design a tissue adhesive with high adhesion strength and improved swelling property for cerebral dura closure.

Experiment: C10-ApGltn was synthesized by reacting the lysine residue in ApGltn with decanal to form the Schiff base, followed by reductive amination with 2-picolineborane. αCD/C10-ApGltn inclusion complexes prepared by dissolving αCD and C10-ApGltn in buffer solution while heating. αCD/C10-ApGltn adhesives were prepared by mixing αCD/C10-ApGltn inclusion complex solutions with 4S-PEG solutions. The swelling ratios were evaluated by immersing the cured adhesive discs in saline. The burst strength was evaluated using collagen casing and porcine brain dura mater according to ASTM F2392-04.

Results and Discussion: The resulting αCD/C10-ApGltn adhesive had improved swelling property in saline and showed significantly higher burst strength than fibrin-based and Org-ApGltn adhesives and equivalent strength to commercial adhesive for cerebral dura mater (Fig.2). From quantitative analysis of αCD, improved swelling property of resulting αCD/C10-ApGltn adhesive was induced by the release of αCD from cured adhesives subsequent assembly of decyl groups in saline.

These results suggest that developed adhesives using αCD/C10-ApGltn inclusion complex have great potential for the application in the field of brain surgery.

References

[1] Mizuno, Y.; Mizuta, R.; Hashizume, M.; Taguchi, T. Biomater Sci, 2017, 5, 982.

[2] Mizuta, R.; Ito, T.; Taguchi, T. Colloids Surf B, 2016, 146, 212.

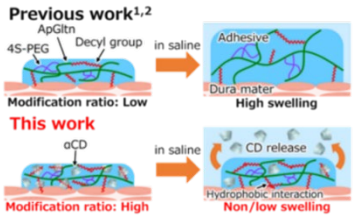


Fig.1 Improved swelling property of cured αCD/C10-ApGltn adhesive in saline environment.

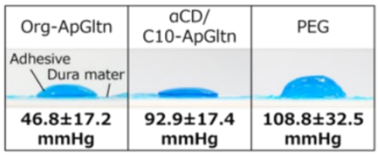


Fig.2 Swelling behaviors of Org-ApGltn, αCD/C10-ApGltn and commercial PEG adhesives applied on porcine dura mater.

PoA.10.08

Development of a dual chamber device for therapeutic cell transport using biomaterial at ambient temperature

Abhik Mallick[1], Daniel Domingo Lopez[2], Benjamin Brennan[1], Ruth Tarpey[1], Garry Duffy[1,2,3]

[1]CÚRAM, Science Foundation IrelandFI Research Centre for Medical Devices, University of Galway, Ireland, Galway, Ireland. [2]Discipline of Anatomy and Regenerative Medicine Institute (REMEDI), School of Medicine, College of Medicine, Nursing and Health Sciences, University of Galway, Ireland, Galway, Ireland. [3]Science Foundation IrelandFI Advanced Materials and BioEngineering Research Centre (AMBER), Trinity College Dublin & University of Galway, Ireland, Galway, Ireland

Abstract

Cell and gene-based therapies or advanced therapy medicinal products (ATMPs) have revolutionized the biopharma industry, with more and more FDA approvals in the burgeoning market. Therefore, cells, genes and/or other ATMPs are commonly sent worldwide using cryopreservation and cold-chain transport, which is associated with some challenges and limitations. Shipping cryopreserved products on dry ice is a widely used method. However, dry ice is classified as a hazardous material because of its explosive potential. Alternatively, cryogenic ATMPs are often shipped with liquid nitrogen and using cold chain logistics, which is less hazardous than its alternative but expensive in terms of both economic cost and energy consumption. In addition, cryopreservation of cells can have negative effects on cell viability and functionality and can degrade the quality of the cell product. Therefore, it is important to provide alternative shipping and transfer technologies to cryopreservation.

We have designed and developed a dual chamber transportation device that aims to sustain cell viability and quality throughout the ambient shipment cycle. The device consists of two chambers: an oxygen supply chamber containing oxygenated perfluorodecalin emulsion (PFD) and a cargo chamber containing native hyaluronic acid encapsulating the cell product, such as insulin producing beta cells (INS-1E) in the specific media separated by a gas-permeable membrane.

A pilot study on the transfer of INS -1E cells under ambient conditions in a dual chamber device was completed from the University of Galway (Ireland) to Explora Biotech (Italy,Rome). Device fabrication, sterile hyaluronic acid hydrogel production with INS -1E cells, preparation of oxygen-enriched PFD emulsion, device filling, and packaging were initially performed at the University of Galway. After 48 hours of shipment, cell viability of INS -1E cells was evaluated. Around 85% of the INS -1E were viable assessed quantitatively by trypan blue assay. This data was supported using live dead assay by confocal microscropy and insulin production ability by GSIS assay, demonstating proper survival and functionality.

In conclusion, we report that our dual chamber invention, when filled with oxygen PFD and a cargo chamber filled with cells, provides a better solution for transporting cells with biological material at ambient conditions than the current standard of cryopreservation or liquid nitrogen. Thus, our dual-chamber bag technology can streamline the shipping process and reduce reliance on cold chain transportation, which is impractical in rural and developing countries with less robust infrastructure and resources.

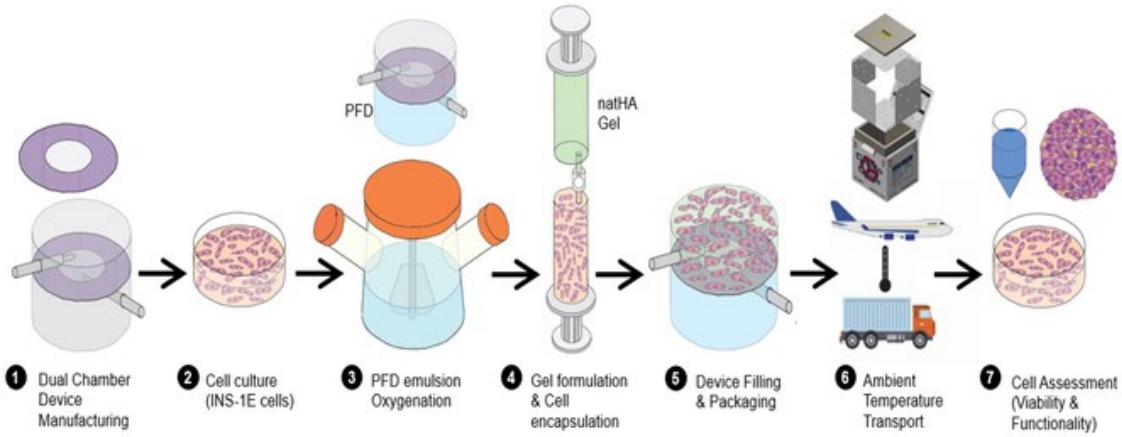


Figure 1: Workflow schematic of ambient transfer process, where the fabrication, formulation, filling, and packaging of devices occurs in University of Galway, Ireland (1)-(5). The devices are then couriered (6), and after a 48-hour transportation period the INS-1E cell viability and functionality was assessed in Explora Biotech Rome, Italy.

PoA.10.10

Biomimetic antibacterial gelatin hydrogels with multifunctional properties for biomedical applications

Hengzhi Ruan[1], Marko Bek[1], Santosh Pandit[2], Alexandra Aulova[1], Jian Zhang[2], Philip Bjellheim[3], Martin Lovmar[2,3], Ivan Mijakovic[2,4], Roland Kádár[1]

[1]Department of Industrial and Materials Science, Chalmers University of Technology, Göteberg, Sweden. [2]Department of Biology and Biological Engineering, Chalmers University of Technology, Göteberg, Sweden. [3]Wellspect Healthcare AB, Mölndal, Sweden. [4]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby, Denmark

Abstract

Gelatin hydrogels have been used in the last decades for different biomedical applications due to the excellent biocompatibility, easy processability, bioactivities to mimic the extracellular matrix (ECM). However, the poor mechanical properties and thermostability limited their potential applications. Herein, a facile and economical approach of introducing dopamine and [2-(methacryloyloxy) ethyl] dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA) via in situ synthesis into gelatin hydrogels with the existence of ZnSO4 was applied to overcome these disadvantages. This fabrication method allows the obtaining of gelatin-based hydrogels (GSDZ) with fatigue resistance and mechanical stability from -100 to 80 ℃. Moreover, the hydrogels showed adhesive, self-healing, electrical and excellent antibacterial properties leading to their potential use as wearable monitoring sensors and antibacterial coatings. In particular, the hydrogels showed adhesion to various types of surfaces such as paper, skin, wood, plastic, rubber and steel, as well as 99.99% and 100% of antibacterial efficiency against Gram-positive and Gram-negative bacteria respectively, indicating widespread applications in many biomedical areas.

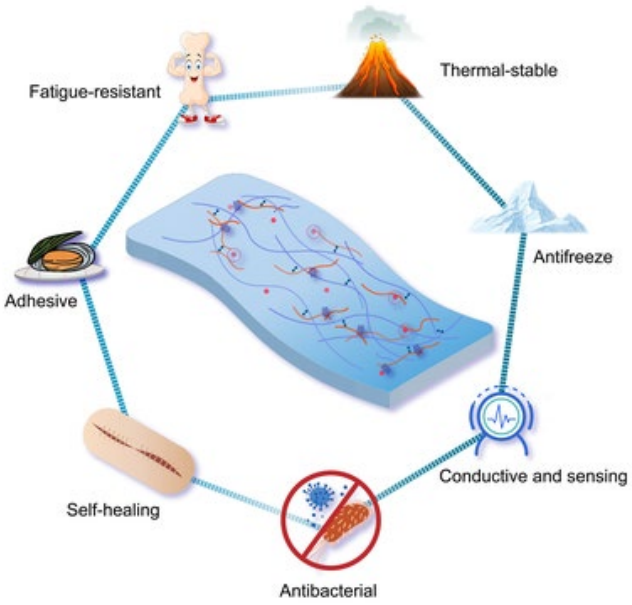


Figure 1: Schematic diagram of GSDZ hydrogels with multiple performances and applications.

PoA.10.11

Glycosaminoglycan-Functionalized Hydrogels as Tools to Modulate Angiogenesis

Fabian Junker[1,2], Toni Radanovic[1,2], Emmanuel Ampofo[3], Matthias W Lascke[3], Wolfgang Metzger[4], Sandra Rother[1,2]

[1]Preclinical Centre for Molecular Signalling, Saarland University, Homburg, Germany. [2]Institute for Pharmacology and Toxicology, Saarland University, Homburg, Germany. [3]Institute for Clinical & Experimental Surgery, Saarland University, Homburg, Germany. [4]Department of Trauma, Hand and Reconstructive Surgery, Saarland University, Homburg, Germany

Abstract

Purpose: In chronic wounds, the angiogenic capabilities of the tissue are decreased due to a state of chronic inflammation. This leads to an increased protease secretion causing extracellular matrix and growth factor degradation. Therefore, biomaterials that increase the angiogenic potential are required. Functional biomaterials with adjustable growth factor interaction are promising for fostering wound healing. Methacrylated gelatine (GelMA) can be functionalized with glycosaminoglycans, which influence vascular endothelial growth factor A (VEGF-A) signalling as well as tissue inhibitor of matrix metalloproteinase 3 (TIMP-3) [1]. This study aims to develop bioinspired sulphated hyaluronan (sHA)/gelatine hydrogels that promote angiogenesis in chronic wounds by binding and controlled release of VEGF-A, and by controlling extracellular matrix remodelling and growth factor degradation via TIMP-3. To study the interaction of endothelial cells and pericytes with artificial extracellular matrices, 3D spheroid culture was used to mimic the *in vivo* conditions.
Methods: Hydrogels were prepared by cross-linking different GelMA concentrations using UV irradiation. For *ex vivo* evaluation, spheroids of human dermal microvascular endothelial cells (HDMEC) or human placental pericytes (HPC) were embedded into the hydrogels before cross-linking. Spheroid proliferation and migration was evaluated over time. The hydrogels were further characterized regarding their stability, binding and release capacities for bioactive proteins, and material properties.
Results: Cell proliferation and migration of both HDMEC and HPC could be tuned by varying the GelMA concentration of the hydrogels in which the spheroids were embedded. Biochemical analyses showed the stabile incorporation of glycosaminoglycans into the hydrogels. Functionalizing these hydrogels with cross-linkable hyaluronan (HA) and sHA fostered HPC migration compared to collagen control gels (*Fig. 1*). Furthermore, the addition of sHA allowed to modulate the VEGF-A and TIMP-3 interaction profiles of the gels leading to a controlled release of bioactive proteins.

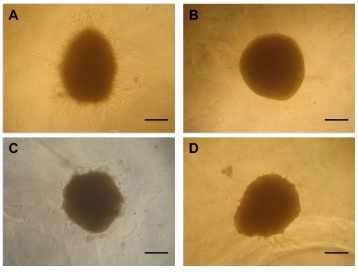


Figure 1: HPC spheroids after 48 hours. (A) GelMA hydrogels allow cell migration. (B) Collagen control gel showing few signs of migration. (C) GelMA/sHA and (D) GelMA/HA hydrogels promote HPC migration. Scale bar: 200 µm.

References

[1] S. Rother, S. A. Samsonov, S. Moeller, M. Schnabelrauch, J. Rademann, J. Blaszkiewicz, S. Köhling, J. Waltenberger, M. T. Pisabarro, D. Scharnweber, V. Hintze, *ACS Appl Mater Interfaces* **2017**, 9, 9539.

Acknowledgements
We thank Caroline Bickelmann for her excellent technical support during the cell culture experiments.

PoA.10.12

β-Tricalcium Phosphate-loaded Chitosan-based Thermosensitive Hydrogel for Periodontal Regeneration

Naiwen Tan[1], Maja Sabalic-Schoener[1], Linh Nguyen[2], Francesco D'Aiuto[1]

[1]UCL Eastman Dental Institute, Periodontology Unit, London, United Kingdom. [2]UCL Eastman Dental Institute, Biomaterials and Tissue Engineering, London, United Kingdom

Abstract

Background & Aim: Current treatment of periodontitis is aimed at resolving gingival inflammation whilst complete periodontal tissue regeneration is not always predictable and it shows a therapeutic challenge. Injectable biomaterials show tremendous potential in dental tissue regeneration and attract great attention. This study aimed to investigate the ability of injectable β-TCP-loaded chitosan-based thermosensitive hydrogel as an effective substrate to carry cells in periodontal regenerative therapy.
Methods: Different concentrations of β-TCP-loaded chitosan hydrogels were prepared (0%, 2%, 4% or 6% β-TCP, 10% β-glycerol phosphate and 1.5% chitosan). The characteristics of the hydrogels were tested by rheological, Scanning Electron Microscope, X-ray diffraction, degradation, spectroscopic and biological analysis.
Results: The new biomaterials showed sol-gel transformation ability at body temperature and exhibited excellent chemical and physical characteristics whilst the existence of inorganic composition enhanced the structure and the properties of the hydrogels (Figure 1). Biological tests confirmed the remarkable biocompatibility of the hydrogels with MC3T3-E1 and HGF cells for 14 days and this result was validated by confocal imaging (Figure 2).
Conclusion: In conclusion, a new kind of β-TCP-loaded thermosensitive chitosan hydrogel was successfully synthesised. This hydrogel showed thermosensitive ability, appropriate degradation excellent physical, chemical and biological properties. A combination of inorganic composition with chitosan hydrogel can strengthen the structure and properties of the hydrogel effectively. These findings suggest the β-TCP-loaded thermosensitive chitosan hydrogels have great potential as a scaffold in periodontal regeneration.

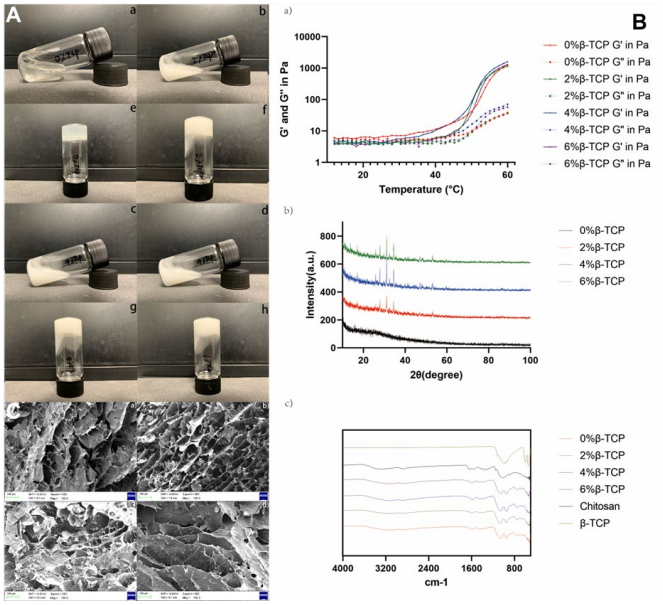


Figure 1. The gelification process of β-TCP-loaded chitosan hydrogel samples (A). 0%, 2%, 4% and 6% β-TCP-loaded chitosan hydrogels was observed under room temperature (a,b,c,d) and at 37℃ (e,f,g,h) separately. The viscoelastic moduli G' and G'' of tested hydrogel samples as a function of temperature, the X-ray diffraction characteristics and FTIR spectra of tested samples are shown in (B) a, b and c separately. The different structure of β-TCP-loaded chitosan samples (C), 0% β-TCP (a), 2% β-TCP (b), 4% β-TCP (c) and 6% β-TCP (d) can be observed in SEM images.

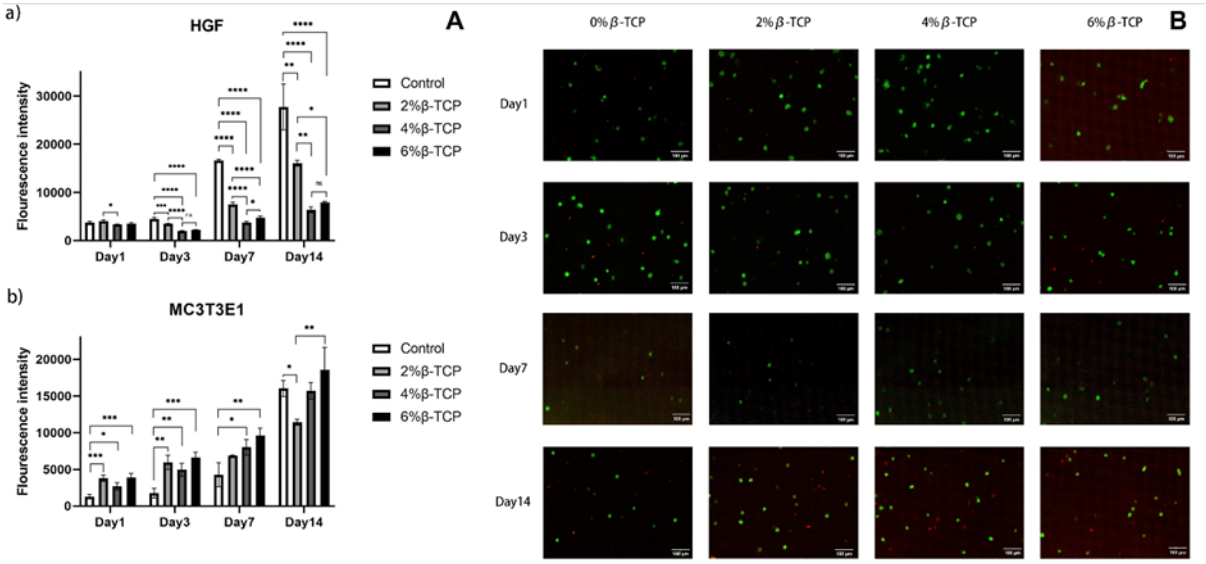


Figure 2. Alamar Blue assay on HGF (a) and MC3T3-E1 cells (b) 3D cultured in the hydrogel samples (A). Control group is the hydrogel without β-TCP. Confocal images of live cells (green) and dead cells (red) show the viability of HGF encapsulated in the hydrogel samples after 1, 3, 7 and 14 days in culture (B). Scale bars: 100μm.

PoA.10.13

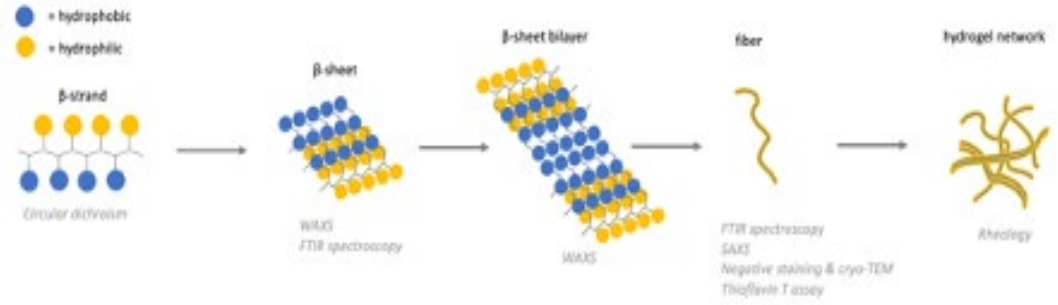
Linking stiffness of peptide-based hydrogel networks to their fiber morphology

Tess De Maeseneer[1], Thibault Cauwenbergh[2], Charlotte Martin[2], James Gardiner[3], Steven Ballet[2], Ruth Cardinaels[1], Paula Moldenaers[1]

[1]Soft matter, Rheology and Technology, Department of Chemical Engineering, KU Leuven, Leuven, Belgium. [2]Research Group of Organic Chemistry, Departments of Chemistry and Bioengineering Sciences, Vrije Universiteit Brussel (VUB), Brussels, Belgium. [3]CSIRO Manufacturing, Clayton, Australia

Abstract

Throughout the past decades, amphipathic peptide-based hydrogels have proven to be promising biomaterials for drug delivery and tissue engineering due to their biocompatibility, biodegradability, cytocompatibility and injectability. These hydrogels are composed of amphipathic peptide strands that self-assemble into β-sheets and form fibers through bilayer stacking. Entanglement of these fibers, in addition to potential hydrophilic association, eventually leads to the formation of a hydrogel network. To ensure the successful use of these gels in biomedical applications, the gels' viscoelastic properties need to reach certain target values, e.g. to match those of actual tissue in tissue engineering applications, but they also need to restructure on an appropriate time scale after structure breakdown during injection into the human body. These viscoelastic properties are inextricably linked to a multitude of fiber characteristics, such as the amount of fibers, the bundling of fibers and the morphology of a single fiber. The latter one heavily relies on the sequence of amino acids that make up the peptide strands. Therefore, the current work systematically investigates how multiple peptide sequence alterations cause the formation of very distinct fibers and consequently, the formation of hydrogel networks of strongly differing stiffness. Hereto, seven amphipathic peptides of different sequence and length, based on the hexapeptide H-Phe-Gln-Phe-Gln-Phe-Lys-NH2, have been synthesized, five of which have not been reported on previously. Small oscillatory shear rheological measurements were performed to probe the viscoelastic properties of all hydrogels. In-depth fiber characterizations were performed using circular dichroism, FTIR spectroscopy, small angle and wide angle X-ray scattering, Thioflavin T assays as well as negative staining and cryo-TEM. In addition, the effect of flow through a needle on the fiber and hydrogel properties has been investigated to ease the transition of this material to clinical use. The results show that the elastic modulus of the hydrogel network reduces if the peptide length is doubled and if additional hydrophilic amino acids are incorporated into the sequence. In addition, structural breakdown by injection of the gel through a 25G needle occurs, which reduces the stiffness of the hydrogel. This reduction can be correlated to the formation of shorter or less fibers in addition to less fiber bundling.



PoA.10.14

Biocompatible Hydrogel materials for wound coverage on oral mucosa

Lucy Kind[1], Elisa Gianfreda[1], Yasmin Grether[2], Oliver Germershaus[3], Falko Schlottig[4], Michael M. Bornstein[5], Eva M. Kulik[6], Hedwig Wariwoda Lütolf[6], Andrea Venturato[7], Adriana Euler[7]

[1]University of Applied Sciences and Arts Northwestern Switzerland (FHNW); School of Life Sciences; Institut of Chemistry and Bioanalytics, Muttenz, Switzerland. [2]affiliation during conduction of experiments: University of Applied Sciences and Arts Northwestern Switzerland (FHNW); School of Life Sciences; Institut of Pharmatechnology, Muttenz, Switzerland. [3]University of Applied Sciences and Arts Northwestern Switzerland (FHNW); School of Life Sciences; Institut of Pharmatechnology, Muttenz, Switzerland. [4]University of Applied Sciences and Arts Northwestern Switzerland (FHNW); School of Life Sciences, Muttenz, Switzerland. [5]University Center for Dental Medicine Basel UZB; Department of Oral Health and Medicine; University of Basel, Basel, Switzerland. [6]University Center for Dental Medicine Basel UZB; Department Research; University of Basel, Muttenz, Switzerland. [7]vVARDIS, Windisch, Switzerland

Abstract

Oral mucosa defects can have various causes (injuries, infections, or reduced immune reactions) which can drastically impair patients' normal oral function by causing pain during eating, chewing, and talking. The wet and highly dynamic environment of the mouth makes local treatment of oral mucosal diseases challenging. The primary focus of therapy therefore lies on the treatment of symptoms. In most of the cases comprises the topical application of pain-reliving oral gels that are mixed with an anesthetic and/or with anti-inflammatory drugs. The disadvantage of these oral gels is, that they only stick to the mucous membrane until the anesthetic has penetrated the wound area and then detach from the oral mucosa within minutes. If the anesthetics wears off, the gel must be applied to the affected area again. It is therefore a recurring process where the gel is only effective for a short period of time.

The use of a biocompatible, synthetic non-collagen-based hydrogel that are easy to apply for the patient and protect the sensitive wound-area by adhesion as a patch for a longer time, still flexible enough to adapt smoothly to the mucosa surface, supporting the healing process, and being able to act as a drug delivery system could be very beneficial (Fig.1). Synthetic self-assembling peptides (SAPs - derived from P11-family) have already been established in other areas of oral medicine such as guided enamel regeneration, desensitization and protection of natural teeth, bone regeneration, periodontal treatment and periimplantitis treatment. The SAPs arrange themselves into well-ordered nanofibers and form three-dimensional hydrogel structures under physiological conditions.

In this project, we studied the optimization of the peptide-hydrogel network from P11-family to serve as a biocompatible stable mucosa-adhesive patch. We established hybrid materials based on peptide hydrogels (SAPs) (Fig.2; Group A), which are either biocompatible physico-chemical modified (Fig.2; Group B) and/or stabilized by a secondary hydrogel network (Fig.2; Group C). The correlation of stiffness to adhesion and stability can be determined through the adapted optimized analytical methods, like rheology, swelling ratio, biocompatibility, and adhesion- and scratch-tests. The construction of a co-hydrogel network with lignin-hydrogels in a biocompatible formulation shows immense progress in relation to the parameters mentioned for use as an oral hydrogel-patch.

Thus, in this project, an iterative process is created to generate new advantageous combinations that meet the objective.

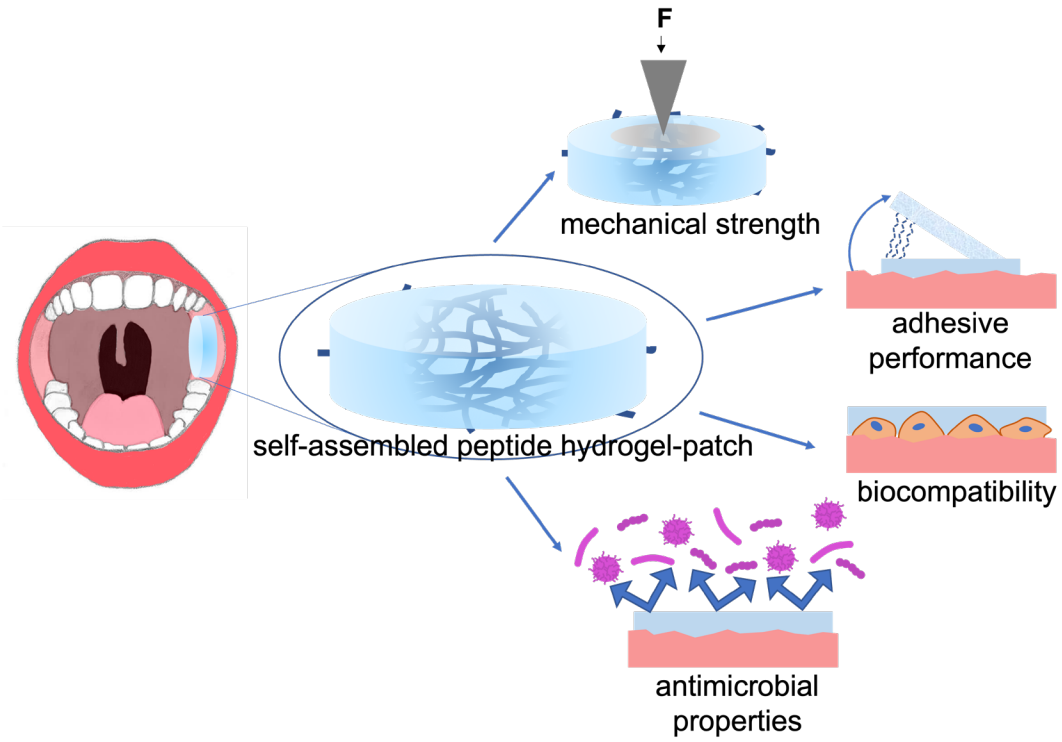


Fig.1: Key requirements of hydrogel for use as an oral mucosa patch.

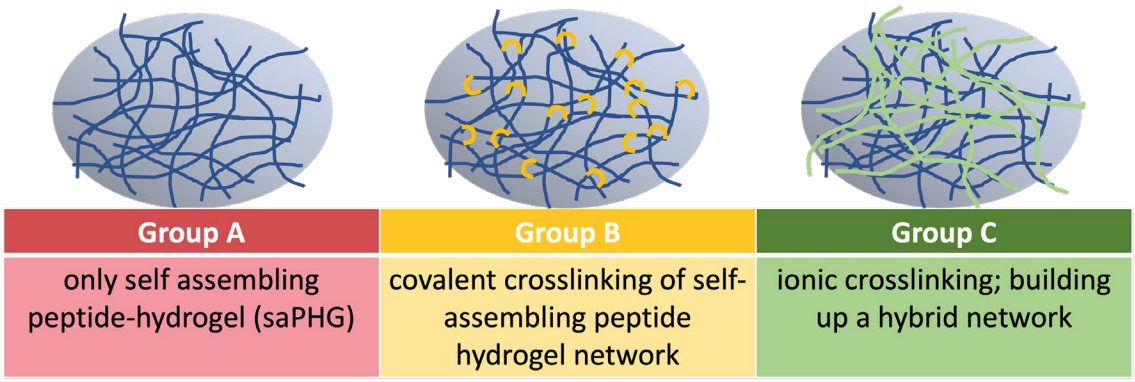


Fig.2: Classification of the different hydrogel candidates.

PoA.10.15

Influence of a hydrophobic copolymer on colon-targeting performance of a pH-responsive hydrogel carrier system.

Mohmmad E Rabeh, Matthew P Wylie, Jessica V Moore, Colin P McCoy

Queen's University Belfast, Belfast, United Kingdom

Abstract

Introduction: Colon-targeted drug delivery systems are a promising strategy for improved treatment of local diseases affecting the colon, such as inflammatory bowel disease (IBD). More than 6.8 million individuals worldwide suffer from IBD complications, which is associated with a significant increase in patient morbidity. The physiology of the gastrointestinal tract (GIT), which has variable pH environments, has encouraged the development of pH-responsive delivery systems able to protect water-soluble drugs from the harsh gastric environment before release in the small intestine but many are unable to specifically deliver high drug payload to the colon for localised colonic therapies.
Methods: A free radical polymerisation method was used to prepare four tri-copolymer hydrogel films of hydroxyethyl methacrylate, methyl methacrylate (MMA) and methacrylic acid, using ethylene glycol dimethacrylate as a crosslinker of 4 different compositions denoted H1-H4. ATR-FTIR was used to confirm the polymerisation of networks. A model, BCS Class I drug, was employed in the study. The swelling diffusion method was used for drug loading. Swelling and in-vitro release studies were performed in three different buffer conditions, mimicking pH conditions of the stomach, colon, and small intestine (pH 1.2, 6.5 and 7.4), respectively.
Results: Tri-copolymer pH-responsive hydrogels were successfully synthesised to assess the effect of MMA on colon drug delivery from a pH-responsive hydrogel system. ATR-FTIR confirmed hydrogel polymerisation of different compositions. All hydrogels have a significantly higher swelling degree at pH 7.4 than at pH 6.5 and 1.2, revealing the hydrogels pH responsivity. In addition, the swelling study shows that increasing MMA content leads to a decrease in the swelling degree, attributed to the hydrophobic nature of the MMA that reduces polymer chain water intake. In-vitro drug release studies were performed in conditions mimicking both pH and average GIT transit time of the drug reaching colon, which is estimated to be 5 h. Figure 1 shows limited and controlled drug release at acidic and neutral pH after 5 hr. The data illustrate the ability of these hydrogels to limit the premature release of a drug during transit through the stomach and small intestine and ultimately improve the availability of a drug payload for release in the colon.
Conclusion: Developing drug delivery systems to control and target drugs to the lower GIT, particularly the colon, is essential to improve treatment of colonic diseases. The results show that tri-copolymer hydrogels are a promising carrier for colon-specific delivery of the model, clinically relevant, water-soluble drugs.

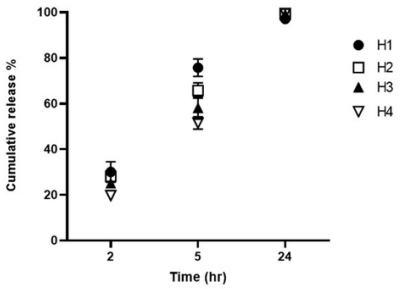


*Fig. 1. The release profile of model drug (mean ± SD) from H1, H2, H3 and H4 hydrogels at different buffers mimicking pH and transit time of stomach **pH 1.2 for 2h**, small intestine **pH 7.4 for 3h** and colon **pH 6.5 up to 24h**. n=3.*

PoA.10.16

3D printing of high-resolution hydrogels based on photosensitive cellulosic formulation dedicated to biomedical applications

lénaïc Soullard[1], Angélique Schlepp[2], Béatrice Labat[3], Sébastien Rolere[1], Guillaume Nonglaton[1], Isabelle Texier[1], Bruno Jean[4]

[1]CEA, Grenoble, France. [2]Microlight3D, Grenoble, France. [3]Université Rouen Normandie, Rouen, France. [4]CERMAV, Grenoble, France

Abstract

Current developments of innovative medical devices are mainly based on the use of biomaterials. These materials usually display high biocompatibility, controlled biodegradability, and advantageously specific biological properties. However, these natural materials must be shaped to fit the mechanical properties and the dimensions of human tissues. In this regard, additive manufacturing technologies are particularly well-suited to respond to the technical specifications of biomedical devices. Notably, Digital Light Processing (DLP) allows the 3D printing of complex parts using UV-curing of liquid photo-crosslinkable materials. Herein, DLP was explored to develop biomedical devices based on carboxymethylcellulose (CMC), a water-soluble cellulose derivative.

CMC was first chemically modified with methacrylic anhydride to obtain carboxymethylcellulose methacrylate (mCMC). The latest was then solubilized in water with a biocompatible photoinitiator. After molding and UV-curing, obtained hydrogels did not exhibit any cytotoxicity, and showed viscoelastic properties (G' = 5.6 ± 0.89 kPa at 25 °C, 1 Hz and 1 % strain) compatible with an application in soft tissues reparation such as brain or lung. When printed with DLP, the hydrogels present a x- and y- resolution lower than a hundred micrometers (Fig. 1a). In order to preserve the objects lifetime, the hydrogels were then freeze-dried into cryogels. The pore size of the resulting materials was in the range of 10 – 20 µm (Fig. 1b), which could limit the growth of cells for extracellular matrix (ECM). Two photons polymerization (TPP), another printing technology, is currently considered to obtain patterns with larger pores to developed ECM applications.

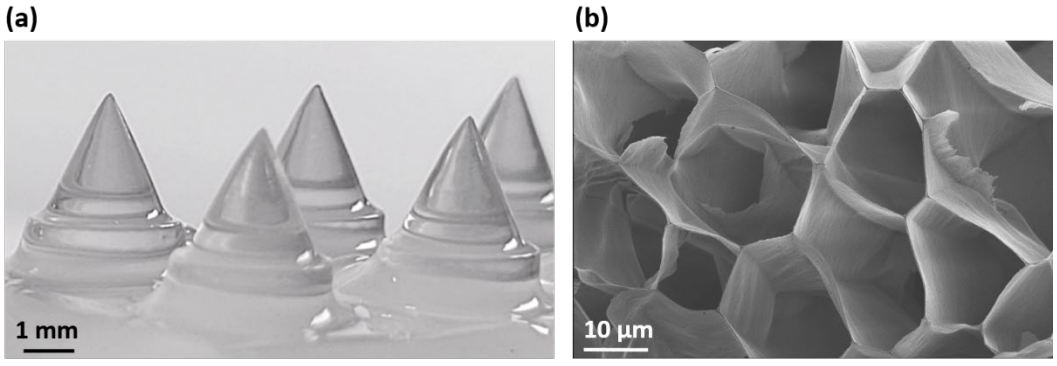


Fig 1. (a) Carboxymethylcellulose methacrylate hydrogel tips printed by DLP using 50 microns thickness increments with 4 s of UV light exposure (385 nm) at 18 mW/cm². The object was post-cured under UV at 9 mW/cm² during 3 min and (b) SEM image displaying the cryogel porosity.

PoA.10.17

Estimation of the swelling kinetics of cross-linked hydrogels in solvents using compressive cyclic-loading tests

Nada Abroug[1], Hermann Seitz[1,2]

[1]Chair of Microfluidics, Department of Mechanical Engineering, University of Rostock, Rostock, Germany. [2]Department of Life, Light and Matter, University of Rostock, Rostock, Germany

Abstract

The water absorption capacity of biopolymer-based hydrogels affects their intrinsic properties such as lowering their mechanical resilience and toughness or affecting certain functions such as drug release rate. It is thus of importance to be able to predict the hydrogels' swelling behaviour.
For that purpose, we assessed a simple experimental and modelling approach based on [1]. Alginate-gelatine hydrogels (1/2.5 wt. % alg-GEL) were prepared and ionically cross-linked using 0.1M $CaCl_2$ solution. We then performed unconfined compressive cyclic tests under constant strain-rate at 100µm/s up to 10% strain. First, the E-moduli E_0 of the specimens in as-prepared state were determined. Then, they underwent additional 10 compressive tests while being immersed in distilled water, with a recovery phase of 5 minutes between each two compressions. The compressive E-moduli were then determined and utilized in order to estimate the evolution of the polymer fraction in the hydrogels based on the variation in the E-moduli during an hour of swelling. For validation purposes, we performed equilibrium swelling experiment. All experiments were performed at 20°C.
The results of the compressive tests indicated a decrease of the E-modulus with swelling. The obtained average polymer volume fractions were fitted to an exponential decay in order to establish the swelling kinetic. The latter describes the swelling-ratio as a function of time and displayed ranges of equilibrium swelling at about 60-80% and 50-70% for consecutively volumetric and mass swelling-ratio. The swelling experiments showed an equilibrium reached at an average mass swelling-ratio of about 60% as shown in Figure 1. It is important to note that several simplifications were employed in the modelling, and thus, some uncertainties were present in the current applied approach. Besides, the equilibrium swelling ranges obtained by modelling required initial estimates of the polymer volume fractions at equilibrium to be made. Additionally, the effect of strain-softening was neglected, and the evolution in E-modulus was considered to be solely due to swelling.
This preliminary study presents a potential method for predicting the swelling of ionically cross-linked alginate-gelatine hydrogels based on the evolution of their mechanical properties, in particular the elastic modulus. This approach will be further developed to create a reliable predictive model for the swelling behaviour of similar hydrogels.

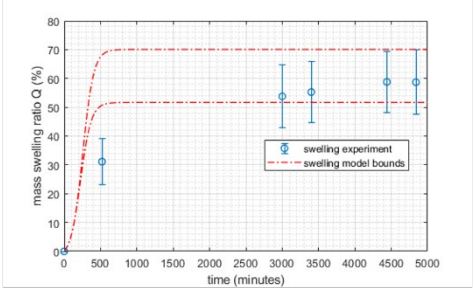


Fig. 1: mass swelling ratio obtained experimentally and through modeling.

References:
[1] Sakai et al., Soft Matter, 2012. 8, DOI: 10.1039/c2sm07043j

Acknowledgements: This research was funded by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – SFB 1270/2 – 299150580.

PoA.10.18

Enzymatically Triggered Deprotection and Cross-Linking of Thiolated Polymers for Generating Dynamic and Modular Bioinks

Sajjad Naeimipour, Fatemeh Rasti Boroojeni, Robert Selegård, Daniel Aili

Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden

Abstract

Utilizing hydrogels composed of biologically derived biomacromolecules to cultivate cells in 3D is a fascinating technique for the development of tissue and disease models. However, many of the hydrogels employed in these applications are characterized by their complexity, difficulty in handling and processing, and significant batch-to-batch variability. Additionally, the utilization of animal-derived materials poses ethical considerations. There is an increasing need for advanced semi-synthetic hydrogel technologies that can offer greater control over material properties. Thiol-based chemistries are widely employed for producing synthetic and semi-synthetic hydrogels. However, the highly reactive nature of thiol groups makes these hydrogels susceptible to oxidation upon exposure to air, resulting in the formation of disulfide bonds that can adversely impact the shelf-life and complicate the application of these hydrogel systems. We propose a novel approach to create tunable modular hydrogels by utilizing both synthetic and natural polymer backbones via thiol chemistry. Cysteine residues were modified with a thiol-protecting group (Phacm) that is enzymatically labile and conjugated to the polymers. Upon the addition of penicillin g acylase (PGA), Phacma was released, initiating the hydrogel formation. The unshielded thiol groups form dynamic covalent disulfide bonds and can also interact with multi-arm poly(ethylene glycol)-maleimide crosslinkers. This chemistry allows for the adjustment of the mechanical characteristics of the hydrogels by modifying the crosslinker concentration. Moreover, we have shown that the hydrogels exhibit a reduction in stiffness in a reducing environment. The degree of softening can be regulated by modifying the proportion of disulfide and thiol-maleimide crosslinking.
In addition, we demonstrate that this chemistry facilitates the 3D bioprinting of complex multi-material and multi-cellular structures. We achieved this by printing hydrogel-based bioinks into a gelatin slurry containing PGA. Cell-laden structures, containing primary human fibroblasts and breast cancer (MCF7) cells, were printed, and high cell viabilities were observed. This tunable and modular cross-linking technique allows for easy processing and 3D bioprinting and presents a promising alternative to animal-derived hydrogel systems for 3D cell culture and biofabrication.

PoA.10.19

Fine-tuning the processability mechanical properties of hydrogels for load-bearing tissue engineering.

Sofie Houben[1], Mahsa Ebrahimi[1], Mariana Arreguin Campos[1], Ana Aldana[2], Matthew Baker[2], Louis Pitet[1]

[1]Hasselt University, Hasselt, Belgium. [2]Maastricht University, Maastricht, Netherlands

Abstract

The preparation of contemporary hydrogels has become an extremely sophisticated endeavor. Innovative efforts to design smart, responsive, and cytocompatible scaffolds having risen to impressive new heights in recent years. However, tuning the mechanical properties in order to match those of certain load bearing tissues remains a challenge, particularly if adaptiveness, responsiveness and processability are to be retained. Our group will highlight several related strategies for accessing mechanically robust scaffolds, built on principles of molecular design. In order to retain all of these key characteristics, we employ well-defined building blocks that lead in some cases to uniform molecular scaffold structures. Such structures exhibit impressive strength and resilience. Meanwhile, functional handles are introduced for the attachment of bioactive motifs, promoting cytocompatibility that is essential in regenerative medicine. Lastly the connectivity of the crosslinks can be modified to exhibit specifically triggered reactions that enable ready processing through various additive manufacturing techniques. For example, we show that light-induced crosslinking can be used in DLP and that base-catalyzed crosslinking is suitable in extrusion based printing. We have employed thiol-yne crosslinking combined with ionic crosslinking in hybrid hydrogels comprised of poly(ethylene oxide) and alginate. The variations in physical behavior are brought about by careful molecular design, ultimately showing a highly tunable platform that is promising for various tissue engineering applications, such as cartilage or blood vessel replacement.

PoA.10.20

Development of a lung tumour hydrogel phantom model: A simulation test-bed for procedural planning

Tapas Mitra, Laura B Gallagher, Aoife Lowery, Garry P Duffy

University of Galway, Ireland, Galway, Ireland

Abstract

Introduction: Lung cancer is the most common cause of global cancer incidence and mortality, accounting for an estimated 2 million diagnoses and 1.8 million deaths [1]. In the European Union, it is the third most prevalent cancer with 388,000 annual cases. Despite advances in surgery, chemotherapy, and radiotherapy, the 5-year survival rate remains as low as 18% [2].
Thermal ablations have been shown to be effective therapies for minimally invasive treatment of tumours. In this process, the tumour is irreversibly damaged by the local application of extreme temperatures and eventual apoptosis of the tumour. It is mainly used to treat small tumours in patients who are not suitable for surgery [3]. The procedure is very similar to the surgical process in which the tumour is removed with a 5-10 mm margin of normal tissue [4]. To date, clinicians do not have meaningful data sets for real-time monitoring of lung tumour ablation.
In the present study, we developed a clinically relevant size tumour phantom with hyaluronic acid-tyramine hydrogel for real-time monitoring of the ablation process.
Materials and Methods: The phantom model consisted of two parts: a normal tissue mimicking phantom and a tumour mimicking phantom. A microwave generator was used to irradiate the cell-loaded tumour phantom using 45 Watts of power for a treatment time of 7 minutes. Fibre optic cables were used as sensors to monitor temperature changes throughout the tumour phantom. Cancer cell viability was subsequently analysed by LIVE/DEAD staining using confocal microscopy.
Results:
Discussion: Fibre-optic monitoring demonstrated temperature differences throughout the tumour phantom. Approximately 83°C, 53°C, and 45°C were recorded at a distance of 5, 10 and 15 mm respectively. Confocal microscopy images demonstrated the geometry of the ablation zone at different depths throughout the tumour phantom.
Conclusion: This study reports a novel cancer cell-loaded phantom model capable of generating real-time temperature data and visualising ablation zones during microwave ablation. This phantom model is a useful tool for clinicians to more accurately predict ablation boundaries in terms of power and timing settings, using a clinically relevant sized phantom model for the treatment of lung cancer and other cancers.

References:

1. 10.5114/wo.2021.103829
2. 10.1007/s00270-020-02432-6
3. 10.3949/ccjm.79.s2.04
4. 10.1109/MPUL.2011.942603

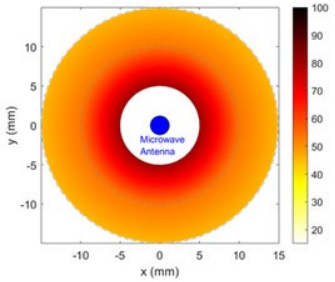


Figure 1: Post ablation heat map of tumour phantom

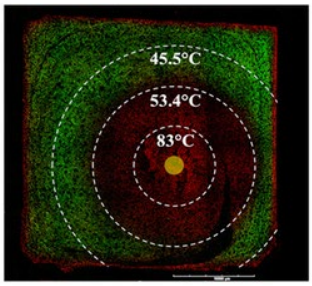


Figure 2: Overlap of heat map and Live/dead image of A549s encapsulated in hydrogel slice. Dimensions of slice = 3cm x 3cm x 2mm. tumour phantom

PoA.10.21

Enhancing the Durability of Hydroxyethylacrylamide-based Adhesives through PEG-NHS Incorporation

Ece Uslu[1], Vijay Kumar Rane[1], François Gorostidi[2], Kishore Sandu[2], Dominique Pioletti[1]

[1]Laboratory of Biomechanical Orthopaedics, Institute of Bioengineering, School of Engineering, EPFL, Lausanne, Switzerland. [2]Airway Sector, Médecine Hautement Spécialisée, Department of Otorhinolaryngology, University Hospital, CHUV, Lausanne, Switzerland

Abstract

Biocompatible adhesive hydrogels can be used as effective sealants to prevent any air/fluid interaction in the wounded area. Due to their intrinsic adhesive properties, they do not require huge efforts or time to be placed on wounded surfaces compared to conventional suturing and stapling. While they offer many advantages over conventional techniques, they are still in their infancy because of their poor durability in wet environments. Hydrogels are 3D hydrophilic polymer networks, imbibing large amount of water under wet conditions. This can lead to lose their mechanical and adhesive properties over time, decreasing their efficacy in the body. Additionally, degradation is a concern that must be addressed for hydrogels aimed for an extended use in human body. Swollen hydrogels can also loose network integrity over time due to chemical or oxidative reactions in the body. Therefore, durability of the adhesive hydrogels is critical and must be addressed in order to provide an effective sealing option.

Herein, biocompatible hydroxyethyl acrylamide (HEAam)-based adhesive hydrogels were developed employing a two-steps photopolymerization technique. These hydrogels exhibited excellent adhesive properties on the various tissue surfaces. However, the adhesion properties of these hydrogels were further enhanced (up to 50%) after the inclusion of 2 wt% PEG-NHS into their network. This inclusion lowered the swelling ratio of respective hydrogels and improved their degradation profile under the enzymatic and oxidative conditions. Based on their adhesive and mechanical properties, HEAam-based adhesive hydrogels with and without PEG-NHS were employed to correct (mild) tracheomalacia, a clinical condition characterized by a malacic trachea. During an ex-vivo study, it was observed that HEAam-PEG-NHS hydrogel provided stronger support to the sub-malacic trachea and significantly reduced the risk of collapsing compared to the HEAam-based hydrogel alone. Therefore, this study has the potential to overcome current limitations and open up new treatment options in clinical situations.

PoA.10.22

Topography-mediated muscle engineering using Alginate-based scaffold

Tianqi Feng

W.J. Kolff Institute, Groningen, Netherlands

Abstract

Skeletal muscle tissue engineering is a promising alternative to traditional volumetric muscle loss surgical treatments that use autogenic tissue grafts, and rather use isolated stem cells with the myogenic potential to generate new skeletal muscle tissues to treat volumetric muscle loss. Biological scaffolds used for medical implants, and tissue engineering scaffolds, not only provide structural support for the promotion of cellular ingrowth but also impart potent modulatory signaling cues that may be beneficial for tissue regeneration. Among the different kinds of materials, Alginate act as an efficient and popular natural material that could form egg-box model in micro-size and gelate in the presence of calcium ions.
The main idea is essentially to combine bio-interfaces to explore the full potential of existing clinical biomaterials currently serving as implants and tissue scaffolds. Using modified scaffolds would offer control over the cellular arrangement, differentiation, and functions and yield a construct that closely resembles natural striated muscle. Combining applicable physical surface parameters with alginate-based hydrogels will drive differentiation and align all three essential constituents of muscle i.e. myofibers, vasculature, and neurons and bring these into contact with each other.
Imprint technology is used to prepare hydrogel wrinkle topography to form the topography surface on hydrogel. This approach will function as a screening platform together with altering the stiffness via cross-linking to determine the influence on cells of combined parameters and thereby offering a superior choice of biomaterial properties. The approach will explore the full potential of alginate-based material, which could increase new function and diminish implant-associated medical complications as a secondary problem and thereby reducing implant impairment as well as lowering patient morbidity and mortality.

PoA.10.23

Ionic liquid-based antimicrobial hydrogel coating

Xiao Teng, Shuai Zhang, Matthew P Wylie, Caoimhe G Clerkin, Jessica V Moore, Colin P McCoy

Queen's University Belfast, Belfast, United Kingdom

Abstract

Background: A polymerizable antimicrobial imidozolium-based ionic liquid (VBIm) was copolymerized with a hydrogel acrylate copolymer based on 2-(hydroxyethyl) methacrylate in varying proportion to synthesize non-releasing antimicrobial hydrogel coatings (HMV0 and HMV1 and HMV3 and HMV5). Contact-killing effect and cytocompatibility were tested here.
Methods: 1. Bacteria adherence: *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were used in bacteria adherence assay. The samples were soaked in bacteria suspensions at an inoculum size of 106 CFU/mL. After 4 hr and 24 hr, the samples were taken out washed followed by sonication, after overnight incubation of bacteria suspension on MHA plates at 37℃ in a static incubator, the number of adherent bacteria were counted.
2. Cell viability test: The cytotoxicity of the samples was analysed through the MTT following the guidelines outlined in ISO-10993-12-2021. Adherent cells were incubated with samples for 24 hours, after which the extractions were carefully removed and MTT was added. After 4 hours, the MTT and media were quickly removed and rinsed with PBS. DMSO was added to solubilise the formed crystals. The plates were left for half an hour before, then UV analysis was conducted to determine cell viability after treatment with the samples.
Results and discussion: From **Figure 1**, it can be observed that as the mass of the ionic liquid involved in the hydrogel polymerization increased, the reduction in adherent bacteria also increased. This indicates that the contact-killing effect of VBIm was related to its mass, with an increase in anti-adherent ability as more VBIm was incorporated into the hydrogel. According to **Figure 2**, cell viability of over 70% was achieved for all hydrogels incorporating ionic liquid. This demonstrates that VBIm is a biocompatible monomer. In conclusion, VBIm is a promisingly candidate monomer for developing antimicrobial hydrogel coatings with high contact-killing ability but low cytotoxicity.

1. Bacteria adherence

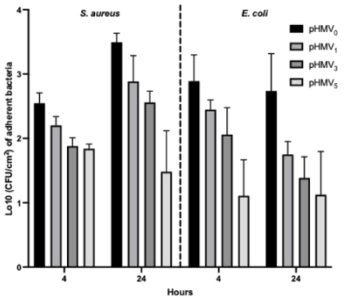


Figure 1. The log10 (CFU/cm2) of numbers of adherent S. aureus and E. coli to surfaces of pHMV0, pHMV1,pHMV3 and pHMV5 after 4 hr and 24 hr in 106 CFU/mL inoculum at pH 7.3. Columns and error bars represent means ± s. d (n=5).

2. Cell viability test

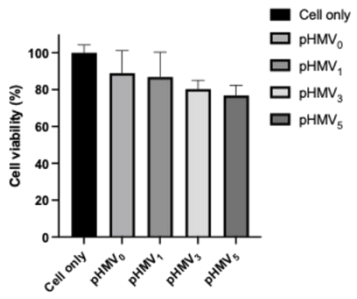


Figure 2. The cytotoxicity of the test materials, pHMV0, pHMV1, pHMV3, and pHMV5. All materials are compared to the control well, which contained culture media. Error bars represent standard deviation (n=5).

PoA.10.24

Fabrication of mechanically robust hydrogels for 3D printing and injection: The role of the thiol-yne/ene chemistry and the network architecture

Mahsa Ebrahimi[1,2], Mariana ARREGUIN CAMPOS[1,2], Agustina Aldana[2], Matthew B Baker[2], Louis M Pitet[1]

[1]Hasselt university, Hasselt, Belgium. [2]MERLN institue-Maastricht university, Maastricht, Netherlands

Abstract

As a result of their unique characteristics, hydrogels have received enormous attention targeting diverse applications, including tissue engineering. However, a challenge that currently limits their widespread application as candidates for commercial biomaterials is their insufficient mechanical strength and toughness which prevent them to fulfill their role as load-bearing tissues. Additionally, despite the fact that injectable and printable hydrogels have shown promise in a number of regenerative medicine applications, there is still a major gap in their capability to be processed into 3D printed or injected structures with mechanical characteristics resembling load-bearing living tissues. Our present work has focused on the role of Thiol-Yne/Thiol-ene chemistry in establishing a link between composition, processing, and properties for developing polyethylene glycol (PEG) based hydrogels in which the network architecture and makeup are systematically varied with target applications for injection and 3D printing in order to make them competitive in the arena of regenerative medicine.

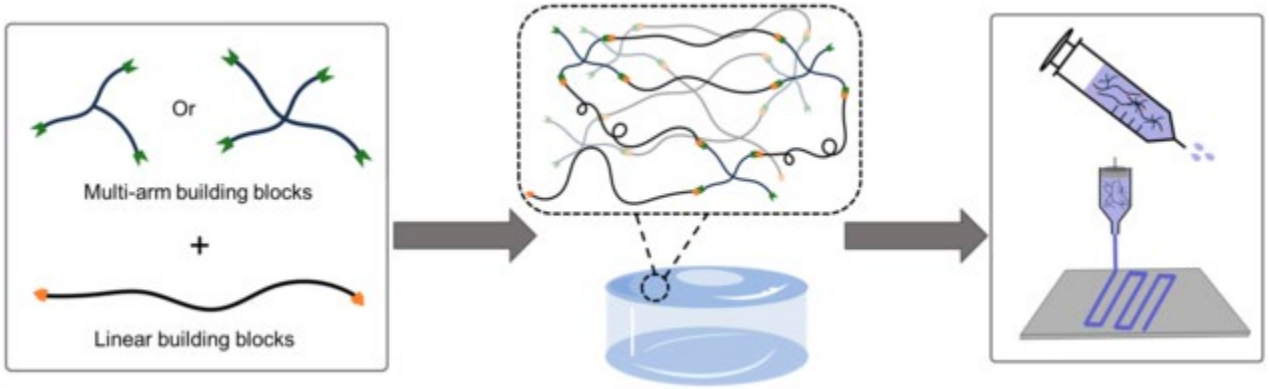


Figure 1. Schematic illustration of the thiol-yne/ene chemistry utilized for fabrication of the mechanically robust hydrogels for 3D printing and injection.

PoA.10.25

Dynamic cucurbit[8]uril-based supramolecular alginate hydrogels

Nataliya Debera, Pascal Jonkheijm

TechMed Centre and MESA+ Institute University of Twente, Enschede, Netherlands

Abstract

The intrinsic properties of hydrogels make them good candidates to mimic the extracellular matrix (ECM). The ideal material would be a dynamic network that can relax and dissipate stress and simultaneously provide biochemical cues for cell-ECM communication.
Alginate hydrogels are used in numerous biomedical applications including tissue engineering. Alginate's versatility as a biomaterial comes from the available range of crosslinking methods. The hydrogels can be prepared by covalent or non-covalent crosslinking approaches. Covalent methods, such as photo-crosslinking, produce alginate hydrogels with non-reversible bonds, stable overtime, but brittle and non-flexible. To address this problem but still keep the advantages of photo-crosslinking, we propose the introduction of dynamic non-covalent host-guest interactions. These interactions are more stable and easily controlled in comparison to other non-covalent crosslinking.
In this approach we functionalize alginate with aminoethyl methacrylate groups through EDC/NHS chemistry. The methacrylate groups have two purposes: form covalent bonds through free radical polymerization and participate in thiol-click chemistry. As a supramolecular host cucurbit[8]uril (CB[8]) is a suitable crosslinker. It possesses a large cavity volume (489 $Å^3$) that can accommodate simultaneously two planar and hydrophobic guests [1]. We use the peptide sequence FGGC as guest, the aromatic side group of phenylalanine can form a 2:1 complex with CB[8] and the thiol side group of cysteine can react with the methacrylate group on the alginate. The hydrogel preparation is depicted in Figure 1. To overcome CB[8] poor solubility we premix it first with the guest, this also forms more homogeneous hydrogels [2].
The thiol-click chemistry allows to graft functional molecules to the alginate backbone, such as cell adhesion sequences to increase the functionality of the alginate backbone. The host-guest chemistry not only adds dynamic and self-healing properties but also makes the hydrogels responsive to external stimuli such as temperature, pH, electric potential and light. The binding affinity of the guest can influence the mechanical strength and flexibility of the hydrogels [1]. This way we hope to expand the toolkit for alginate hydrogels crosslinking methods and improve their ability to mimic the native ECM for cell culture.

[1] Z.Wang et al *RSC Med Chem*,12,no.5, 722-729, 2021.
[2] A.C.Madl et al *ACS Macro Letters*,9, no.4, 619-626, 2020.

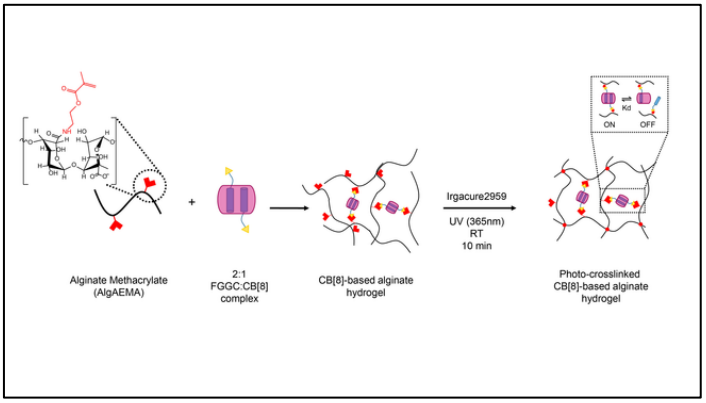


Figure 1 - Schematic representation of dynamic cucurbit [8]uril-based supramolecular alginate hydrogels formation. AlgAEMA is first mixed with the preassembled CB[8]:FGGC complex and then irradiated with UV light.

PoA.10.26

COL/ACE-based hydrogels to promote wound healing with enhanced angiogenesis

Lluís Oliver-Cervelló, Delphine Gourdon, Cristina Gonzalez-Garcia

Centre for the Cellular Microenvironment, Advanced Research Centre, University of Glasgow, Glasgow, United Kingdom

Abstract

Introduction: Chronic wounds, ulcers, scar formation and poor vascularisation during wound healing are major concerns that impede soft tissue repair [1]. Thus, there is scope for further advancements in developing new strategies to promote wound healing. Herein, we engineered novel collagen (COL) hydrogels, which incorporated acemannan (ACE), a bioactive polysaccharide derived from Aloe Vera with intrinsic immunodulatory and tissue repair properties [2]. The COL/ACE hydrogels presented tuneable physicochemical and mechanical properties and were able to orchestrate fibroblast behaviour while promoting angiogenesis.

Methodology: COL/ACE hydrogels were developed by cross-linking COL with 8-arm-PEG-NHS and physically entrapping ACE (0.1 or 0.2% concentration) within the COL network. Physicochemical and mechanical properties of the hydrogels were assessed by means of rheology, scanning electron microscopy, swelling, degradation, and ACE retention studies. The biological performance of the system was evaluated in terms of viability, proliferation, and migration of neonatal human dermal fibroblasts (nHDFs) within the hydrogel. Furthermore, the potential of the ACE to guide angiogenesis was investigated *in vitro* using human vascular endothelial cells (HUVECs) and performing nHDFs/HUVECs co-cultures, as well as *in vivo* using a chorioallantoic membrane (CAM) assay.

Results: The hydrogels had the capacity to retain ACE within the COL network, while presenting the desired mechanical properties, swelling and internal structure and porosity to develop biomaterials for wound healing applications, allowing the culture of nHDFs and HUVECs within the network. In this regard, control hydrogels without ACE supported nHDFs survival and viability but failed to trigger the rest of cell functions, such as cell migration. On the contrary, the presence of ACE (specially at the highest concentration), significantly improved fibroblast spreading within the hydrogel as well as their proliferation, migration (Figure 1) and wound closure rate.

Moreover, although all hydrogel conditions supported HUVECs viability, only the ones presenting ACE promoted angiogenesis.

Conclusions: The incorporation of ACE within the COL network resulted in novel biomaterials with the required mechanical properties and biochemical cues to enhance nHDFs spreading, proliferation and migration, which plays a critical role in modulating the angiogenic process. Thus, these COL hydrogels incorporating ACE might be a potential approach to develop new biomaterials for wound healing with improved wound rate and angiogenesis.

References:

[1] X. Kong et al., Acta Biomaterialia, 2019, 100, 255-269.

[2] C. Liu et al., Molecules, 2019, 24, 1554.

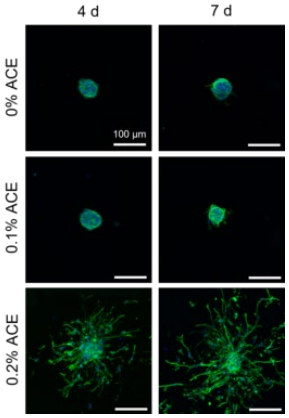


Figure 1. nHDFs migration from spheroids laden in the hydrogels. Actin and nucleus staining of nHDFs.

PoA.10.27

How to prepare a defined methacryloyl modification (GelMA) hydrogel. Factors influencing the hydrogel properties

Jos Olijve, Thomas Van Gansbeke, Jan-Philip Zegwaart

Rousselot BV, Gent, Belgium

Abstract

Gelatins are widely used in the biomedical and tissue engineering fields, due to their biocompatibility, biodegradability and cell-interactivity. Gelatin can be chemically crosslinked to originate hydrogel constructs that are stable at body temperature. A popular way to crosslink gelatin in tissue engineering and additive manufacturing is methacryloyl modification. The cross-linking is initiated by photo irradiation of the chemically introduced methacryloyl groups. It is well know that cell biocompatibility (proliferation, differentiation) depends very much on the hydrogel mechanical properties. A defined and reproducible hydrogel mechanical structure is therefore important for tissue engineering applications. We identified that gelatin characterization (molecular weight (Mw), degree of modification (DoM) and purity) and also resin formulation conditions (polymer and photo-initiator (PI) concentration, amount of salt, temperature conditions) are important factors to guarantee the hydrogel mechanical reproducibility and predictability and are essential for both in-vitro in-vivo translations.

Different GelMA's with two different molecular weights and three different DoM, werre dissolved at various concentrations in PBS. The PI concentration, amount of salt and temperature were varied. The crosslinking kinetics and the hydrogel strengths of the GelMAs were studied using photo-rheology (MCR 302e, Anton Paar, Belgium) compression testing, and dynamic mechanical analysis.

The storage modulus was associated with the DoM and the gelatin concentration. Surprisingly, either a power or linear correlation was found for the correlation between gelatin concentration and storage modulus, depending on the GelMA molecular weight. This indicates that the GelMA molecular weight affects cross-linking efficiency. The salt concentration of the solvent has a large effect and influences GelMA hydrogel strength within a 90 % range selection. The temperature at which photo-crosslinking is performed causes a 95 % difference in hydrogel strength. This effect depends on, the temperature history in the preparatory and application phase, the concentrations and types of PI, the MW, the DoM, and the GelMA concentration.

In conclusion, the results give insight in critical GelMA resin formulation aspects to produce a reproducible and predictable GelMA hydrogel. In short, the interplay between the various aspect of a GelMA resin is diverse, but it is mappable and hence insightful decisions can be made, greatly improving GelMA hydrogel reproducibility and related tissue engineering and delivery applications.

PoA.10.28

A Universal Nanogel-Based Coating Approach for Medical Implant Materials

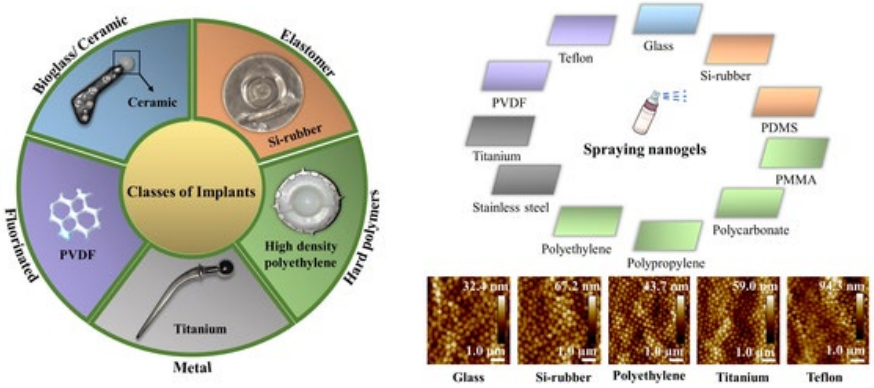
Devlina Ghosh[1], Damla Keskin[1], Abigail M. Forson[1], Colin W.K. Rosman[1], Guangyue Zu[1], Clio Siebenmorgen[1], Reinier Bron[1], Theo G. van Kooten[1], Max J.H. Witjes[2], Alessia Lasorsa[3], Patrick C.A van der Wel[3], Jelmer Sjollema[1], Henny C. van der Mei[1], Patrick van Rijn[1]

[1]University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering, Groningen, Netherlands. [2]University of Groningen, University Medical Center Groningen, Department of Oral and Maxillofacial Surgery, Groningen, Netherlands. [3]Zernike Institute for Advanced Materials, University of Groningen, Groningen, Netherlands

Abstract

INTRODUCTION: Biomaterials (implants) have revolutionized the healthcare system by enhancing drug delivery at targeted sites or by restoring tissue/ organ functions. However, secondary complications such as infection and inflammation due to biofilm formation and immune response in the host body can lead to implant failure.

Coatings have been regarded as an excellent possibility to induce desired responses or prevent complications, as the bulk implant material does not need to be altered. Owing to the diverse physicochemical properties of implants, the coating strategy required for polymeric materials is generally different than ceramics, or metal-based implants. In order to enhance the coating applicability, a more relevant and better translatable universal method for coating most implant materials, regardless of their composition, will tremendously impact the field of biomedical coating developments (Scheme 1).



Scheme 1. *Different classes of implant materials. AFM images show the formation of homogeneous coating on the surfaces, using the universal coating approach.*

EXPERIMENTAL METHODS: p(NIPAM-*co*-APMA) nGel particles were synthesized by precipitation polymerization reaction. The particles were characterized by DLS and zeta potential, while the coating was visualized by AFM. The coating was labeled with FITC for successful detection and imaging by IVIS. The stability of the coating was determined *in vivo* by implanting MRB-labeled nGel-coated PVDF hernia mesh in mouse model.

RESULTS AND DISCUSSION: The DLS measurements showed the hydrodynamic diameter was 540.63 ± 11.2 nm and positive zeta potential, 15.83 ± 0.11 mV at 24°C was attributed to the presence of protonated primary amine groups, introduced by APMA. The homogeneous coating formation was achieved by using electrostatic interactions between oxygen plasma-activated surfaces and positively charged nGels combined with a spraying method for nGel deposition. AFM images showed a closely packed nGel-layer on all the distinct surfaces, irrespective of their varied physicochemical properties. As a proof of concept, FITC was conjugated to the NH_2 groups of the coating and IVIS was used for easy traceability of the nGel-coated surfaces. The short-term *in vivo* test showed that the coatings on PVDF mesh were stable for up to 13 days, after which the fluorescent signal was decreasing (data not shown).
CONCLUSION: The coating strategy was successfully translated to all 11 unique medically relevant materials; hence we call it a universal coating approach. In the future, different functions such as antibacterial properties, or imaging modalities can be incorporated into the coating, thereby creating a highly functional and potentially multimodal system depending on the desired application.

PoA.10.30

Monophasic Hyaluronic Acid-Silica Hybrid Hydrogels with Improved Cell Proliferation and Adhesion for Articular Cartilage Regeneration

Huijun Zhang, Aldo R. Boccaccini

Institute of Biomaterials, Friedrich-Alexander-Universität Erlangen-Nuremberg, Erlangen, Germany

Abstract

Introduction: One way to treat cartilage degeneration is autologous matrix-induced chondrogenesis (AMIC). AMIC is a technique that combines microfracture and an exogenous scaffold[1]. These scaffolds should support cell attachment, proliferation, and migration to achieve cartilage regeneration. Hydrogels have been widely researched for cartilage tissue regeneration because of their similar properties to the extracellular matrix (ECM). In particular, hyaluronic acid (HA), an FDA-approved injection polymer and component of the ECM, is a great candidate for hydrogel scaffolds[2]. However, crosslinked HA scaffolds have poor mechanical and cell support properties. In this study, we develop a monophasic, stiff, and stable hybrid hydrogel with improved cell adhesion and proliferation by forming covalent bonds between HA and (3-Glycidyloxypropyl)trimethoxysilane (GPTMS).

Experiment and methods: HA solution was mixed with GPTMS at different molar ratios then the solution was stirred at 40 ºC for 4 hours. The sol solution was transferred to silicon molds and placed in the incubator overnight . The morphologies of the cross-section of the freeze-dried hydrogels were assessed using SEM. The compressive strength and Young's modulus were assessed through a compression test. Rheological measurements were conducted using a Discovery HR-3 rheometer at different frequencies and strains. Cell proliferation was evaluated by WST-8 assay by indirect and direct tests with MC3T3-E1 cells (n=6). The morphology of the cells attached to the hydrogels was examined using fluorescence microscope also to evaluate cell adhesion.

Results and Discussion: All hydrogels exhibited interconnected microporous microstructures (Figure 1). Young's modulus and storage modulus increased as the GPTMS ratio increased, showing that a denser and stronger crosslinking network can be established. The higher crosslinking density and the branching of the silica structure give higher support to the material and limit the flexibility of the matrix and 3D network. A higher level of cell spreading and proliferation was observed on the surface of the hybrid materials with increased silica content. The addition of silica content could reduce the hydrophilicity of HA hydrogel and provides interaction sites for better cell attachment.

Conclusions: HA-silica hybrid hydrogels were synthesized by one-pot sol-gel method. Adding GPTMS improved the porosity, stability, stiffness, and cell adhesion property of the hybrid hydrogels. These properties indicate that HA-silica hydrogels are capable of supporting cartilage cell adhesion and proliferation.

References
1. Giannini, S. et al., Injury 2010, 41, 1196.
2. Jeong, S.H. et al., Biomacromolecules 2016, 17, 841-851.

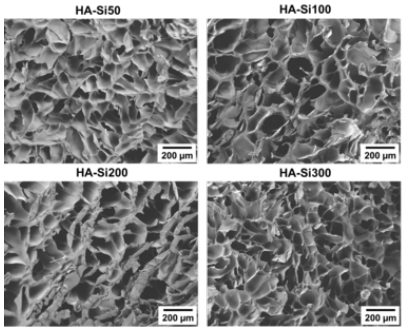


Figure 1. SEM images of freeze-dried HA-silica hybrid hydrogel cross sections.

PoA.10.31

Engineered nano- and microstructured hydrogels for regenerative medicine

Daniele Marciano[1], Clemence Nadal[1], Jordi Gonzalez Molina[2], Julien Gautrot[1]

[1]Queen Mary University of London, London, United Kingdom. [2]Karolinska Institutet, Solna, Sweden

Abstract

Physical properties of the native extracellular matrix (ECM), such as mechanics and nano- to micro-topography, are known to have an important impact on cell adhesion, migration, proliferation and cell fate [1]. Synthetic hydrogels, such as those based on photo-crosslinkable PEG and hyaluronic acid, modified with acrylates, methacrylates, norbornene and thiol residues, allow the fine control of the mechanics and degradability of the cell microenvironment [2], but typically display porosity in the nanometer range, resulting in relatively slow cell spreading and migration compared to matrices based on fibrinogen or collagen. Recently, a range of granular hydrogels have been proposed to remediate to this issue and facilitate the processing and 3D printing of synthetic hydrogels [3,4]. However, these systems only allow the control of the microstructure over a relatively narrow range (e.g. 10-200 µm). Here, we report nano-to-microstructured hydrogels featuring porosity over a very broad range of length scales, from 100 nm to several 100 µm, suitable for in situ cell encapsulation. We develop a simple and scalable process to achieve pores over a wide range of sizes. Using a combination of AFM, photorheology and interfacial rheology, we demonstrate the control of local nanoscale mechanics, macroscale mechanics and porosity, with inherently cell adhesive and cell remodellable hydrogels. The control of the porosity of these hydrogels and the morphology of the resulting network is characterised by a combination of confocal microscopy, environmental SEM and small angle neutron scattering. Direct encapsulation of mesenchymal stem cells (MSCs) resulted in rapid cell spreading, comparable to phenotypes observed in fibrin or collagen gels, modulated by matrix mechanical, degradable, adhesive, and structural properties (varying crosslinker types, concentrations, the presentation of cell adhesive ligands, pore size). The mechanism of cell adhesion and protrusion in these materials is explored and compared to that of nanoporous hyaluronic acid-based hydrogels. Finally, we show that the secretory profile of MSCs is influenced by the structure and design of these materials, displaying attractive potential for application as scaffolds for soft tissue engineering.

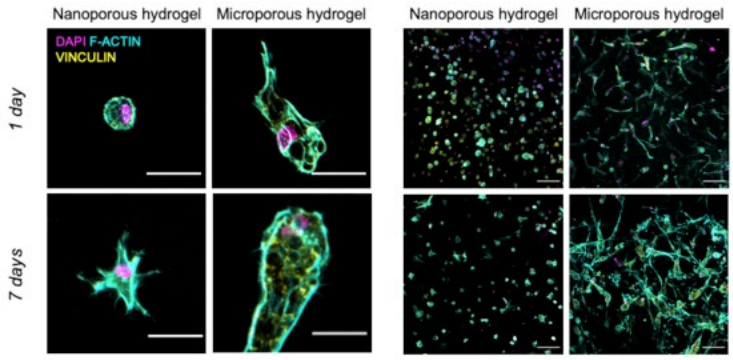


Figure 1. MSCs spreading in nanoporous hydrogels and microporous hydrogels after 1 day and 7 days of culture (scalebars: left 30 µm, right 100 µm).

[1] S. Di Cio et al. Acta Biomater. 30 (2016) 26–48.
[2] M. Guvendiren et al. Curr. Opin. Biotechnol. 24 (2013) 841–846.
[3] L. Riley et al. Curr. Opin. Biotechnol. 60 (2019) 1–8.
[4] T.H. Qazi et al. Biomater. Biosyst. 1 (2021) 100008.

PoA.10.32

Development of a novel plant-derived polysaccharide-based hydrogel for bone tissue engineering

Xinyu Li, Aleixandre Rodrigo-Navarro, Lluís Oliver-Cervello, Oana Dobre, Manuel Salmeron-Sanchez, Cristina Gonzalez-Garcia

Centre for the Cellular Microenvironment, Advanced Research Centre, University of Glasgow, Glasgow, United Kingdom

Abstract

Introduction: The healing of critical-size bone defects is a major challenge in bone tissue engineering. The controlled delivery of growth factors (GFs) has shown the potential to induce tissue regeneration [1]. However, high doses of GFs are required to obtain significant improvement in clinical settings, leading to severe side effects [2]. Herein, a plant-derived polysaccharide (acemannan) possesses immunomodulatory properties and drives stem-cell osteogenic differentiation [3], has been incorporated in PEG-based hydrogels to develop novel 3D carriers with tuneable physicochemical properties. Such hydrogels enabled the controlled delivery of GFs, promoting osteogenic differentiation of stem cells.
Methodology: Biological activity of acemannan was tested in 2D by viability, adhesion, and osteogenic differentiation of human mesenchymal stem cells (hMSCs). Afterwards, PEG hydrogels incorporating acemannan (0.1% or 0.2%) were developed. PEG-MAL polymer was crosslinked with PEG-diSH and a protease-degradable peptide (VPM), physically trapping the acemannan (ACE) into the PEG network. The mechanical properties of the hydrogels were measured by rheology; and swelling, degradation and acemannan retention were also tested. Then, BMP2 GF was encapsulated in the hydrogel and the released evaluated. The biological response of these hydrogels was evaluated through viability, proliferation, and differentiation studies *in vitro*.
Results: The biological characterisation in 2D showed positive effect of acemannan on both viability and adhesion of hMSC. Moreover, Alizarin red staining assays (Figure 1) and OCN staining by In-Cell Western demonstrated the capacity of acemannan to promote hMSCs osteogenic differentiation.
Then, PEG/ACE-based hydrogels were engineered. Such hydrogels presented tuneable mechanical properties, as shown by swelling, degradation, and rheology measurements, while retaining the acemannan in the hydrogel network. Fibronectin-fragments with affinity for GFs were successfully incorporated to increase the sustained release of the GF. The biological characterisation showed the influence of acemannan in hMSCs osteogenic differentiation as demonstrated by the ALP activity and qPCR results.
Conclusion: Biological characterisation in 2D demonstrated the potential of acemannan to promote hMSCs osteogenic differentiation. Such results were translated to 3D, where PEG hydrogels loaded with acemannan and BMP2 GF also triggered the osteogenic differentiation of hMSCs, suggesting the potential of the system as a promising approach for bone tissue engineering.

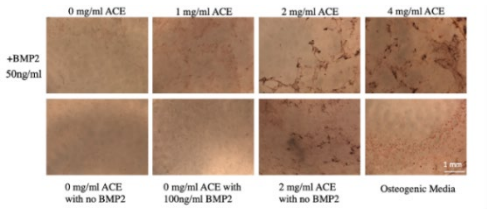


Figure 1: Alizarin red staining of MSCs after 21 days in culture with different concentrations of acemannan.

References:
[1] Dalby, M.J. et al., Nat. Rev. Mater. 3, 17091(2018)
[2] James, A.W. et al., Tissue Eng. Part B Rev. 22, 284-297 (2016)
[3] Boonyagul, S. et al., Odontology, 102, 310-317(2014)

PoA.10.33

Dynamic G-quadruplex based perfusable supramolecular hydrogels embedded in photocrosslinkable matrices for bioapplications

João Borges, Vera Sousa, Adérito J. R. Amaral, Edgar J. Castanheira, Igor Marques, João M. M. Rodrigues, Vítor Félix, João F. Mano

CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Aveiro, Portugal

Abstract

Nature provides us with an unprecedented toolbox of stunning supramolecular systems formed through the molecular self-assembly of fundamental biological molecules. Among them, G-quadruplexes, ubiquitous noncanonical four-stranded supramolecular structures formed in guanine-rich DNA and RNA sequences which regulate essential biological processes,[1] have inspired the development of supramolecular architectures for bioapplications owing to their unique self-assembling features.[2,3] Such bioinspired supramolecular structures are developed by the self-recognition of guanines into stacked tetrads, formed by Hoogsteen-type hydrogen bonding interactions between four guanines and stabilized by alkali metal cations. Herein, a novel dynamic hyaluronic acid (HA)-functionalized G-quadruplex supramolecular hydrogel will be presented via a combined experimental-computational study by exploring the hydrogen bonding and π-π interactions between four guanosines coupled via dynamic boronate ester bonds to 3-aminophenylboronic acid-functionalized HA and stabilized by K+. The self-healing, thermo-responsive, injectable, and conductive properties of the hydrogel will be discussed, and its well-known instability explored to produce interconnected, size and shape tunable perfusable microchannels embedded in photocrosslinkable supporting matrices (Figure 1). The higher number of viable cells denoted by the microchannel-embedded 3D constructs when compared to the 3D bulk construct and their migration towards the perfusable microchannels will be showcased aiming for being use as artificial vessels for enabling the diffusion of nutrients and oxygen essential for cell survival. The versatility imparted by the proposed approach is expected to open up new avenues in drug/therapeutics delivery, tissue engineering and regenerative medicine.

Acknowledgments:
This work was funded by the European Union's Horizon Europe research and innovation programme under the grant agreement No. 101079482 ("SUPRALIFE"), as well as by the Programa Operacional Regional do Centro-Centro 2020 (FEDER) and national funds (FCT/MCTES) in the scope of the project "SUPRASORT" (PTDC/QUI-OUT/30658/2017, CENTRO-01-0145-FEDER-030658). The financial support by FCT through the PhD grants (2020. 06771.BD, V.S.; SFRH/BD/144880/2019, E.J.C.), and Junior (CEECIND/01363/2018, J.M.M.R.) and Assistant Researcher contracts (2020.00758.CEECIND, J.B.) is gratefully acknowledge. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC). Part of the MD simulations were performed on the BOB supercomputing cluster installed at MACC, under FCT, I.P. Advanced Computing Project CPCA/A1/470074/2021.

References:
[1] D. Rhodes, H.J. Lipps, *Nucleic Acid Res.* 2015, *43*, 8627.
[2] J. Spiegel, S. Adhikari, S. Balasubramanian, *Trends Chem.* 2020, *2*, 123.
[3] G.M. Peters, J.T. Davis, *Chem. Soc. Rev.* 2016, *45*, 3188.

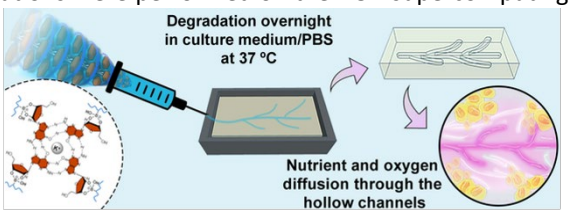


Figure 1. Schematic representation of bioengineered perfusable 3D constructs

PoA.10.34

Injectable Polypeptide-Based Hydrogels for Local Antibacterial Therapy

Artemijs Sceglovs[1,2], Ingus Skadins[3], Valdis Pirsko[4], Juta Kroica[3], Kristine Salma-Ancane[1,2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia. [3]Department of Biology and Microbiology, Riga Stradins University, Riga, Latvia. [4]Institute of Oncology, Riga Stradins University, Riga, Latvia

Abstract

Introduction: Polypeptide-based hydrogels are one of the promising strategies for developing highly effective antibacterial tools which can act against antibiotic-resistant pathogens. In the current study, we developed injectable hydrogels according to the recent report (Salma-Ancane et al. 2022) and evaluated their injectability, bactericidal activity, as well as bacteria potential for resistance development and human cell viability.

Materials and Methods: Hydrogels based on ε-polylysine (ε-PL) and hyaluronic acid (HA) with different mass ratios were fabricated according to the synthesis method described previously with minor changes (Salma-Ancane et al. 2022). Within rheological studies, amplitude and frequency sweeps, viscosity-shear rate and recovery tests were performed. To evaluate *in vitro* bacteria potential for resistance development and antibacterial efficacy were investigated using reference bacterial strains - *E.coli* and *S.aureus*, and clinically isolated multi-resistant bacteria strains – ESBL *E.coli* and MR *S.aureus*. To evaluate *in vitro* cytotoxicity, hydrogels were evaluated by indirect tests on human fibroblasts HDFa cell line.

Results: Amplitude and frequency sweeps of the fabricated ε-PL/HA hydrogels revealed viscoelastic curves with stable solid-like (G'> G'') material behaviour. The difference between G' and G'' at the stable region, as well as relatively high values of matrix transformation, confirmed chemical entanglement dominance in materials structure. Further rheological properties revealed materials shear-thinning properties, as well as matrix recovery feature. Antibacterial tests revealed MIC values of ε-PL against bacteria strains which were as follows 37 μg/mL against *E.coli*, *S.aureus* and MRSA, and 18 μg/mL against ESBL *E.coli*. After resistance potential studies studied bacteria were not able to develop resistance against ε-PL. Furthermore, significant bacteria reduction ($p<0.05$) was obtained for all ε-PL/HA hydrogel within 24h. However, cytotoxicity tests demonstrated that ε-PL/HA hydrogel compositions with 70:30 and 80:20 wt% mass ratios showed a significant reduction in cell viability.

Conclusions: Obtained results were used as defining criteria to find optimal amino acid-to-polysaccharide mass ratio to achieve sufficient antibacterial and cell-friendly effect. It was found that ε-PL/HA hydrogels with ε-PL to HA mass ratios of 50:50 and 60:40 showed the most favourable mechanical properties and biocompatibility for developing multifunctional antibacterial and injectable biomaterials for tissue engineering applications.

Acknowledgement: The authors acknowledge financial support from the Latvian Council of Science research project No. lzp-2020/1-0072 "Injectable bioactive biocomposites for osteoporotic bone tissue regeneration (inBioBone)".

The authors acknowledge financial support from the European Union's Horizon 2020 research and innovation programme twinning project RISEus2 under the grant agreement No 952347.

PoA.10.35

3D bioprinted, perfused and vascularized bone organoids to study extravasation and metastasis of cancer

Alain Plüss[1,2], Adam Aleksander Korczak[1,2], Martin Ehrbar[1,2]

[1]University of Zurich, Zurich, Switzerland. [2]University Hospital Zurich, Zurich, Switzerland

Abstract

Introduction: Metastasis is a leading cause of cancer-related death. Bone is the most common site for metastasis. The understanding of the pathophysiological mechanism is incomplete and models to study invasion and colonization of cancer cells in the bone are of great importance. 3D-bioprinting allows to reproducibly create spatially organized 3D tissue constructs by depositing a cell-laden biomaterial layer-by-layer. The overall aim of this project is to establish an *ex vivo* model based on printed, vascularized and perfused bone organoids that allows the investigation of the pathophysiology of cancer metastasis. Therefore, in a first step we aim to engineer printable poly(ethylene glycol) (PEG) formulations that support the formation of bone and blood vessels.
Methods: Previously established transglutaminase cross-linked PEG hydrogels were used to encapsulate mesenchymal stromal cells (MSCs) and human umbilical vein endothelial cells (HUVEC). Osteogenic differentiation and vessel-like network formation of co-cultures was assessed in response to bone morphogenetic protein 2 (BMP-2) and fibroblast growth factor 2 (FGF-2) treatment [1],[2]. To enable extrusion of the cross-linked material, PEG formulations were modified using viscosity enhancing agents. Rheological measurements were performed to characterize changes in the mechanical properties. Then, MSCs and HUVECs were encapsulated in the bioink and their survival, spreading and function was analysed by microscopy post-extrusion.
Results: In the bulk hydrogel, alkaline phosphatase (ALP) staining revealed osteogenic differentiation of MSCs cultured in medium containing BMP-2. FGF-2 promoted the network formation of HUVECs. Addition of viscosity enhancing agents enabled the extrusion of stable PEG hydrogel fibers. Rheological characterization indicated a significant increase of storage and loss modulus when adding viscocity enhancer to PEG. The modification in the PEG formulation reduced the amount of dead cells in the extrusion process. In the extruded fibers osteogenic differentiation and cell spreading of MSCs and HUVECs were observed similar as in the bulk hydrogel.
Conclusions and Outlook: HUVECs and MSCs were successfully co-cultured in the bulk PEG hydrogel. The PEG formulation could be modified so that extrusion of cell-laden fibers was possible. Bioink formulations will further be optimized and more complex bone organoids with a central perfusion will be printed. The central perfusion will be of great importance to mimic the processes of metastasis *in vitro*.

[1] Ehrbar, Martin, et al. "Biomolecular hydrogels formed and degraded via site-specific enzymatic reactions." Biomacromolecules 8.10 (2007): 3000-3007.
[2] Ehrbar, Martin, et al. "Enzymatic formation of modular cell-instructive fibrin analogs for tissue engineering." Biomaterials 28.26 (2007): 3856-3866.

PoA.10.36

Developing an *in vitro* ovary model for the prolonged culture of follicles

Mira Jacobs[1,2], Martin Ehrbar[1,2]

[1]University of Zurich, Zurich, Switzerland. [2]University Hospital Zurich, Zurich, Switzerland

Abstract

Background: Most chemo- and radiotherapeutic cancer treatments are toxic to the ovaries, which can lead to impaired fertility and/or endocrine function in female patients (1). The state-of-the-art method of fertility preservation is to cryopreserve the ovaries, which for some types of cancer poses the risk to reintroduce malignant cells. Thus, current approaches in research aim to develop an artificial ovary for the temporary and safe culture of the patients' follicles (2). This engineered scaffold would be fabricated using different biomaterials and celltypes to ensure follicle survival and growth. In the present study we develop 3D scaffolds by combining natural and synthetic biomaterials to culture follicles and ovary cells for several days. We will evaluate cell viability, morphology, and functionality in response to different biomaterial combinations.

Methods: Hydrogels as 3D scaffolds were fabricated from the synthetic polymer poly(ethylene glycol) (PEG) and different natural biomaterials, such as collagen type I and fibronectin (PEG-ECM). The ECM molecules were incorporated to promote cell adhesion in the otherwise bioinert PEG matrix. First, a protocol for the sequential isolation of mouse follicles and ovary cells from the same sample was established. Next, ovary cells, follicles or a combination of both were embedded in PEG-ECM hydrogels and observed for several days regarding cell viability and morphology.

Results: With the help of established protocols to embed cells into PEG hydrogels, follicles and ovary cells were successfully incorporated into PEG-ECM scaffolds. Follicles remained viable in pure PEG hydrogels and some increased in size during a culture period of five days. Ovary cells embedded in PEG-Collagen hydrogels spreaded faster compared to the cells in other PEG-ECM hydrogels.

Conclusions and Outlook: A protocol for the sequential isolation of mouse follicles and ovary cells from the same sample was established and both were successfully incorporated into different PEG-ECM scaffolds. Next experiments will evaluate different concentrations of ECM molecules in PEG-ECM hydrogels regarding their effect on ovary cell viability and morphology over one week. Additionally, immunofluorescence staining will be used to detect matrix deposition of cells and possible differences in cell morphology. Eventually, PEG-ECM hydrogels will be adapted for the encapsulation of bovine and human ovary cells, to investigate their effect on follicle growth and maturation.

References:

[1] Donnez J and Dolmans MM (2017), N Engl J Med 377, 1657-1665.

[2] Meirow D et al., (2008), Hum Reprod, 23, 1007-1013.

PoA.10.37

Tough PEG-based hydrogels for 3D printing and tissue engineering

Mariana Arreguín Campos[1,2], Mahsa Ebrahimi[1,2], Matthew Baker[2], Louis Pitet[1]

[1]Institute for Materials Research (IMO) and Advanced Functional Polymers (AFP) Group, Hasselt University, Hasselt, Belgium. [2]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

Abstract

As humans, injuries, diseases, and congenital malformations have always been part of our lives. Despite significant progress, pharmaceutical treatments often have limited efficacy. It is here where regenerative medicine and tissue engineering emerge, trying to provide solutions for treating severe injuries where the body's response is not sufficient to restore comprehensive functionality. Nevertheless, mimicking native tissue is a formidable task.

Hydrogels are hydrophilic materials that can take up to thousands of times their dry weight in water. Due to their soft consistency and permeability to small molecules, hydrogels are attractive materials for regenerating tissue. Although they can already be found in some applications, such as contact lenses, drug delivery, and wound dressing, achieving mechanical integrity still hinders their application as structural materials. There are several approaches being studied to improve their mechanical strength; here, we propose the incorporation of a 3-arm molecule into a PEGDMA network to create a more homogeneous architecture, thus, providing increased mechanical performance. Several formulations are explored so that hydrogels with targeted predefined and specific properties can be made and their application can be further expanded. We employ thiol-ene crosslinking chemistry for a controlled and fast reaction, which can benefit their processability into complex structures.

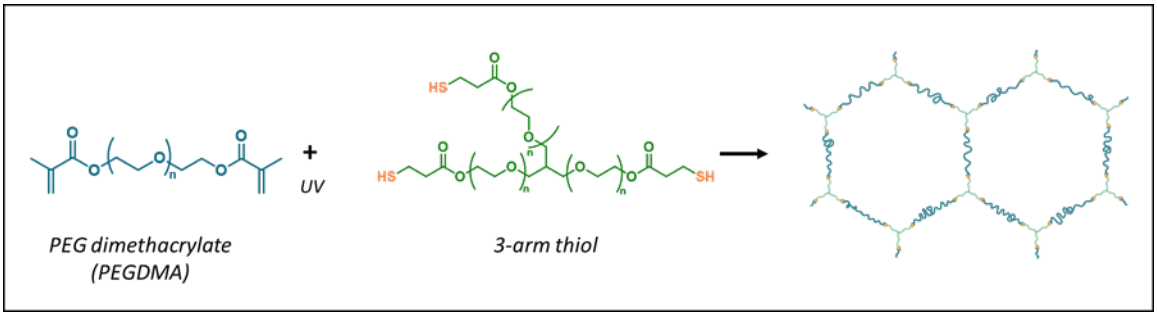


Figure 1. Thiol-ene crosslinked PEG based network.

PoA.10.39

Fucoidan/Chitosan Hydrogels for Sustained Delivery of Platelet-Rich Fibrin Containing Growth Factors

Karina Egle[1,2], Eva Dohle[3], Verena Hoffmann[3], Ilze Salma[2,4], Sarah Al-Maawi[3], Shahram Ghanaati[3], Arita Dubnika[1,2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia. [3]FORM, Frankfurt Oral Regenerative Medicine, Clinic for Maxillofacial and Plastic Surgery, Johann Wolfgang Goethe University, Frankfurt am Main, Germany. [4]4Institute of Stomatology, Riga Stradins University, Riga, Latvia

Abstract

PRF is a second-generation platelet concentrate obtained from blood without the use of anticoagulants that includes important growth factors improving tissue regeneration [1]. Thus, it would be beneficial to store these growth factors at the target site by the using a carrier such as a hydrogel. In this content, we combined PRF with a hydrogel carrier composed of fucoidan (FU) and chitosan (CS) due to their biocompatibility and ability to form polyelectrolyte complexes via self-assembly [2]. Aim of this study was to determine and compare the release kinetics of different bioactive molecules from PRF and PRF/FU_CS hydrogel matrices.

Samples were prepared by combining FU_CS hydrogels with PRF. PRF was obtained using low RCF protocol (600 rpm and 8 min). After centrifugation, PRF was collected from tube and then 200 µL added to the FU_CS hydrogels, pure PRF matrix was used as control. The gel fraction, degree of swelling and microstructure were determined for FU_CS hydrogels and PRF/FU_CS hydrogels. Also, the histology and release kinetics of bioactive molecules (transforming growth factor beta-1 (TGF-β1), human interleukin 6 (IL-6), platelet-derived growth factor BB (PDGF-BB) and epidermal growth factor (EGF) were determined using ELISA method.

The results showed the swelling equilibrium of FU_CS hydrogel matrices was reached between 1 and 2 hours and the gel fraction was 95.66 ± 2.01%. The release kinetic results show that there is a difference between the amount of bioactive molecules released from the pure PRF and PRF/FU_CS hydrogel at different time points. A higher amount of growth factor TGF-β1 is released from pure PRF than from FU_CS hydrogel with PRF after 6 hours. Similar trend can be observed for other studied molecules. The present study shows that the incorporation of PRF into the FU_CS hydrogel matrix delays the release of growth factors, thus ensuring a longer delivery of the growth factors. Overall, the fucoidan/chitosan-based hydrogel can serve as a controlled release system for the sequential delivery of multiple growth factors and cytokines to accelerate tissue repair and regeneration. Histology showed that the liquid PRF penetrated the entire hydrogel matrix.

ACKNOWLEGMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 952347 (RISEus2) and the Latvian Council of Science research project No. lzp-2020/1-0054 (MATRI-X).

REFERENCES:

1. Narayanaswamy, R. et.al. *Bioengineering*, 2023,*10*(1), 58.
2. Venkatesan, J. et.al. *Molecules,* 2018,*23*(6):1429

PoA.10.41

Glycosaminoglycan-gelatin hydrogels to promote fibroblast proliferation, migration and angiogenesis

Toni Radanovic[1,2], Fabian Junker[1,2], Wolfgang Metzger[3], Sandra Rother[1,2]

[1]Preclinical Centre for Molecular Signaling, Saarland University, Homburg, Germany. [2]Institute for Pharmacology and Toxicology, Saarland University, Homburg, Germany. [3]Department of Trauma, Hand and Reconstructive Surgery, Saarland University, Homburg, Germany

Abstract

Impaired wound healing in multimorbid patients poses a potential threat to the patient's life. Functionalized hydrogels represent a promising treatment for chronic wounds by providing targeted support to dysfunctional phases of wound healing (Scharnweber et al. 2015). Gelatin methacryloyl offers a basis for a tunable scaffold that can be functionalized with glycosaminoglycans, proteins, and seeded with cells to support wound healing (Yue et al., 2015). This work focused on establishing 3D gelatin-based scaffolds for incorporation of primary cells, spheroids and microvascular fragments to identify proliferation, migration and angiogenesis promoting conditions.

A cell proliferation assay based on metabolic capacity activity was performed using single primary cells in a gelatin scaffold with varying gelatin methacryloyl concentrations and cell numbers. In addition, hydrogel digestion was optimized for isolation and subsequent quantification of cell number and viability. Cell migration was elaborated by quantifying the spatial occupancy by cells migrating out of spheroids over time.

Primary normal human dermal fibroblast (NHDF) survival, proliferation and migration strongly depended on the percentage of gelatin methacryloyl of the hydrogel. Optimized gelatin methacryloyl hydrogels allowed comparable NHDF behavior to collagen hydrogels, but with simplified handling and multiple functionalization options with cross-linkable glycosaminoglycans. Furthermore, we are aiming to study angiogenesis using a 3D sprouting assay. Adipose tissue-derived microvascular fragments embedded in hydrogels and cultured to mimic the vascular system provide a promising system to rate angiogenesis efficiency based on sprouting events. This tunable hydrogel system serves as a versatile tool to study the role of growth factors, glycosaminoglycans, and different cell types on the wound-healing efficiency

References

Scharnweber, D., et al. (2015). "Glycosaminoglycan derivatives: promising candidates for the design of functional biomaterials." J Mater Sci Mater Med 26(9): 232.

Yue, K., et al. (2015). "Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels." Biomaterials 73: 254-271.

PoA.10.43

3D polysaccharides-based hydrogels for improved extracellular matrix deposition and cardiac repair

Vanessa Morais Lima[1], Albane Carré[1], Emmanuelle Poque[2], Christelle Harscoat-Schiavo[2], Raphaëlle Savoire[2], Pascale Subra-Paternault[2], Mathilde Maillard[3], Teresa Simon-Yarza[1]

[1]Université Paris Cité, Université Sorbonne Paris Nord, Laboratory for Vascular Translational Science INSERM U1148, Paris, France. [2]Université de Bordeaux, Institut CBMN UMR 5248, Pessac, France. [3]Université Paris Cité, Paris, France

Abstract

Biomaterials with the incorporation of cellular components have the potential to improve cardiac function by induction of endogenous repair or by replacement through tissue grafts. Decellularized extracellular matrix (ECM) hydrogel is a promising biomaterial for repairing cardiovascular tissue, by maintaining the complex array of proteins, glycosaminoglycans, proteoglycans, and other matrix features. In this study, we optimized polysaccharide-based scaffolds to obtain bioactive pore surfaces able to support cardiac fibroblast 3D cell culture and thus cardiac ECM in situdeposition. For this, PUDNA hydrogels were obtained by mixing dextran and pullulan polysaccharides and chemically cross-link them with sodium trimetaphosphate. Gelatin or collagen type I (1mg/ml) coating mediated by freeze-drying were used (fig 1). Then, their ability to support fibroblasts cell culture at 7, 14 and 21 days and the ECM deposition were evaluated by cell staining and confocal imaging. Confocal microscopy observations revealed that in PUDNA-ColI (fig 2B) and PUDNA-Gel (fig 2C) hydrogels, cells attached and spread to form a layer on the surface of the pores, which was not observed in PUDNA-Ctrl (fig 2A) without coating. Besides that, in situ ECM deposition was demonstrated by immunostaining of ECM proteins, including Col IV (fig 2D), Col I (fig 2E), and Fibronectin (fig 2F). Our next step was to decellularize the hydrogels to maintain the cell secreted ECM using super-critical CO2 (SC-CO2) fluid extraction. After a short treatment using SC-CO2 (90min under 170bars at 45°C), on the PUDNA-Gel matrix, confocal microscopy observations confirmed the cells elimination. In addition, macroscopic aspect and porosity of the samples remained unchanged after the treatment. These preliminary tests demonstrated that our samples were compatible with the SC-CO2 method of decellularization. Altogether, the results demonstrated that the coatings facilitate the cells adhesion, migration and proliferation within the pores, and encourage us to continue to adapt this new decellularization method to engineer a 3D biohybrid cardiac scaffold for tissue repair.

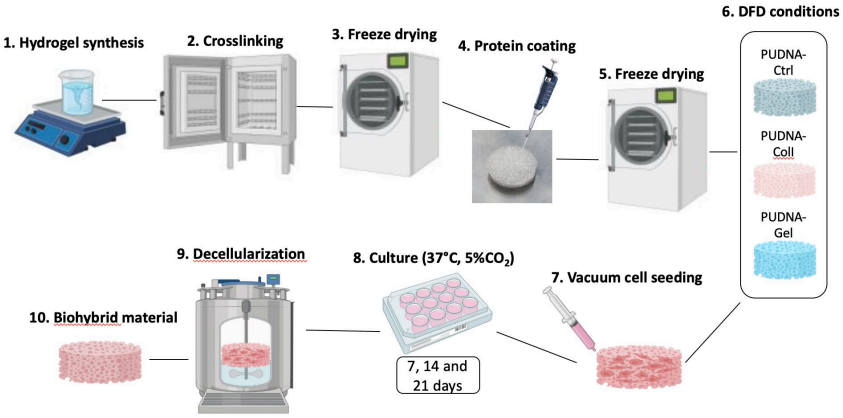


Figure 1: Schematics of the preparation methods to develop a hybrid material to mimic the physico-chemical features and composition of the cardiac ECM to serve as bioactive scaffold for ischemic heart repair.

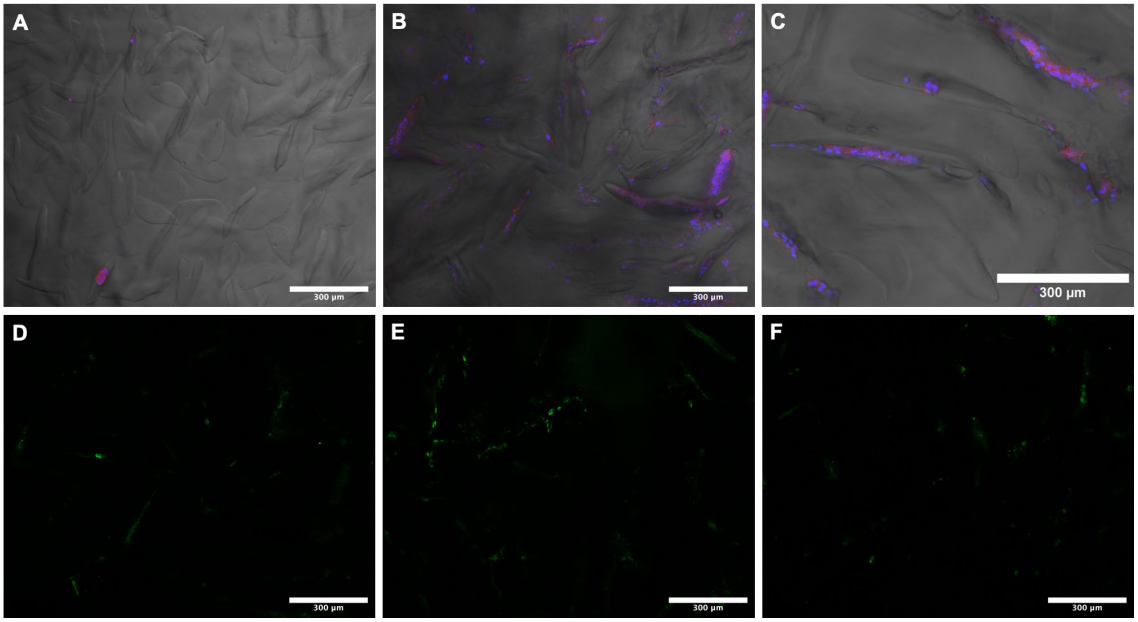


Figure 2: Immunofluorescent staining of 3T3 Balb/c fibroblasts after 21 days of culture within the PUDNA-Ctrl (A), PUDNA-Coll (B), and PUDNA-Gel (C) hydrogels. Cells are stained with DAPI (blue) and Phalloidin (red). Extracellular matrix secreted by cells (green) in PUDNA-Coll: Col IV (D), Col I (E), and Fibronectin (F). Scale bar: 300µm.

PoA.10.44

Development of hyaluronic acid-based hydrogel platforms for delivery of immunotherapeutic agents in cancer treatment

Bita Mahdavi Firouzabadi[1], Cristina Casadidio[1], Mariarosa Gigliobianco[1], Roberta Censi[1], Piera Di Martino[2]

[1]University of Camerino, Camerino, Italy. [2]University of Chieti, Chieti, Italy

Abstract

Introduction: Finding new strategies for the delivery of protein-based therapies is of great importance specially in cancer treatment as most of the recently developed therapeutic agents have a protein-based structure, such as monoclonal antibodies [1]. Hyaluronic acid based hydrogels are considered suitable delivery systems for these agents due to their high biocompatibility, biodegradability and modifiable properties [2].
Methods: In this project we synthesized a thiolated hyaluronic acid (HA-SH, 38 kDa) with different degrees of substitution (30, 50 and 70% DS) and formulated multiple nano and macro hydrogel platforms for delivery of protein-based therapeutic agents. Immunoglobulin G (IgG) (150 kDa) was physically entrapped in the hydrogel and its encapsulation efficiency and release were studied *in vitro*. The redox-responsiveness of the hydrogels was studied by exposure to PBS buffer containing 10mM of L-glutathione reduced. The size and zeta potential of the particles were measured by dynamic light scattering (DLS) instrument and the morphological properties were studied by scanning electron microscopy (SEM).
Results: The synthesized nanohydrogels with HA-SH of 30% DS, demonstrated a size of 170±15nm and zeta potential of -42 ±3mV whereas the nanoparticles with HA-SH of 50% DS had a bigger size of 430± 25 nm and zeta potential of -32 ±1mV (Figure1). The HA-SH with the 70% DS was capable of forming micro-sized particles and macrogels (Figure2). The HA-SH polymeric chains are cross-linked by disulphide (S-S) bonds which are redox-labile, making these hydrogels a promising system for the delivery of therapeutics in reductive tumour microenvironments. Furthermore, high encapsulation efficiency and sustained release of the IgG at different time points were recorded.
Conclusion: HA-SH based hydrogels can provide a stimuli sensitive platform for delivery of protein-based drugs in cancer therapy. The thiolation degree of HA plays an important role in the properties such as particle size, zeta potential and degradation time of the hydrogels. By considering the different parameters such as DS, cross-linking time and the molecular weight of hyaluronic acid, the optimum formulation for the desired release mode of protein-based drugs can be selected.
Acknowledgements: This research was granted by European Commision H2020-MSCA-ITN-2020-CAST (grant number 857894).
References: [1] Zinn S. et al. Nature Cancer (2023), 1-16. [2] Trombino S, et al., Pharmaceutics. (2019).

PoA.10.45

Microencapsulation of mesenchymal stem cells in covalent hydrogel for joint therapy

Mathilde AMBROSINO[1], Fabien NATIVEL[1], Nathan LAGNEAU[1], François LOLL[1], Boris HALGAND[1], Arnaud TESSIER[2], Jérôme GUICHEUX[1], Vianney DELPLACE[1], Catherine LE VISAGE[1]

[1]Nantes Université, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, Nantes, France. [2]Nantes Université, CNRS, Laboratoire CEISAM-UMR 6230, Nantes, France

Abstract

Mesenchymal stem cells (MSCs) have been proposed as a relevant therapeutic approach for cell therapy of diseased joints. These cells can secrete anti-inflammatory, immunomodulatory, and pro-regenerative factors. Considering their low cell viability after injection and cell leakage outside the injection site, encapsulating MSCs in hydrogels could protect them and provide a suitable 3D microenvironment supporting their biological activities. Alginate is the most studied and characterized polymer for cell encapsulation. Crosslinking alginate with calcium ions has been widely investigated, but its *in vivo* stability is unknown. On the other hand, the covalent crosslinking of alginate via strain promoted alkyne-azide cycloaddition (SPAAC) could provide better *in vivo* stability. Hence, this work aims to generate injectable microparticles from SPAAC-alginate using a micromolding technique and evaluate the encapsulation and biological activity of human MSCs.

The two SPAAC-alginate hydrogel precursors were obtained by functionalizing alginate with an azide (N3) and a strained alkyne (bicyclononyne, BCN), respectively. Alginate modifications were confirmed via elemental analysis. SPAAC-alginate hydrogels were then obtained upon mixing of the two alginate components in culture medium (Dulbecco's Modified Eagled Medium, DMEM). Gelation time, stiffness, and stability in DMEM were analyzed via rheological measurements, compression tests, and swelling/stability evaluation, respectively. Furthermore, the diffusion of pro-inflammatory cytokines TNF-α and IFN-γ through the SPAAC-alginate hydrogels was investigated. SPAAC-alginate microparticles were then obtained using a micromolding process (polydimethylsiloxane molds; diameter 150 µm, height 100 µm).

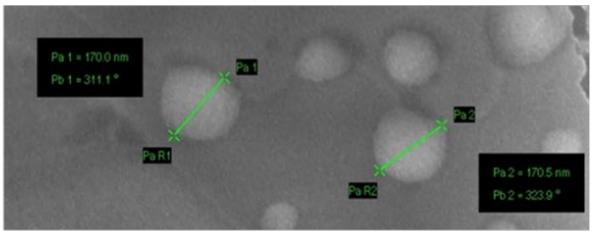


Figure1- SEM picture of nanohydrogels with 30% DS

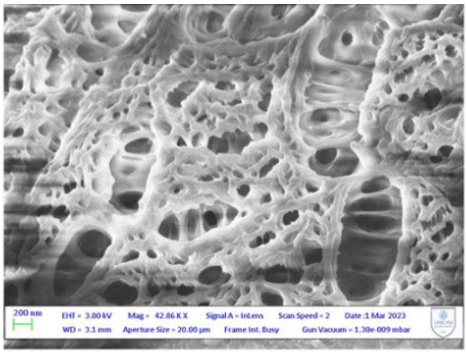


Figure2- SEM picture of the network of hydrogels with 70% DS

We first showed, after optimization, that SPAAC-alginate hydrogels have a gelation time of ≈ 20 min at 37°C. SPAAC-alginate hydrogel stiffness increased after gelation, reaching a peak value of ≈ 1kPa after 24 hours and ≈ 3kPa after 7 days. Bulk and micromolded hydrogels crosslinked for 1 hour at 37°C were stable for over 2 months in basal cell culture medium at 37°C. Concerning the diffusion of molecules through the hydrogel, while TNF-α (17 kDa) diffused well through the SPAAC-alginate hydrogel (70 % in 3 days), the diffusion of IFN-γ was limited (5% in 3 days). Human MSCs were then microencapsulated in SPAAC-alginate hydrogels. Live/dead confocal imaging, cell metabolism assay, and DNA content analysis confirmed the cell viability both immediately after microencapsulation and after 14 days of culture.
These results confirm the suitability of SPAAC-alginate hydrogel to microencapsulate human MSCs for injectable cell therapy approaches.

PoA.10.46

Dendritic-Linear-Dendritic (DLD) Based Materials and Their Potential Use in Biomedical Applications

Noemi Molina, Natalia Sanz del Olmo, Daniel J Hutchinson, Michael Malkoch

KTH Royal Instute of Technology, Stockholm, Sweden

Abstract

Dendrimers are structurally perfect branched nanomaterials synthesized in a step-by-step fashion by taking advanced organic chemistry to a macromolecular level. Dendritic-linear-dendritic (DLDs) molecules are hybrid polymers consisting of the combination of linear polymers (L) and dendritic moieties (D) that can be synthesized in large scale reactions involving simple purification steps.

Bis-MPA based dendritic systems have much potential for use in biomedical applications, as they are biocompatible and biodegradable.[1] A library of polyester based DLD materials comprising linear poly(ethylene glycol) and dendritic blocks based on bis-MPA has been successfully synthesized and postfunctionalized in order to obtain homofunctional[2] or heterofunctional[3] DLDs. When decorated with allyl groups, the crosslinking of these systems with thiol-containing crosslinkers via High Energy Visible Light Thiol-Ene Coupling chemistry (HEV-TEC) results in highly customizable, biocompatible and biodegradable hydrogels.[4] DLD architectures have therefore been studied as promising polymeric scaffolds for the creation of advanced hydrogels suited for biomedical applications.[5]

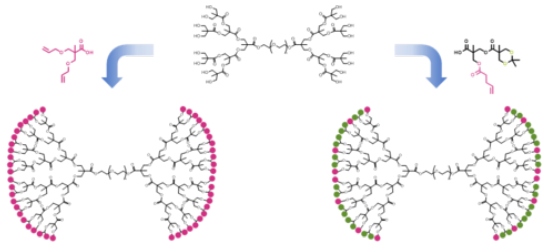


Figure: Post-functionalization of bis-MPA DLDs with allyl groups.

References:

1. Feliu, N.; Walter, M. V.; Montañez, M. I.; Kunzmann, A.; Hult, A.; Nyström, A.; Malkoch, M.; Fadeel, B. Stability and biocompatibility of a library of polyester dendrimers in comparison to polyamidoamine dendrimers. *Biomaterials*, **2012**, *33*, 1970–1981
2. Namata, F.; Sanz del Olmo, N.; Molina, N.; Malkoch, M. Synthesis and Characterization of Amino-Functional Polyester Dendrimers Based On Bis-MPA with Enhanced Hydrolytic Stability and Inherent Antibacterial Properties. *Biomacromolecules*, **2023**, *24* (2), 858-867
3. García-Gallego, S.; Stenström, P.; Mesa-Antunez, P.; Zhang, Y.; Malkoch M. Synthesis of Heterofunctional Polyester Dendrimers with Internal and External Functionalities as Versatile Multipurpose Platforms. *Biomacromolecules*, **2020**, *21* (10), 4273–4279
4. Stenström, P.; Fan, Y.; Zhang, Y.; Hutchinson, D.; García-Gallego, S.; Malkoch, M. UV-Cured Antibacterial Hydrogels Based on PEG and Monodisperse Heterofunctional Bis-MPA Dendrimers. *Molecules*, **2021**, *26*, 2364
5. Oberg, K.; Hed, Y.; Rahm, I. J.; Kelly, J.; Lowenhielm, P.; Malkoch, M. Dual-purpose PEG scaffolds for the preparation of soft and biofunctional hydrogels: the convergence between CuAAC and thiol-ene reactions. *Chem. Commun*, **2013**, *49* (62), 6938–6940

PoA.10.47

Gellan Gum as promising material for human adipose tissue engineering

Franziska B Albrecht, Petra J Kluger

Reutlingen University, Reutlingen, Germany

Abstract

Adipose tissue fulfills important functions besides energy storage, it protects internal organs, and secretes numerous hormones, influencing the whole body's metabolism. Setting up *in vitro* models with long-term stability is still challenging due to the lack of suitable biomaterials and adequate adipogenic maturation. Due to its biological comparability and easily tunable material properties, we chose the bacterial exopolysaccharide Gellan Gum (GG) to create manual, bioprinted, or dynamic functional and flexible applicable human adipose tissue models.
Human primary adipose-derived stem cells (ASCs) and mature adipocytes (ACs) were isolated from skin biopsies, encapsulated in 1% GG or 0.5% GG, respectively. The ASCs were adipogenically differentiated, and cultured for up to 98 days. Further, bioprinted and non-bioprinted models were compared regarding viability and function. The mature adipocytes were cultured in a defined medium and co-cultured with monocytes, to simulate inflamed conditions in static and dynamic cultures.
Adipocytes, as the characteristic cell of adipose tissue, exhibit a roundish, univacuolar cell morphology and secrete hundreds of adipokines. The cells used for *in vitro* models should resemble adipocytes as closely as possible. Within GG hydrogels, it was possible to differentiate the encapsulated ASCs into univacuolar cells after 42 days. After both manual and additive setup, they had high cell viabilities and exhibit a roundish, univacuolar morphology, displaying their similarity to adipocytes. As the amount of lipid-positive and univacuolar cells increase, it can be concluded that the GG hydrogels successfully support adipogenesis. In ACs, viability and intracellular lipid content were high and morphological (actin, perilipin A) analyses showed similar results for both culture methods (static and dynamic). Inflammatory stimuli induced morphological changes independently of the culture condition. After activation, monocytes exhibited membrane protrusions, and ACs showed decreased perilipin A integrity.
Our work showed that GG is a promising material for adipose tissue engineering, as it allows the use of ASCs and ACs under various culture conditions. The hydrogels were non-toxic, non-monocyte activating and long-term stable. It enabled the culture of viable human primary ASCs for 98 days, successful adipogenic differentiation, and maintenance with univacuolar morphology. The established extrusion-based bioprinting of pre-crosslinked GG, showed no process-related effects on cell viability or functionality over 42 days compared to manual models. ACs exhibited also high viability, maintained intracellular lipids, and secreted pro-inflammatory cytokines upon stimulation. The advanced possibility for perfusion culture makes our model applicable for studying the influence of inflamed adipose tissue on other tissue.

PoA.10.48

Injectable hydrogels based on Schiff-base linkages as promising therapeutic platform for *in situ* drug delivery

Roberta Pappalardo[1,2], Monica Boffito[1], Claudio Cassino[3], Valeria Chiono[1], Gianluca Ciardelli[1]

[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy. [2]Department of Surgical Sciences, Università degli Studi di Torino, Turin, Italy. [3]Department of Science and Technological Innovation, Università del Piemonte Orientale, Alessandria, Italy

Abstract

In situ forming injectable hydrogels represent promising systems for tissue engineering (TE) applications thanks to their high-water content, which provides a physiologically similar environment to the native extracellular matrix, and the capability of encapsulating and transferring their payload to the surrounding tissues in a minimally invasive manner. Hydrogels based on Schiff-base bonds exploit the advantages of chemical hydrogels (i.e., favorable stability under physiological conditions) without any toxic crosslinking agent required for their preparation. Furthermore, injectability, self-healing ability and pH-responsiveness make them attractive to prepare therapeutic platforms for *in situ* drug delivery. In this scenario, we designed new injectable hydrogels based on Schiff-base linkages exploiting the versatility of polyurethanes (PURs) as constituent materials.

Two water-soluble poly(ethylene glycol)-based PURs were synthesized, bearing primary amino groups along each polymeric chain and aldehyde end groups, respectively. A high molecular weight PUR-NH_2 (M_n 24 kDa, D 1.7) was synthesized using N-Boc serinol as chain extender and it was subjected to an acidic treatment to remove Boc protecting groups, thus exposing primary amines. The complete cleavage of Boc groups was confirmed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, and the exposed primary amines were quantified to be around 10^{20} units/g_{PUR}. A low molecular weight PUR-CHO (M_n 4 kDa, D 1.5) was synthesized by end-capping the prepolymer with 4-hydroxybenzaldehyde. PUR successful synthesis was proved by the appearance of the signal ascribed to the proton on the carbonyl carbon at 9.8 ppm in its ^{1}H NMR spectrum and aldehyde groups were quantified to be around 10^{20} units/g_{PUR}. Hydrogels were prepared by mixing SHE3350 and AHE1500 aqueous solutions and characterized to assess their key physico-chemical and rheological properties. The formation of Schiff-base bonds was assessed through infrared and Carbon-13 solid-state NMR spectroscopies. Rheological characterization confirmed the formation of hydrogels with high resistance to applied strain. In contact with buffers at different pH, hydrogels exhibited a high swelling ability and stability in physiological-like conditions up to 27 days; at pH 5, dissolution phenomena occurred at 13 days, due to the Schiff-base hydrolysis in acidic conditions. Moreover, they showed high permeability, controlled and sustained release of a model molecule (i.e., Fluorescein isothiocyanate dextran, FD4) until 21 days. Lastly, hydrogels exhibited easy injectability and self-healing ability.

Overall, our results highlighted that PUR chemistry could be exploited to engineer new injectable hydrogels based on Schiff-base linkages with promising properties for the design of therapeutic platforms for TE applications.

PoA.10.49

Overcoming photoinitiator limitations. Self-crosslinking material for bioprinting application.

Agnieszka Zakrzewska, Sylwester Domański, Marta Klak, Michał Wszoła

Polbionica Ltd., Warszawa, Poland

Abstract

Main aim of presented research is to develop photocurable material basing on gelatin derivatives which do not demand polymerization initiator for efficient crosslinking of polymer fibers.
Gelatin is a naturally occurring polypeptide soluble in water. The content of individual amino acids and their order in the peptide chain depends on the origin of the gelatin.
Due to presence of functional groups such as primary amine groups and hydroxyl groups in the peptide chain, it is possible to occur nucleophilic substitution reactions with the appropriate acid anhydride or activated carboxylic acid. Modified gelatin such as GELMA is a photocurable and biocompatible polymer. It is an important substrate for the creation of materials used in tissue engineering and regenerative medicine.
One of the stages of creating 3D scaffolds from hydrogels is polymer cross-linking using a photoinitiator such as LAP or Irgacure. It is commonly known that the by-products of photoinitiator degradation show cytotoxicity, which is a very undesirable effect in biomaterials engineering.
Facing the problem of photoinitiators cytotoxicity we initiated research on materials capable of crosslinking without using of photoinitiator (Fig.1.). The key of the research was to find compounds containing appropriate groups sensitive to UV-Vis irradiation, incorporated them into polymer structure and optimize working parameters for application in bioprinting.
Moreover, the use of gelatin fibers as the basic biopolymer paved the way for the expansion of a new group of materials that do not require the use of a photoinitiator. It is possible to find new applications for the above materials, which would allow for a significant development of the chemistry of biomaterials.
In addition, using various methods of synthesis, it is possible to functionalize the most important part of the polymers in bioprinting such as chitosan or hyaluronic acid.

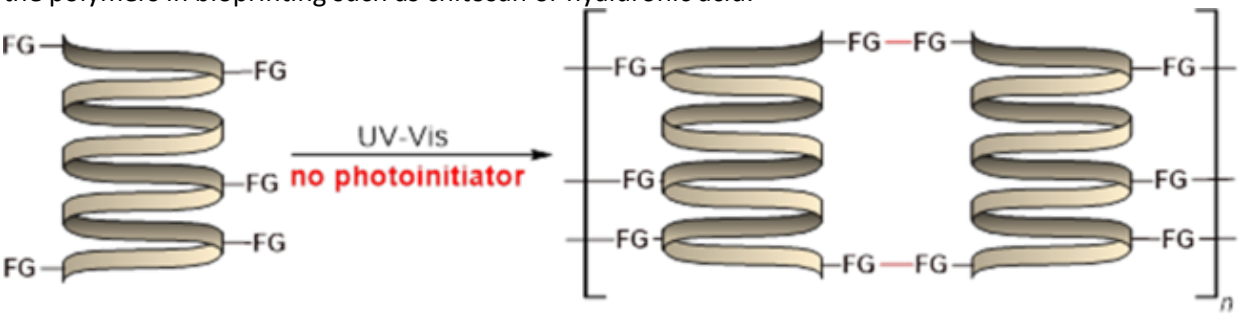


Fig.1. Polymer cross-linking

PoA.10.51

Cold-water fish gelatine-based hydrogels for tissue engineering applications

Tobias Hammer[1], Ke Yang[2], Tobias Spirig[1], Markus Rottmar[2], Katharina Maniura-Weber[2], René M Rossi[1], Kongchang Wei[1,2]

[1]Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biomimetic Mem-branes and Textiles, St. Gallen, Switzerland. [2]Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biointerfaces, St. Gallen, Switzerland

Abstract

Amidst the different materials employed in the field of tissue engineering, hydrogels have been identified as especially suitable candidates due to their intrinsic extracellular matrix (ECM)-like properties and tuneable nature. In particular, gelatine represents one of the most commonly used natural polymers for cell culture, given its intrinsic cell-adhesive nature. With porcine or bovine origin as the dominating source, gelatin from cold water fish skin (fGel) has been discovered as a valuable alternative owing to a number of favourable properties such as lower gelling and melting points as well as lower degree of immunogenicity [1]. In order to form stable hydrogels from biopolymers, chemical modifications are often necessary to allow for covalent and dynamic-covalent cross-linkings [2]. Photoinitiated thiol-ene click-chemistry is a suitable method for biomedical applications given its highly specific and fast cross-linking under cytocompatible reaction conditions [2]. Nonetheless, the weak mechanical properties of biopolymers often necessitates blending with synthetic, biologically inactive polymers [3].

Here, hydrogels were fabricated from chemically modified cold-water fish gelatin (fGel) via click chemistry-mediated cross-linking. By synthesizing two functional derivatives in the form of thiolated (fGel-SH) and norbornene-functionalized (fGel-NB) fish gelatin, stable hydrogels with a purely gelatine-based network were obtained after exposure to UV-light (Fig. 1A). Rheological evaluation of the mechanical properties of the resulting hydrogels revealed a rapid increase in storage modulus shortly after UV-exposure, with hydrogels reaching their maximum modulus within one minute (Fig. 1B). Such cold-water fish gelatine-based hydrogels demonstrated promising properties for rapid 3D cell encapsulation and *in vitro* culture. Encapsulation of human dermal fibroblasts (HDFs) within the hydrogels was achieved by curing the cell-suspended precursor solution of both fGel-NB and fGel-SH in the presence of photoinitiator (I2959). The cytocompatibility of the material was revealed by high cell viability during the 7-day culture (Fig. 1C), thus making it a promising candidate for a variety of tissue engineering applications.

Acknowledgements

The authors would like to acknowledge funding by Skintegrity.CH, SNSF Spark grant (CRSK-2_190493) and Empa, Swiss Federal Laboratories for Materials Science and Technology.

References

[1] Levato, Riccardo, et al. Materials Today Bio (2021): 100162.

[2] Muir, Victoria G., and Jason A. Burdick. Chemical reviews 121.18 (2020): 10908-10949.

[3] Reddy, M. Sai Bhargava, et al. Polymers 13.7 (2021): 1105.

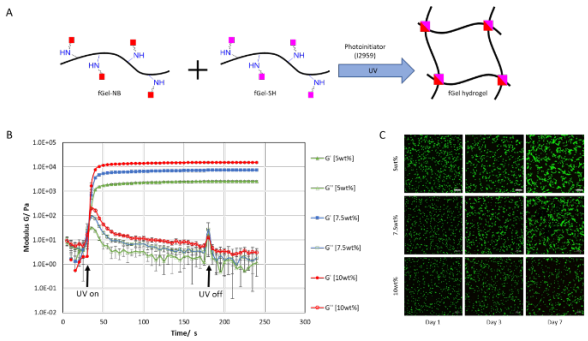


Figure 1. Reaction scheme for the formation of fGel hydrogels (A), rheological time-sweep measurements (B) and live/dead assay of HDFs (C) for fGels of varying concentrations. Scale bar represents 200 µm.

PoA.10.52

Cell-patterned temperature-sensitive hydrogels for endochondral ossification

Athanasia Pylostomou[1,2], Jennifer R. Weiser[3], Matteo D'Este[2], Jacek K. Wychowaniec[2], Junxuan Ma[2], Riccardo Tognato[2], Tiziano Serra[2], Dagnija Loca[1], Andrea J. Vernengo[2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of Riga Technical University, Faculty of Materials Science and Applied Chemistry, Baltic Biomaterials Centre of Excellence, Riga, Latvia. [2]AO Research Institute, Davos, Switzerland. [3]3The Cooper Union for the Advancement of Science and Art, New York, USA

Abstract

INTRODUCTION: Large bone defects are typically treated by autologous bone grafts, which, present challenges such as donor site morbidity and limited availability [1]. Endochondral ossification, the process of forming long bones, involves precursor cell aggregation and proliferation along the bone longitudinal axis [2]. Cell alignment and proximity are crucial components for mimicking the process of endochondral ossification *in vitro*. Hydrogels are ideal biomaterials due to their customizable architectures and high-water content similar to natural tissues [3]. In this study, polyacrylic acid microgels (Carbopol® 940), gelatin, and poly(N-isopropylacrylamide)-chondroitin sulfate (pNIPAAM-CS) were combined to support freeform extrusion of high-density cellular bioinks into embedded channels within the multicomponent hydrogel. The unique properties of this 3D system provide a temperature-responsive environment for on-off cell attachment. It was hypothesized that this could be exploited for stimulating the formation of closely associated and oriented cellular patterns within the hydrogel.

METHODS:Bone marrow derived human mesenchymal stromal cells (BM-hMSCs) were suspended in gelatin and DMEM and 3D printed into the hydrogel at 25°C as embedded channels. The cells were cultured for 5 weeks under static (37°C) or dynamic conditions (cycling between 37°C and 25°C for 10 min every 5 days) in chondropermissive medium. Cell morphology was examined using phalloidin and DAPI counterstain.

RESULTS: Confocal images revealed that the bioprinted pattern was maintained over 5 weeks of culture. After 5 weeks, the BM-hMSCs that were cultured in static conditions displayed a tendency for elongation and migration within the hydrogels (Figure 1A), while BM-hMSCs cultured in dynamic conditions presented an aggregated channel pattern with round cell nuclei (Figure 1B). The results provide preliminary evidence that our approach enables control over cell morphology and proximity, which could be used to modulate the process of chondrogenesis. Current studies are examining gene expression for chondrogenic hypertrophic markers with real-time PCR.

ACKNOWLEDGEMENTS: This project has received funding from the AO Research Institute Davos and European Union's Horizon 2020 research and innovation program under grant agreement No 857287 (BBCE). J.K.W. would like to acknowledge the European Union's Horizon 2020 (H2020-MSCA-IF-2019) research and innovation programme under the Marie Skłodowska-Curie grant agreement 893099 — ImmunoBioInks.

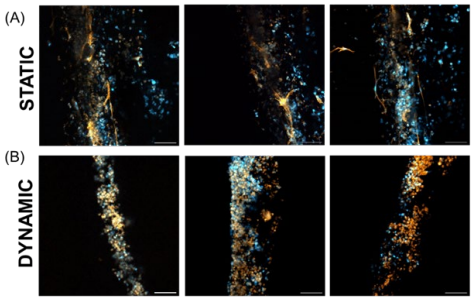


Figure 1. Cell nuclei stained in blue (DAPI) and cytoskeleton stained in orange (phalloidin) of hMSCs cultured for 5 weeks in chondropermissive medium under (A) static temperature of 37°C and (B) dynamic cycled temperature between 25°C and 37°C. Scale bars = 100 µm.

REFERENCES:

[1] Rodríguez-Merchán, E. C. (2022). International Journal of Molecular Sciences, 23(6), 3352.

[2] Galea, G. L., et al. (2020). Developmental Dynamics.

[3] Sheehy, E. J., et al. (2019). Materials Today Bio, 100009.

PoA.10.54

Development of gellan gum-based bioinks utilizing a two-step photo-crosslinking approach for extrusion-based 3D bioprinting

Vijay Singh Parihar, Hatai Jongprasitkul, Sanna Turunen, Christine Gering, Minna Kellomäki

Tampere University, Tampere, Finland

Abstract

Introduction: Bioprinting applications are expanding in basic tissue engineering, regenerative medicine, personalized medicine, and organ-on-chip technology. Bioink plays an important role in the fabrication of suitable 3D scaffolds and is crucial to achieving physiologically more relevant *in vitro* models. An ideal bioink should possess the desired physicochemical properties, such as proper mechanical, rheological, chemical and biological characteristics. Gellan gum (GG)-derived hydrogels show excellent viscoelastic properties which make this material a potential candidate for bioinks. In our previous studies, we utilized the dual crosslinking approach to develop gallic acid functionalized GelMA (GelMA-GA)[1] and GGMA[2]-based bioinks. In both cases, we achieved excellent printability by utilizing a two-step crosslinking approach: metal chelation in the first step to control the ink viscosity, followed by photo-crosslinking after the printing.

Results and discussion: In the current work, we omitted the ionic crosslinking step and utilized controlled photo-crosslinking in a two-step manner to modify GGMA and GGMA-GA printable (Figure 1A). The purified GG was methacrylated at basic pH and gallic acid (GA) was functionalized using EDC coupling on GGMA (Figure 1B).

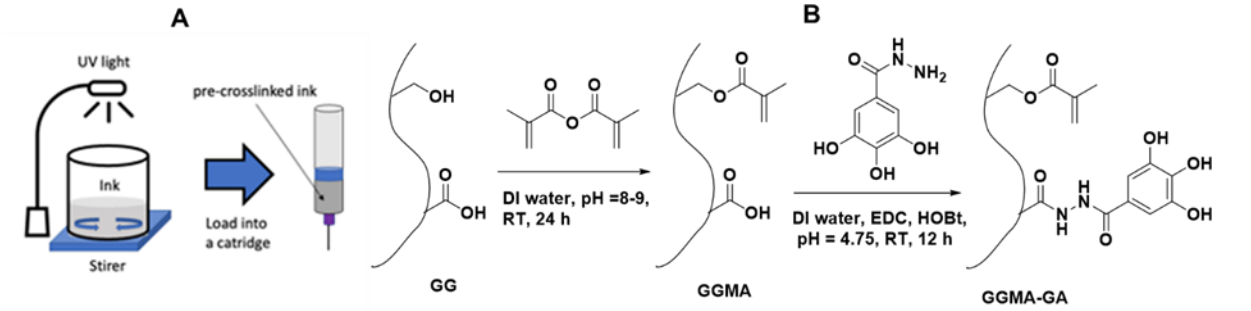


Figure 1: (A) Controlled photo-crosslinking to obtain printable ink; (B) Synthesis of gellan gum methacrylate (GGMA) and GGMA-GA.

^{1}H-NMR spectroscopy confirmed the degree of methacrylation and conjugation of GA to the GG backbone. The printable inks were obtained by controlled photo-crosslinking of GGMA and GGMA-GA by UV exposure of 10 mW/cm2 for 120 seconds with constant stirring (Figure 1A). The results show that upon extrusion, the GGMA-GA produced a smoother fiber with less die swelling compared to GGMA (Figure 2A). The inks were pre-characterized via rheology and then printed into multi-layered grid structures (Figure 2B) and 3D tubular structures. Moreover, GA conjugation provides tissue adhesion and antioxidant properties and shows several advantages over conventional bioink for extrusion-based 3D bioprinting. However, the swelling tests showed that in the cell culture medium, GGMA-GA started to shrink rapidly on the first day, whereas GGMA hydrogels remained stable. Both GGMA and GGMA-GA hydrogels were stable until the end of the observation period, but the hydrogels containing GA shrank slightly more. The developed two-step strategy can be easily adapted to create a library of bioinks.

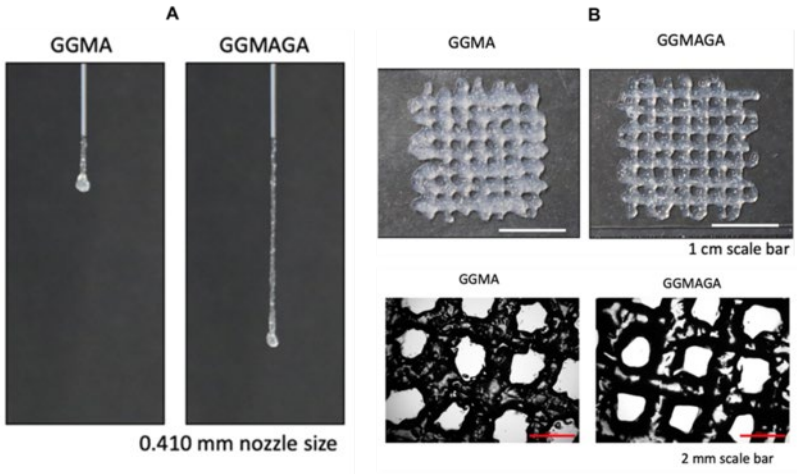


Figure 2: (A) Filament extrusion test of controlled photo-crosslinked precursors; (B) Pictures of the two-layered grid structures obtained after printing.

References:
1. H. Jongprasitkul, et. al, Biomacromolecules, 2023, 24, 1, 502–514.
2. H. Jongprasitkul, et. al, Bioprinting, 2022, 25, e00185.

PoA.10.55

Chemical warfare decontamination: strong potential of an innovative phyllosilicate-based film-forming hydrogel for wounded skin decontamination.

Magaly Misbach, Kardelen Durmaz, Jean-Paul Salvi, Alix Danoy, Bernard Verrier, Jérôme Sohier

IBCP, LBTI, CNRS UMR 5305, Lyon, France

Abstract

Introduction: Organophosphate neurotoxins, as VX, are chemical agents that can enter the body through skin absorption1. Phyllosilicate (PS), as Fuller's Earth (FE), are used in powder form as decontamination tool but cannot be applied on damaged skin due to potential hazard. Therefore, we elaborated novel Film-Forming composite Hydrogels (FFH) composed of hydrophilic polymers (HP), FE and surfactants (S), which allow an easy application and removal on the skin. We hypothesized that the FE-embedded films would allow for an efficient adsorption, sequestration and disposal of organophosphorus compounds, even on wounded skin. The FFH were therefore evaluated in regard of their cytotoxicity structure, sequestration, and decontamination capacities on glass slides (*in vitro*) and on pig skin explants (ex vivo). Furthermore, other PS (PS-A,PS-B) than FE were investigated to broaden the FFH use to other chemical agents.
Materials and methods: FFH were prepared by mixing various concentrations of HP, PS and S (HPxPSySz in w/w%) in a solvent composed of 10% ethanol at 90°C. Their toxicity was evaluated on normal human dermal fibroblast (NHDF) with metabolic test (ISO-standard 10993-5). Structure of the formed films and sequestration of paraoxon (POX, simulating the neurotoxic VX) were assessed with EDX-SEM. Decontamination efficiency (DE) of POX was evaluated and quantified (UV-HPLC) on glass slides and FFH showing DE>95% were tested through pig ear skin explant (VitroPharma® miniaturized Franz cell).
Results: Films containing FE showed a homogeneous dispersion of FE particles within the hydrogel bulk (Fig1A). POX, representing by phosphorus, was both sequestrated in hydrogel and PS phase (representing by aluminium, silicium and magnesium), underlining the decontamination capacities of the films on their own (Fig1B). None of the tested FFH compositions, with and without FE, showed cytotoxic effect on NHDF (viability>90%, Fig2A). PS incorporation, however, increased DE of glass slides, regardless of the PS used (Fig2B). Ex vivo assays showed a DE>80% and up to 92.5% (Fig2C), which is higher that RSDL sponge (gold-standard of skin decontamination). Of utmost interest, application and removal of FFH to decontaminate the skins did induce any damage to the skin tissue and left the stratum corneum intact.
Conclusions: We here demonstrate that FFH containing PS are non-cytotoxic and allow for a highly efficient decontamination and sequestration of organophosphate toxic agents. Their ability to leave the skin intact foster their potential for wounded skins, which is currently in progress in ex vivo and *in vivo* cut and burn models.

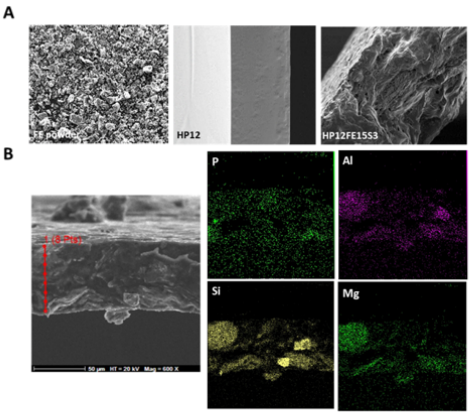
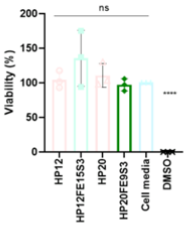


Figure 1. Structure and POX sequestration assessment in the FFH with EDX scanning electron microscopy. (A) Structure of Fuller's Earth, FFH with 12% HP and FFH with 12% HP, 15% FE and 3% S (HP12FE15S3). (B) Elemental mapping of HP12FE15S3 highlighting phosphorus (P) only present in POX and alumium (Al), silicium (Si) and magnesium (Mg) only present in FE.

A



B

Formulation	*Quantity of POX found in hydrogel (%)*	*Quantity of POX found on the*
HP12 S3	82 ± 12	18 ± 12
HP20 S3	76.8 ± 4.8	23.2 ± 4.8
HP12 FE15 S3	97.9 ± 0.9	2.1 ± 0.9
HP20 FE9 S3	96.6 ± 0.3	3.4 ± 0.3
HP12 PS-A15 S3	90.4 ± 5.3	9.6 ± 5.3
HP20 PS-A9 S3	93.6 ± 5.1	6.4 ± 5.1
HP12 PS-B15 S3	97.6 ± 1.3	2.4 ± 1.3
HP20 PS-B9 S3	95 ± 1.5	5 ± 1.5

C

Formulation	**HP12 FE15 S3**	**HP20 FE9 S3**	**HP12 PS-B15 S3**	**HP20 PS-B9 S3**	**RSDL**
Decontamination efficacity (%)	80.9	82.9	85.3	92.5	83.3

Figure 2. (A) Percentage of NHDF viability (n=3). (B) FFH decontamination efficiency on glass slides depending on quantity of POX found in hydrogel bulk and on a glass surface (*in vitro*). (C) Decontamination efficiency on pig ear skin explant (*ex vivo*) of hydrogels showing. HP: hydrophilic polymer; S: surfactant; FE: Fuller's Earth; PS-A: Phyllosilicate A ; PS-B : Phyllosilicate B

PoA.10.58

Combination of Platinum-doped $CaCO_3$ and Amylopectin-based Gel to Synergize with Radiotherapy for High-grade Glioma

Jason Lin[1], Wei-Yang Hong[1], Hsiang-Kuang Liang[1,2,3], Feng-Huei Lin[1,4]

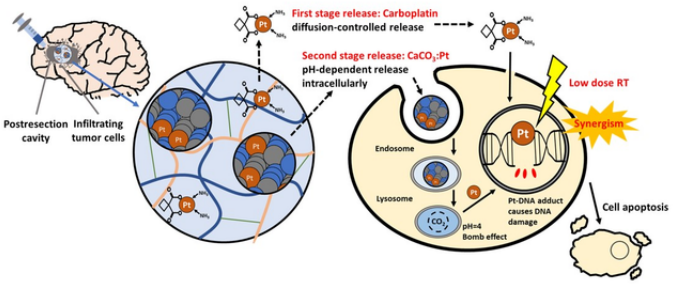
[1]Department of Biomedical Engineering, National Taiwan University, Taipei, Taiwan. [2]Division of Radiation Oncology, Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan. [3]Radiation Science and Proton Therapy Center, National Taiwan University College of Medicine, Taipei, Taiwan. [4]Division of Biomedical Engineering and Nanomedicine Research, National Health Research Institutes, Taipei, Taiwan

Abstract

High-grade glioma (HGG) poses a formidable challenge clinically with poor prognosis and high recurrence rate. Nowadays, standard treatment of chemoradiotherapy is shown incurable but merely improve the median survival, because it still failed to tackle the problem of infiltrating nature of tumor, which causes the tumor relapse within several months inevitably.

The time interval between postresection and radiotherapy is usually 3 weeks for standard treatment of HGG, so it is crucial to block tumor infiltration during this time interval and prevent from recurrence. Accordingly, this study is aim to design a long-term drug delivery system (DDS) for local treatment and further synergize with radiation. The combination of Platinum-doped $CaCO_3$ ($CaCO_3$:Pt) and Carboplatin-loaded Amylopectin-based gel (CPG) is treated locally and divided into 2 releasing stages. Firstly, Carboplatin, released initially from gel by diffusion, is able to cause anti-proliferation effect of cancer cells. Secondly, $CaCO_3$:Pt could cause apoptosis of glioma cells via endocytosis by releasing platinum from $CaCO_3$:Pt intracellularly based on acidic degradation in endosome-lysosome complex, where alkylating agent, platinum could cause cancer cell apoptosis by crosslinking onto DNA.

As for material analysis, Spherical microparticles $CaCO_3$:Pt is synthesized as vaterite form and it was validated by TEM, XRD. The drug release profile of the designed DDS is measured by ICP-MS and was shown to release alkylating agents up to 21 days. As for in-vitro test, both the WST-1 assay and live/dead cell staining assay results indicate the CPG and $CaCO_3$:Pt are both cytotoxic to ALTS1C1 glioma cells. Furthermore, the clonogenic assay validates that both $CaCO_3$:Pt and radiation could inhibit glioma cells from proliferation and both of which could reach a synergistic effect by calculating the combination index. As for in-ovo test, the combination of CPG and $CaCO_3$:Pt with radiation was shown to be effective and could suppress the tumor growth in the chicken chorioallantoic membrane model. Lastly, as for in-vivo study, glioma cells are grafted intracranially in mice brain, and the CPG combined $CaCO_3$:Pt is intratumoral delivered locally, where the tumor growth was assessed by IVIS imaging system and there were shown to be decreasing in tumor size for treatment groups. As a whole, the combination of CPG and $CaCO_3$:Pt were shown to cause apoptosis of glioma cells and could synergize with radiation so as to block the tumor infiltration, which makes it a potential co-treatment with radiation in addition to TMZ/RT.



PoA.10.59

Nanofibrillar cellulose hydrogel with stem cells, organoids and wound care

Lauri J Paasonen

UPM Biomedicals, Helsinki, Finland

Abstract

New biologically relevant materials and methods are needed for creating three-dimensional (3D) multicellular structures for disease modelling, drug development and tissue engineering. Currently there is wide transition from animal-derived materials to synthetic or other natural ones. Nanofibrillar cellulose (NFC) hydrogel is a highly biocompatible material that has been shown to provide an effective support matrix for culturing various cell types in 3D. As an animal-free matrix, NFC enables possibilities also for clinical applications.
(A) Pluripotent stem cells were embedded in NFC hydrogel and cultured up to 26 days. Pluripotency was analysed with OCT4 and SSEA-4 marker expression, *in vitro* EB-mediated differentiation, and teratoma assay. Cells proliferated in NFC without feeder cells, and the cells retained their pluripotency without changes in karyotypes. (B) Renal organoids can mimic the structure and function of *in vivo* kidneys. Organoids were cultured from metanephric mesenchymal cells followed by chemical induction to undergo nephrogenesis. Cells were embedded in NFC which reduced the distortion or stress-induced affects. This allowed the organoids to grow in conditions mimicking better the physiological environment. (C) Wound healing is a complex and continuous process which involves a variety of cells, soluble factors, and extracellular matrices. The potential of NFC for wound treatment was studied as hydrogel-based dressing and hydrogels. In addition, its potential as a cell scaffold for human adipose-derived mesenchymal stromal cells (hASCs) was studied. Clinically, NFC wound dressing provided efficient wound healing at skin graft donor sites, and as a hydrogel it did not affect wound closure rate *in vivo* or altered a normal healing process. Foreign-body reaction was also not observed. hASCs cultured on top of NFC dressing presented to maintain their undifferentiated state, immunological properties and high cell viability. These findings offer a good platform to continue the development of the cell-based wound treatment. Nanofibrillar cellulose is a biocompatible material that offers a well-defined 3D culture matrix for various cell types *in vitro* and enables opportunities also in regenerative medicine applications. Authors would like to thank Yan-Ru Lou, Ulla Saarela, and Jasmi Snirvi for performing the experiments.

PoA.10.61

Unlocking the Potential of Thiazolidine and Disulfide-Crosslinked Hyaluronic Acid Hydrogels for 3D Bioprinting

Shima Tavakoli, Oommen Varghese

Uppsala University, Uppsala, Sweden

Abstract

One of the concerns regarding stem cell-derived therapies is the survival of the transplanted cells into the host tissue. Overall, approximately 1–20 % of the transplanted cells survive, considerably limiting their therapeutic potential. Recently, 3D bioprinting for stem cell delivery has significantly improved the capacity to administer stem cells in a precise and controlled manner, however, the development of an efficient biomaterial to fulfill all the requirements for a successful bioink during formulation, injection, post-injection, and long-term survival phases remains a significant challenge. To address this challenge, we developed dynamically crosslinked hyaluronic acid-based hydrogel with customizable properties. For this purpose, we developed dual crosslinked hydrogel by incorporating cysteine and aldehyde functional groups. The cysteine moiety undergoes disulfide bonds as well as thiazolidine crosslinkages when mixed with aldehyde-modified polymers. Unlike the general conception that thiazolidine formation is slow and forms a thiohemiacetal intermediate that slowly converts to a stable thiazolidine product, we show for the first time that formation of thiazolidine product is instantaneous. Taking advantage of the differences in the kinetic rates of disulfide and thiazolidine formation reaction, we could fine-tune the gelation time of hydrogels by employing different ratios of disulfide and thiazolidine chemistry in the system. While hydrogels solely relying on disulfide crosslinking suffer from slow gelation at physiological pH and poor long-term mechanical stability, the presence of thiazolidine linkage increased the gelation rate and hydrogel stability, significantly. We observed that hydrogels formed solely from either disulfide or thiazolidine crosslinking alone did not meet the requirements necessary for a promising bioink for the delivery of stem cells. However, our investigation revealed that when disulfide and thiazolidine crosslinking were combined, a remarkable hydrogel system emerged as an exceptional bioink, it supported cell survival throughout extrusion from the needle. It showcases rapid gelation kinetics, shear-thinning, and shape-maintaining capabilities, ensuring the preservation of high cell viability throughout the printing. Furthermore, the disulfide-bonded part of the system contributes to a self-healing hydrogel, effectively safeguarding the encapsulated cells post-printing. Moreover, the inclusion of disulfide linkages facilitates cell migration, while the presence of thiazolidine chemistry promotes long-term stability and cell proliferation, which can preserve the long-term survival of cells. In this context, our research presents a promising solution to overcome these critical challenges, thus opening up new avenues for advancements in tissue engineering and regenerative medicine.

PoA.10.62

REVERSIGEL: REVERSIbly crosslinked hydroGELs for effective cardiac stem cell delivery

Ken Princen, Wanda Geudens, Geert-Jan Graulus, Peter Adriaensens

Hasselt University, Diepenbeek, Belgium

Abstract

During a myocardial infarction, heart tissue dies due to hypoxemia, disturbing the heart's function and potentially leading to heart failure, which is often fatal. Cardiac Atrial appendage Stem Cells (CASCs) are promising stem cells for heart tissue repair. However, stem cell-based therapies are limited by poor stem cell retention at the transplantation site. This work concentrates on developing an adaptable hydrogel to facilitate CASCs transplantation and retention. The envisioned hydrogel will mimic the heart's extracellular matrix via two components: Elastin-Like Proteins (ELPs) and oxidised Hyaluronic Acid (HA) derivatives. ELPs are thermoresponsive proteins with $(VPGXG)_5$ as the core sequence, where X denotes any amino acid except proline. These ELPs can also contain short peptide sequences for cell adhesion. However, HA offers synthetic versatility via its free carboxylic acids and alcohol groups, along with favourable biological properties like promoting angiogenesis and wound healing. Both components can be crosslinked by dynamic hydrazone bonds and via the ELPs' inherent LCST transition. Moreover, the hydrogels' mechanical properties can be further improved via in situ Diels-Alder cross-linking. To this end, SuFEx chemistry was applied to introduce orthogonal furan moieties along the ELP backbone being chemical handles for ultimately cross-link tuning with maleimide-decorated HA.

PoA.10.63

From Complementary Components to Advanced Biocomposites based on Fibrin and Silk Fibroin

Ikram El Maachi[1], Stavroula Kyriakou[1], Stephan Rütten[2], Marius Köpf[3], Stefan Jockenhoevel[1,4], Alicia Fernández-Colino[1]

[1]Department of Biohybrid & Medical Textiles (BioTex), AME-Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany. [2]Electron Microscopy Facility, Uniklinik RWTH Aachen, Aachen, Germany. [3]Fibrothelium GmbH, Aachen, Germany. [4]AMIBM-Aachen-Maastricht-Institute for Biobased Materials, Faculty of Science and Engineering, Brightlands Chemelot Campus, Maastricht University, Geleen, Netherlands

Abstract

In the context of tissue engineering, achieving a fully functional substitute for native tissue often requires of the combination of multiple materials, as a single material alone falls short of meeting all needed properties. While fibrin is a natural polymer known for its excellent bioactivity, versatility, and processability, its biomedical application for the fabrication of tissue-replacements is hindered by cell-mediated contraction and insufficient mechanical strength. To overcome these limitations, we present a novel approach, in which we combine fibrin with silk fibroin, another extraordinary natural polymer, using a simple and reproducible biofabrication method [1].

To fabricate composites of fibrin and silk fibroin, we employed an injection-molding technique that encompassed the simultaneous co-injection of fibrin precursors and silk fibroin. The resulting scaffolds' internal structure was examined using scanning electron microscopy. Rheology and burst strength measurements were conducted to characterize the mechanical properties. The fibrin/silk fibroin scaffolds were then seeded with primary human venous endothelial cells and human arterial smooth muscle cells, to assess their bioactivity and resistance to cell-mediated contraction, respectively. Cell adhesion and morphology were analyzed using confocal microscopy. Additionally, we explored the feasibility of fabricating tubular-shaped composite scaffolds. The fabrication of homogeneous, porous composite scaffolds comprising fibrin and silk fibroin was successful. These composite scaffolds exhibited significantly enhanced mechanical properties compared to scaffolds of only fibrin. Additionally, they experienced minimal cell-mediated contraction even when cultured with smooth muscle cells. The scaffolds were suitable for the culture of endothelial cells, which formed a confluent endothelial cell layer, highly desirable for tissue-replacements in contact with blood (vascular grafts, valves). We also demonstrated the possibility to fabricate tubular-shaped composites that featured remarkable flexibility, capable of being bent up to 180° without kinking. Our study presents a simple and scalable biofabrication approach for obtaining composite scaffolds that leverage synergistic effects between fibrin and silk fibroin. By combining the exceptional bioactivity of fibrin with the mechanical stability offered by silk fibroin, we address the limitations associated with single material approaches in tissue engineering. The developed composite scaffolds, with their outstanding flexibility and the ability to be molded in various shape, hold great promise for cardiovascular tissue engineering, advancing the field and offering potential solutions to challenges in single-material constructs.

1. El Maachi *et al.*; Polymers 2022.

The authors acknowledge the NanoMatFutur Program (German Ministry of Education and Research, grant number 13XP5136).

PoA.10.64

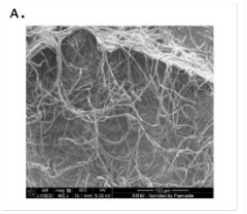
Fibrin Gel as tissue-mimicking material: synthesis optimization and characterization

Sabrina Caria[1], Jessica Petiti[2], Sara Venturi[3], Mattia Pegoraro[2], Federica Celegato[2], Andrea Lapini[4,2], Marco Pisani[2], Francesca Orso[5], Federico Picollo[1], Carla Divieto[2]

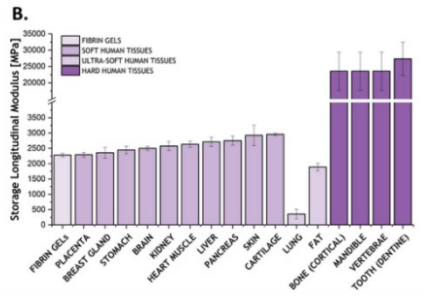
[1]Università degli Studi di Torino, Turin, Italy. [2]Istituto Nazionale di Ricerca Metrologica (INRiM), Turin, Italy. [3]European Laboratory for Non-Linear Spectroscopy (LENS), Florence, Italy. [4]Università di Parma, Parma, Italy. [5]Università del Piemonte Orientale, Novara, Italy

Abstract

Fibrin gel is a promising biomaterial for biomedical research thanks to its intrinsic porosity, shape-shifting capability, visco-elasticity, and biodegradability. Currently, it is rarely used in research because of stability issues, in terms of durability and mechanical properties. Final aim of this work is fibrin gel synthesis optimization and characterization to overcome its main drawbacks and demonstrate its potential and advantages as tissue-mimicking material. *In vitro*, fibrin gel formation occurs through a sol-gel reaction of fibrinogen in presence of thrombin, Na^+, and Ca^{2+} ions. For fibrin gel synthesis, fibrinogen is dissolved in 0.9% NaCl solution and cell culture medium (1:1 ratio), and thrombin and $CaCl_2$ are dissolved in MilliQ-H_2O. This protocol allows repeatable and reproducible synthesis of long-lasting fibrin gel respecting physiological concentration of each reagent.Physical and biological, i.e. cytocompatibility, characterization gave the results described below.Morphological characterization by low-vacuum SEM highlighted that fibrin gel consists of fibers made of fibrin that aggregate together forming a 3D network where cell nutrients and oxygen can pass through (Fig.A). Bulk density measurements using pycnometer together with acoustic characterization, performed using transient grating spectroscopy, showed up compatibility between fibrin gel and human soft tissues in terms of density and speed of sound. Furthermore, density and sound velocity propagation results allowed calculation of storage longitudinal modulus (M'), and again a correlation between fibrin gel and human soft tissues was observed (Fig.B).Preliminary cytocompatibility tests, by resazurin-based cell viability test, revealed that human tumor cells (A549), whose doubling time is about 24 hours in 2D culture (from literature), almost triplicate in 5 days when embedded in fibrin gel. Moreover, the effect of cell culture medium as component of fibrin gel was also evaluated to show its importance in supporting cell survival and proliferation within the gel. Long-term experiments of 21-day and 60-day showed that fibrin gel, synthesized by following this protocol, did not degrade in presence and absence of cells, respectively. All physical parameters considered, including M', are repeatable and reproducible emphasizing that repeatable and reproducible protocol results in stable physical properties. In conclusion, this work has led to significant progress in solving the two major problems that currently limit fibrin gel use in research. Results highlight fibrin gel ability to mimic soft human tissues, from both physical and biological perspectives, and pave the way to design novel studies exploiting fibrin gel's intrinsic advantageous properties as 3D scaffold.



Morphological characterization result.



M' results.

PoA.10.65

Defined Substrate Induced Fibroblast Epigenetic Reprogramming

Kang Lin, Kristopher Kilian

UNSW, Sydney, Australia

Abstract

Induced pluripotent stem cells (iPSC) revolutionized the field of regenerative medicine and disease modelling in recent years, but its clinical viability has been hindered by limited reprogramming efficiency and the usage of exogenous factors. Here I will present a vector free approach to induce plasticity in somatic cells through epigenetic reprogramming[1]. A combination of extracellular matrix (ECM) proteins are covalent conjugated on a thin layer of polyacrylamide (PA) gel to create controlled biophysical and biochemical cues, and defined topography promotes epigenetic reprogramming and pushes varied cell types towards pluripotent phenotypes in the absence of exogenous factors. The reprogrammed cells express pluripotent markers, forming spheroids or aggregates that are prone to 3D bio-assembly in various biomaterial substrates, and are capable of performing trilineage differentiation. The onset of mechanical induced epigenetic reprogramming correlates with elevated autophagy activities, which promote cytoplasmic remodeling by reducing cell size and complexity. Moreover, confined substrate priming improves fibroblasts reprogramming efficiency substantially and shorten the reprogramming process when incorporate with standard reprogramming through viral transduction.

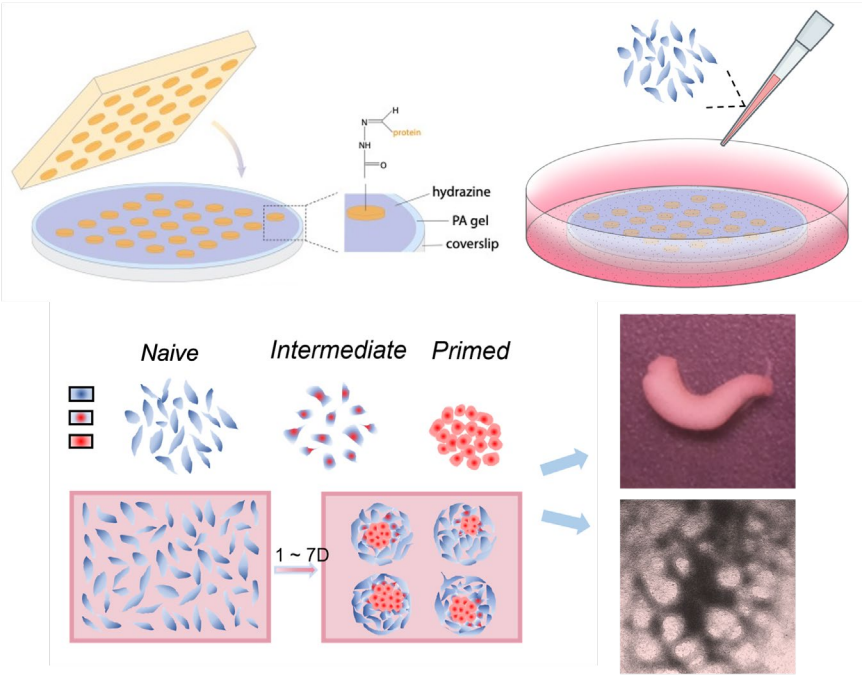


Figure 1. Schematic representation of polyacrylamide (PA) gel patterning and subsequent tissue self-assembly and differentiation.

Reference
1 Romanazzo, S. et al. Adv. Drug Deliv. Rev., 2020, 161, 124-144

PoA.10.66

Development of Poly(acrylic acid)-Cysteine-Based Hydrogels with Tailorable Mechanical Properties for Advanced Cell Culture Applications

Ellen Kennedy

University of Limerick, Limerick, Ireland

Abstract

Accurate mimetic *in vitro* cell models have become crucial in drug development, disease research, and regenerative medicine. However, traditional cell culture studies have primarily focused on 2-D environments, despite cells existing in a 3-D extracellular matrix in their natural state, surrounded by proteins and growth factors. Human tissues exhibit a stiffness of 1 to 50 kPa, while standard cell culture flasks are much stiffer at 1x107 kPa (fig. 1). This significant difference in stiffness greatly influences cell behaviour, including proliferation, differentiation, and survival. Therefore, replicating these physiological conditions is essential for obtaining accurate and reliable results in in-vitro studies.

With this objective in mind, a project was initiated to develop a hydrogel capable of bridging the gap between 2-D and 3-D cell culture for various tissues. The main focus of this study revolved around creating a customizable hydrogel with adaptable properties to enhance advanced cell culture applications.

Poly(acrylic acid) has been extensively used in hydrogel development over the years. However, its inherent biological compatibility is limited. To overcome this challenge, the study enhances the biocompatibility of poly(acrylic acid) by introducing cysteine onto the polymer. This modification creates a polymer with mucoadhesive properties, facilitating cell adhesion.

Upon creation of a biocompatible base polymer, this study aims to tailor three major properties of the hydrogel system: tissue architecture, biomechanics, and biological factors. Tissue architecture will be addressed through the utilization of 3-D inkjet printing techniques, enabling the creation of intricate structures resembling *in vivo* architecture. Biomechanics will be manipulated by adjusting the crosslinking process and incorporating surface treatments to modify the material's elasticity and topography, respectively. Furthermore, soluble protein factors commonly found in native tissues will be incorporated into the hydrogel by conjugating them to free thiol groups.

This project is a step towards revolutionizing cell culture studies by producing a hydrogel capable of accurately replicating the 3-D environment in which cells naturally exist. This work enhances our understanding of biological processes and makes significant contributions to the advancement of drug therapies and regenerative medicine techniques.

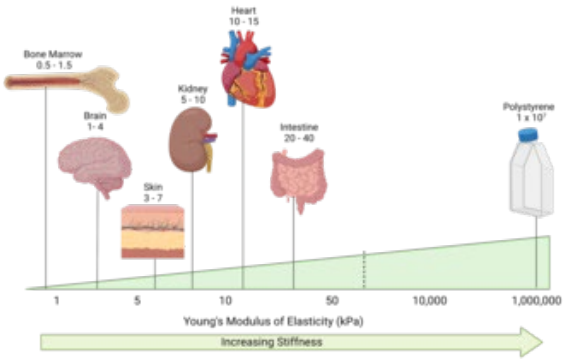


Fig 1: Demonstrating the difference in stiffness, given as Young's Modulus, between polystyrene culture flasks and in vivo organs.

PoA.10.67

Development of Photo-Cross-Linkable Catechol Conjugated hybrid Tissue Sealant Hydrogels

Ahmet Erdem[1], Dilara Küçük[1], Feyzanur Şentürk[1], Aygün Iseyeva[1], Umit Gülyüz[2], Ufuk Yıldız[1], Gökhan Duruksu[1]

[1]Kocaeli University, Kocaeli, Turkey. [2]Kırklareli University, Kırklareli, Turkey

Abstract

Gelatin-based hydrogels derived from extracellular matrices have emerged as promising candidates for sealing leaks in seamless wound closure surgical procedures. However, gelatin-derived hydrogels exhibit low mechanical properties and weak adhesion. To enhance the performance of these hydrogels, hybrid structures have been created using various materials, as well as by incorporating catechol modifications inspired by mussel adhesion. Nevertheless, the impact of the catechol group on the bioadhesive capacity of methacrylate gelatin (GelMA)-based hydrogels, which can crosslink through free radical-mediated reactions, remains a topic of discussion. In this study, new hybrid bioadhesive formulations were developed by incorporating bifunctional methacrylate and catechol-modified alginate (AlgMaC) into GelMA. The tissue adhesion properties, physical characteristics, biocompatibility, and ex vivo performance of the created hydrogels were examined. The effects of post-crosslinking ionically with Fe3+ ions and oxidatively with NaIO4 solution on the adhesion performance were compared for the bioadhesive compositions obtained via photopolymerization. It was observed that an increase in the content of highlighted catechol molecules led to lower adhesive performance for typical wet adhesion in bioadhesive materials. Furthermore, the use of NaIO4 in the bioadhesive formulations was found to enhance the adhesion performance compared to ionic crosslinking slightly. This study provides an essential foundation for the future use of bifunctional materials in wound closure applications.

PoA.11.01

A biodegradable polycarbonate with immunomodulatory properties

Fei Chen, Stuart Young, Brian G Amsden

Queen's University, Kingston, Canada

Abstract

Introduction: Macrophages play a central role in the nature of the inflammatory response and several strategies aimed at encouraging their transition from a pro-inflammatory to a pro-regenerative phenotype are being explored. Herein, the effect of butyrate conjugation to a degradable polycarbonate backbone on macrophage polarization and fibrous capsule formation following subcutaneous implantation in rats was examined.

Methods: Poly(trimethylene carbonate-co-benzyloxy carbonate) (P(T-BT) was synthesized and characterized as in [1]. Following removal of the benzyloxy groups the resulting hydroxyls were reacted with butyryl chloride to form: P(HT-T)-30-70, P(BtT-T)-40-60, and P(BtT-HT-T)-15-25-60 (Figure 1). Spherical samples (60-80 mg) of the copolymers were sterilized and implanted in subcutaneously in the dorsa of Wistar rats. The rats were humanely euthanized and the copolymers and surrounding tissue harvested on weeks 1, 2, 6. Degradation of the copolymers was measured via ^{1}H NMR. The tissue and remaining polymer were cryosectioned, stained with Masson's trichrome and immunostained for CD68, CCR7 and CD163. Following imaging, total macrophage (CD68+), M1-polarized (CD68+CCR7+) and M2-polarized (CD68+CD163+) macrophages were counted. Fibrous capsule thickness was measured from Masson's stained images.Results and Discussion: The HT containing polymers degraded via an intramolecular cyclization reaction. The HT molar ratio for both P(HT-T)-30-70 and P(Bt-HT-T)-15-25-60 decreased with time while the molar composition for P(BtT-T)-40-60 did not change. The BtT content of the retrieved P(BtT-HT-T)-15-25-60 samples remained constant. Other than week 2, where the fraction of CD68+CCR7+ cells present in the inflammatory zone was higher for the P(BtT-T)-40-60 implants, there was no significance difference in the fraction of M1 macrophages present. However, the fraction of CD68+CD163+ cells present in the inflammatory zone was significantly greater for the P(BtT-HT-T)-15-25-60 implants than the other two copolymers at weeks 1 and 2, and the P(BtT-T)-40-60 implants at week 6. A thin fibrous capsule was present around all polymers by week 2. The thickness of the capsule was significantly greater at week 6 around the P(HT-T)-30-70 and P(BtT-T)-40-60 than around the P(BtT-HT-T)-15-25-60. The non-degradable P(BtT-T)-40-60 had the thickest capsule at all time points.

Conclusion: Collectively, the data indicates that soluble oligomer degradation products bearing a pendant butyrate group induced macrophage polarization towards an M2 phenotype.

S. Mohajeri et al. Mol. Pharm. 2020, 17:1363.

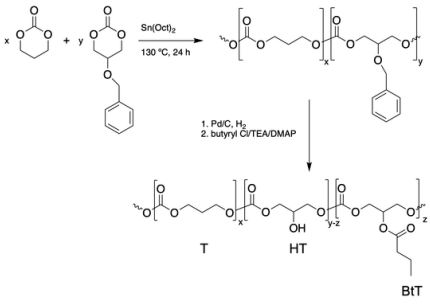


Figure 1. Synthesis route to P(BtT-HT-T)

PoA.11.02

Using Alginate Hydrogel Niches to Steer Monocyte Derived Macrophage Polarization by Viscoelasticity

Raphael S. Knecht[1], Georg N. Duda[1], Katharina Schmidt-Bleek[1], David J. Mooney[2]

[1]Julius Wolff Institut at Charite – UniversitätsmedizinBerlin, Berlin, Germany. [2]Harvard University, Boston, USA

Abstract

INTRODUCTION: A misdirected or imbalanced local immune composition is often one of the reasons for unsuccessful regeneration resulting in scarring or fibrosis. While biomaterial-based approaches to control local immune responses are emerging as potential new treatment options, the extent to which biophysical material properties themselves play a role in modulating local immune reactions has so far been considered only occasionally.

METHODS: Here, immunologically inert alginate hydrogels were used to specifically mimic the elastic and viscoelastic properties of tissues. To ensure a controlled cell-matrix interaction, the alginate polymer was modified with a cell adhesive motif (RGD) using distinct ways of carbodiimide chemistry. Monocyte-derived macrophages were then placed and encapsulated (3D) in alginate hydrogels with distinctly different elastic and viscoelastic properties. Subsequently, the expression of macrophage polarization markers was analyzed using flow cytometry. In addition, a multiplex ELISA was performed to assess cytokine secretion.

RESULTS: High molecular weight alginate (MVG) or low molecular weight alginate (VLVG) alginate with Ca2+ concentration of 28mM or 32mM for crosslinking showed elastic modulus of 13 kPa and 38 kPa, respectively. The stress relaxation half time depends mostly on the molecular weight of the alginate. VLVG alginate showed faster stress relaxation (thalf,VLVG = 40 s) than MVG alginate (thalf,MVG = 524 s). Within this study we were able to show that macrophage polarization can be influenced by material stiffness. 48h after encapsulation of the macrophages in the alginate hydrogels, the expression of the cell surface markers CD45, CD206, CD64, CD80, CD86 and HLA-DR seems to be modulated by the mechanical niche. While this effect was observed in macrophages, monocyte derived dendritic cells exposed to the same mechanical cues, did not show changes in their surface markers.

DISCUSSION & CONCLUSIONS: Native materials are characterized by distinct material properties. Viscoelastic material properties have more recently been introduced as a relevant key parameter determining cell fate. Alginate hydrogels can be used to mimic a wide range of viscoelastic properties of biological tissues, and thus allow to investigate and control the impact of mechanical niche properties on cells to be studied more comprehensively. Here we show that the mechanical niche alters the macrophage phenotype in a different manner than the classical cytokines for macrophage polarization (IL-4+ IL-13 for M2 and IFN-y + LPS for M1). Our data illustrates the potential of ECM and its biophysical properties, specifically the viscoelastic properties to impact macrophage polarization in their distinct 3D niche.

PoA.11.03

Modulation of Macrophage Phenotypes by Wound Dressings of Different Biomaterial Composition

Shirin Saberianpour[1], Gianluca Melotto[2], Lucy Redhead[2], Rachel Forss[2], Matteo Santin[1]

[1]1 Centre for Regenerative Medicine and Devices, University of Brighton 2School of Applied Sciences, University of Brighton, Brighton, United Kingdom. [2]1 Centre for Regenerative Medicine and Devices, University of Brighton 3 School of Sport and Health Sciences, University of Brighton, Brighton, United Kingdom

Abstract

Introduction: Wound dressings are medical devices for the treatment of severe wounds such as chronic, non-healing wounds. Dressings are developed using biomaterials that can fulfil these requirements while being suitable for different types of wounds, i.e., those relatively dry (e.g.e.g.,thetic polyesters), those with producing copious exudate (e.g. carboxymethyl cellulose) and those presenting bleeding (e.g. alginate). This *in vitro* study provides a systematic analysis of the host response towards wound dressings made of different biomaterials.

Methods: Commercially available wound dressings were chosen and categorised in three groups: synthetic polymer-based (Melolin, Atrauman, N-A, for dry wounds), CMC-based (Aquacell, Keramax and Keracell, for wounds with copious exudate) and alginate (Kaltostat, for bleeding wounds). U-937 cells including monocytes/macrophages and lymphocytes were incubated (1 x 105 cell/2ml) with wound dressing coupons (1.5×1.5cm) in RPMI medium enriched with 10% foetal bovine serum (FBS), 24h, 37oC, static conditions. Wound dressings were fixed with 4% paraformaldehyde and immuno-stained with antibodies against CD68, CD19 and CD206 markers and analysed by confocal microscopy. ELISA tests for IL-6, TGF-a, CXCR4, FGF markers were performed on the supernatants. Data were analysed in relation to the chemical composition (FTIR analysis) and swelling properties of the dressings in simulated body fluids (SBF) and FBS.

Results: The combination of swelling experiments and FTIR revealed the relative hydrophobicity of the dressings based on synthetic polymers and of the CMC-based Keramax. Aquacell was the dressing able to absorb the highest amounts of fluids (85% w/w), while Kaltostat swelling was visually visible, but difficult to measure because of its relatively fast degradation process. Aquacel, Melolin and Kaltostat showed the highest number of adhering cells, but with different percentages of post-inflammatory, pro-regenerative phenotype (CD206+, M2) (Aquacell: 66%, Melolin: 22.6%, Kaltostat: 47%) (Figure 1, Table 1). These data correlated to the highest levels of TGF-b release induced by these dressings. ELISA showed that Kerramax induced the highest release of the pro-inflammatory cytokine IL-6 as well as of the cell migration factor CXCR4 and FGF (Table 1).

Conclusions: The present study suggests that although the total number of adhering monocytes/macrohpages did not relate to the biomaterial physicochemical properties, relatively more hydrophilic materials such as Aquacel and Kaltostat showed a high ratio of adhering M2 macrophages releasing growth factors able to stimulate wound regeneration.

Acknowledgment: This work has been supported by the UKRI EPSRC grant n. EP/W023164/1

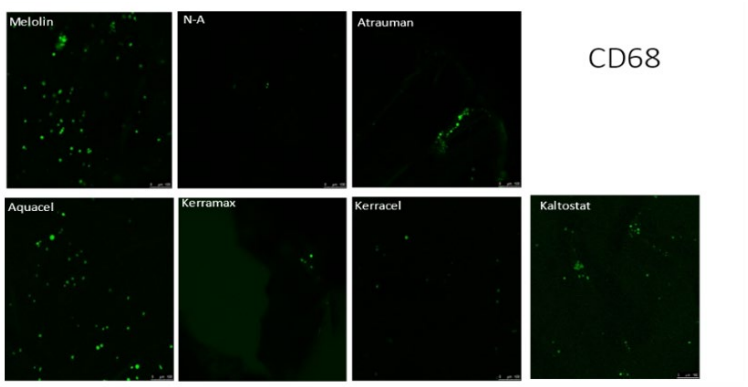


Figure 1. CD68+ U937 cell adhesion on wound dressings.

Table 1	Pro-inflammatory markers			Post -inflammatory markers		
Wound dressing	CD68 Mean cell n/field ±SD	IL-6 Mean O.D (450nm)/mg of protein ±SD	CXCR4 Mean O.D (450nm)/mg of protein ±SD	CD206 Mean cell n/field ±SD	TGF-b Mean O.D (450nm)/mg of protein ±SD	FGF Mean O.D (450nm)/mg of protein ±SD
Kerracel	4±1.41	1.014±0.59	0.633±0.053	4.5±0.70	0.093±0.034	0.7955±0.29
Kerramax	5±1.41	2.075±0.28	1.646±0.418	2.5±0.70	0.086±0.01	1.477±0.089
Aquacel	32.5±3.53	0.52±0.14	1.364±1.411	21.5±12.02	0.833±0.150	0.681±0.021
Melolin	62±2.82	0.776±0.13	0.602±0.111	14±1.41	0.57±0.066	0.77±0.201
N-A	10.5±6.36	0.533±0.031	1.3565±0.67	10.5±6.36	0.195±0.166	1.122±0.206
Atrauman	4.5±0.70	0.63±0.038	0.8255±0.106	4±1.41	0.109±0.018	0.81±0.004
Kaltostat	40.5±4.94	0.3235±0.086	0.5135±0.033	19±5.656854 249	0.620.629	0.74±0.192

PoA.11.04

Engineered amphiphilic cyclodextrin nanoparticles for macrophage repolarization.

Felix Brettner, Stefanie Gier, Annika Haessler, Sarah Vogel-Kindgen, Maike Windbergs

Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany

Abstract

Macrophages (MΦ) play key roles in innate and adaptive immune responses as well as in tissue development and function. They can display a wide range of phenotypes, orchestrated by surrounding microenvironmental (patho)physiological stimuli and signals. Resting MΦ0 can be activated to the pro-inflammatory MΦ1 or anti-inflammatory MΦ2 phenotype, having different functions as well as transcriptional profiles. Precise control of macrophage phenotype is crucial for tissue homeostasis, remodeling, repair or regeneration. Bioactive compounds from natural sources could be an appealing alternative to synthetic drugs for macrophage modulation and polarization. It has been shown, that cyclodextrins (CDs), naturally occurring oligosaccharides and FDA-approved pharmaceutical excipients, exert anti-inflammatory effects, with the underlying mechanisms being not completely understood yet. After administration of hydroxypropyl-beta-CD solutions, reduced levels of pro-inflammatory cytokines, increased cholesterol efflux and macrophage reprogramming were observed.

In this work, we engineered immunoinstructive, drug-free amphiphilic cyclodextrin nanoparticles to control macrophage polarization. With the aim to decipher structure-function relationships, a library of amphiphilic CD derivatives was synthesized, nanoparticles in the size range of 150 to 300 nm were fabricated by CD self-assembly upon nanoprecipitation and their potential to polarize macrophages was investigated *in vitro* (Fig 1A).

Incubation of MΦ1 with the nanoparticles resulted in altered levels of pro-inflammatory cytokines compared to untreated control cells with the effect being dependent on the structure of the CD derivative (Fig. 1B). Further, mRNA coding for pro-inflammatory cytokines and MΦ1-specific surface markers was significantly reduced compared to untreated control cells. Lipidomic profiling using label-free Raman microscopy revealed restoration of lipid abundance similar to MΦ0 after particle treatment of MΦ1 (Fig. 1C). While the lipid spectra of MΦ1 revealed high amounts of polyunsaturated fatty acids (PUFA), both, MΦ0 and particle treated MΦ1, displayed larger Raman signal intensities for saturated fatty acids compared to the PUFA signal intensity.

In conclusion, exploitation of the immunomodulatory properties of amphiphilic CD nanoparticles revealed their potential as drug-free biotherapeutics for macrophage repolarization. Based on their versatile chemistry, amphiphilic CD derivatives provide a platform for engineering nanocarriers with tailor-made control over inflammatory processes.

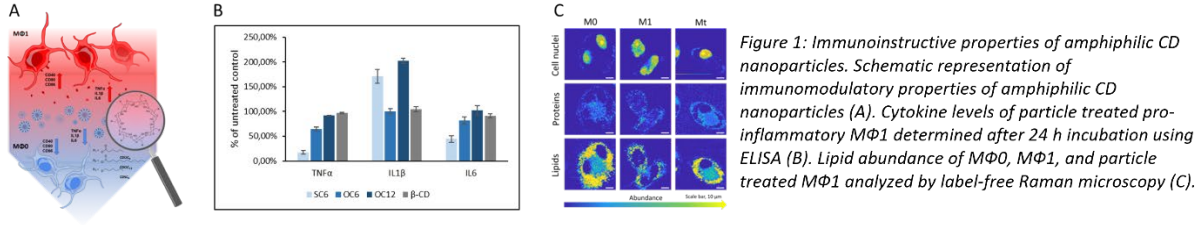


Figure 1: Immunoinstructive properties of amphiphilic CD nanoparticles. Schematic representation of immunomodulatory properties of amphiphilic CD nanoparticles (A). Cytokine levels of particle treated pro-inflammatory MΦ1 determined after 24 h incubation using ELISA (B). Lipid abundance of MΦ0, MΦ1, and particle treated MΦ1 analyzed by label-free Raman microscopy (C).

PoA.11.05

Maresin-1 Loaded Zein Nanoparticles as Inducers of Pro-regenerative Microenvironments for the Treatment of Chronic Wounds

Ana Beatriz Sousa[1,2,3], Judite Novais Barbosa[1,2,3], Mário Adolfo Barbosa[1,2,3]

[1]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [2]INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [3]ICBAS – Instituto Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Abstract

Introduction: The incidence of chronic wounds is escalating worldwide. The main goal of this research work is to develop zein nanoparticles (NPs) loaded with maresin-1 (Mar1) to induce a pro-regenerative microenvironment that will accelerate wound healing, ultimately providing an effective strategy for the rapid healing of chronic wounds. Zein is an insoluble prolamin protein that is extracted from corn. The use of zein has been reported for several applications, such as platforms for drug delivery and for tissue engineering. Maresin-1 is a potent immunoresolvent, biosynthesized in inflammatory exudates. To control inflammation, it stimulates the resolution programs, limits polymorphonuclear (PMN) leukocytes infiltration, enhances macrophage uptake of apoptotic PMNs, and affects immune cells polarization.
Methods: The Dolomite Microfluidics® chip was used as a platform to load Mar1 into zein NPs. Briefly, the nanoparticles were produced by flow-focusing the organic central stream (containing a mixture of 1% (w/v) of zein and Mar1 in 70% ethanol) with the aqueous outer fluid (Milli-Q water). Three different concentrations of loaded Mar1 were tested: 10, 50, and 100nM. The ability of the developed NPs to affect macrophage viability and polarization was assessed in primary human macrophages (isolated from buffy coats of healthy blood donors). Cell viability was evaluated through the LDH assay, and macrophage polarization was assessed by flow cytometry analysis, using CD14 as a pan macrophage marker, CD86 as an M1 marker and CD163 as an M2 marker.
Results**:** The obtained results showed that the NPs (whose characteristics are illustrated in Figure 1) induced an increase in macrophage polarization towards an M2-like phenotype, when compared with zein nanoparticles without Mar1. In addition, the higher concentration of Mar1 loaded into the NPs led to a higher number of M2-like macrophages out of all experimental conditions. Moreover, the developed NPs did not present cytotoxicity.
Conclusion: The developed NPs, in particular the ones with a higher concentration of Mar1, were able to modulate macrophage polarization towards an M2 anti-inflammatory and pro-regenerative phenotype. As such, the herein described NPs will be able to promote pro-regenerative microenvironments.

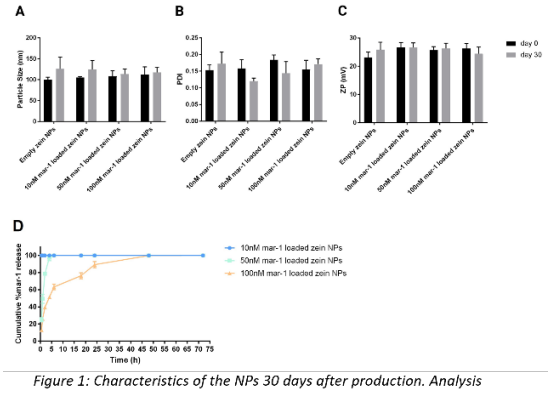


Figure 1: Characteristics of the NPs 30 days after production. Analysis performed for (A) size, (B) PDI, (C) zeta potential, and (D) release profile.

Acknowledgments
The authors would like to thank Norte Regional Operational Program: Structured R&D&I Projects, Unorte.pt. HEALTH-UNORTE; NORTE-01-0145-FEDER-000039 for providing financial support to this research work, and to the BiotechHealth PhD Program for funding their attendance at the congress.

PoA.11.07

Magnetic responsive hydroxyapatite scaffold modulated macrophage polarization through PPAR/JAK-STAT signaling and enhanced fatty acid metabolism

Xiaoqing Sun, Yao Wu

National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, China

Abstract

Despite the widespread observations on bone repair of magnetic cue, the diverse roles of magnetic cue in macrophage response during bone healing have not been systematically investigated. Herein, by introducing magnetic nanoparticles into hydroxyapatite scaffolds, an appropriate and timely transition from proinflammatory (M1) to anti-inflammatory (M2) macrophages during bone healing is achieved. The combined use of proteomics and genomics analysis reveals the underlying mechanism of magnetic cue-mediated macrophage polarization form the perspective of protein corona and intracellular signal transduction. Our results show that intrinsically-present magnetic cues in scaffold contribute to the upregulated peroxisome proliferator-activated receptor (PPAR) signals, and the activation of PPAR signal transduction in macrophages results in the downregulation of the Janus Kinase-Signal transducer and activator of transcription (JAK-STAT) signals and the enhancement of fatty acid metabolism, thus facilitating M2 polarization of macrophages. Magnetic cue-dependent changes in macrophage benefit from the upregulation of adsorbed proteins associated with "hormone" and "response to hormone", as well as the downregulation of adsorbed proteins related to "enzyme-linked receptor signaling" in the protein corona. In addition, magnetic scaffolds may also act cooperatively with the exterior magnetic field, showing further inhibition of M1-type polarization. This study demonstrates that magnetic cues play critical roles on M2 polarization, coupling protein corona, intracellular PPAR signals and metabolism.

PoA.11.08

Size Matters: The Immunoregulatory Role of Hyaluronan Molecular Weight on Dendritic cells within 3D biomimetic microenvironments.

Brian C Quartey, Jiranuwat Sapudom, Jeremy CM Teo

New York University Abu Dhabi, Abu Dhabi, UAE

Abstract

Dendritic cells are a type of immune cell and are vital as they serve as the key intermediates between the innate and adaptive immune response. They are highly specialized for antigen presentation and hence able to scavenge tissues, recognize foreign antigens, process them and efficiently present them to T and B lymphocytes to activate the adaptive immune response. Increasing evidence suggests that the immune potency of dendritic cells are regulated by various biological factors, particularly the various components in the extracellular matrix. Among these different extracellular matrix molecules, is hyaluronan (HA), a glycosaminoglycan which has been shown to exhibit immunoregulatory properties. What makes HA interesting is that it exhibits immunoregulatory effects on macrophages, and the magnitude of this immunoregulation is dependent on the molecular weight of the glycosaminoglycan. Under normal physiological conditions HA is synthesized by cells such as fibroblasts in high molecular weight forms. However, during certain pathological conditions such as in inflamed tissues, wound or cancer tissues, these high molecular weight HA may be broken down into smaller molecular weight fragments. In this work we first established 3D fibrillar collagen matrices with immobilized HA of different molecular weights. Further, we characterize the cytokine binding capabilities of each of our collagen-HA matrix conditions to provide understanding how collagen as well as the different molecular weight HA within tissues may act as a reservoir for cytokines, thereby controlling their availability and activity. Using our established 3D models, we investigated how the different molecular weight hyaluronan namely low molecular weight HA (LMW-HA;8-15kDa), medium molecular weight HA (MMW-HA; 500-750kDa), and high molecular weight HA (HMW-HA; 1250-1500kDa) within physiologically relevant 3D biomimetic microenvironments differentially impact dendritic cell differentiation, maturation and functions, including antigen uptake and migration. We found that LMW-HA preferentially promotes pro-inflammatory immune response of DCs, while HMW-HA maintains DC homeostasis. Overall our results demonstrate that the different molecular weights of HA distinctively regulate immune response of immature and mature dendritic cells, revealing the immunoregulatory role of HA in physiological and pathological tissues.

PoA.11.09

Dual Responsive Nanoparticles for Precision Therapy of Rheumatoid Arthritis

Gizem Erensoy, Luca Dirk Menges, Tilia Selldén, Alexandra Stubelius

Chalmers University of Technology, Gothenburg, Sweden

Abstract

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that causes cartilage and bone destruction. Despite the improvement in clinical outcomes, a third of patients still fail to respond [1]. To achieve early and rapid disease remission, systemic glucocorticoids are used as adjunctive therapies, however, the risk of side effects may not outweigh the treatment benefits. In addition, RA's high pathogenic heterogeneity poses further issues hindering optimized treatment strategies [2]. The goal of this study was to develop more advanced and accurate drug-delivery systems. Our approach is based on the strategy that smart materials can react and release drug based on the pathogenic inflammatory microenvironment. The dynamic microenvironment of inflammation requires highly sensitive systems that can rapidly both turn ON, and crucially, also be turned OFF. Here, as a proof of concept, both pH and/or reactive oxygen species (ROS)-responsive dextran-based nanoparticles (NP) were formulated, tested for ability to turn ON/OFF, and evaluated for their immunomodulatory properties.
Methods: pH-responsive (AcDex) and ROS responsive (PBEDex) polymers were synthesized according to the literature [3,4]. NPs were synthesized by nanoprecipitation and characterized. Cell studies were performed using RAW264.7 macrophages, and subsequently analyzed by FACS for the expression of CD14 (PE, Biolegend) and CD80 (BV510, Biolegend).
Results: Physicochemical characterization showed the hydrodynamic size of AcDex58% particles to be 214.16±1.51 nm, with a zeta potential of 15.1±0.96 mV. Release was measured over time, demonstrating a sensitive system at pH 6 (Fig 1A), and in a proof-of-concept study where pH alternated over time–representing ON/OFF/ON–, a dynamic release system could be shown (Fig. 1B).
However, the study revealed that a more advanced material composition was needed due to a spontaneous release of the cargo at neutral pH. AcDex was therefore blended with PBEDex at a ratio of 1:1 (size 189.4 ±57 nm and charge -2.23 mV). The anti-inflammatory properties of dual-sensitive NPs were investigated by measuring the expression of pro-inflammatory receptors on the cells using flow cytometry. The mean fluorescence intensity for CD14+ and CD80+ receptors decreased after cells were treated with NPs at indicated dosing, displaying a clear anti-inflammatory dose dependency.

References:
1. Taylor PC. Rheumatol Int. 2016;36:685.
2. Lewis WJ. Cell Reports. 2019;28(9):2455-2470.
3. Broaders KE. PNAS. 2009;106(14):5497-5502.
4. Manaster AJ. ACS Appl Bio Mater. 2019;2:3755-3762

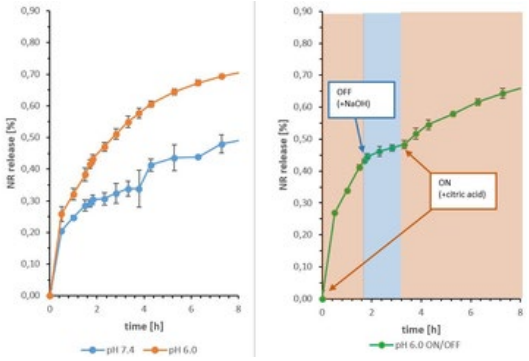


Figure 1: A) Release studies at acidic and neutral conditions B) ON/OFF/ON release system for AcDex58%.

PoA.11.11

Genetic Engineering of Immune Effector Cells using a Peptide Technology

Emma M McErlean[1], Nicholas J Dunne[2], Helen O McCarthy[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Dublin City University, Dublin, Ireland

Abstract

Introduction: Chimeric antigen receptor (CAR) therapy has revolutionised the field of cancer immunotherapy with further immune effector cells including macrophages and natural killer (NK) cells being explored to improve on CAR-T therapy[1]. CAR-Ts have had little success in treatment of solid tumours due to poor infiltration, immune evasion and heterogeneity in tumours[2]. CAR-macrophages (CAR-Ms) are currently under investigation for ability to infiltrate solid tumours[1]. NK cells exhibit potent natural cytotoxicity via unique activating receptors and ADCC[3] and have the advantage over CAR-T cells of unlimited use of allogeneic NK sources without concern of graft-versus-host disease; with potential for an 'off-the-shelf' CAR-NK product[4]. CAR-based immunotherapy necessitates efficient transfer of a CAR transgene, but safety concerns and expense associated with viral vectors warrants the development of non-viral delivery systems[5]. RALA is a 30mer cationic amphipathic peptide which is a highly effective multifunctional delivery system for nucleic acids such as DNA or mRNA, and small molecules, with industry acceptable characteristics at a clinically relevant scale[6,7]. In this study, RALA is investigated for genetic editing of CAR immune effector cells and production of advanced therapeutic medicinal products (ATMPs).

Methods: RALA/mRNA nanoparticles (NPs) were prepared using the automated NanoAssemblr Ignite system and lyophilised. NP physiochemical characterisation was performed by DLS, TEM imaging, encapsulation, and nucleic acid integrity. *In vitro* efficacy of RALA NPs delivering mRNA encoding GFP was assessed by FACS in immune cell lines DC2.4 Dendritic cells, RAW264.7 Macrophages, Jurkat T cells, THP-1 Monocytes and iPSCs.

Results and Discussion: RALA formed cationic NPs with mRNA following automated production, which remain stable and functional following lyophilisation. RALA/mRNA NPs transfected a range of immune effector cell lines including Jurkat T cells, THP-1 monocytes and iPSCs with minimal toxicity; demonstrating the potential of RALA as a non-viral alternative to viral vectors for development 'off-the-shelf' ATMPs.

Conclusion: This work provides proof of concept for the use of RALA as a transfection agent for ex vivo genetic modification of immune effector cells. Future work will evaluate transfection in NK-92 and KHYG-1 NK cell lines and primary NK cells, assessment of toxicity and NK phenotypic functional retention following transfection with RALA/mRNA NPs.

References

1. Pan,K.et al. J.Exp.Clin.CancerRes.2022;41:1–21.
2. Wrona,E.et al. Int.J.Mol.Sci.2021;22:1–19.
3. Stabile,H.et al. Front.Immunol.2017;8:1–8.
4. Liu,S.et al. J. Hematol.Oncol.2021:14.
5. Raes,L.et al. Biotechnol.Adv.2021;49:107760.
6. McCarthy,H.O.et al. J.Control.Release.2014;189:141–9.

PoA.11.12

Effects of low dose BMP-2 on fracture healing and cytokine levels in a femur segmental defect in rats

Maria Schröder[1], Lena Gens[1], Laura Bernhard[1], Daniel Arens[1], Dominic Gehweiler[1], Stephan Zeiter[1], Martin Stoddart[1], Esther Wehrle[1,2]

[1]AO Research Institute, Davos, Switzerland. [2]Institute for Biomechanics ETH Zürich, Zürich, Switzerland

Abstract

Stimulating bone healing using growth factors, delivered via an osteoconductive scaffold, represents an alternative to traditional methods like autologous bone grafting to promote healing1. Clinically applied bone morphogenetic growth factors like BMP-2 show good healing capacity but have been associated with excessive and prolonged pro-inflammatory cytokine release and heterotopic ossification in muscle at high doses2. This study aims to investigate the effect of low dose BMP-2 (1 µg), delivered locally using a collagen scaffold, on callus formation and cytokine levels in the fracture repair tissue (haematoma, callus) and adjacent muscle in a femur segmental defect model in rats. 2 mm segmental femoral defects were created in skeletally mature (19-24 weeks old) female F344 rats (n=3 per group), internally fixed with a 1.25 mm-thick polyetheretherketone plate (animal license: GR/19/2022;nat. number: 35156) using established protocols for analgesia and anesthesia. Animals received either no treatment (empty defect), a Lyostypt collagen sponge, or Lyostypt collagen sponge + 1 µg BMP-2 (InductOs, Medtronic) and were sacrificed at 3, 7 or 14 days. Radiographs were taken throughout the study to determine healing efficacy. In addition, the fracture repair tissue, as well as the adjacent muscle, were collected, snap frozen in liquid nitrogen, and stored at -80°C. Afterwards, the tissues were collected in T-PER tissue protein extraction reagent (Thermo Fisher) and the levels of interleukin 1β (IL-1β) were determined by ELISA (R&D Systems DuoSet). Radiographs demonstrate that collagen scaffolds with 1 µg BMP-2 induced pronounced new bone formation and cortical bridging 2 weeks post-operatively. IL-1β levels in the fracture repair tissue of all groups showed a decline from day 3 to 14 and no significant differences were found between any of the groups. Lower IL-1β levels were detected in the muscle than in the fracture repair tissue. In conclusion, low dose BMP-2 had a strong local effect on new bone formation during fracture healing in a femur segmental defect models in rats without inducing an excessive and pro-longed cytokine expression. Future work may explore the potential of immunomodulation strategies to counteract excessive and prolonged cytokines levels associated with clinically applied higher doses of BMP-2 and different biomaterials for the local delivery of this protein.

Acknowledgements: This project was supported by AO foundation. The author's acknowledge Andrea Further for *in vivo* micro-computed tomography scans.

References:

[1] Dimitriou et al. BMC Med. 2011; 9(66)

[2] James et al. Tissue Eng Part B Rev. 2016; 22(4):284-97

PoA.11.13

Multi-functionality of Dry-Etched Titanium (DETi): Exploring Immune Cell Interactions

Benedictus I.M. Eijkel, Lidy E Fratila-Apachitei, Iulian Apachitei, Amir A Zadpoor

Delft University of Technology, Delft, Netherlands

Abstract

Antimicrobial resistance (AMR) is a global concern driven by the misuse of antibiotics, necessitating the development of alternative approaches [1]. Dry-etched Titanium (DETi) nanopillars have shown bactericidal properties and the ability to prevent biofilm formation in addition to supporting the osteogenic functions of osteoprogenitor cells [2],[3]. The bactericidal effect depends on the physical interaction between bacteria and the surface, making the development of AMR towards these surfaces impossible. Despite its promising biofunctionalities, the impact on immune cell behaviour and its potential for immunomodulation remains unexplored in the context of antibacterial orthopaedic biomaterials [4].
This study investigates the interaction of J774A.1 macrophages with DETi and polished Titanium (pTi) surfaces. We demonstrate that the presence of DETi pillars influences the spatial organization and morphology of macrophages over time (Figure 1). Moreover, DETi has the potential to induce a more elongated morphology in macrophages relative to pTi after 24 hours of culture, indicative of anti-inflammatory macrophage polarization. Preliminary gene expression data reveals an upregulation of anti-inflammatory genes on DETi compared to pTi.
By focusing on the immune response, this study offers novel insights into the multiple biofunctionalities of DETi. The ability to modulate the immune response, directing it towards a short-lived antibacterial effect and subsequently enhancing immune-mediated osseointegration, represents a promising approach for biomaterial development. These findings contribute to the understanding of DETi's potential as a powerful biomaterial in the field of antibacterial orthopaedic biomaterials.

References

[1] World Health Organization, “The evolving threat of antimicrobial resistance Options for action,” 2012. Accessed: Aug. 24, 2022. [Online]. Available: www.who.int/patientsafety/en/.
[2] K. Modaresifar et al., “On the Use of Black Ti as a Bone Substituting Biomaterial: Behind the Scenes of Dual-Functionality,” Small, vol. 17, no. 24, p. 2100706, Jun. 2021, doi: 10.1002/smll.202100706.
[3] J. Hasan, S. Jain, and K. Chatterjee, “Nanoscale Topography on Black Titanium Imparts Multi-biofunctional Properties for Orthopedic Applications,” Sci. Rep., vol. 7, no. 1, p. 41118, Jan. 2017, doi: 10.1038/srep41118.
[4] F. Loi, L. A. Córdova, J. Pajarinen, T. Lin, Z. Yao, and S. B. Goodman, “Inflammation, fracture and bone repair,” Bone, vol. 86, pp. 119–130, May 2016, doi: 10.1016/j.bone.2016.02.020.

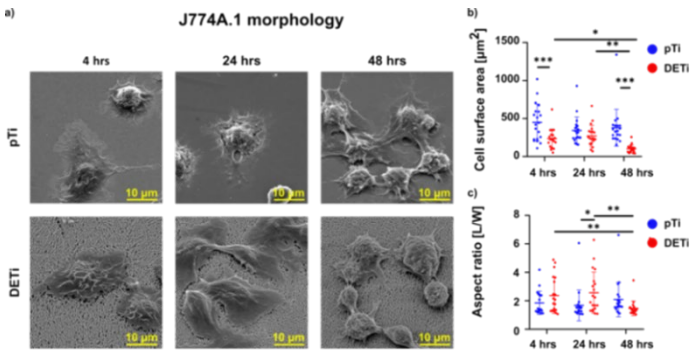


Figure 1. Morphological changes of J774A.1 cells over time: (a) SEM images of J774A.1 macrophages on polished Titanium (pTi) and Dry-Etched Titanium (DETi), 4, 24 and 48 hrs after seeding; (b) Mean cell surface area and (c) aspect ratio of randomly selected cells (n=20) on pTi and DETi surfaces.

PoA.12.01

Textural properties and the mechanism of pore formation in 45S5 bioactive glass microspheres fabricated by alkali activation-flame synthesis

Si Chen[1], Jozef Kraxner[1], Martin Michálek[1], Dušan Galusek[1,2]

[1]FunGlass – Centre for Functional and Surface Functionalized Glass, Trenčín, Slovakia. [2]VILA – Join Glass Centre of the IIC SAS, TnUAD, FChPT STU, Trenčín, Slovakia

Abstract

Nowadays, bioactive glasses are used in a wide range of applications including bone repair [1], soft tissue regeneration [1], wound dressings [2], drug delivery [3], cancer therapy [4] and disease diagnosis [4]. Different applications require different morphological and textural properties of bioactive glass. In the present work, 45S5 bioactive glass (45S5 BG) raw material was flame synthesised after alkali activation treatment to obtain bioactive glass microspheres with micron-sized pores (mPBGMs). The mPBGMs with different porosity and pore size distributions (Figure 1) were obtained by adjusting three parameters: i) activation time (0.5, 1 and 3 h), ii) NaOH concentration (0.5, 1 and 2 M) and iii) temperature (25 and 50 °C) during the alkali activation process. The porosity of the microspheres increased with increasing alkali activation time, temperature and NaOH concentration, with the exception of the sample activated for 3 h (1M-3h-25), which showed an inhomogeneous pore distribution. Samples activated at 50 °C (1M-1h-50) exhibited the highest porosity up to 54 ± 6 %. The size distribution of the pores in the mPBGMs ranged from 1-70 µm, with the majority of the samples showing maximum abundance of pores in the 1-10 µm range. The abundance of large-size pores could be significantly enhanced by increasing the concentration of NaOH. The internal structure, chemical composition, decomposition and thermodynamic behaviour of alkali-activated glass feedstock were analysed using micro-CT, XRD, Raman, FTIR and TGDTA in order to explore the mechanism of pore formation during alkali activation/flame synthesis. The formation of pores was based on a complex process of foaming of highly viscous sodium silicate solutions and the decomposition of carbonates and hydrates at high temperatures during the flame synthesis, while all relevant pore-forming compounds were produced by the alkali activation process.

ACKNOWLEDGMENT

This work is a part of dissemination activities of the project FunGlass. This project has received funding from the European Union´s Horizon 2020 research and innovation programme under grant agreement No 739566. The authors want to acknowledge Prof. Aldo R. Boccaccini and Assoc. Prof. Enrico Bernardo as the project partners. Financial support of this work by the grants APVV-20-0322 and VEGA 1/0057/23 is gratefully acknowledged.

PoA.12.02

Effect of common ion concentrations on bioactive glass dissolution *in vitro*

Minna Siekkinen, Markus Engblom, Leena Hupa

Åbo Akademi University, Turku, Finland

Abstract

Bioactive glasses are clinically used to treat bone defects, e.g., chronic bone infections. Bioactive glasses are highly reactive materials that release ions to the surrounding solution by an ion exchange with the solution, gradually resulting in a silica-rich layer and a precipitated hydroxyapatite layer. The hydroxyapatite then later bonds to bone apatite. The reactions and layer forming can be studied *in vitro* in conditions relevant to the human body to predict *in vivo* reactions.

This work utilized a dynamic *in vitro* experimental set-up, mimicking the dynamic human body, in which the bioactive glass particles were placed in a reactor. The solution was fed through the material. Three reactors were coupled in series and two different flow rates were used to feed the solution through the system. A solution buffered in the same range as the extracellular body fluid (Tris buffer) and an acidic solution (lactic acid) mimicking infected body tissue conditions were used. The experimental time was one day. The three reactors (*Fig. 1*) were assumed to describe different parts of an implanted glass particle bed. The first reactor mimics the outermost part of an implant with the first solution contact. In contrast, the following reactors mimic the implant's inner parts in contact with a solution containing common ions dissolved from the outermost part of the implant.

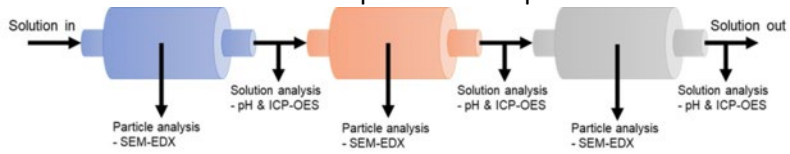


Fig. 1. Experimental set-up for dynamic in vitro experiment.

The results showed that common ions in the Tris solution decreased the bioactive glass dissolution and changed the layer formation on particles. The normalised surface-specific mass loss rate of each ion (*Fig. 2*) decreased with added reactors in the series. In addition, a lower flow rate resulted in a lower mass loss rate. The results indicate that particles inside an implant react slower compared to outer particles. In contrast, the dissolution of bioactive glass in the acidic environment was not notably affected by an increase in the common ion concentrations in the solution. The dissolution of calcium, sodium, and phosphorous was extensive, leaving a residual silica-rich layer on the surface, without the possibility of bone bonding through a precipitated hydroxyapatite layer. The results suggest that bioactive glass implanted at a healthy site would react non-uniformly. In contrast, particles implanted at an infection site would react more uniformly in the particle bed.

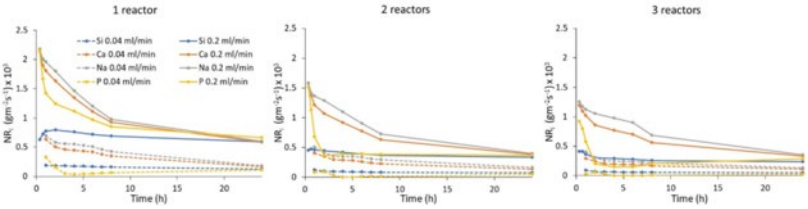


Fig. 2. Normalised surface-specific mass loss rate for bioactive glass particles in dynamic dissolution with Tris buffer for 24 h.

PoA.12.03

Structure and antibacterial activity of strontium silicate nanoparticles

Yun-Ru Huang, Shinn-Jyh Ding

Institute of Oral Science, Chung Shan Medical University, Taichung, Taiwan

Abstract

Nanoparticles have been proven to effectively affect the release of components, control the physicochemical properties of materials, and thus stimulate cellular functions around nanoparticles. Therefore, nanoparticle materials can be used as biomolecular carriers, antibacterial agents, 3D bioinks, and gene therapy vectors. Among these materials, strontium (Sr) has received great attention. *In vitro* and *in vivo* studies have confirmed that Sr can promote bone formation and inhibit bone resorption. Silicon (Si) contributes to bone formation and is an important factor in bone development and repair. Sr is generally used to replace Ca ions in Si-based bioactive glass or hydroxyapatite to increase ion dissolution rate, improve cell attachment and proliferation, and enhance the expression levels of alkaline phosphate, osteonectin and osteocalcin genes. However, pure strontium silicate materials are usually developed for phosphors and rarely used in medical applications. For this reason, it is necessary to prepare strontium silicate (SrSi) nanoparticles to explore its medical application. In this study, we used the commonly used cetyltrimethylammonium bromide-assisted precipitation method to prepare nanoscale SrSi particles by adjusting the pH of ammonia solution and the molar ratio of Sr to Si precursor. Tetraethylorthosilicate (TEOS) and strontium nitrate were used as precursors for the Si and Sr components, respectively. After precipitation, the synthesized SrSi powders were calcined in air at 800 °C to remove the surfactant template. The experimental results showed that when using 2% NH4OH and an equimolar ratio of Sr and Si precursors (2NSrSi), a broad peak appeared around 2θ = 15~30°, indicating the presence of a large amount of Sr-containing amorphous SiO2. Conversely, increasing the Sr(NO3)2 precursor content or the higher NH4OH concentration led to the formation of the SrSiO3 phase. Regarding the morphology, the 2NSrSi powder had a diameter of about 100 nm and presented spherical aggregates. In addition, with the increase of NH4OH concentration and Sr(NO3)2 precursor content, higher Sr/Si ratio and larger average particle size were obtained. Notably, the higher the Sr content in the powder, the greater the antibacterial activity of the powder against E. coli was found. The minimum inhibitory concentration can be low as 100 mg/mL, and the bacteriostatic ratio was 94%. It is concluded that the composition, morphology, and antibacterial activity of SrSi nanoparticles depended on the synthesis parameters and could be tailored for medical applications.

PoA.12.04

In vitro Mineralization Properties of Dental Filling Materials

Timo Peltola[1], Minna Siekkinen[2], Leena Hupa[2], Eija Säilynoja[1,3]

[1]Research Development and Production Department, Stick Tech Ltd – a GC Europe AG Company, Turku, Finland. [2]Johan Gadolin Process Chemistry Centre, Åbo Akademi University, Turku, Finland. [3]Department of Biomaterials Science and Turku Clinical Biomaterial Center (TCBC), Institute of Dentistry, University of Turku, Turku, Finland

Abstract

INTRODUCTION: Collagen and minerals constitute the main components of human dentin. The mineral layer is, unfortunately, destroyed during the caries treatment procedure. Thus, the remineralization of dentin is of great significance for restoring demineralized dentin.
Essential factors for mineralization are an optimal surface structure together with free calcium and phosphate ions originating from the surrounding tissue, the surrounding environment or released from the material itself. The aim of this study was to investigate the *in vitro* mineralization properties of different commercial and experimental dental filling materials.
MATERIALS AND METHODS: Studied commercial materials were Surefil One (Denstply Sirona), TheraCal LC (BISCO Dental) and GC Fuji II LC (GC Ltd). Also, an experimental short fiber reinforced resin-modified glass ionomer cement (SFR RMGIC) was studied.
Sample materials were placed in a reactor, and simulated body fluid (SBF) was fed through the reactor for 72 hours with a peristaltic pump (0.04 ml/min). Ion concentrations in the outflow were measured with inductively coupled plasma optical emission spectroscopy (ICP-OES). Scanning electron microscopy (SEM) examination was done after continuous flowthrough of SBF (top view) to determine the material surface structure.
RESULTS AND DISCUSSION: For TheraCal LC, a distinct calcium phosphate formation on material surface was observed. However, no release of calcium or phosphorus was analyzed. Only experimental SFR RMGIC showed a small burst of calcium and phosphorus at the beginning of solution feed. Surefil One and Fuji II LC were more stable materials in SBF. Both resin-modified glass ionomer materials (Fuji II LC and SFR RMGIC) also showed a small burst of strontium. Strontium is known to provide potential antibacterial activity.
CONCLUSION: Experimental short fiber reinforced resin-modified glass ionomer cement suggested the potential to mineralize surrounding environment, such as dentin with possible antibacterial activity. After these promising results, the next step is to evaluate the material's potential *in vitro* in a dentin environment.

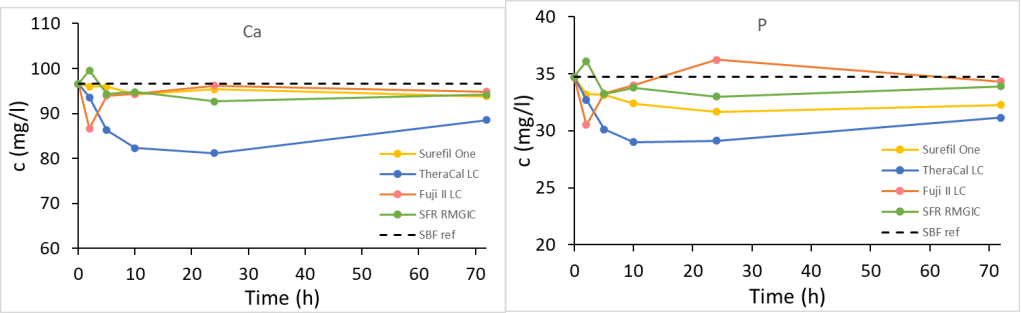


Figure 1. *Changes in Ca and P concentrations (ICP-OES) during continuous flow of SBF.*

PoA.12.05

Fate of preosteblast cell line in contact with a various alpha-tricalcium phosphate-based bone cements *in vitro*

Oznur Demir[1,2], Dagnija Loca[1,2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

Abstract

Calcium phosphate cements (CPCs) are widely recognized for orthopedic and maxillofacial surgeries. α-tricalcium phosphate (α-TCP) is a common reactant for CPCs, which can hydrolyze into calcium-deficient hydroxyapatite (CDHAp) upon contact with water. Due to its lower density, the higher free energy of formation, and greater reactivity and solubility, α-TCP is a more efficient reactant for CPC. In this study, low-temperature α-TCP powder was produced by thermally treating amorphous calcium phosphate with a high specific surface area (>60 m^2/g) at 650-750°C. The resulting α-TCP powder was mixed with sodium-hydrogen phosphate buffers to create an injectable, self-setting bone cement. However, pH of the liquid phase can significantly affect the formation of the final crystalline phase, potentially altering the setting mechanism and the cell's fate. Here, we examined the viability and differentiability of MC3T3-E1 cells upon contact with the bone cements. MC3T3-E1 cells were placed in contact with extractive medium of cement prepared using a liquid phase with different pH values (ranging from 6.0 to 7.4). Compared to the control, extracted medium of all formulations collected on days 1 and 3 showed higher viability. Although cells incubated with extracted medium at days 5 and 7 showed lower viability than control, no cytotoxicity was observed. We monitored the pH levels of cell medium and found that there was a slight decrease in pH in the control group and in cement prepared using liquid with a pH of 7.4 (pH7.4-cement) and pH 7.0. However, there was a more significant decrease in pH levels in other formulations of the cement. These findings explain why the pH7.4-cement had better results in terms of cell viability and cell capture efficiency (~60%). The *in vitro* proliferation of cells continuously grown in the bone cements was evaluated up to 7 days, revealing the same trend as found for cell capture. Further *in vitro* osteogenic differentiation capacity of prepared cement formulations and controls were compared using a typical late osteogenic mineralization marker, alizarin Red S staining. The mineralized area was significantly higher than that for the control group on day 21. ALP activity of the prepared cement samples' was found to increase for pH7.4-cement on day 14 and then decreased on day 21 for all formulations. These results suggest the ability of prepared cements, specifically pH7.4-cement, to promote osteoblast mineralization and differentiation.

Acknowledgement: European Union's Horizon2020 research and innovation programme; grant No. 857287 (BBCE–Baltic Biomaterials Centre of Excellence).

PoA.12.07

Bioinspired Mineralization Strategies for Advanced *in vitro* Bone Models

Bregje W.M. de Wildt[1,2], Nico A.J.M. Sommerdijk[3], Anat Akiva[3], Ralph Müller[1], Keita Ito[2], Sandra Hofmann[2], Xiao-Hua Qin[1]

[1]ETH Zurich, Zurich, Switzerland. [2]Eindhoven University of Technology, Eindhoven, Netherlands. [3]Radboud University Medical Center, Nijmegen, Netherlands

Abstract

Developing *in vitro* models of human bone tissue could facilitate the investigation of bone biology while reducing the need for animal experimentation. To recapitulate the cell-matrix interactions *in vitro*, a bone-mimetic biomaterial in which an organic and inorganic component are integrated is likely essential [1]. While the fabrication of composite materials could be achieved by blending organic materials with inorganic particles or by soaking organic materials in supersaturated solutions, a main challenge is the creation of an interconnected and well distributed mineral network as found in native bone [2]. *In vivo*, non-collagenous proteins are believed to play an instrumental role in the infiltration of mineral precursors into collagen fibrils. Poly-aspartic acid (pAsp) can be used to mimic the functionality of these acidic proteins *in vitro* through the polymer-induced liquid precursor phase (PILP) mechanism (**Figure 1A**). The application of such bioinspired mineralization methods could facilitate the investigation of complex cell-matrix interactions for *in vitro* bone models [3].

Here, we present two applications of bioinspired mineralization for the development of *in vitro* bone models. First, silk fibroin scaffolds were pre-mineralized using a mineralization solution of 10x simulated body fluid and pAsp. As such, pAsp was instrumental to guide mineral crystals into the porous scaffolds. Bone remodeling was mimicked by seeding human monocytes and mesenchymal stromal cells on these scaffolds and by inducing osteoclastic and osteogenic differentiation (**Figure 1B**). As a result, mineralized scaffolds supported osteoclastic differentiation and resorption and enhanced further mineralization. The second application goes to a cytocompatible PILP-induced mineralization method of soft hydrogels which could mimic the embedding of osteoblasts in osteoid during bone formation (**Figure 1C**). Preliminary results to understand 3D cell-material interactions in mineralized hydrogels will be presented.

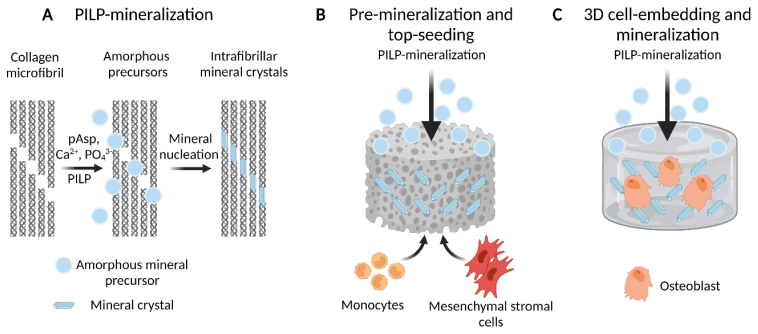


Figure 1. (A) The mechanism of polymer-induced liquid precursor phase (PILP) mineralization. (B-C) Methods to apply PILP mineralization for in vitro bone models. Figure created with BioRender.com.

1. Y. V. Shih and S. Varghese, Biomaterials, 198:107–121, 2019.
2. M. Maher et al., Acta Biomater., 131:41–61, 2021.
3. G. Thrivikraman et al., Nat. Commun., 10: 35202019, 2019.

PoA.12.09

Nature-inspired biomineralization process for the development of a stable phycocyanin - apatite multifunctional system for biomedical applications

Chiara Artusi[1], Elisabetta Campodoni[1], Franco Furlani[2], Rosaria Lauceri[3], Monica Sandri[1]

[1]ISSMC-CNR, Faenza, Italy. [2]University of Udine, Udine, Italy. [3]IRSA-CNR, Verbania, Italy

Abstract

In the last decade Spirulina, also called Arthrospira platensis, has been introduced by the research as one of the most effective biologically active compounds, both for its health benefits and ability to prevent and treat diseases or their symptoms. Spirulina is a photosynthetic cyanobacterium also known as blue-green microalgae. This organism contains a variety of bioactive macromolecules, as well as unsaturated fatty acids and essential amino-acids, which contribute to basic human nutrition. In addition, it contains other compounds, such as chlorophylls, carotenoids, phycocyanins, which give it its typical bright blue color. However, among all the Spirulina components, the most attractive is the active C-phycocyanin. Phycocyanin is a water-soluble phycobiliprotein, known as the only blue food coloring authorized by the FDA and often used in candy, cold drinks, beverages etc. However, recently it has been discovered that phycocyanin has important applications in the biomedical field, such as anti-oxidant, anti-inflammatory, anti-bacterial molecule and immune system stimulator in critical disease. The main using problem of the extracted phycocyanin, however, is its poor stability depending from temperature, pH and light. Indeed, it easily produces precipitates and changing color rapidly deactivating itself and limiting its wide application. To face this issue, through a natural-inspired approach in this work a stable anti-oxidant and antibacterial phycocyanin-based hybrid compound has been developed and tested. This biomimetic multifunctional system was thought for the desing of a new cosmetic and medical generation products, such as for sunscreen and wound healing. Reproducing the natural biomineralization process, phycocyanin molecules were conjugated with nanostructured hydroxyapatite particles (PcHA), obtaining a hybrid compound characterized from a light blue color. It has been fully investigated by chemical-physical and morphological analysis, in particular stability tests have been performed under different conditions of temperature, pH, and light, demonstrating the chemical stability of phycocyanin when conjugated with HA particles. Furthermore, the antioxidant properties evaluation has highlighted the preservation of molecule efficacy even after biomineralization. Its optimal cytocompatibility and antibacterial activity, demonstrated by *in vitro* cellular tests, suggest that PcHA could be promising for many biomedical applications. In particular, its antioxidant effect can be exploited in cosmetic products, especially in sunscreens, to protect skin tissue against free radicals (ROS) damage. While, Pc anti-inflammatory and antibacterial activity can play an important role in the chronic wounds treatment. Its ability to improve skin wounds has already been demonstrated, thus PcHA particles could be functional for dressing patches or topical formulations development.

PoA.12.11

Synergistic combination of artemisinin and cerium mesoporous nanoparticles for the induction of osteogenic properties in human periodontal ligament cells

Ioannis Tsamesidis[1], Georgia K. Pouroutzidou[1,2], Iason Chatzimentor[1], Anastasia Beketova[1], Evgenia Lymperaki[3], Maria Bousnaki[1], Eleana Kontonasaki[1]

[1]School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece. [2]School of Physics, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece. [3]Department of Biomedical Sciences, International Hellenic University, Thessaloniki, Greece

Abstract

INTRODUCTION: One of the most prevalent inflammatory disorders and the main reason for adult tooth loss is periodontitis. Strategies for periodontal tissue regeneration include regenerative surgery and the use of various grafting materials. Co-delivery concept with high synergistic combination indexes has appeared promising in multi-functional materials with the capacity to simultaneously deliver different ions along with bioactive molecules, such as drugs in order to apply therapeutic effects on osteogenesis(1). Artemisinin (ART), presented potential application in various iochemical pathways in different dental cells(2). In this work, cerium doped mesoporous nanoparticles (MSNs) were synthesized and loaded with ART with final target to evaluate their biocompatibility, hemocompatibility and biomineralization profile. EXPERIMENTAL METHODS: Mesoporous silica-based nanoparticles (MSNs) were synthesized as previously described(3). Biological assays: The biocompatibility, hemocompatibility, calcium deposition and ROS

Sample	SiO_2	CaO	CeO
Si	100		
Si^{CaCe}	60	35	5

Table 1. Nominal composition (in % mol)

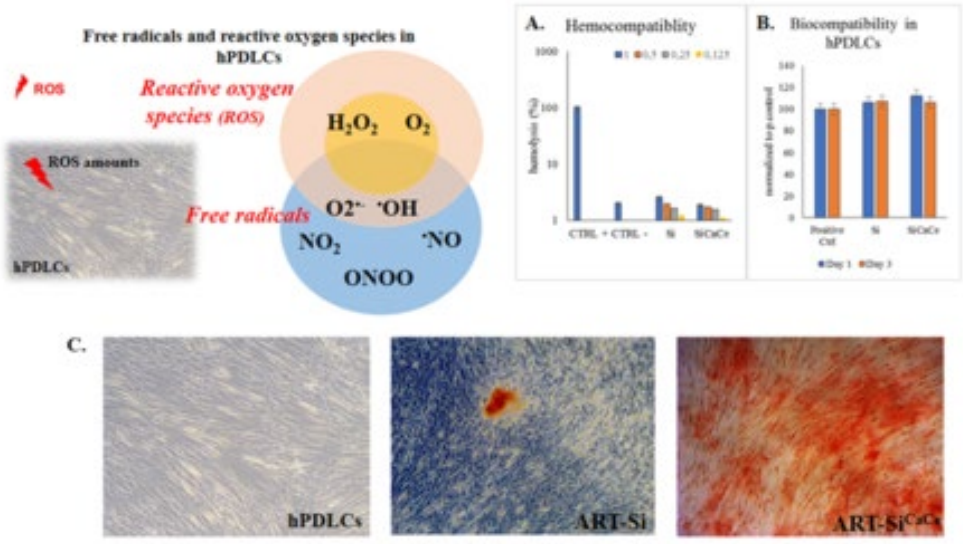


Fig. 1: (A) hemocompatibility of the tested MSNs after 24 hours of incubation (B) Cell viability assay of hPDLCs after 1and 3 days of incubation (C) calcium deposition in hPDLCs alone or cultured with the ART-Si or ART-Si^{CaCe}, at the highest tested concentration

levels were evaluated. Moreover, in order to mimic periodontitis cell injury, hPDLCs were stimulated with different hydrogen peroxide (H_2O_2) concentrations (25- 250 μM) for 1 hour in combination with different concentrations of MSNs with and without ART loading.

RESULTS AND DISCUSSION: The newly synthesized MSNs didn't present hemolytic effect, indicating their compatible profile and potential to be used as nanocarriers of ART. After 1 and 3 days of incubation, the viability assessment of hPDLCs with the tested MSNs demonstrated biocompatibility at all tested concentrations. Once, the MSNs were proven non-toxic, ART was loaded to evaluate its effect on cells' osteogenic differentiation. ART-loaded Ce-doped NPs promoted differentiation but only in combination with SiCaCe NPs, suggesting a synergistic ion-ART effect.

CONCLUSION: Based on the results of the present study, ART can be successfully loaded to cerium-doped MSNs to synergistically improve the calcium deposition of human periodontal ligament cells. Further investigation should be conducted to clarify the underlying mechanisms of this synergistic effect. especially under increased oxidative stress conditions.

REFERENCES

1. Pontremoli C., et all. 14(9): 1890., 2022
2. Hong Mei Hu., et all. 15 ;277:119471., 2021
3. Pouroutzidou GK., et all. 8;22(2):577, 2021
4. Tsamesidis I., et al. Nanomaterials, 26;11(9):218 ,2021

ACKNOWLEDGMENTS: This work is supported by Aristotle University of Thessaloniki AUTH's (2022-2023) (Greece) institutional research committee (ELKE) with the project title: ROS production and osteogenic differentiation of human periodontal ligament fibroblasts under stimulated oxidative stress in direct contact with artemisinin-loaded mesoporous cerium-doped calcium silicate nanopowders

PoA.12.13

Effect of cobalt doping in biphasic calcium phosphate nanoparticles for immunomodulation regulated bone tissue regeneration.

Deepa Negi, Yashveer Singh

IIT Ropar, Rupnagar, India

Abstract

Introduction: Bone abnormalities can result in local impairments and pose major health risks. Autografts and allografts are the most popular therapy options but complications after surgery, graft rejection, and other limitations result in the reduction of their clinical effectiveness[1]. Bioceramics are a promising choice for bone regeneration but their use in clinical settings is limited because these strategies usually ignore the immune responses generated by biomaterial-mediated osteogenesis[2]. Cobalt is a bioactive ion, which has immunomodulatory potential and also plays a role in promoting vascularization in bone tissue. However, its therapeutic concentrations for immune cell response in bone tissue regeneration have not been studied. In this work, we have fabricated biphasic calcium phosphate nanoparticles (CaP) with different concentrations of cobalt ions (CoCaP) for promoting osteogenesis and modulating inflammatory responses in bone regeneration.

Experimental Methods: CaP nanoparticles were fabricated and doped with different mole percentage of cobalt to fabricate cobalt-doped calcium phosphate nanoparticles (CoCaP). They were characterized by FT-IR, XRD, DLS, EDX, AFM, and FESEM, and their cell viability was measured in MC3T3 and RAW 264.7 cells. Osteogenic potential of nanoparticles was estimated by measuring ALP and calcium deposition, and their effect on osteoblast cell migration was estimated by scratch assay.

Results and discussion: The CoCaP nanoparticles were fabricated and characterized (Figure 1). FTIR analysis indicated the presence of peaks at 3568 cm^{-1} assigned to hydroxyl groups and 1010 and 566 cm^{-1}, which were attributed to $PO4_3^-$. EDX data confirmed the presence of cobalt in doped nanoparticles and the DLS data indicated an increase in the particle size from 95.2 ± 5.14 nm to 234.6 ± 8.12 nm. FESEM analysis indicated the round morphology of nanoparticles, and they were cytocompatible to MC3T3 and macrophage cells and induced cell migration and proliferation. Scratch assay indicated the complete healing of scratch and there was an increase in the ALP activity and calcium deposition in the presence of 2% CoCaP nanoparticles.

Conclusions: We have developed biphasic calcium phosphate nanoparticles with cobalt to accelerate bone tissue regeneration by promoting osteogenesis and macrophage polarization, and they showed enhanced ALP and calcium deposition activity along with the ability to promote cell proliferation and migration indicating its potential in bone regeneration.

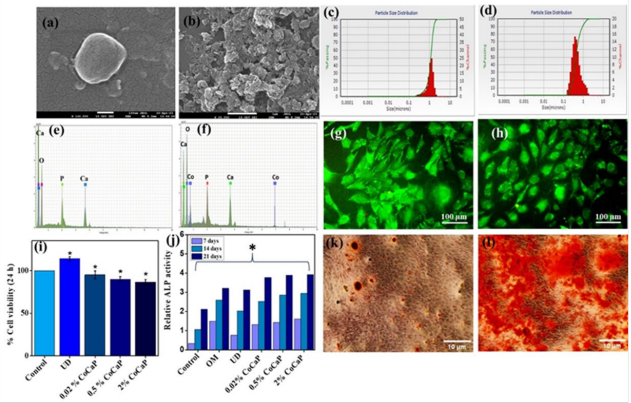


Figure 1. *Biphasic CaP and 2% CoCaP nanoparticles. (a, b) FESEM analysis. (c, d) Particle size analysis. (e, f) EDX analysis. (g, h) Live-dead assay. (i) Percentage cell viability. (j) Relative ALP activity. n = 3, mean ± SD; *p < 0.05. (k, l) Calcium deposition studies. Scale bar: 10 µm*

References

1. Aslankoohi, N et al., ACS Appl. Bio Mater. 3: 3621-3630, 2020.
2. Liang, H et al., ACS Appl. Mater. Interfaces. 11: 41758-41769, 2019.

PoA.12.14

Zinc-doped bioactive glass nanoparticles for tissue regeneration

Zuzana Neščáková[1], José Joaquín Velázquez García[1], Martin Michálek[1], Dušan Galusek[1,2], Aldo R. Boccaccini[3]

[1]Centre for Functional and Surface Functionalized Glass – FunGlass, Alexander Dubček University of Trenčín, Trenčín, Slovakia. [2]Joint Glass Centre of the IIC SAS, TnUAD and FChFT STU, Trenčín, Slovakia. [3]Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany

Abstract

Bioactive glasses are well-known and widely studied inorganic biomaterials used for tissue regeneration. Among them, sol-gel derived glass nanoparticles (NPs) have attracted interest as promising novel therapeutic and regenerative agents. In addition to the advantages of bioactive glass nanoparticles (BGNs) themselves, they offer the possibility to incorporate various therapeutic inorganic ions which increase the specific biological properties (e.g., angiogenesis, osteogenesis). Zinc plays a crucial role in the formation, mineralization, development, and maintenance of healthy bones. It is involved in homeostasis and angiogenesis, has antibacterial properties, and it is strongly connected with wound healing processes.
In this study, zinc-doped system with the composition 62SiO2 – 30CaO – 3P2O5 – 5ZnO (mol%) was synthesized and characterized for its structural, morphological, elemental, and antibacterial properties. Compared to the previous results, the present composition shows a relatively high content of Ca2+ ions (up to 30 mol%) that have been successfully incorporated by controlling the sol-gel synthesis conditions (nature of the solvent, stirring time, order of reagents, etc.). Nanoparticles with a size below 80 ± 20 nm have been produced. The addition of zinc did not influence the amorphous nature of the glass. The BGNs after immersion in simulated body fluid (SBF) mineralized to the apatite phase. The presence of zinc slowed down but did not inhibit the hydroxyapatite formation, confirming that the zinc-doped BGNs retained their bioactivity. The formation of HAp was further confirmed by SEM, XRD and Raman spectroscopy. Antibacterial properties against Gram-negative and Gram-positive bacteria, as well as eukaryotic fungal pathogens, have been evaluated. The antibacterial effect, as well as the inhibition of biofilm formation by E. coli and S. aureus, were confirmed. In addition, an antimicrobial effect against Candida glabrata was observed already at a concentration of 10 mg/mL by direct agar diffusion method. In addition to bioactivity, the prepared glasses thus offer additional biological functions and can serve as a tool for the treatment of infections directly at the site of application.

Acknowledgments
This project has received funding from the European Union´s Horizon 2020 research and innovation program under grant agreement No 739566. This research work has been also supported by the Research Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic, by the project: Advancement and support of R&D for "Centre for Diagnostics and quality testing of materials" in the domains of the RIS3 SK specialization, Acronym: CEDITEK II., ITMS2014+ code 313011W442.

PoA.12.15

Effects of thermal cycling and acidic storage on the surface properties and biocompatibility of different dental restorative materials.

Panagiotis Pandoleon, Anna Theocharidou Theocharidou, Eleana Kontonasaki

Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

INTRODUCTION: The oral cavity constitutes an aggressive environment for restorative materials, as fluctuations in temperature and pH may negatively affect their properties (1). The aim of the present work was to investigate the effect of thermal cycling and exposure to artificial gastric acid on the surface roughness and biocompatibility of various ceramic materials.

EXPERIMENTAL METHODS: Specimens of the following materials were prepared, according to the manufacturer's instructions: Group 1. Katana High Translucent – Kuraray (K), Group 2. Suprinity -Vita (S), Group 3. Enamic-Vita (E), Group 4. IPS e-max CAD- Ivoclar-Vivadent AG (I) and Group 5. LiSi Press -GC Dental Products (L). Twenty specimens were fabricated from each material. Thermal cycling (TC) was performed in distilled water for a total of 10.000 cycles between 37°C – 55°C – 37°C – 5°C. The simulated gastric acid solution (SGA) consisted of 5% hydrochloric acid (pH = 2) and specimens were kept at 37 °C for 91 hours. Surface characterization was performed before and after TC and exposure to SGA through X-Ray Diffraction Analysis-XRD and roughness was measured with a 3D optical profilometer. The MTT test was used to evaluate the biocompatibility of all specimens with human gingival fibroblasts.

RESULTS AND DISCUSSION: Immersion in SGA affected mostly surface roughness parameters of the silica-containing dental ceramics, without being able to significantly affect their mean surface roughness. Based on the combination of surface roughness profile and structural integrity, zirconia specimens presented the least changes, while zirconia-reinforced lithium silicate ceramic presented the most. Lithium disilicate materials presented different degrees of crystallinity and minor surface structure alterations after treatments. Both treatments affected the biocompatibility of most of the materials, except for one of the lithium disilicate ceramics investigated (Figure 1).

CONCLUSION: Although restorative dental materials are considered inert and stable in corrosive environments, compositional differences may contribute to different dissolution processes that may change surface roughness (2). From the materials tested, lithium disilicate in group 5 presented optimum stability and biological performance.

Acknowledgment

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers - 2nd Cycle" (MIS-5033021), implemented by the State Scholarships Foundation (IKY).

References

1. Cruz et al. J Prosthet Dent. 2020;123:483–90.
2. Kulkarni et al. J Prosthodont. 2020; 29:207-218.

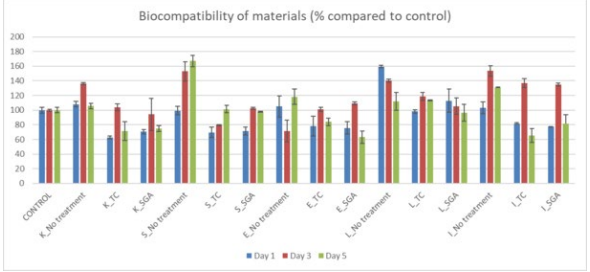


Figure 1. Biocompatibility testing of the materials before and after treatments.

PoA.14.01

Bond Strength and Adhesion Mechanisms of Novel Bone Adhesives

Sarah J Upson[1], Matthew J Benning[2], David A Fulton[3], Ian P Corbett[4], Kenneth W Dalgarno[2], Matthew J German[4]

[1]Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, Bournemouth, United Kingdom. [2]School of Engineering, Newcastle University, Newcastle upon Tyne, United Kingdom. [3]Chemical Nanoscience Laboratory, Chemistry-School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom. [4]School of Dental Sciences, Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

INTRODUCTION: Internal fixation plates (IFPs) made of titanium alloys or resorbable polymers are frequently used to aid healing of cranio-maxillofacial bone fractures. IFPs are attached to bone using screws, however, failure rates can be high. As the composition of bone is similar to dentine, we hypothesise that monomers used to make dentine adhesives might be an alternative to affix IFP's. The ability to attach a biodegradable IFP to porcine bone was assessed for 10-methacryloyloxydecyl dihydrogen phosphate (MDP), used as a primer (P), homopolymer or a copolymer with urethane dimethacrylate (U).

METHODS: Primer and adhesive formulations were prepared (MDP+/-U)[1]. Porcine mandible sections were set in resin. Hydroxyethyl methacrylate-terminated poly(lactic-co-glycolic acid)(HT-PLGA), discs were synthesised by ring opening polymerisation of lactide and glycolide[2] and compression moulded. Polymer-adhered bone samples were stored in chloramine T solution (1wt%, 37°C) for up to six weeks. Shear bond tests were performed with a universal testing machine (one-way ANOVA, post-hoc Tukey's test). Bone sections were also visualized by TEM.

RESULTS: Shear bond strength significantly reduced over time irrespective of adhesive ($p<0.001$). The addition of U to MDP improved bond strength up to week 1, this was further improved with a priming stage (Fig.1).

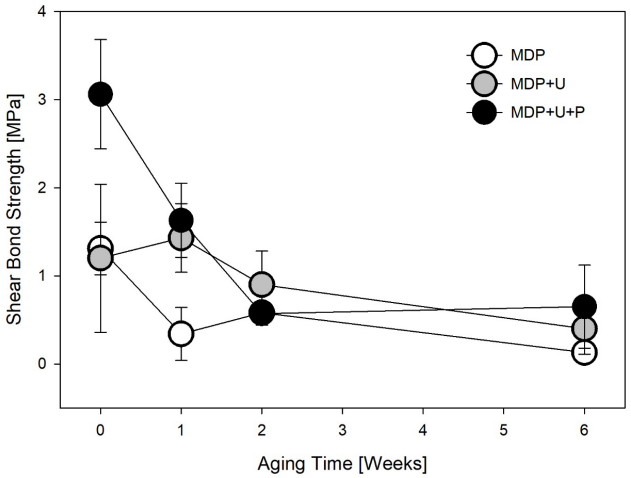


Fig.1.Mean shear bond strength of adhesive formulations over time, +/- SD.
MDP primer exhibited nano-layering on the surface of bone (Fig.2), spacing correlated with photomicrographs of MDP on dentine and hydroxyapatite samples[3].

CONCLUSION: TEM revealed the formation of nano-layered structures with the MDP primer, something not previously reported on bone. In a 6-week study both MDP+U and MDP+U+P demonstrated shear bond strength to affix IFPs above 0.2MPa, previously identified[4] as required to maintain fracture reduction for healing to occur.

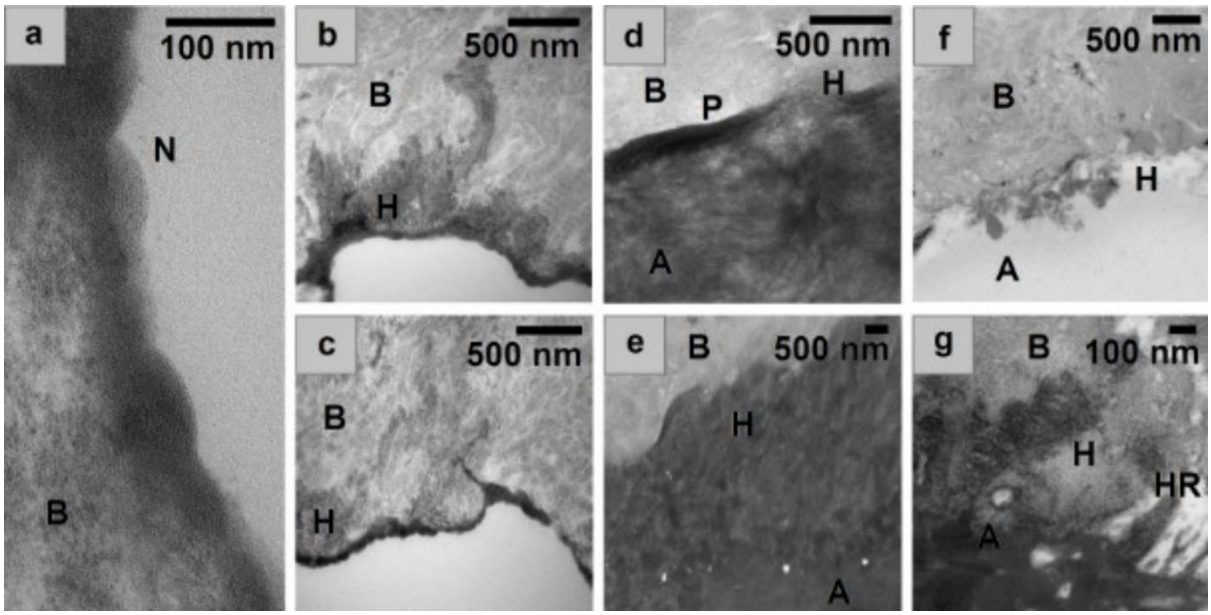


Fig.2.(a) nano-layering exhibited on the surface of bone by P, spacing of layers is equal to ~3.5nm, (b,c) P, showing integration into the collagen network of bone, (d) MDP+U+P, (e) MDP, (f) MDP+U, (g) expansion of (e). B=Underlying bone, H=Hybrid layer, N=Nano-layering, HR=Phenomenon similar to hybrid resin tag, P=Primer layer, A=Adhesive.

Funded by: EC FP7 RESTORATION and MeDe Innovation.
1.Upson,S.J.; et al., Bioengineering, 2023, 10(1),78.
2.Upson,S.J.; et al., Mater. Sci. Eng. C., 2016, 69,470-477.
3.Yoshihara,K.; et al., Acta biomater., 2010, 6(9),3573-82.
4.Weber,S.C.; Chapman,M.W., Clin. Orthop. Relat. Res., 1984, (191),249-61.

PoA.14.02

Recycled polysulfone-based composites with potential for load-bearing applications

Shinn-Jyh Ding, Chi-Nan Chang

Chung Shan Medical University, Taichung, Taiwan

Abstract

Metal materials (titanium and stainless steel) are widely used in artificial joints, dental implants, bone fixation devices and other fields due to their excellent mechanical properties. However, metal implants can cause bone atrophy because of stress shielding caused by a higher modulus of elasticity than cortical bone. This has inspired the development of non-metallic load-bearing materials. In addition to excellent mechanical properties, non-metallic polymer matrix composites also need osteogenic properties in order to achieve rapid bone healing in a load-bearing environment. Moreover, the implant and device not only reduce the stress shielding effect, eliminate subsequent surgery that may need to remove the metallic material, but also avoid metal artefacts, enabling postoperative diagnostic imaging. Among polymer materials, polysulfone (PSF) is a high-performance thermoplastic transparent plastic with heat resistance, chemical stability, high permeability, biocompatibility and X-ray resistance, and is the most widely used membrane material in hemodialysis. In addition, the elastic modulus of PSF is close to that of cortical bone, which can reduce stress shielding effect. With the widespread recognition of environmental protection issues, the recycling of materials has become imminent. Based on this, the reuse of PSF fibers in the recycled dialysis tube as polymer-based materials is worth exploring. To this end, this study used recycled PSF fibers to prepare high-strength and excellent osteogenic composites after adding bioactive calcium silicate (CaSi) ceramics. Among them, calcium silicate has osteogenic ability and antibacterial properties, and is used as a biofunctional reinforcing filler. This biofunctional PSF/CaSi composite bone material may be used for long-term hard tissue repair such as compact bone repair and fracture internal fixation. Experimental results showed that the recycled PSF fibers were hollow. After mixing with CaSi nanoparticles and heat treatment, this PSF/CaSi composite exhibited a PSF-specific broad diffraction peak at $2\theta=14–20°$. The granular particles in the surface morphology of composite materials were CaSi particles, while the smooth structure was due to PSF. When 20 wt% CaSi was included in the composite, the obtained three-point bending strength was about 80 MPa, which fell within the reported bending strength range of compact bone (50–150 MPa). However, the heat treatment temperature had no impact on the strength value. In conclusion, this PSF-calcium silicate composite would show great potential for load-bearing implant applications.

PoA.14.03

Evaluation of bone fusion capability of interface free HA PEEK cage

Minseong Chae[1], Kang-Sik Lee[1], Sung Tan Cho[1], Seung Hyun Baek[1], Yu-Chan Kim[2], Hojeong Jeon[2], Seung-Hoon Um[2], Hwachul Jung[3], Dongkyu Koo[3], Dong-Ho Lee[1]

[1]Biomedical Engineering Research Center, Asan Medical Center, Seoul, Korea, Republic of. [2]Biomaterial Research Center, Korea Institute of Science and Technology (KIST), Seoul, Korea, Republic of. [3]Innosys Co.,Ltd., Uijeongbu, Gyeonggi-do, Korea, Republic of

Abstract

Polyetheretherketone (PEEK) is a widely accepted implantable material that has relatively higher strength and superior durability than other polymers. However, due to its bioinertness, PEEK is not able to integrate with bone tissue. Recently, a various bioactive coating was developed to improve bone-to-PEEK bonding ability.
Nevertheless, since the coating layer are easily peeled off during the procedure, research is continued to strengthen the interfacial bonding force of the coating layer. It was tried to overcome this drawback by using a specific process that involves the use of nanosecond lasers to synthesize interface-free HA/PEEK surface. The aim of this study was to prove bioactivity of the interface free PEEK cage in canine anterior cervical fusion model.
HA/PEEK cage (W10 x D6 x H4 mm) is a specimen treated to prevent the interface from being separated from HA with Interface free laser on both sides in contact with the residential bone.
In three beagles weighing with 10 -15 Kg, both interface free HA/PEEK cage and pure PEEK cage (control) were implanted into the cervical intervertebral space (C3/4 or C4/5) with auto graft. After the 12-weeks survival period, interbody fusion activity evaluated based on histology and histomorphometry by measuring bone volume (BV).
The new bone volume of interface free HA/PEEK cage group was found to be 40.04%, while the new bone volume of pure PEEK cage group was only 13.57%. The new bone volume (%) was significantly greater for interface free HA/PEEK cage group than for the pure PEEK cage group (showing a 26.47% difference; $p<0.005$).
The results of this study indicate that interface free HA/PEEK cage exhibits more active bony conductibility into the cage and fusion rate than pure PEEK cage, suggesting that interface free HA/PEEK cage may offer greater clinical benefits for spinal interbody fusion surgeries.

This study received a grant from Korea Medical Device Development Fund Project (detailed project number: RS-2020-KD000061).

PoA.14.04

Biomaterial coatings with lubricious, slippery properties for improved urinary catheter performance

Jasmine L Ross, Colin McCoy, Matthew Wylie

Queen's University Belfast, Belfast, United Kingdom

Abstract

Introduction: Intermittent catheterisation is used to facilitate urine drainage. Although designed as single use, the reuse of intermittent catheters (ICs) is a common practice to reduce environmental impact and healthcare costs[1]. The repeated insertion and removal of ICs increases the risk of urethral trauma, with non-sterile reuse also increasing patient susceptibility to catheter-associated urinary tract infections (CAUTIs)[2]. This project aims to develop a surfactant-incorporated urinary catheter coating to impart the unique tribological and antimicrobial properties of cationic surfactants to the resulting coating[3]. A range of polymerizable phosphonium-based surfactants with varying hydrocarbon pendant and spacer groups have been synthesized. The optimal pendant and spacer group size was investigated to enhance both lubricious and wettability properties of hybrid coatings incorporated with 2-hydroxyethyl methacrylate (HEMA).

Methods: Phosphonium-based surfactant monomers were synthesized via nucleophilic substitution of bromoalkenes with trialkylphosphines. Ionic liquids were synthesized and characterised using nuclear magnetic resonance (NMR) and Fourier-transform infrared (FTIR) spectroscopy. Thermal polymerization was used to synthesise copolymer films of HEMA and surfactant monomers, using ethylene glycol dimethacrylate (EGDMA) crosslinker and azobisisobutyronitrile (AIBN) initiator (Table 1). ATR-FTIR confirmed successful polymerization. Copolymer swelling studies were performed at 37 °C, pH 7.4 to mimic physiological conditions. Contact angle measurements were determined via captive bubble. Adherence assays investigated the anti-adherent properties of the copolymer gels against *Staphylococcus aureus* ATCC 29213.

Table 1: Feed composition to prepare 10 g films.

Hydrogel symbol	Component weight / g			
	HEMA	$P_{888allyl}Br$	EGDMA	AIBN
H1	9.95	0.00	0.10	0.10
H2	8.80	1.00	0.10	0.10
H3	7.80	2.00	0.10	0.10

Results: Swelling studies have shown that increasing copolymer composition of surfactant monomer results in a decrease in swelling ability of the gel, with 34.6% (H1) compared to 29.4% (H3). Contact angle measurements have shown that increasing copolymer composition of surfactant monomer results in a decrease in contact angle, with 33.9° (H1) compared to 33.2° (H3). Preliminary adherence observations have shown a reduction in viable *S. aureus* by 36.9-60.5% on surfactant-incorporated films compared to p(HEMA).

Conclusion**:** Surfactant-incorporated films have different properties to p(HEMA). Developing a urinary catheter coating including surfactant-based functionality may increase catheter lubricity and bacterial anti-adherent properties leading to improved patient quality of life and reduced risk of CAUTI development.

References:

1 Wilks, S.A. *et al*. *Neurourol. Urodynam*. 39:907-15,2020.

2 Prieto-Fingerhut, T. *et al*. *Rehabil. Nurs*. 22:299-302,1997.

3 Anvari, S. *et al*. *J. Mol. Liq*. 221:685-90,2016.

PoA.14.05

POST-OPERATIVE CLINICAL EFFECTS OF INTRA-SOCKET CONCENTRATED GROWTH FACTOR (CGF) AFTER THIRD LOWER MOLAR EXTRACTION: A RANDOMIZED CONTROLLED CLINICAL TRIAL

Alberto Pispero, Elena Maria Varoni, Giovanni Sacilotto, Giovanni Lodi

University of Milan, Milan, Italy

Abstract

Introduction: The surgical extraction of impacted third molars is one of the most common procedures in oral surgery, associated with several postoperative sequelae (pain, oedema, trismus) that compromise the patient's quality of life in the days after the intervention. This randomized controlled clinical trial (RCT) aims at assessing the role of concentrated growth factor (CGF) on improving postoperative sequelae after the extraction of impacted lower third molars.
Materials and Methods:This RCT included 39 patients who needed a single mandibular third molar extraction. The patients were randomly divided into two groups: the first one included 18 patients undergoing dental extraction using CGF, positioned inside the post-extraction socket (Fig. 1); the second group included 21 patients undergoing the same surgical procedure, but without receiving CGF.

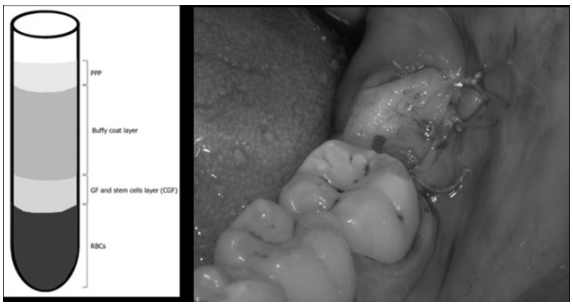


Figure 1. Post-operative intraoral image. CGF placed in the post-extraction socket and 4/0 absorbable suture.

Patients did not receive antibiotic prophylaxis. The postoperative outcomes were evaluated after seven days by recording pain (VAS Scale), number of pain medications taken (NSAID) and postoperative symptom severity scale (PoSSe Scale), which assesses the impairment in quality of life. A p-value ≤ 0.05 was considered statistically significant.
Results: VAS and PoSSe scale showed a slight improvement in the pain perception of the test group (VAS: 4.48; PoSSe Scale: 31.71) compared to the control group (VAS: 4.79; PoSSe Scale: 33.31), without a statistically significant difference. More relevant was the difference in the NSAIDs taken by the patient of the test group (mean N=8) compared to the control group (mean N=11.2), although not achieving statistical significance. Three patients in the control group experienced postoperative complications (one case of alveolar osteitis and two cases of wound infections with suppuration, oedema and pain), while no complications were recorded in the test group. The duration of the surgery was comparable in the two groups, with a mean difference of 1 minute.
Conclusions: This study showed a slight improvement, not statistically significant, in the use of CGF in the extraction of the lower third molar concerning the number of NSAIDs taken, pain perceived and patients' quality of life after one week. A possible beneficial effect of CGF is in reducing the incidence of postoperative complications.

PoA.14.06

Critical size defect model in the sheep tibia with locking compression plate fixation for bone regeneration research: partial results

Geissiane M Marcondes[1], Nicole F Paretsis[1], Anderson F Souza[1], Joice Fülber[2], Danielli CB Silva[1], Grazieli CM Silva[1], Thamires SPM Koga[1], Marília N Cardoso[1], Silvia RG Cortopassi[1], Ana MG Plepis[3], Virginia' CA Martins[3], André LV De Zoppa[1]

[1]Department of Surgery, School of Veterinary Medicine and Animal Science, Universidade de São Paulo, São Paulo, Brazil. [2]Department of Clinical Medicine, School of Veterinary Medicine and Animal Science, Universidade de São Paulo, São Paulo, Brazil. [3]Department of Chemistry and Molecular Physics, Institute of Chemistry of São Carlos, University of São Paulo, São Carlos, SP, Brazil

Abstract

Comminuted fracture treatment represents an orthopedic challenge and only osteosyntheses procedures are often insufficient for ideal bone regeneration. The use of composites associated with mesenchymal stem cells (MSC) filling bone defects can be indicated to enhance osteoconductivity. Prior to clinical trials, animal model designs are used following the “3Rs” principles. Critical size defects in sheep models are widely used for tissue engineering studies. Several techniques are used for ostectomy stabilization, however, the use of a single locking compression plate (LCP) fixation has benefits such as easy access to the bone defect and minimizing the risks of post-operative complications (fractures), especially concerned about animal welfare. The aim of this study is to evaluate a single LCP fixation in critical size defects in the sheep tibia for bone regeneration research. This study was approved by the Ethics Committee (CEUA/FMVZ – 2958110219). Twelve Suffolk ewes were submitted to a 30mm right tibial ostectomy stabilized with osteosynthesis using a 4.5mm narrow LCP. The animals were separated into two groups: Group 1 (hydroxyapatite, chitosan, and carbon nanotube biomaterial) and group 2 (biomaterial associated with MSC applied four weeks after surgery). The MSC (heterologous) of bone marrow was collected from a lamb. The cells were cultivated and prepared in a 1 x107 cell concentration (1.5 mL solution), for a three-point sites bone defect administration. The animals were maintained in lateral recumbency, and non-invasive guided ultrasound has been carried out with a hypodermic needle to apply the cells. Animals were confined in pairs throughout the experimental period. A full limb cast applied was maintained for 60 days and bivalve casts were replaced by a plantar splint allowing foot contact with the ground. This was kept for 30 days. The animals received nursing care and were evaluated with behavior indicators and pain scores developed for the species. Radiographic images were taken in the transoperative and monthly. Until now, five months after the ostectomy, no physiological parameters alteration (pain-related), lameness, wounds, or fractures were presented. The radiographs show the integrity of the osteosynthesis device. No procedure complications were observed and related (including ostectomy, composite, or MSC application). After twelve months the animals will be submitted to euthanasia for a micro-CT scan and immunohistochemistry assay, comparing the groups. The osteosynthesis with a single LCP fixation in a sheep tibial bone defect model is secure and viable for bone regeneration assays, with minimal animal welfare impact.

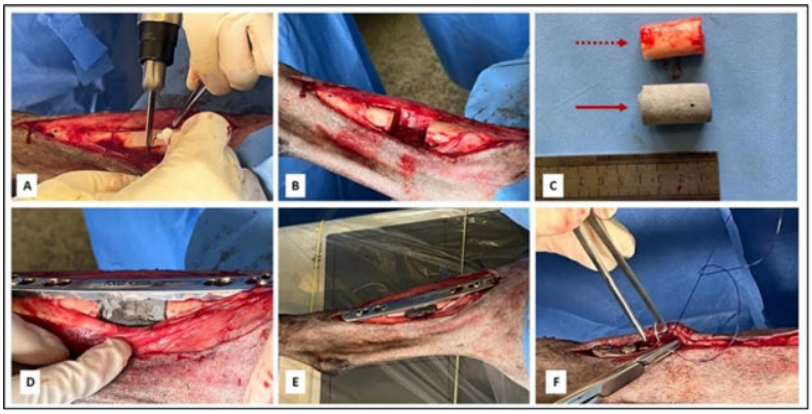


Figure 1.

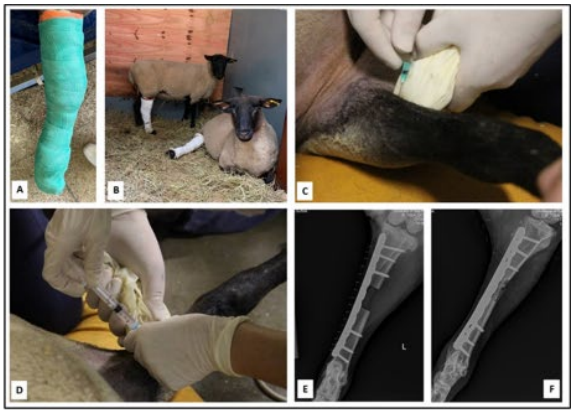


Figure 2.

PoA.14.07

The effect of PET meshes on the mechanical and structural properties of a photo-curable hydroxyapatite composite intended as internal fixation in complex bone fractures.

Guillaume Patt-Lafitte[1], Sarah Rasolonjatovo[1], Daniel J. Hutchinson[2], Michael Malkoch[2], David Eglin[1]

[1]Mines Saint-Etienne, Univ Jean Monnet, INSERM, U 1059 Sainbiose, 42023, Saint-Etienne, France. [2]Division of coating technology, Royal Institute of Technology, Stockholm, Sweden

Abstract

Complex phalanx fractures are often managed with open reduction internal fixation (ORIF) metal implants. While these implants provide stability to the healing fracture, the mechanical mismatch between the bone and metal and the formation of soft tissue adhesions can result in complications at a rate as high as 64% (Guerrero E. M. et al., Hand (N Y). 16:248-252, 2019). To address this problem, a customisable and non-adhesive polymeric fixation has been proposed. It is composed of a primer formulation for adhesion to bone, a photo-curable resin composite based on a thiol-ene coupling reaction (monomers and a photo-initiator) and two reinforcing phases (hydroxyapatite particles and poly(ethylene terephthalate) (PET) fiber meshes). While the effect of the hydroxyapatite on the mechanical properties of the resin is well understood, the role and impact of the PET meshes on the curing, structural and mechanical properties of the fixation device has not been fully explored. Thus, the following work aims to systematically study the mechanical and structural properties of the photo-curable resin hydroxyapatite composite, augmented with PET meshes to provide boundary conditions for the design of future biodegradable reinforcing meshes. Prepared fixation devices (N = 3 to 10) were characterized by Raman spectroscopy, water uptake analysis, three-point bending and tensile tests, microcomputed tomography (µcT) and scanning electron microscopy (SEM). The effect of the meshes on the curing efficiency, the water uptake, the structural homogeneity, the presence of voids, the moduli, maximum strength and toughness values as well as mode of failure of the fixation device samples was assessed and statistical analysis was performed.

Raman spectroscopy and water uptake quantification showed the absence of effect of the PET meshes insertion on the curing efficiency. µcT and SEM analysis indicated a good impregnation of the meshes by the composite resin during sample formulation. The insertion of PET mesh did not increase the moduli and strength values of the cured fixation device with hydroxyapatite under bending or tension. However, mechanical tests revealed a new failure mode. Indeed, the PET meshes increased significantly the toughness, most likely by a mechanism of deflection of crack propagation, allowing the fixation to withstand 60 % more stress along more strain. Thus, addition of meshes influenced positively the toughness of the polymeric fixation and likely its fatigue behaviour, indicating its importance in future biodegradable design of customisable polymeric fixation.

PoA.14.09

Diffusion doping of analgesics into UHMWPE for pain management of total joint replacement

Nicoletta Inverardi[1,2], Sashank Lekkala[1], Keith K Wannomae[1], Brad Micheli[1], Hany Bedair[1,2], Orhun K Muratoglu[1,2], Ebru Oral[1,2]

[1]Massachusetts General Hospital, Boston, USA. [2]Harvard Medical School, Boston, USA

Abstract

The delivery of non-opioid analgesic drugs from an ultrahigh molecular weight polyethylene (UHMWPE) implant is desirable to address postoperative pain after total joint arthroplasty. Blending drugs into UHMWPE can be limiting to the strength and toughness at high concentrations.[1] We investigated an alternative process for lidocaine or bupivacaine incorporation into UHMWPE to improve the mechanical properties of these therapeutic-eluting UHMWPEs.

METHODS: Blocks of UHMWPE (50x85 mm^2, thickness ranging between 3 and 15mm) were compression molded and a subset of the blocks irradiated under vacuum (100kGy e-beam). Strips machined from the irradiated blocks (3×5×20 mm^3) were doped by soaking at 120°C for 4h (the melting point for lidocaine and bupivacaine is ~ 68 °C and ~ 108 °C, respectively). Samples were weighed and the doping profile was characterized by FTIR. The drug stability was investigated by ^{1}H NMR of eluted compounds in DMSO-d_6. The drug release was investigated by elution into 1.7ml de-ionized water and UV spectroscopy. Type V tensile samples (ASTM D638-10) were die-cut and tested at a crosshead speed of 10 mm/min.

RESULTS AND DISCUSSION: The amount of doped drug normalized to surface area is shown in Table 1. NMR spectra for the unprocessed drugs agree well with those of the eluted drugs, suggesting the stability of the drugs after doping. The ultimate tensile strength (UTS) and the elongation at break (EAB) were not changed compared to those of UHMWPE without doping for non-irradiated and irradiated materials (Table 1). When extrapolating the drug release rates to the surface area of a tibial component in a hypothetical knee implant (100cm^2), the resulting day 1 dose was 16 to 91 mg/day (Table 1).

	Irradiation Dose (kGy)	Doped drug normalized to surface area (mg/cm^2)	Elution per day from 100cm^2 implant at day 1 (mg/day)	UTS (MPa)	EAB (%)
Virgin UHMWPE	0	-	-	50.8 ± 2.5	448 ± 34
	100	-	-	49.3 ± 3.5	300 ±17
Lidocaine doped	0	6-9	91 ± 17	49.2 ± 2.2	419 ± 14
	100	4-6	44 ± 5	49.7 ± 1.7	324 ± 7
Bupivacaine doped	0	6-7	16 ± 1	53.8 ± 2.3	427 ± 14
	100	4-6	17 ± 1	50.5 ± 1.4	299 ± 13

CONCLUSION: Incorporation of non-opioid analgesics into UHMWPE implant materials by diffusion retained high mechanical strength and ductility, which may enable a new tool for pain relief in joint replacement.

1. Grindy et al., Acta Biomaterialia 93 (2019) 63–73

FUNDING Office of the Assistant Secretary of Defense for Health Affairs (Peer Reviewed Medical Research Program, Award No. W81XWH-17-1-0614). Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

PoA.14.10

PHAsT: Sustainable and Renewable Materials for Biomedical Applications

Andrea Mele, Ipsita Roy, David Alexander Gregory, Daniel Syed Mohammad Syed Mohamed

University of Sheffield, Sheffield, United Kingdom

Abstract

PHAsT, a team of research entrepreneurs, have developed a sustainable bacteria-driven manufacturing process that leads to the production of highly pure biopolymers called Polyhydroxyalkanoates (PHAs), with significant potential for biomedical applications. The utilisation of polymers as biomaterials has greatly impacted the advancement of modern medicine. A wide range of polymers has been investigated in the last decades, showing promising results, while exhibiting limitations in degradation with toxic byproducts, biocompatibility, processability and physicochemical properties, leading to questionable results from *in vivo* experiments. In addition, currently used biomaterials are derived from fossil fuel-based resources and are environmentally persistent. Hence, there is an urgent demand to replace these materials with high-performance alternatives that are also environmentally friendly. PHAs have recently attracted huge interest due to their sustainability, wide variety of mechanical properties, tunable and controlled biodegradability, and excellent biocompatibility. However, they have occupied a small niche of the biomedical market, due to difficulty in manufacturing, high cost, inadequate purity and lack of processability. At PHAsT, we are able to produce medical grade rigid and brand new elastomeric PHAs, currently unobtainable commercially. In addition, our process allows us to provide different blends using our PHAs to target a wide range of biomedical applications. Our innovation is currently at TRL 5 and has been extensively validated within our laboratory. The team is able to produce medical-grade rigid and elastomeric PHAs, via pilot plant production in a 30L bioreactor with excellent yields of approximately 1 Kg/20L of fermentation working volume. Furthermore, the team has characterised the materials to determine their physicochemical properties. The PHAs have been successfully tested with respect to cytotoxicity and cell viability, using several types of cell lines including myoblasts, neuronal, keratinocytes, bone, pancreas and kidney cells. Additionally, a significant amount of *in vivo* data has been obtained confirming their excellent biocompatibility. Finally, the team is able to process PHAs via electrospinning, 3D printing, melt extrusion, laser ablation, dip moulding, oil in water emulsions, and solvent casting, to produce structures of varying shapes, porosity and mechanical properties. The team has also carried out a preliminary Life Cycle Analysis that proves the sustainability of the material and the manufacturing process.
Having the aim to incorporate a spinout company, we are looking for collaborations and partnerships in order to develop new biomedical products using our materials and translate our research to the medical market improving healthcare quality.

PoA.14.11

Application of Computer Vision for the Assessment of Degradation-related Microstructural Changes within Injectable Cements

Ghayadah Al-kharusi[1], Tanya Levingstone[1], Chiara Vitale Brovarone[2,3], Sonia Fiorilli[2], Ilaria Corvaglia[2], Federica Banche Niclot[2], Nicholas Dunne[1], Suzanne Little[1]

[1]Dublin city university, Dublin, Ireland. [2]Politecnico di Torino, Torino, Italy. [3]RU Politecnico di Torino, Firenze, Italy

Abstract

The microstructural properties of ceramic-based cement have a significant impact on their mechanical and degradation properties. Pores in a cement reduces its strength, durability, and resistance to chemical attack, which can lead to premature failure of the structure. Therefore, the accurate detection and measurement of pore size and porosity is crucial for understanding the material behaviour over time and developing strategies to improve its durability and performance. To address this issue, computer vision image analysis function was developed using OpenCV (Python 3.9) and validated using experimentally measured pore size and porosity measurements. The function consists of pre-processing steps, including grayscale conversion, resizing, noise reduction, and edge enhancement through thresholding, morphological operations, such as dilation and erosion, followed by contour detection to measure pore dimensions by calculating the distance between pore boundaries and determining pore diameters (Fig.1A). This approach was applied to two calcium sulphate (CSH)-based injectable cements designed for the treatment of vertebral compression fractures, one containing 100% CSH (100CSH) and the other was enriched with strontium-containing mesoporous bioactive glasses (Sr-MBG) and zirconia nanoparticles (ZrO2) (75CSH-MBG). To assess degradation behaviour of the cements, solid cylindrical cement samples (13 x 5 mm) were placed in tris-HCL solution (0.1M, pH 7.4) on an orbital shaker at 37°C for up to 28 days in accordance with ISO 5833-2002. Micro-CT imaging was performed at Days 0, 3, 7, 14, 21 and 28, and the images were assessed using the image analysis function to quantify pore size and porosity. The data showed a 19.1% and 17.2% increase in porosity (Fig.1B) for the 100CSH and 75CSH-MBG cements, respectively, over the 28-day period. Pore size distribution data for the CSH100 cements showed a higher percentage of pores in the 100-150 µm range at Day 28 compared to Day 0 (Fig.1C), with an increase in average pore size of 20%. Similarly, for the 75CSH-MBG cements, the percentage of pores measuring > 50 µm increased between Day 0 and Day 28 (Fig.1D) with a 60% increase in average pore size. The application of computer vision offers several advantages over existing techniques, including faster and more accurate pore size and porosity measurements, providing greater insights into the degradation-related microstructural changes of a biodegradable cements. Ultimately, this approach will pave the way for biomaterials development in terms of understanding relationship between changes in microstructural features and mechanical-degradation property trade-off, which can be applied more widely to materials research.

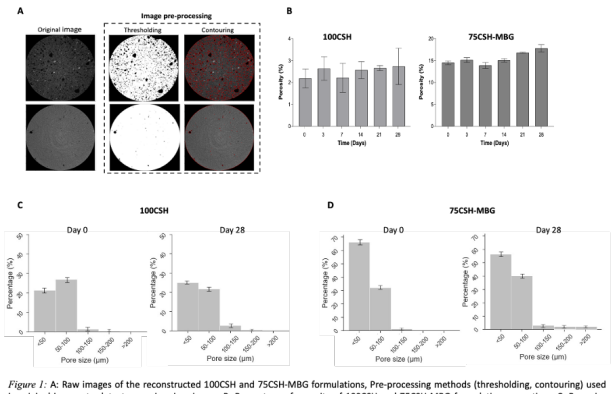


Figure 1: A: Raw images of the reconstructed 100CSH and 75CSH-MBG formulations, Pre-processing methods (thresholding, contouring) used in original images to detect pore sizes in microns. B: Percentage of porosity of 100CSH and 75CSH-MBG formulations over time. C: Pore size distribution for 100CSH. D: Pore size distribution for 75CSH-MBG.

PoA.14.13

Comparative Evaluation of the mechanical and optical properties of Zircon-Zirconia Multiphase Ceramics in Simulated body fluid

Gulsan A S Kazi, Bashir Ahmmad

Yamagata University, Yonezawa, Japan

Abstract

Nowadays, composite materials are being widely employed across various fields such as biology, technology, and industry. Notably, recent developments in materials engineering have focused on enhancing the properties of ceramics by utilizing composite materials instead of single-phase materials. In this context, we have successfully produced a composite material composed of Zircon-Zirconia-Spodumene ceramic using a straightforward solid-state reaction method. The fabricated composite exhibits desirable mechanical and optical characteristics. To assess the potential application of these multiphase ceramics in biology, in the present study we have conducted an in-vitro analysis to evaluate their bioactivity in simulated body fluid (SBF).

Materials and Methods: Raw materials for ceramic synthesis explained in Table 1 were mixed (1:1) and sintered at different temperatures.

Table 1 The ratio of the raw materials

	$Z_rO_2(\%)$	$Y_2O_3(\%)$
Three wt% YSZ	97.0	3.0
Six wt% YSZ	94.0	6.0

	$Li_2CO_3(\%)$	$K_2CO_3(\%)$	$Al_2O_3(\%)$	$SiO_2(\%)$	$NH_4-H_2PO_4(\%)$
LDS	7.6	8.4	9.0	70.0	5.0

The powder was pressed with a manual hydraulic press and prepared a 10 mm x 2 mm size disc. Finally, the disc was sintered at 1450℃ temperature for 2 hours. A simulated body fluid (SBF) absorption test was carried out according to ISO specifications 1567-2000. The specimens were soaked in SBF and weighed at time intervals. Then calculate the weight gain and loss of the samples by the following equations: Mass weight gain = {(W1-W0)/W0} x 100% , Mass weight loss = (W0-W1) *100%

Diametral tensile strength (DTS), Vickers hardness, and fracture toughness were measured and evaluated. Density and porosity were also evaluated using Archimedes' principle.

Results and Discussion: X-ray diffraction (XRD) analysis confirmed the presence of zircon, monoclinic and tetragonal zirconia, as well as spodumene crystalline phases. Among the samples containing six wt% yttria, the highest value of DTS was recorded at 29 MPa. Furthermore, minimal mass weight gain of approximately 0.01-0.1% and weight loss ranging from 2.3% to 6.4% indicated that the composite did not undergo significant soaking or drying processes and did not exhibit macroscopic degradation over time.

The optical properties of the synthesized composite were assessed, revealing that the total transmittance and optical bandgap of the samples confirmed their translucent nature. Notably, the Ra and Rz values of the samples with six wt% yttria were lower compared to the samples with three wt% yttria. These findings suggest that the composite containing six wt% yttria holds promise in terms of mechanical and optical performance.

These valuable insights contribute to the advancement of further research and practical applications of these composites in the field of biology.

PoA.14.14

Development of Metallic Biomaterials: Processing and Surface Modification for Improved Biomedical Response

Ana Lúcia do Amaral Escada[1], João Pedro Aquiles Carobolante[1], Kerolene Barboza da Silva[1], Rita de Cássia Reis Rangel[2], Danielle Duque Domingues[2], Celso Bortolini Jr[1], Andre Luis Reis Rangel[1], Ana Paula Rosifini Alves[1]

[1]Unesp, Guaratinguetá, Brazil. [2]Unesp, Ilha Solteira, Brazil

Abstract

Metallic biomaterials have a wide range of applications in the biomedical field, including dental implants, stents, joint replacements, and other medical devices. According to your application, both permanent and temporary implants may be used. Several types of metals have been studied for this purpose. For example, titanium and cobalt-based alloys are commonly used as substitutes for hard tissues, whereas iron-based alloys can be employed in temporary stents. Adverse effects may arise from the use of permanent implants, such as inadequate integration, inflammation, mechanical instability, necrosis, and infection. These factors can lead to increased patient suffering, pain, and reduced functionality. As these materials are considered bioinert, modifying their surface can be a solution to these problems while also enhancing their functional response. On the other hand, the degradation rate of temporary implants remains a limitation associated with their use. However, surface treatments can minimize this drawback and provide specific responses, such as immunomodulatory and bactericidal properties. In recent years, our research group has focused on studying the processing and surface modification of biometallic materials for both permanent and biodegradable applications. In terms of surface properties, the utilization of anodic oxidation in titanium-based alloys has demonstrated outstanding outcomes in the formation of TiO_2 nanotubes, which have been found to improve cellular response and stimulate the growth of bone tissue. The use of this technique in absorbable metals results in changes in the degradation rate and an improved biological response. Our research evaluating the use of anodic oxidation with metals that have antibacterial and immunomodulatory activity has also presented excellent results.

PoA.15.01

Green processing of SAIB/Chitin based membranes: Structural and Functional Analysis

Cristiana Gonçalves[1,2], Simone S. Silva[1,2], Hajer Radhouani[1,2], Rui L. Reis[1,2], J. Miguel Oliveira[1,2]

[1]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Guimarães, Portugal. [2]ICVS/3 B's – PT Government Associate Laboratory, Guimarães, Portugal

Abstract

Membranes for tissue engineering are an active area of research in biomedical engineering, constantly evolving [1]. Membranes can be produced by several production techniques and using various materials, which is critical to their effectiveness as tissue engineering tools.

Sucrose acetate isobutyrate (SAIB) is a clear and very viscous liquid. It is a high molecular weight material obtained by sucrose molecules esterification with two acetic acids and six isobutyric acid moieties [2]. SAIB is a potential material for tissue engineering due to its biocompatibility, biodegradability, and controlled delivery of bioactive molecules. However, it is a challenging material to process due to its restricted solubility properties. Even in its processing with ionic liquids (ILs, as solvents), the SAIB-based structures (scaffold or membrane) lack strength and durability.

Chitin, a natural polysaccharide derived from crustacean shells, has excellent properties for membrane applications (e.g., good mechanical properties and the potential to promote cell adhesion and proliferation). Thus, chitin emerged as an excellent candidate to provide better mechanical properties to the SAIB structures. In fact, combining two polymers into a single structure can provide a more tailored and effective solution for tissue engineering.

In this study, we used ILs as common solvents to dissolve chitin and SAIB in a single system and then cast the mixture into membranes. Further, the membranes were submitted to freeze-drying to create SAIB/chitin porous membranes. We also investigated the possibility of using genipin, a natural crosslinking agent derived from the fruit of Gardenia jasminoides Ellis [3], to improve the membranes' mechanical properties. The effect of genipin at different reaction conditions on the properties of the membranes was also evaluated by various techniques, for instance, by Scanning electron microscope - SEM (Figure 1). Genipin can form covalent bonds with the hydroxyl groups to enhance the mechanical strength of the membranes.Our results demonstrated that ILs could effectively dissolve chitin and SAIB and produce membranes with improved properties (such as iocompatibility, permeability, mechanical strength, and cost-effectiveness) for medical applications, and adding genipin can further enhance the membranes' mechanical properties.

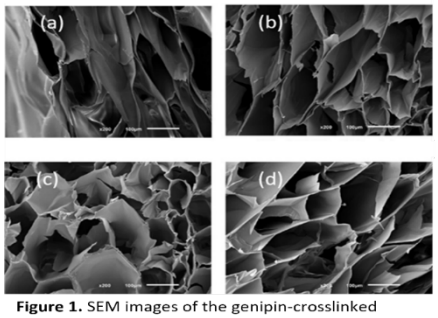


Figure 1. SEM images of the genipin-crosslinked SAIB/chitin-based porous membranes at room temperature (a, c) and 37 ºC (b,d) and with 10 mM (a,b) and 20 mM (c,d) of Genipin.

Acknowledgments

The authors thank the financial support from FCT-Portugal, with references SFRH/BPD/94277/2013-CTTI-58/18-I3Bs (4); CEECIND/00111/2017, and CEECIND/01306/2018.

References

1. doi.org/10.1002/0470020393
2. doi.org/10.1016/j.ijpharm.2016.06.012
3. doi.org/10.1533/9781845697198.3.589

PoA.15.02

An *in vitro* blood-air barrier model based on electrospun poly(Ɛ-caprolactone) meshes as the basement membrane mimic

Neval Sevinc Ozdemir[1,2,3], Halime Kenar[1,4,5], Vasif Hasirci[1,4,5,6]

[1]ACU Biomaterials Center, Acibadem Mehmet Ali Aydinlar University (ACU), Istanbul, Turkey. [2]Department of Medical Biotechnology, Graduate School of Health Sciences, ACU, Istanbul, Turkey. [3]Department of Pharmaceutical Basic Sciences, School of Pharmacy, ACU, Istanbul, Turkey. [4]Department of Biomedical Engineering, School of Engineering, ACU, Istanbul, Turkey. [5]Grad. Department of Biomaterials, ACU, Istanbul, Turkey. [6]BIOMATEN, METU Ctr. of Excellence in Biomaterials and Tissue Engineering, Ankara, Turkey

Abstract

The blood-air barrier is a pulmonary structure that acts as a barrier between the air inside the alveoli and the blood in the surrounding capillaries. In pneumonia caused by distal lung infections, including COVID-19, damage to the barrier due to inflammation can result in fluid accumulation in the lungs, making it harder for oxygen to enter the bloodstream and leading to respiratory distress. The main goal of this study was to develop a physiologically relevant *in vitro* blood-air barrier model to test the efficacy of potential treatments and investigate the mechanisms underlying COVID-19's effects on the barrier. Poly(Ɛ-caprolactone) (PCL) meshes were prepared by electrospinning to mimic the basement membrane in the barrier. Electrospinning parameters were optimized to obtain micro- and nanofibrous meshes, and fiber diameter and pore size were characterized by SEM. Meshes were coated with collagen type IV and laminin, and placed in 24-well plates. Primary human alveolar epithelial cells (phAECs) and primary human umbilical vein endothelial cells (pHUVECs) were seeded on opposite sides of the meshes and cultured for two weeks. The barrier models created with micro (~1.5 µm) and nano (200-300 nm) thick fibered electrospun meshes were studied in terms of cellular attachment to mesh surfaces and infiltration through them. Expression of the cellular phenotypic markers and formation of intercellular junctions were studied by immunostaining and confocal microscopy. High cell permeability of the microfibrous meshes was not observed with nanofibrous meshes which allowed formation of single layers of epithelial and endothelial cells on opposite sides. The co-culture conditions for generating the model with confluent monolayers of cells on both sides will be optimized, while monitoring barrier resistance through measurements of trans-epithelial electrical resistance (TEER), and studying the barrier's permeability to oxygen and molecules of varying sizes.

Funding Information: This study is funded by the Scientific and Technological Research Council of Turkey (TUBITAK) within the scope of Southeast Asia - Europe Joint Funding Scheme for Research and Innovation (SEA-EU JFS) (TUBITAK 1071, Grant No: 121N817). We thank ACU Biomaterials Center for the use of the facilities.

PoA.15.03

Unraveling the Transcriptome Profile of Pulsed Electromagnetic Field (PEMF) Stimulation In Bone Regeneration Using an *In vitro* Investigation Platform Based on a Perfusion Bioreactor and 3D-Printed Bone-like Scaffolds

Farah Daou[1], Beatrice Masante[2], Stefano Gabetti[2], Giovanni Putame[2], Eleonora Zenobi[3], Federico Mochi[3], Cristina Bignardi[2], Federica Dell'Atti[1], Francesco Favero[1], Costantino Del Gaudio[4], Diana Massai[2], Andrea Cochis[1], Lia Rimondini[1]

[1]Dept. of Health Sciences, Center for Translational Research on Autoimmune and Allergic Diseases (CAAD), Università del Piemonte Orientale (UPO), Novara, Italy. [2]Dept. of Mechanical and Aerospace Engineering and PolitoBioMed Lab, Politecnico di Torino, Turin, Italy. [3]Hypatia Research Consortium, Rome, Italy. [4]Italian Space Agency, Rome, Italy

Abstract

INTRODUCTION: Bioreactors are being investigated as experimental platforms for bone biology research [1]. Pulsed electromagnetic field (PEMF) stimulation has gained increased attention in bone and cartilage repair [2], and is widely applied in clinical practice [3]. Several signaling pathways related to its osteogenic, chondrogenic, and anti-inflammatory effects were determined, but most of the pathways are still unknown [4]. Rigorous testing is required to gain deeper insights into the associated signaling pathways and to optimize and standardize clinical protocols in terms of waveform, amplitude, frequency, and exposure dose. This work aimed to uncover the signaling pathways elicited by PEMF using a novel *in vitro* investigation platform that exposes 3D bone-like models to physiological-like perfusion and PEMF stimulation.

EXPERIMENTAL METHODS: *Bioreactor.* An automated perfusion bioreactor for culturing in parallel up to 3 bone-like models under tunable direct perfusion (0.006-24 mL/min) with supplemental PEMF stimulation (1.5 mT, 75 Hz), was adopted (Fig. 1A) [5].

Scaffolds. Polylactic acid (PLA) scaffolds (total porosity = 60%, average pore size = 600 µm) were 3D printed to resemble bone microarchitecture.

Biological evaluations. Scaffolds were seeded with human mesenchymal stem cells (hMSCs) and exposed to perfusion (0.3 mL/min) with and without PEMF stimulation (4 h/day) for 21 days in basal or osteogenic medium (Fig. 1B). Static cultures served as control. RNA sequencing (RNA-Seq) and real-time qPCR were conducted to detect the signaling pathways elicited by PEMF.

RESULTS AND DISCUSSION: The PEMF effects on cells are evident in the absence of biochemical stimuli. RNA-Seq revealed that PEMF stimulation in basal medium targets the four phases of bone healing: inflammatory, fibrovascular, bone formation, and bone remodeling phases, even in the absence of a pathological state (Figure 1C).

CONCLUSION: The proposed *in vitro* investigation platform allowed for uncovering the signaling pathways activated by PEMF and represents a powerful tool for bone biology research.

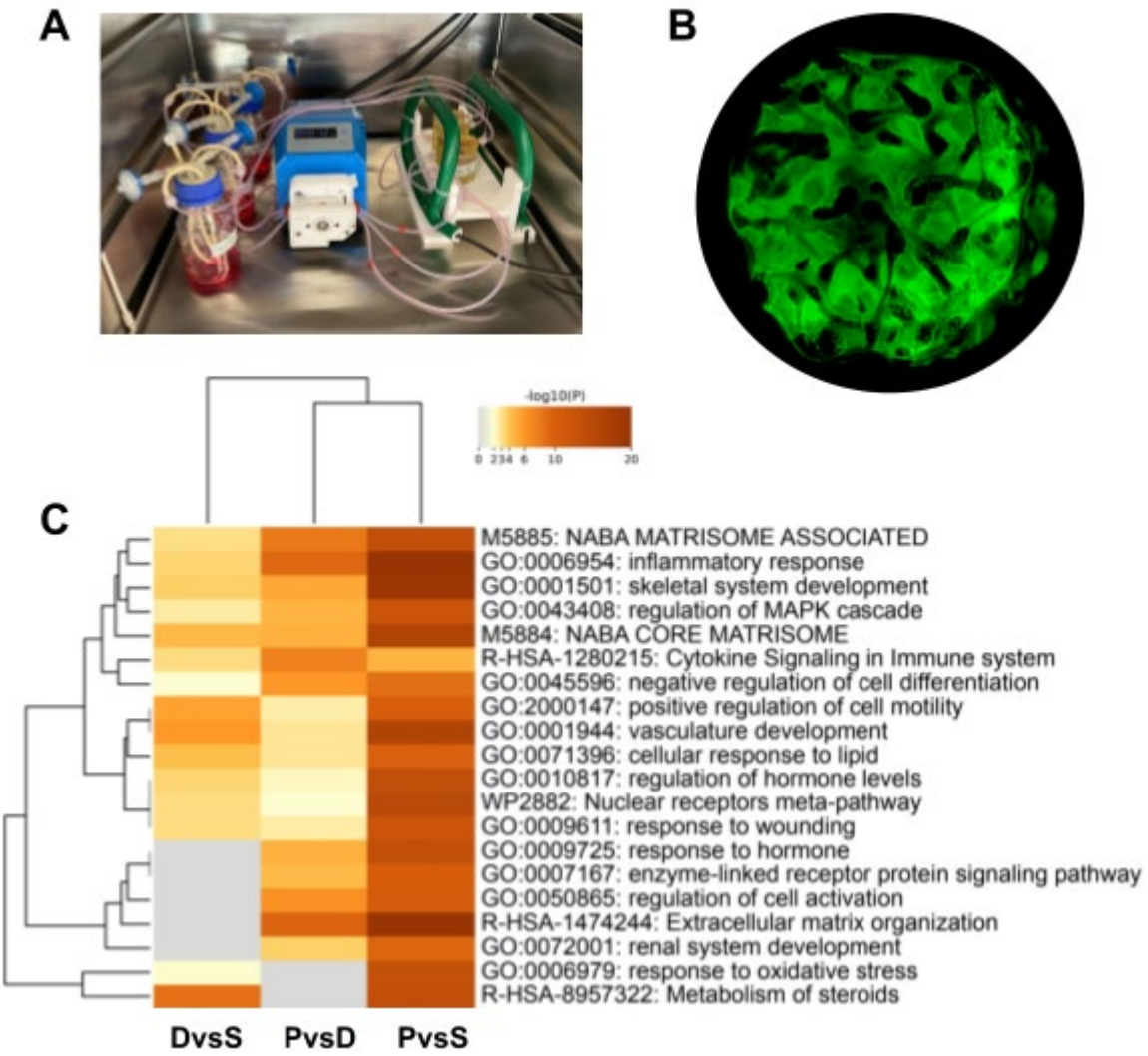


Figure 1: (A) In vitro investigation platform; (B) Live/dead assay after 21 d of direct perfusion in basal medium; (C) GO enrichment analysis. S: static; D: direct perfusion; P: Direct perfusion with PEMF stimulation.

REFERENCES

1. Selden, C. et al. Bioengineering, 5(2), 32, 2018
2. Varani, K. et al. Int. J. Mol. Sci., 22(2), 809, 2021.
3. Massari, L. et al. Int. Orthop., 43(3), 539–551, 2019.
4. Cadossi, R. et al. JAAOS Glob. Res. Rev., 4(5), e1900155, 2020.
5. Gabetti S. et al. Sci. Rep, 12(1):13859, 2022.

PoA.15.04

Dynamic Bioreactor for Studying Mechanobiological Responses of Periodontal Ligament Cells during Orthodontic Tooth Movement

Kuo-Hui Chiu, Mert Karpat, Johannes Hahn, Sanja Aveic, Horst Fischer

Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany

Abstract

The periodontal ligament (PDL) is a specialized connective tissue that encompasses and supports the teeth and is essential for proper tooth function and movement. During orthodontic tooth movement (OTM), mechanical force is exerted on the periodontal tissue, leading to tissue remodeling. Mechanical stretching of the PDL can alter cell shape, gene expression, and extracellular matrix production. However, the mechanisms underlying intracellular signaling during PDL regeneration in response to mechanical stimuli remain poorly understood. The *in vivo* studies of PDL stretching are difficult due to the intricate nature of native PDL tissues and the challenge of regulating mechanical loading in living systems. In this study, we propose a novel dynamic bioreactor that mimics the native dynamic loading on PDL-derived fibroblasts (PDLFs). We used collagen-based hydrogels as matrix for the embedded cells that offer an optimal microenvironment for cell cultivation and mechanotransduction, characterized by high biocompatibility, permeability, and mechanical stretchability. Controlled cyclic stretching on PDLF-embedded collagen-based hydrogels lead to an aligned collagen matrix and promoted cell proliferation and spreading. Furthermore, we confirmed effective mechanotransduction to the embedded cells through a three-fold increase in Periostin expression level after seven days of cultivation. Additionally, glycosaminoglycan production analysis revealed extensive extracellular matrix (ECM) remodeling under dynamic conditions. Collagen type I and Collagen type III which are the major components of periodontal tissue were illustrated by immunofluorescence to visualize the ECM remodeling process. Moreover, we observed an increase in hydrogel stiffness during the dynamic cultivation period as a result of collagen fiber orientation and ECM remodeling. Our findings demonstrate that long-term dynamic loading is essential for a physiological behavior of PDLF cells. Thus, our novel dynamic bioreactor can help to unravel mechanobiological cues induced in the periodontal ligament during orthodontic tooth movement.

PoA.15.05

An innovative *in vitro* microfluidic and three-dimensional blood-brain-barrier model able to mimic the neurovascular unit environment

Maria Cristina Ceccarelli[1,2], Matteo Battaglini[1], Gianni Ciofani[1]

[1]Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera, Italy. [2]The Biorobotics Institute, Scuola Superiore Sant'Anna, Pontedera, Italy

Abstract

The blood-brain barrier (BBB) is a highly selective and dynamic physiological barrier that separates the brain extracellular fluid from the bloodstream, regulating the exchange of nutrients and other molecules. This barrier is composed of specialized endothelial cells, astrocytes, pericytes, and basement membrane components with a crucial role in protecting the brain from toxins, pathogens, and drugs.1 However, it also poses a significant challenge for drug delivery to the central nervous system (CNS), the efficacy of which is limited by low BBB penetration. In this context, several *in vitro* models has been introduced2 to provide a platform for studying the transport and permeability of drugs across the BBB.

In our recent work, we developed a BBB-on-chip using microfabrication techniques. In particular, the microfluidic device was made using polydimethylsiloxane (PDMS) via photolithography and soft-lithography. The plane-parallel design consist of a channel in communication with three different chambers by a row of pillars spaced 5 µm each. Endothelial cells (HCMEC/d3) were seeded in the channel coating the pillars, in order to form a 3D barrier, while microglia cells (HMC3) were seeded in the three chambers within a 3D Matrigel matrix. Afterwards, we evaluated the BBB permeability with fluorescent tracers.

Our analysis confirmed the obtainment of a microfluidic device able to mimic the BBB structure, where the endothelial cells efficiently adhere to the pillars and grow in a cohesive three-dimensional layer (Figure 1a, 1b) offering resistance to the passage of fluorescent tracer (Figure 1c).

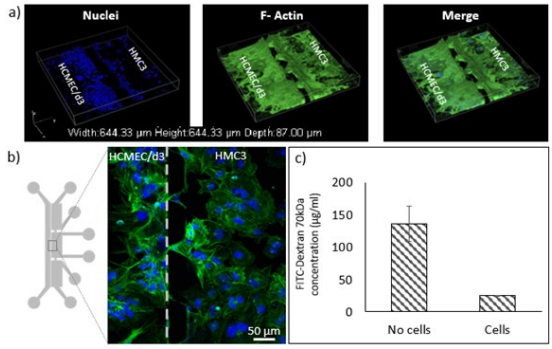


Figure 1. a) Confocal images of cells seeded on the microfluidic device (in blue nuclei, in green F-Actin); b) scheme of the microfluidic device; c) passage of FITC-dextran 70 kDa in the presence or absence of endothelial cells.

In conclusion, our BBB-on-chip model offers a system for studying under flow conditions the physiology of the BBB. Specifically, our aim will be the integration of the various components of the neurovascular unit (NVU) such as pericytes, astrocytes, endothelial cells, neurons and glia. In addition, we can evaluate the cell migration by the communication channels between the chambers, and the BBB crossing of either drug or nanostructures.

References

1. R. Daneman, A. Prat, Cold Spring Harb Perspect Biol, 2015, 7:a020412
2. B.Peng, [...], W. Huang, Lab Chip, 2022, 22, 3579-3602

PoA.15.06

Decoupling the effects of pore size and extracellular matrix stiffness on 3D stem cell mechanosensation

Samuel J Maher[1], Chrissie Astell[1], Sebastian E Amos[1], Vihara Ellepola[1], Yongsung Hwang[2], Jennifer L Young[3,4], Yu Suk Choi[1]

[1]School of Human Sciences, The University of Western Australia, Perth, Australia. [2]Soonchunhyang Institute of Medi-bio Science, Soonchunhyang University, Cheonan-si, Chungcheongnam-do, Korea, Republic of. [3]Mechanobiology Institute, National University of Singapore, Singapore, Singapore. [4]Department of Biomedical Engineering, National University of Singapore, Singapore, Singapore

Abstract

The microenvironment provides a vast array of signalling cues to cells that regulate cell behaviour and phenotype, all of which play a vital role in homeostasis. Given the complexity and ever-changing presentation of the extracellular matrix (ECM) *in vivo*, it remains challenging to effectively model and subsequently identify the primary drivers of cellular mechanosensitive responses in 3D. Furthermore, current biomaterial platforms such as hydrogels are coupled in the sense that to increase stiffness, porosity must be decreased, which may limit cell volume expansion. As such, this study aims to develop and utilise tunable hydrogel platforms to draw conclusions about the independent effects of pore size and ECM stiffness on 3D stem cell mechanosensation. Human adipose-derived stem cells (ASCs) encapsulated in phototunable gelatin methacryloyl (GelMA) hydrogels were cultured for six days at either a static or gradient stiffness, which aimed to achieve differential cell volume expansion. Following this, hydrogels were 'on-demand' stiffened using UV photomasks to a desired stiffness and after a further six days, observations of cell morphology and protein expression were made. Cell volume was successfully limited using the phototunable hydrogel platform with cells being significantly larger in areas of lower substrate stiffness. Furthermore, the gradient expansion model, which allowed for differential volume expansion whilst being exposed to the same stiffness showed a significant decrease in nuclear localisation of both YAP and MRTFa in stiffer regions. In contrast, the equal expansion model, which allowed for equal expansion with differential stiffnesses exposure showed no significant changes in cell morphology or mechanomarker expression. However, in both platforms, a correlation between cell volume and nuclear mechanomarker localisation was observed. Similarly, stem cell differentiation showed a clear correlation with volume expansion, rather than the surrounding hydrogel stiffness. Local elasticity also appeared to be elevated in cells with larger volume expansion, which was quantified using optical coherence elastography (OCE). The current results from this platform suggest that 3D stem cell mechanosensation and differentiation are more closely correlated with volume expansion, rather than hydrogel stiffness. The findings may prove useful in controlling stem cell fate in 3D and informing biomaterial design for regenerative medicine purposes.

PoA.15.07

Role of Serum Protein Acidic and Rich in Cysteine and Human Serum Albumin in Cancer Drug Delivery

Ramakrishna Prasad Are, Anju R Babu

National Institute of Technology Rourkela, Rourkela, India

Abstract

The main challenge in designing drugs for cancer is treating patients without affecting the patient's quality of life. Our study focuses on how to utilize the external microenvironment surrounding the cancer cells for efficient drug delivery. Cancer cells have more serum protein acidic and rich in cysteine (SPARC) and collagen than normal cells. SPARC protein plays a crucial role in maintaining the integrity of the ECM by modulating the basal lamina and collagen in the ECM. In our present study, an in-depth analysis was performed to determine the subdomains of HSA (IA, IB, IIA, IIB, IIIA, IIIB) that interacts with the SPARC and SPARC-Collagen. The binding affinity of the 3D structures of the individual subdomains of HSA with SPARC and SPARC-Collagen complex were analyzed in ClusPro 2.0 and ZDOCK servers. Molecular dynamics were performed in GROMACS V.2021.2 for a period of 10 ns. The SPARC-Collagen complex has shown a higher affinity with the HSA than the SPARC, as shown in table 1.

HSA	IA	IB	IIA	IIB	IIIA	IIIB
SPARC	-751.6	-1071.6	-973.3	-808.1	-787.5	-831.9
SPARC-Collagen	-766.4	-1019	-1042.9	-797.6	-806.7	-775

Table 1: ClusPro 2.0 binding affinity scores of each domain of HSA, SPARC, and SPARC-Collagen.

The SPARC and SPARC-Collagen complex has a high affinity with the sub-domain IB and IIA. Sub-domain IIA consists of the drug binding site 1 of HSA; because of that, SPARC-Collagen has shown more binding affinity, and fatty acid site 2 overlaps with drug binding site 1. Root mean square deviation (RMSD) plots show that the SPARC-Collagen-HSA complex is more stable during the time course than the SPARC-HSA complex, as shown in figure 1. The radius of gyration of the SPARC-Collagen-HSA complex has shown the same compactness as that of the SPARC-HSA complex.

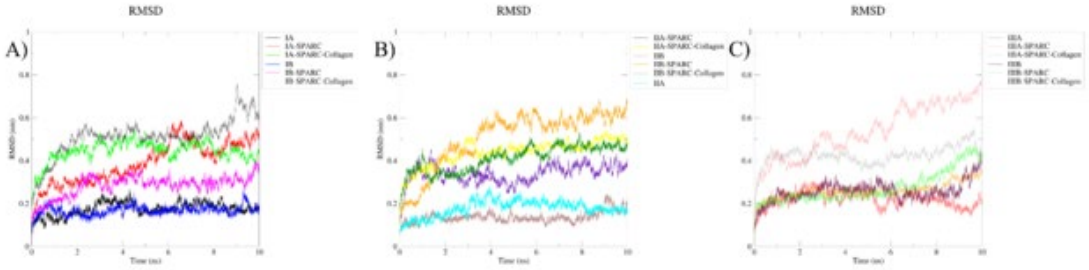


Figure 1: RMSD plot of each sub-domain of HSA, SPARC, and SPARC-Collagen.

Cancer cells develop resistance over time to drugs makes it challenging to design the drugs and treat patients. Designing drugs to deliver to the cancer cells with the help of HSA and SPARC bypassing the lysosome degradation system will efficiently deliver the drugs to the cancer cell. In case cancer cells develop resistance to this mechanism, the cancer cells may decrease the SPARC and collagen influx into the cells, resulting in decreased cancer cell proliferation and metastasis rate, providing extra time for the health care workers to provide better therapy to the patients.

PoA.15.08

Decellularized extracellular matrix as a scaffold for a 3D model of Dupuytren´s disease

Jarmila Knitlova[1,2], Martina Doubkova[1], Adam Eckhardt[1], Roman Stachon[3], Elena Filova[1]

[1]Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic. [2]Faculty of Science, Charles University, Prague, Czech Republic. [3]Department of Orthopaedics, Bulovka Hospital, Prague, Czech Republic

Abstract

Fibrosis is characterized by hyperproduction and accumulation of extracellular matrix which may lead to morphological changes and dysfunction of the organ or affected tissue. Dupuytren´s disease (DD) is a contractile and fibroproliferative diasese with unknown etiology which affects mainly men over 50 years of age. The symptoms of DD are abnormal thickening and the contracture of the fascia of the palm and fingers. These symptoms develop over time into thick fibrotic nodules and cords under the skin, causing bending of fingers with inability to straighten. The standard treatment is surgical fasciectomy which brings improvement but has a high recurrence rate. There is a tremendous amount of research on antifibrotic treatment for similar diseases in other tissues and thus a necessity for relevant *in vitro* and *in vivo* models. We believe that the outcomes of DD patients surgeries could be enhanced with a properly selected adjunctive pharmacological treatment. However, there is no relevant and/or available animal or 3D *in vitro* model for necessary preclinical testing of potential drugs for this disease.

The aim of our study is to establish a 3D model of DD based on decellularization of stiff and contracted nodular tissue of DD. This matrix (if decellularized carefully) can keep its original microenvironment and mechanical properties and serve as a scaffold for subsequent repopulation with cells isolated from the original tissue.

We tried different decellularization protocols using ionic and non-ionic detergents. The best result were obtained by using 0,5% sodium dodecylsulfate (SDS) followed by DNAse I treatment and extensive washing steps. This protocol efficiently removes cellular and nucleic acid debris. We observed a decrease in amount of glycosaminoglycans, total collagens and fibronectin after decellularization compared to mock-treated controls, however, the substantial quantity of the main matrix components was still present. The natural architecture of collagen network visualized by imaging of SHG signal of collagen stayed preserved. Decellularized matrix was reseeded with fibroblasts isolated from the fibrotic nodules of DD. The matrix showed no toxicity and the cells proliferated and produced collagen type I.

We are going to build on these results to create a complex 3D model of DD. Such a model can be utilized for testing of pharmacological compounds with possible antifibrotic effects.

This study was supported by Czech Science Foundation of the Ministry of Health of the Czech Republic (NU22-10-00072), and by the Academy of Sciences of the Czech Republic (AP2202).

PoA.15.09

The in-vitro efficacy of GAG-collagen scaffold in induction of cell growth with/without growth factor

Shuanfu Liu, Changhui Chen, Kevin Yen

Han Biomedical Inc., New Taipei, Taiwan

Abstract

Tissue regeneration for chronic wounds is still challenging and until now researchers are still finding an effective solution. There are unmet needs of optimal treatment option for chronic wound repair due to complicated over time clinical situations. Convincing data had been reported over the years that collagen scaffolds including GAG-collagen scaffolds play the important role for wound repair by mimicking the environment of extracellular matrix (ECM). Besides, growth factors were studied for accelerating the tissue repair. We performed the in-vitro study of GAG-collagen scaffolds in induction of cell proliferation with or without growth factor. The study results showed that GAG-collagen scaffold could induce the cell growth into the scaffold, and the growth factor used can further strengthen the effect of GAG-collagen scaffolds.

The GAG-collagen scaffolds were the composite of collagen and chondroitin-6-sulfate (C6S). For further strengthen the structure of GAG-collagen, the scaffolds were crosslinked with either GA (glutaraldehyde) or EDC/NHS. We compared the in-vitro cell viabilities of those scaffolds with pure collagen scaffold. While culturing, cell viability assay was performed for more detailed access of cell proliferation trends and cell cycle behavior. After culturing, samples were sectioned and Goldner trichrome dyed in view of distinct cell appearance.

Both GAG-collagen scaffolds and pure collagen scaffolds, with or without growth factor showed a promising result of section layout with variant cell distributions. GAG-collagen scaffolds, however, had an overwhelming cell growth comparing to pure collagen scaffold under 28 days culturing. But despite all, all sorts of scaffolds were shown to be a reassuring material for cell proliferation and cell mitosis had been observed on sample sections under several microscope scenes, especially in C6S-EDC collagen scaffolds and pure collagen scaffold. ES135b growth factor had shown it can accelerate proliferation. All GAG-collagen scaffolds and collagen scaffolds had better cell viability assay data and degradation profile after adding ES135b.

In conclusion, C6S-collagen scaffolds had successfully induced the cell growth in the in-vitro study. Adding ES135b growth factors will pull those properties to a further extent.

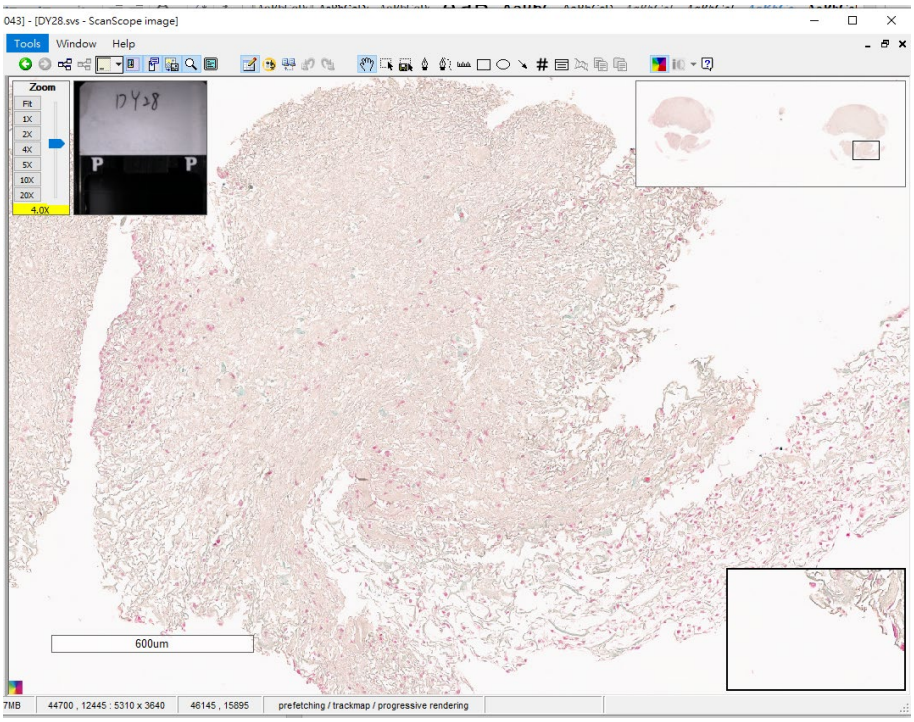


Fig 1. Growth factor productively induced cell proliferation in GAG-scaffolds

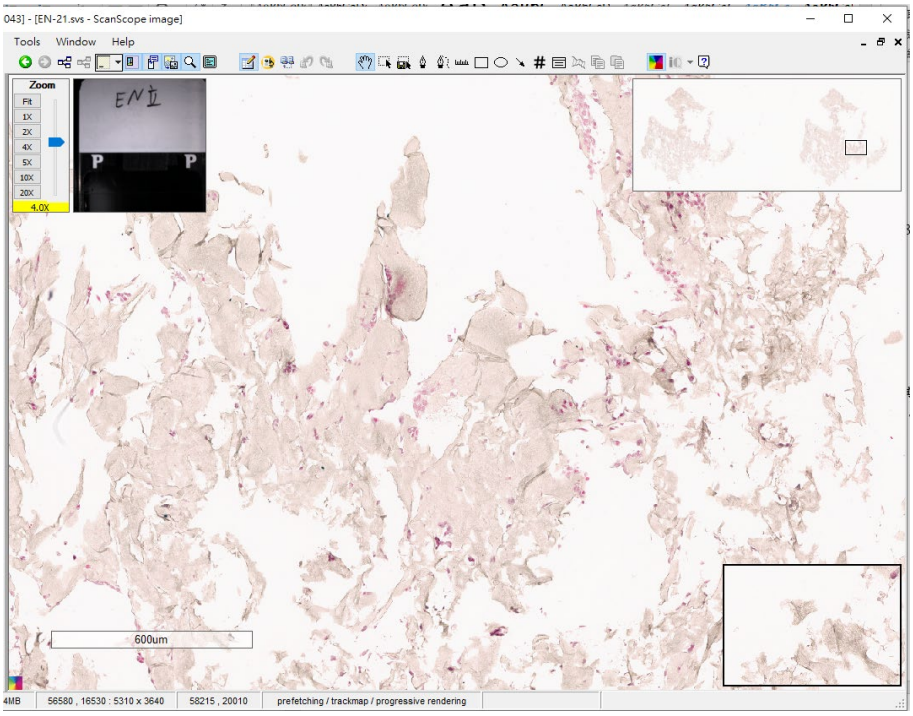


Fig 2. Clear, complete and comparatively more cells could be spotted in EDC-scaffolds

PoA.15.10

Vascularised Cardiac Spheroids-on-a-Chip for Testing the Toxicity of Therapeutics

Stefania Di Cio[1], Malcolm Haddrick[2], Julien E Gautrot[1]

[1]Queen Mary University of London, London, United Kingdom. [2]Medicines Discovery Catapult, Cheshire, United Kingdom

Abstract

Microfabricated organ-on-a-chip tissue models are rapidly becoming the gold standard for the testing of safety and efficacy of therapeutics. A broad range of designs has emerged, but recreating microvascularised tissue models remains difficult in many cases. This is particularly relevant to mimic the systemic delivery of therapeutics, to capture the complex multi-step processes associated with trans-endothelial migration, uptake by targeted tissues and associated metabolic response. In this report, we describe the formation of microvascularised cardiac tissue spheroids embedded in microfluidic chips. The embedding of spheroids within vascularised multi-compartment microfluidic chips was investigated to identify the importance of the spheroid processing, and co-culture with pericytes on the integration of the spheroid within the microvascular networks formed. The architecture of the resulting models, the expression of cardiac and endothelial markers and the perfusion of the system was then investigated. The ability to retain beating over prolonged periods of time was quantified, over a period of 25 days, demonstrating not only perfusability but also functional performance of the tissue model. Finally, as a proof-of-concept of therapeutic testing, the toxicity of one therapeutic associated with cardiac disfunction was evaluated, identifying differences between direct *in vitro* testing on suspended spheroids and vascularised models.

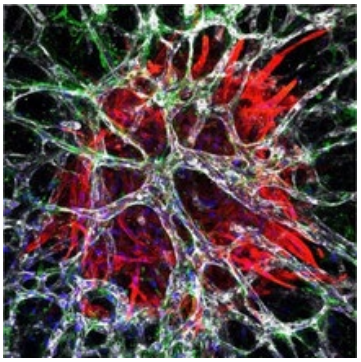


Image of cardiac spheroid embedded in a microvascularised chip. White, CD31; Green, NG2; Red, TNNT2.

REFERENCES

1. Dibble, M.; Di Cio, S.; Luo, P.; Balkwill, F. R.; Gautrot, J. E., Impact of Pericytes on the Stabilisation of Microvascular Networks in Microfluidic Systems in Response to Nanotoxicity. BioRxiv 2022, doi.org/10.1101/2022.05.03.490457.
2. Jones, C. F. E.; Di Cio, S.; Connelly, J. T.; Gautrot, J., Design of an Integrated Microvascularised Human Skin-on-a-Chip Tissue Equivalent Model. Front. Bioeng. Biotechnol. 2022, 10, 915702.
3. Di Cio, S.; Haddrick, M.; Gautrot, J. E., Vascularised Cardiac Spheroids-on-a-Chip for Testing the Toxicity of Therapeutics BioRxiv 2023, https://doi.org/10.1101/2023.01.20.524970.

PoA.15.11

Biofabrication of a bone model for breast cancer bone metastasis drug screening

Anaïs Lamouline[1,2,3], Simone Bersini[1,2,4], Giuseppe Talo[1,5], Chiara Arrigoni[1,2,4], Matteo Moretti[1,2,4,5]

[1]Regenerative Medicine Technologies Laboratory, Laboratories for Translational Research (LRT), Ente Ospedaliero Cantonale (EOC), Bellinzona, Switzerland. [2]Service of Orthopaedics and Traumatology, Department of Surgery, EOC, Lugano, Switzerland. [3]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milano, Italy. [4]Faculty of Biomedical Sciences, USI, Lugano, Switzerland. [5]Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milano, Italy

Abstract

Breast cancer cells can metastasize to bone tissues and hijack the bone remodeling process in a vicious cycle of bone resorption, causing fractures and limiting the patient's life expectancy [1]. Bone metastases are incurable, in part due to the drug resistance generated by the microenvironment [2]. It is now becoming clearer that drug screening would benefit from being carried out in 3D *in vitro* models, where the tumor architecture and composition can be faithfully replicated.

Despite the unmet clinical need of more effectively treating bone metastases, there is currently no pre-clinical model able to test in a high-throughput manner the efficacy of cancer treatments on breast cancer bone metastases. Hence, the goal of this project is to create a human bone model that can be used to predict which drug combinations will be the most effective. Based on our previous bone models[3], [4], this study includes the cell types involved in the vicious cycle of bone metastases: breast cancer cells, osteoblasts and osteoclasts (i.e. endosteal niche). The model also includes the perivascular niche composed of endothelial cells and bone marrow mesenchymal stromal cells (BM-MSCs), allowing for the creation of a vascular network. The endosteal and perivascular are designed within a resin chip created using CAD and digital light processing (DLP) 3D printing. These two niches are developed in hydrogels containing components from the bone matrix, i.e. collagen I and hydroxyapatite nanoparticles, to recreate as accurately as possible the bone microenvironment with a composition optimized to guarantee cell viability and tissue maturation.

The chip structure has been designed and the hydrogels for both niches have been optimized to allow for vascular development and bone cell activity, as observed using immunofluorescence staining of bone markers (e.g. SPARC, TRAP). Cancer cells have been added to the model and increase in both micrometastases size and number have been observed within the device (figure 1). Succeeding to create a high-throughput version of this model could improve the way pre-clinical trials are conceived, and reduce the discovery pipeline time and cost by including a physiologically-relevant tumor architecture as well as the influence of the microenvironment on cancer aggressiveness and drug resistance.

[1] F. Salvador et al. J Pathol, Sep. 2019;249(1):6–18.

[2] K. Venetis et al., Cells, Jun. 2021;10(6):1377.

[3] M. Bongio et al., Nanomedicine, May 2016;11(9):1073–91.

[4] M. V. Colombo et al., Biofabrication,Apr. 2021;13(3):035036.

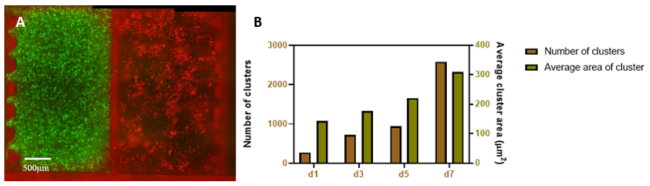


Figure 1: A: Presence of breast cancer cell clusters (in red) and vascular network (in green) 5 days after seeding. Cells in the left/perivascular niche (GFP-HUVECs and BM-MSCs) are embedded in a fibrin gel, cells in the right/endosteal niche (osteoblasts, osteoclasts and RFP-MDA-MB-231) are embedded in a fibrin, collagen, and hydroxyapatite nanoparticles gel. B: Evolution of the number and size of cancer cell clusters.

PoA.15.12

Gellan gum-based hydrogels optimized with lignin or resveratrol: investigating cartilage-like tissue maturation under a normal state or oxidative stress.

Alessandro C Scalia[1], Stefania Cometa[2], Andrea Cochis[1], Francesco Busto[3], Piergiorgio Gentile[4], Lia Rimondini[1], Elvira De Giglio[3]

[1]Università del Piemonte Orientale, Novara, Italy. [2]Jaber Innovation S.r.l., Roma, Italy. [3]Department of Chemistry, Università di Bari "Aldo Moro", Bari, Italy. [4]Newcastle University, Newcastle, United Kingdom

Abstract

Osteoarthritis (OA) still represents a clinical burden affecting millions of people worldwide involving cartilage, subchondral bone, synovial membrane, and joint capsule; it causes pain, disability, and a reduction of patients' quality of life [1,2]. Clinically, OA's pharmacological administration by non-steroidal anti-inflammatory drugs (NSAID) is often not sufficient, thus requiring surgical intervention to reduce pain and restore the tissue functionality. Tissue engineering represents a promising field aimed to develop implantable scaffolds being able to temporary or definitively replace the injured tissue; moreover, the combination of biomaterials resembling the mechanical properties of the target tissue and biomolecules boosting the healing process can represent a winning strategy for OA's treatment. Hydrogels represent very promising materials for cartilage defects repair [3,4]; among them, Gellan gum (GG), a natural polysaccharide produced by the bacteria *Sphingomonas elodea*, is gaining attention thanks to the tuneable mechanical properties and its cells' friendly behavior [5]. Accordingly, here we report about two innovative GG-based hydrogels composites aimed at cartilage repair. In the first composition, resveratrol (RESV) has been coupled with manuka honey (MH) and diatomaceous earth (DE) to improve mechanical properties and to release in situ anti-inflammatory and anti-bacterial compounds. In the second composition, the GG-based hydrogel has been enriched with the polyphenolic compound lignin (LIG) making the hydrogel suitable for 3D printing in view of personalized medicine.
After physical-chemical characterization, the GG-MH-DE/RESV systems were evaluated for their cytocompatibility, antibacterial properties, and ability to support chondrogenesis of 3D cultures both in the physiological and oxidative stress-induced environment; results confirmed the ability of RESV to protect cells from oxidative stress allowing for cartilage-like tissue maturation under inflammation, whereas MH prevented scaffolds from infection (figure 1).

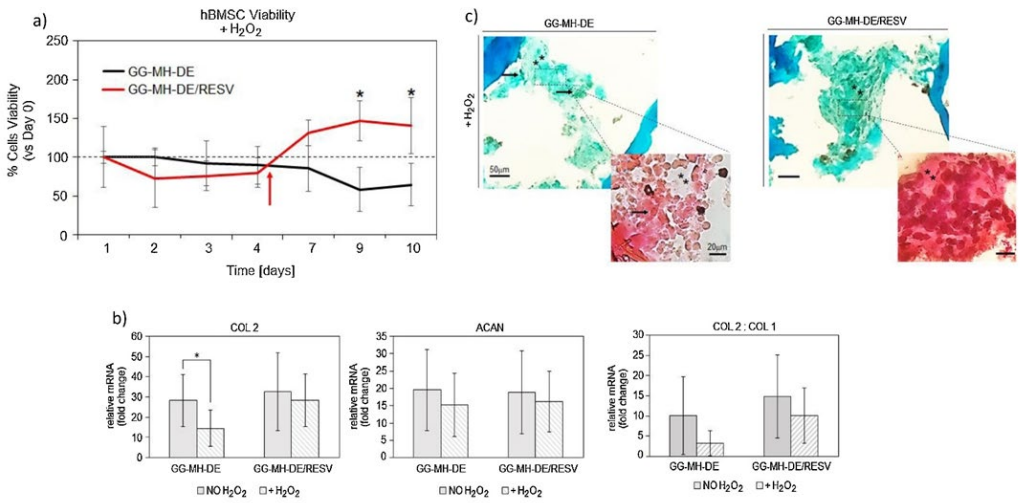


*Figure 1. Biological evaluation of GG-MH-DE/RESV. a) cytocompatibility in presence of H_2O_2; b) Expression of Col2, ACAN and Col2/Col1 ratio; c) Histological analysis after chondrogenesis. *p<0.05*

The second GG-lignin composites were evaluated for physical-chemical properties too, whereas the biological evaluation confirmed scaffolds' cytocompatibility, their ability to support chondrogenesis as well as they were successfully applied as cellularized bio inks for 3D printing (figure 2).

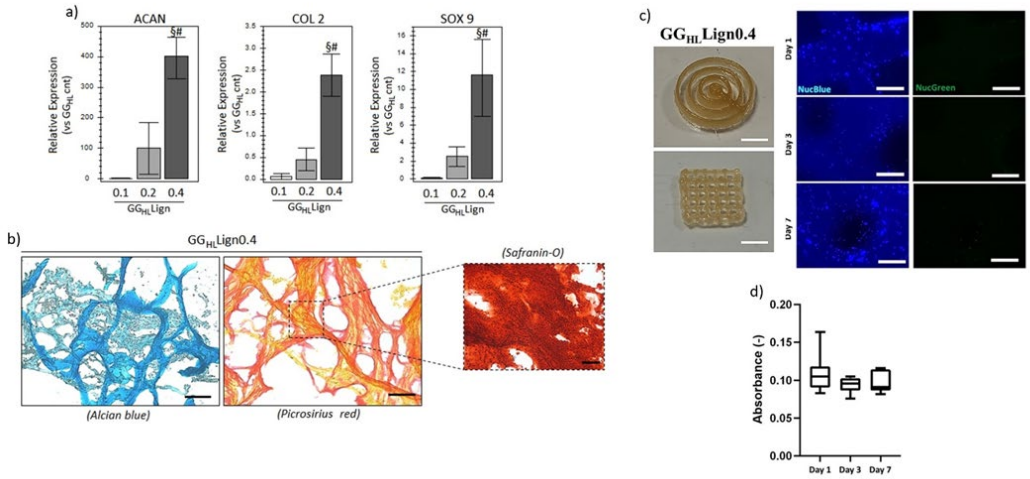


Figure 2. Biological evaluation of GG-lignin. a) Expression of ACAN, Col2 and Sox9; b) Histological analysis after chondrogenesis; c-d) Evaluation of the bio-printed constructs. § #, p<0.05

In conclusion, these innovative hydrogels composites represent promising tools for cartilage tissue engineering.

Bibliography

1. 10.1007/s40744-021-00327-7.
2. 10.1186/s12889-020-09140-3.
3. 10.3390/membranes10110348.
4. 10.3389/fbioe.2021.603444.
5. https://doi.org/10.1016/j.msec.2019.110444.

PoA.15.13

Breaking the Barrier: Investigating the Influence of ECM Stiffness on Lung Cancer Spheroid Invasion

Mahdiyeh Nouri[1], Zaid Rahman[1], Kayeu Hau[1], Ziqi Deng[1], Peter Ten Dijke[2], Pouyan E. Boukany[1]

[1]TUDelft, Delft, Netherlands. [2]Leiden University Medical Center, Leiden, Netherlands

Abstract

Lung cancer is a major cause of cancer-related deaths globally, with the process of epithelial-to-mesenchymal transition (EMT) playing a significant role in tumor invasion and metastasis. The stiffness of the extracellular matrix (ECM) is an essential factor in the tumor microenvironment, which affects EMT and tumor progression. Tissue stiffness increases significantly with disease progression, with lung cancer tumors being stiffer than normal lung tissue (20-30kPa vs. 0.5-5kPa)[1]. Studies on cervical cancer cells in 2D models have shown that cells on a 20 kPa substrate exhibit higher EMT ability and vimentin levels than those on a 1 kPa substrate[2]. However, there is limited information on EMT activation in 3D models, particularly in lung tumor models. To address this gap, this study utilized bioprinting technology to develop a 3D lung tumor model and examined whether changes in ECM stiffness affect EMT in lung tumor spheroids.

A wide range of elastic modulus (*E*) values of healthy (soft) and cancerous (stiff) lung tissue was generated using gelma with different concentrations (GELMA-3wt%, GELMA-5wt%, 0.8<*E*<3 kPa) and alginate with varied crosslinking time (Alg-4min, Alg-15min, 10<*E*<30 kPa, Figure.1a). Stiffening of the ECM was associated with slow tumor growth and stable spheroid morphology, while softer ECM caused broken spherical symmetry and spheroid invasion (Figure.1b,c). When TGFβ3, an EMT-driven stimulus, was added to the culture medium, spheroids were disseminated only in the softest gel (GELMA-3%), possibly due to matrix metalloproteinases (MMPs) being produced more in GELMA-3% and degrading ECM proteins to allow cancer cells to invade and migrate through the ECM (Figure.1c) [3]. This study highlights the crucial role of the ECM stiffness and TGFβ3 in regulating EMT, MMPs production, and ultimately, tumor growth and invasion, which may have important implications for cancer therapy.

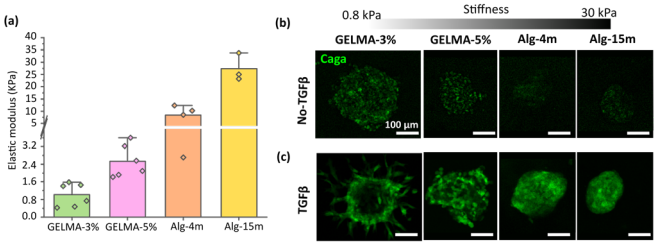


Figure1.(a) Hydrogel stiffness. (b,c)Caga expression without and with TGFβ3 in scaffolds.

Methods: Cylindrical scaffolds were printed with gelma (SigmaAldrich) and alginate (CellinkBioink). Elastic modulus measured using a mechanical compression device (CellScale). 2000 A549 lung adenocarcinoma spheroids were formed in a low adherent spheroids microplate (Corning), mixed with 1 mL gel and 3D-printed/molded into tumor models. EMT response was evaluated by adding 10 ng/mL TGFβ3 to the culture medium and observing the expression of caga and vimentin markers using confocal microscopy on day 3.

[1] Ishihara,S., et al. (2022). Cancers, 14(4), 1049.
[2] Piao,J., et al. (2017). Int.J.Oncol., 50(6), 2033-2042.
[3] Nouri,M., et al., unpublished.

PoA.15.14

Co-culture spheroids as *in vitro* models to replicate the inflammatory microenvironment of the vulnerable atherosclerotic plaque.

Ibukunoluwa Naiyeju[1], Stephanie Lehoux[2], Maryam Tabrizian[3,4]

[1]Department of Biological & Biomedical Engineering, McGill University, Montreal, Canada. [2]Lady Davis Institute for Medical Research, Montreal, Canada. [3]Department of Biomedical Engineering, McGill University, Montreal, Canada. [4]Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Canada

Abstract

INTRODUCTION: Atherosclerosis is a chronic, systemic, inflammatory disease associated with the build-up of fatty deposits ("plaques") in arteries. An enormous global health burden, severe atherosclerosis has been reported to commence when low-density lipoproteins (LDL) and cholesterol gain access into the subendothelial space due to the compromised nature of injured arterial endothelium. The presence of these foreign macromolecules kickstarts an inflammatory signalling cascade, due to the recruitment of immune cells, and this cascade contributes significantly to fast-tracking plaque rupture and precipitating adverse cardiovascular events such as heart attacks and strokes. In existing literature, there is a dearth of characterized co-culture spheroid (3D) models of the atherosclerotic plaque which could provide more insight into this inflammatory process. To address this knowledge gap, we have developed a novel co-culture spheroid model using cells representative of those found within the plaque microenvironment.
METHODS: Co-culture spheroids were fabricated using THP-1-derived M0 macrophages and human aortic smooth muscle cells (SMCs) at biomimetic co-culture ratios of 1:1 and 1:2 under varying co-culture conditions (varying culture media ratios, immediate co-culture versus step-wisely, and supplementation with or without extracellular matrix). Fluorescent labelling of the cells with cell trackers and subsequent confocal microscopy imaging was conducted to assess the morphology and location of the cell populations within the spheroids over time. Furthermore, quantification of pro-inflammatory cytokine expression (IL-1β, IL-6, IFN-γ, TNF-α) via ELISA was conducted to assess the model's feasibility to replicate the inflammatory microenvironment.
RESULTS: The formation of spheroids which retain their functionality and compactness for over 10 days was observed, with the supplementation of extracellular matrix (collagen Type 1 within the concentration range of 5-7.5 µg/mL) aiding compactness. Furthermore, the use of fluorescent cell trackers promoted the identification of the cell sub-types in within the spheroids over time, and the monitoring and quantification of cell distribution within the spheroids. ELISA quantification revealed the expression of pro-inflammatory cytokines which was attributed to the effect of the presence of the immune cells such as macrophages in the model.
CONCLUSION: Through the development of this *in vitro* model, the promising potential of spheroid models for atherosclerosis studies has been demonstrated. Furthermore, vital knowledge about the relevant parameters which enhance model design efficiency and reproducibility will be gained be as further investigations into protein expression, proliferation, and apoptosis within the model are conducted.

PoA.15.15

The Influence of WNT Signaling on Migration of Isolated Fetal Membrane Cells

Katharina Gegenschatz-Schmid[1,2], Eva Luzia Müller[1,3], Lukas Moser[4,2], Nicole Ochsenbein-Kölble[1,5,2], Martin Ehrbar[1,2]

[1]Department of Obstetrics, University Hospital Zürich, Zürich, Switzerland. [2]University of Zurich, Zürich, Switzerland. [3]University of Basel, Basel, Switzerland. [4]Department of Obstetrics, University and University Hospital Zürich, Zurich, Switzerland. [5]The Zurich Center for Fetal Diagnosis and Therapy, Zürich, Switzerland

Abstract

Introduction: In 30% of the cases, preterm premature rupture of the fetal membranes (iPPROM) is observed after minimally invasive surgeries, leading to premature birth of babies. The most likely reason for iPPROM is the insufficient potential of the fetal membranes (FM) to heal spontaneously. Interestingly, the amniotic layer of the FMs, shows a healing triggering effect when used to treat chronic skin wounds and when used as a biological bandage in eye surgeries. Therefore, to solve this enigma, the aim of this project is to investigate how different growth factors and cytokines influence the healing of FMs. Recent proteome analysis of the amnion reported the presence of several members of the WNT signaling cascade. In this work we analyzed the effect of WNT signaling and external factors on migration of cells isolated from the different FM layers.

Methods: Amniotic mesenchymal cells (AMCs), amniotic epithelial cells (AECs) and chorionic cells (CCs) were isolated from term FMs and cultured. Spheroids with individual FM cell populations were formed by hanging drop and encapsulated in TG-PEG hydrogels. To induce the three-dimensional migration of cells, the TG-PEG hydrogels were cultured in medium supplemented with PDGF-BB and were further supplemented with WNT activator (BML284), WNT inhibitor (WNTC59), 0.1% DMSO as negative control, or diluted 1:1 with conditioned medium from AMCs, AECs or CCs. Migration was assessed during a culture period of 7 days and quantified by measuring the increase in cell populated areas using ImageJ (Fiji). For confocal imaging fixed samples were immunostained for actin, fibrinogen and with DAPI.

Results: Cell migration of AMCs was stimulated by addition of PDGF-BB and conditioned medium from AECs and CCs. WNT activator BML284 inhibited AMC migration. WNT inhibitor C59 supported AMC migration insignificantly. No significant change in CC migration was observed upon addition of WNT-inhibitor, -activator or growth in conditioned medium.

Conclusion: Based on our results we can conclude that AECs and CCs rather support migration of AMCs. The effect of additional external cues such as the presence of amniotic fluid will be tested in the future. Furthermore, our results showed an unknown inhibiting effect of WNT signalling on AMCs migration. In a next step, specific WNT activators will be tested and the physiological role of WNT signalling further characterized. This could lead us one step closer to the understanding, why FMs heal insufficiently after a prenatal surgery, and could help to design FM healing biomaterials.

PoA.15.16

Engineering brain ECM-like hydrogels with Schiff-base dynamic covalent cross-links

Marta Sacchi[1,2], Antoine Hoang[1], Heloise Castiglione[3,4], Lucie Madrange[3], Frank Yates[2,3], Jean-Philippe Deslys[2], Isabelle Texier[1]

[1]Université Grenoble Alpes, CEA, LETI-DTBS, Grenoble, France. [2]Université Paris-Saclay, CEA, SEPIA-Jacob, Fontenay-aux-Roses, France. [3]CellTechs Laboratory, Sup'Biotech, Villejuif, France. [4]NETRI, Lyon, France

Abstract

Engineered 3D culture models that better mimic neural tissue are emerging tools to decipher the structural and functional complexity of the nervous system and facilitate the translation from *in vitro* to *in vivo* studies. Notably, the accurate exploration of phenotypes in 3D neural cultures demands morphological, molecular, and especially functional readout (i.e. electrophysiological characterization). Tissue-derived extracellular matrices, such as mouse-tumour-derived Matrigel™, have allowed the successful generation of cerebral organoids, efficiently recapitulating tissue and disease pathology *in vitro*. However, the mechanical and biochemical variability within a single material batch and between batches, as well as the potential tumorigenicity of Matrigel™ could lead to experimental uncertainty and lack of reproducibility. Moreover, such matrices are poorly conducive to physical or biochemical manipulation, thus further hindering the translational potential of these systems.
Advances in synthetic scaffolds have led to the development of well-defined biomimetic hydrogels with controlled features (i.e., crosslinking chemistry, polymer compositions) and physicochemical properties, such as mechanics [1]. Recent studies have shown how hydrogels with bioinspired covalent dynamic crosslinks can closely mimic the dynamics and functions of natural extracellular matrix in soft tissues, fostering organoid growth and morphogenesis *in vitro* [2]. Herein, we hypothesized that the combination of different dynamic crosslinks (Schiff bases, hydrazone and oxime bonds) in a network of polysaccharide (hyaluronan and chondroitin sulfate) hydrogels, could give rise to new biochemically controlled materials, able to sustain cerebral organoid development and morphogenesis, under dynamic, physiological-like environments. The mastering of such dynamic covalent crosslinks allowed fine control over the stiffness and relaxation behaviour of the resulting hydrogels. Notably, by varying hydrazone-to-oxime ratio, it was possible to obtain cytocompatible, self-healing hydrogels with Young's moduli and characteristic relaxation times typical of those of soft tissues.
Following the biomaterial validation as supportive matrix in the culture of human neural organoids, the introduction of an electroconductive polymer to the hydrogel formulation is under development, in order to obtain an intrinsically electroconductive, biomimetic hydrogel for *in situ* neural tissue electrophysiological analysis [3].

1. Aisenbrey E.A. and W.L. Murphy, *Nature Reviews Materials* **5**(7), 539-551 (2020).
2. Elosegui-Artola A. et al., *Nature Materials* **22**(1), 117-127 (2023).
3. Leprince M. et al., *Carbohydrate Polymers* **301**, 120345 (2023)

PoA.15.17

Growth and Metabolic Activity of Preosteoblast Cells in Meta-Biomaterials with Controlled Poisson's Ratio

Ebrahim Yarali[1,2], Amir A. Zadpoor[1], Maria Klimopoulou[1], Urs Staufer[2], Lidy E. Fratila-Apachitei[1], Angelo Accardo[2], Mohammad J. Mirzaali[1]

[1]Department of Biomechanical Engineering (BME), 3mE, Delft, Netherlands. [2]Department of Precision and Microsystems Engineering (PME), 3mE, Delft, Netherlands

Abstract

Stiffness of the scaffolds is a well-known mechanical cue that significantly impacts cells, modulating their growth, focal adhesions, growth directionality, and differentiation. Moreover, other mechanical cues, such as Poisson's ratio, regulate cellular responses in terms of proliferation and differentiation of stem cells. However, from a solid mechanics standpoint, stiffness and Poisson's ratio are mutually exclusive. Therefore, to elucidate the effect of Poisson's ratio or stiffness, they should be isolated from other mechanical and morphological cues (e.g., porosity and pore size). Mechanical meta-biomaterials are potential materials for decoupling Poisson's ratio from stiffness because of their intrinsic geometrical properties. Here, we aimed to determine the effect of Poisson's ratio on the responses of preosteoblast cells by isolating it from other mechanical and morphological properties of the meta-biomaterials.

The rationally designed meta-biomaterials (Figure 1.a.i) were additively manufactured via two-photon polymerization (2PP) at meso-scale (Figure 1.a.ii). The preosteoblasts initially settled on the bottom of the meta-biomaterials (i.e., the pedestal), Figure 1.b, day 3) and then covered the entire meta-biomaterials within 17 days of culture (Figure 1.b, days 6 and 17). It shows that the direction of the meta-biomaterials also affects the preosteoblasts directionality. Moreover, they induced a local bending in the struts, more specifically in the meta-biomaterials with negative Poisson's ratio (=auxetic, NPR) (Figure 1.b, day 10). The metabolic activity of the preosteoblast cells not only increased over 17 days of culture, but, in the presence of the meta-biomaterials with a positive Poisson's ratio (PPRs), it was higher than that of cells cultured within NPR scaffolds (Figure 1.a.iii). The differences observed in cellular responses may be associated with the different geometry of the unit cells in the PPRs (positive-angle tilted struts) and NPR scaffolds (negative-angle tilted struts). This study showed that Poisson's ratio, as a mechanical cue, should be also taken into account in the development of meta-implants.

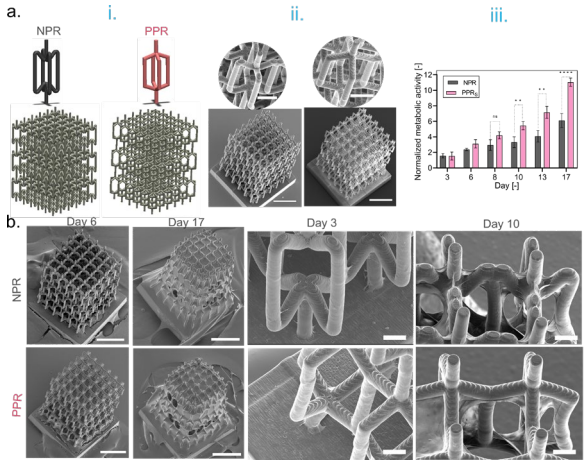


Figure 1. a.i. Schematic showing the final design of the meta-biomaterials. ii. SEM images of the 3D printed meta-biomaterials via 2PP. Scale bars for the original structures and the unit-cells are 500 and 100 µm, respectively. iii. Metabolic activity of pre-osteoblasts on the meta-biomaterials. b. SEM images of the meta-biomaterials seeded with pre-osteoblasts, showing their attachment on different days of culture. The scale bars of the images of days 6 and 17 are 500 µm and for days 3 and 10 are 50 µm.

PoA.15.18

Novel methods to analyze cell-generating tractional and intracellular force for understanding the mechanotransduction

Sung Sik Hur[1,2], Ji Hoon Jeong[3,2], Kyung Mu Noh[1,2], Jin Kwon Chung[2,4], Jae Hong Park[2,5], Yongsung Hwang[1,2]

[1]Soonchunghyang Institute of Medi-Bio Science (SIMS), Cheonan, Korea, Republic of. [2]Soonchunhyang University, Asan, Korea, Republic of. [3]Soonchunghyang Institute of Medi-Bio Science (SIMS), Cheoan, Korea, Republic of. [4]Soonchunhyang University Seoul Hospital, Seoul, Korea, Republic of. [5]Soonchunhyang University Cheonan Hospital, Cheonan, Korea, Republic of

Abstract

Cell-generating tractional and intracellular forces within physiological and pathological microenvironments and their transduction to regulate various cell behaviors have been of great interest. Although these mechanical forces exerted cells have been traditionally analyzed by utilizing polyacrylamide (PAAm) hydrogel-based traction force microscopy (TFM) due to its good linear elastic and transparent optical properties, PAAm hydrogel was failed to support three-dimensional (3D) cell encapsulation to understand the cellular forces within 3D microenvironment. Here, we aim to develop a novel method to assess cell-matrix (traction) and intracellular (and intercellular) stresses using an alternative candidate, methacrylated gelatin (GelMA), to overcome the current limitations. Our preliminary results demonstrated that viscoelastic and elastic properties of both PAAm and GelMA-based hydrogels were successfully matched, which were confirmed by measuring Young's modulus, dynamic modulus (G', G'', and tanδ), stress relaxation, and strain recovery. Next, we evaluated various cell-generating forces of either tonsil-derived stem cells in a single cell level or conjunctiva-derived epithelial cells as a monolayered cell sheet using GelMA-based traction force microscopy (TFM), intracellular force microscopy (IFM), and monolayer stress microscopy (MSM), where the results were also compared and validated using PAAm-based methods. Our initial findings indicate that our GelMA-based TFM, IFM, and MSM methods exhibited cell-generating forces and mechanotransduction in a spatio-temporal manner. Taken together, these results suggest GelMA can be a good candidate for the cell-ECM and intracellular stress measurement platform and this technique can offer insight into the important roles of dynamic cell-matrix and intracellular stresses in regulating stem cell lineage commitment and disease progression within 3D microenvironments.

PoA.15.20

SERS sensing and imaging in 3D printed *in vitro* cancer models

Clara García-Astrain[1,2], Malou Henriksen-Lacey[1,2], Elisa Lenzi[1], Paula Vázquez-Aristizabal[1,3,4], Patricia González-Callejo[1], Dorleta Jimenez de Aberasturi[1,2,5], Luis Liz-Marzán[1,2,5]

[1]CICbiomaGUNE, Donostia-San Sebastián, Spain. [2]CIBER-BBN, Donostia-San Sebastián, Spain. [3]Biodonostia, Donostia-San Sebastián, Spain. [4]Universidad del País Vasco, Donostia-San Sebastián, Spain. [5]Ikerbasque, Bilbao, Spain

Abstract

Bioprinting is an innovative technique that holds immense potential for fabricating scaffolds that support tissue or tumor growth [1]. A wide range of biopolymer and hydrogel-based inks are now available for creating increasingly complex 3D cell models. However, there is a crucial need for detection tools that can effectively monitor cell behavior in a non-invasive way within 3D microenvironments over extended periods [2]. To address this issue, 3D printable inks can be modified to create scaffolds with sensing properties that can monitor tissue growth or disease evolution [3]. In this study, we explore the potential of incorporating plasmonic nanoparticles to different ink formulations to perform sensing and imaging within 3D printed tumor models by means of Surface-Enhanced Raman Scattering (SERS). This technique utilizes the remarkable optical properties of noble metal nanoparticles, which exhibit Localized Surface Plasmon Resonances (LSPR) that enable them to absorb and scatter light at specific wavelengths, generating high local electric fields on the surface [4]. These electric fields enhance the Raman scattering of molecules near the metal surface and permit extremely low detection limits and multiplex detection ability. Additionally, the excitation wavelength can be tuned to the near infrared range (NIR) that corresponds to the biological transparency window (650-1350 nm), enhancing light penetration into tissues [5]. For this work, different 3D models were fabricated using biopolymers (methacrylated hyaluronic acid), synthetic polymers (polyethylene glycol diacrylate) or decellularized extracellular matrix (dECM)-based inks containing gold nanostars or gold nanorods for *in vitro* sensing and imaging. For sensing applications, bare gold nanoparticles were employed for detection of cell-secreted metabolites whereas SERS imaging was performed using gold nanoparticles decorated with SERS tags that can be also internalized by cells (Figure 1). Different material compositions, printing configurations and nanoparticle combinations were explored in order to perform sensing as well as multiplex imaging of different cell populations in a 3D fashion.

1. P. Vázquez-Aristizabal et al., ACS Omega, 7, 2022, 16236
2. C.S. Hansel et al. Biomateirals, 226 (2020), 119406
3. C. García-Astrain et al., Advanced Functional Materials, 30 (2020), 2005407
4. J. Langer et al., ACS Nano, 14 (2020), 28
5. E. Lenzi, et al. ACS Sensors, 4 (2019), 1126

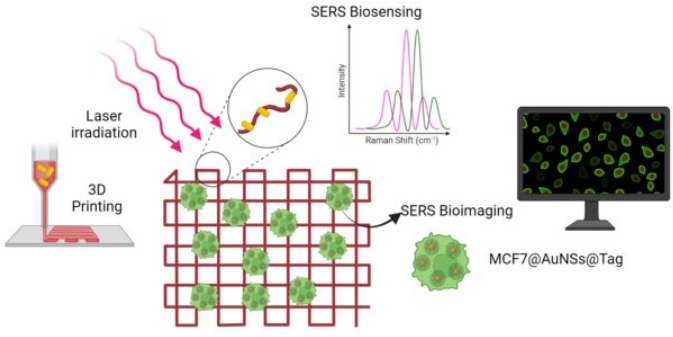


Figure 1. Schematic of 3D printing of plasmonic scaffolds for SERS sensing and imaging of cancer cells

PoA.15.22

Anisotropic 3D hydrogels with spatial patterns in degradation guide hMSC differentiation

Claudia Garrido[1,2], Daniela Garske[1,2], Shahrouz Amini[2], Katharina Schmidt-Bleek[1], Georg N Duda[1], Amaia Cipitria[3,2]

[1]Julius Wolff Institute, Charité - Universitätsmedizin Berlin, Berlin, Germany. [2]Max Planck Institute for Colloids and Interfaces, Potsdam, Germany. [3]Biodonostia Health Research Institute, San Sebastian, Spain

Abstract

Encapsulation of stem cells encapsulation in biomaterials offer a novel therapeutic technique. Patterned materials allow to integrate multiple characteristics in a single material which better mimic the anisotropy of biological tissues. We hypothesize that spatial patterning of matrix biophysical cues (ex. Degradation) could modulate stem cell behavior and differentiation. Therefore, this study aims to evaluate cell response in 3D soft alginate-based hydrogels with spatial patterns in degradation.

Degradation patterns in the material were formed using photopatterning and the combination of two crosslinking methods: MMP-sensitive peptide with UV crosslinking to form degradable regions (Deg) and norbornene-tetrazine with Diels Alder click chemistry for non-degradable regions (nonDeg). Each bulk material was mechanically characterized based on rheology (for single phase materials) and surface microindentation (for patterned materials). To analyze cell differentiation in both Deg and nonDeg phases, primary human mesenchymal stem cells (hMSCs) were encapsulated in the 3D patterned hydrogels and analyzed with respect to growth, adipogenic and osteogenic differentiation. Cell viability (live/dead staining), cell morphology (DAPI/phalloidin), proliferative state (Ki67), mechano-sensation (YAP expression and nuclear translocation) and differentiation (mineralization using OsteoImage, osteocalcein expression and oil droplets stained with Nile Red) were evaluated at days 1 and 14. All statistical evaluations were performed using Wilcoxon Signed Rank test ($p < 0.05$).

The mechanical characterization in bulk and nano-indentation showed comparable elastic modulus in both Deg and nonDeg phases (1-2 kPa). hMSC viability remained high (>85%) over the 14 days in both phases. Differences in hMSC morphology within patterned materials were observed, with a significant increase in cell spreading (projected area) and decrease in circularity in the Deg phase. Mechanotransduction markers such as YAP have a different localization in the two phases, the nuclear translocation of YAP is evident in Deg phase, compared to higher cytoplasmic expression in noDeg phase. hMSC osteogenic differentiation under growth media was more pronounced in Deg compared to nonDeg phase, as higher osteocalcein expression is evident in Deg phase. The previously mentioned differences in cell behaviour between Deg and nonDeg phases, were enhanced when the hydrogels were cultured in osteogenic media.

Patterned hydrogels were fabricated with spatially tunable degradation properties determined by the crosslinking type. Differences in degradation properties influenced 3D hMSCs proliferation, morphology and differentiation. Spatially segregated osteogenic differentiation was achieved as higher osteogenic differentiation was promoted in degradable areas compared to non-degradable phases. Ongoing research is focused on exploring patterned materials under dynamic conditions subjected to fluid flow.

PoA.15.24

Alginate and hyaluronic acid-based hydrogels loaded with antioxidant nanoentities as human dental pulp stem cells carriers to improve cell therapy in central nervous system.

Sara Martín-Colomo[1], Gaskon Ibarretxe[2], Jose Ramon Pineda[2], Aitor Larrañaga[1]

[1]University of the Basque Country, Mining-metallurgy Engineering and Materials Science, Bilbao, Spain. [2]University of the Basque Country, Cell Biology and Histology, Leioa, Spain

Abstract

Central nervous system (CNS) lesions are characterized by the overproduction of reactive oxygen species (ROS), which provoke cell loss, thus having detrimental consequences in the damaged tissue regeneration. Stem cell-based therapies have shown poor success due to reduced cell survival and compromised cell differentiation capabilities when transplanted within this hostile environment. Herein, we followed a combinatorial approach by exploiting i) the accessibility of human dental pulp stem cells (hDPSCs) showing potential to differentiate towards neural-like cells and ii) a brain-mimetic protective microenvironment based on alginate/hyaluronic acid hydrogels loaded with antioxidant nanoentities as cell carriers (Figure 1).

To fabricate hydrogels, sodium alginate was physically crosslinked by using Ca^{2+} ions. Hyaluronic acid (HA) of either low-100 KDa or high-1 MDa molecular weight was incorporated to mimic the stiffness and composition of the extracellular matrix (ECM) of CNS. Finally, our hydrogels were loaded with antioxidant nanoentities (e.g. MnO_2 and CeO_2 nanoparticles) to protect the cells from the excess of ROS.

To use our hydrogels as cell carriers, we assessed their mechanical properties and then encapsulated our cells within, testing their compatibility with astrocytes (the main cells in CNS with a supportive role for neurons) of murine origin, and human stem cells isolated from dental pulp. hDPSCs present the advantage of their neural crest origin, and thus potential differentiation capabilities for CNS therapy, and of their isolation from third molars, concerning accessibility and a reduction of ethical concerns as dental pieces are considered biological waste. We characterized their interaction with the hydrogels before and after adding the antioxidant nanoentities. Furthermore, we compared our results with different concentrations and molecular weights of HA into the alginate matrices, and analyzed cell viability, metabolism and expression of CNS markers for hDPSCs upon differentiation.

Our results suggested that adding increased concentrations of HA caused slightly softer structures and allowed cell interaction contrary to only the alginate matrix. Moreover, incorporating antioxidant nanoentities supported the cells viability and differentiation.

In conclusion, our hydrogels are a new useful tool to carry cells, being a potential approach to deliver stem cells for the damaged CNS regeneration.

This work has been funded by UPV/EHU (COLAB22/07) and Basque Government (IKUR, IT1751-22, IT1766-22). Sara Martín-Colomo also acknowledges the predoctoral grant from the UPV/EHU (PIF22/119).

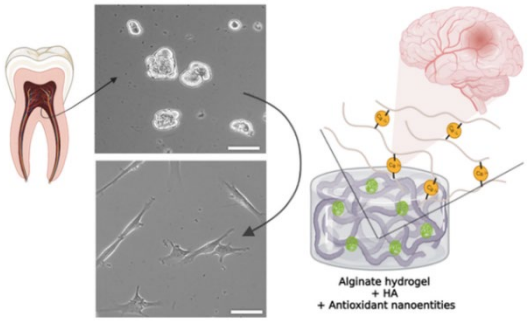


Figure 1: Scheme of research work. hDPSCs forming-floating dentospheres as a control culture (upper) vs hDPSCs culture in proliferative conditions with surface-coated extracellular matrix (down). Scale bar 50mm.

PoA.15.25

Correlation between cell-material and cell-cell interactions and the formation of spheroids during culture

Roberta Teixeira Polez[1,2], Ngoc Huynh[1], Chris S. Pridgeon[2], Juan José Valle-Delgado[1], Riina Harjumäki[2], Monika Österberg[1]

[1]Aalto University, Espoo, Finland. [2]University of Helsinki, Helsinki, Finland

Abstract

The US Food and Drug Administration (FDA) has recently eliminated the need for animal testing in the development of novel drugs and products, creating an opportunity for spheroids and organoids to replace, decrease, and refine regulatory animal testing. Compared to monolayer culture, these 3D spheroid cell cultures demonstrate greater *in vivo*-like cellular functions. The use of biomaterials in culture methods offers several advantages, such as mimicking the natural cell environment and improving the models' metabolic relevance. To enhance 3D cell culture systems and discover new potential materials for these cultures, it is crucial to quantify cell-biomaterial and cell-cell interactions and identify the types of interactions that allow cell spheroid formation. This study employed an AFM-based technique that utilizes colloidal probe microscopy (CPM) to evaluate adhesion forces between cells and biomaterials and single-cell force spectroscopy (SCFS) to quantify the adhesion between cells and relate them to spheroid formation. Within 72 hours, two different cell lines (HepG2 and iPS(IMR90)-4) formed rounded spheroids when cultured in cellulose nanofibrils (CNF)-embedded culture. Matrigel-embedded culture, however, produced looser aggregations with different morphology than spheroids. The adhesion force between HepG2 cells was greater than the adhesion force between CNF and HepG2 cells. However, CNF provided an environment that was favourable for the formation of cellular spheroids, most likely due to strong but non-specific interactions (hydrogen bonds) with the cells. Regarding the stem cells, it was observed that the iPS(IMR90)-4 interaction with Matrigel was the strongest. The weakest interactions were observed between CNF and stem cell self-interaction. These findings suggest that cell-cell interactions played a dominant role in the formation of spheroids in CNF-embedded culture, whereas cell-matrix interactions played a significant role in hindering spheroid formation in Matrigel-embedded culture. Therefore, characterizing cell adhesion to biomaterials and using force spectroscopy measurements can help identify the underlying molecular mechanisms to enhance *in vitro* drug toxicity testing.

PoA.15.26

The antifibrotic effect of minoxidil administration on clubfoot-derived cells in a crowded microenvironment

Martina Doubkova[1,2], Jarmila Knitlova[1], Adam Eckhardt[1], Martin Ostadal[3], Tomas Novotny[4,2], Elena Filova[1]

[1]Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic. [2]Charles University, Second Faculty of Medicine, Prague, Czech Republic. [3]First Faculty of Medicine, University Hospital Bulovka, Charles University, Prague, Czech Republic. [4]Masaryk Hospital Usti nad Labem, Usti nad Labem, Czech Republic

Abstract

Idiopathic clubfoot (CF) is a complex congenital orthopedic deformity causing rotation of the foot. A stiff, contracted tissue in the medial part of the foot shows signs of fibrosis. Standard treatment relies on physiotherapy, repeated casting, and tenotomy. However, antifibrotic agents could be administered to enhance the treatment. Minoxidil (MXD), a drug with an inhibitory effect on lysyl hydroxylases, influences the extracellular matrix (ECM) crosslinking. We assessed its antifibrotic potential in a model of CF fibrosis induced by macromolecular crowding. We added polymer polyvinylpyrrolidone and a differentially sized sucrose co-polymers (Ficoll70, Ficoll400) to cell culture medium to crowd it. In order to mimic macromolecular crowding (MMC) of the physiological environments corresponding to the fractions of the volume occupied (FVO) by the macromolecules in the media, we tested concentrations in the range of 4%-54% FVO. We assessed the changes in ECM production of human dermal fibroblasts (NHDF, control) and clubfoot fibroblasts (CF) in culture media with tested MMC concentrations, ascorbic acid and 5 or 10% of fetal bovine serum. The CF cells were then grown in culture media with or without the presence of optimal MMC and MXD (0.25 - 2 mM) for 3 to 16 days. We assessed cell proliferation, cell metabolic activity/viability (resazurin), quality and quantity of deposited ECM collagen and its crosslinking by immunofluorescent staining, SHG imaging, RT-qPCR, pyridinoline EIA and SIRCOL assays.
The fastest deposition and maturation of ECM were consistently achieved with Ficoll at 18% FVO in cell culture media with 10% serum, without a significant increase in cell proliferation. The total amount of newly produced acid-soluble and insoluble collagen in ECM on day 7 and later were at least 1.5x and 2x higher, respectively, than in the control. Concentration-dependent inhibition of cell proliferation by MXD in the control culture was shifted, making the higher concentrations of MXD better tolerated by the cells in MMC. Gene expression of lysyl hydroxylases, lysyl oxidase, but also collagen type I and III, fibronectin, alpha actin were reduced in MMC and MXD groups after 3 days. MMC is an important tool for the *in vitro* screening of potential antifibrotic drugs, such as minoxidil, for adjuvant pharmacological therapy in the standard treatment of relapsed CF.
Support: The Czech Science Foundation, Ministry of Health of the Czech Republic (NU22-10-00072, NU22-06-00016), Charles University (project GA UK, No. 410121), The Academy of Sciences of the Czech Republic, Praemium Academiae (grant No. AP2202).

PoA.15.28

A Synthetic Void-Forming Hydrogel for *In vitro* Generation of Functional Bone Cell Networks

Doris Zauchner[1], Monica Z. Müller[1], Leana Bissig[1], Feihu Zhao[2], Sung Sik Lee[3], Ralph Müller[1], Xiao-Hua Qin[1]

[1]Institute for Biomechanics, ETH Zürich, Zurich, Switzerland. [2]Department of Biomedical Engineering and Zienkiewicz Institute, Swansea University, Swansea, United Kingdom. [3]Scientific Center of Optical and Electron Microscopy, Institute of Biochemistry, ETH Zürich, Zurich, Switzerland

Abstract

During osteogenesis, osteoblasts create a soft organic matrix, which gradually mineralizes. Embedded osteoblasts differentiate into osteocytes which form a 3D cell network within the lacuno-canalicular system where they sense fluid shear stresses (FSS) induced by mechanical loading. This dynamic microenvironment is essential for bone formation, yet *in vitro* generation of a functional 3D bone cell network to study human osteogenesis remains difficult. To address this challenge, we advance a previously reported macroporous poly(ethylene glycol) (PEG) hydrogel[1] with cell-degradability and tunable porosity to promote rapid formation of 3D bone cell networks and subsequent osteogenic differentiation for future application in microfluidic perfusion culture.

Herein, we report a synthetic, matrix metalloproteinase (MMP)-sensitive hydrogel that undergoes in situ pore formation via polymerization-induced phase separation (PIPS). The hydrogel comprises 4-arm-PEG-vinylsulfone, an MMP-degradable di-thiol peptide crosslinker, an RGD peptide to promote cell attachment, and dextran (**Figure 1a**). Confocal microscopic imaging demonstrated that PIPS resulted in a composition-dependent pore size distribution ranging from 2 to 20 µm (**Figure 1b**). After embedding human mesenchymal stem cells (hMSCs), 3D cell network formation was observed after 24h under osteogenic culture conditions. Importantly, we found that cell-matrix remodeling by proteolysis was crucial for long-term maintenance of embedded bone cell networks. Additionally, we observed enhanced collagen secretion in MMP-degradable hydrogels during osteogenic culture for up to 30 days (**Figure 1c**). Integration of this permissive hydrogel with a microfluidic chip enabled us to mimic interstitial fluid flow within bone tissue with FSS around 2 Pa as determined by computational fluid dynamics (CFD) simulation (**Figure 1d**). Finally, we will present preliminary results on microfluidic perfusion culture.

Altogether, this work highlights a synthetic cell-degradable hydrogel enabling rapid generation of functional 3D bone cell networks and microfluidic perfusion, providing a new platform for future studies of bone (patho-)physiology during osteogenesis.

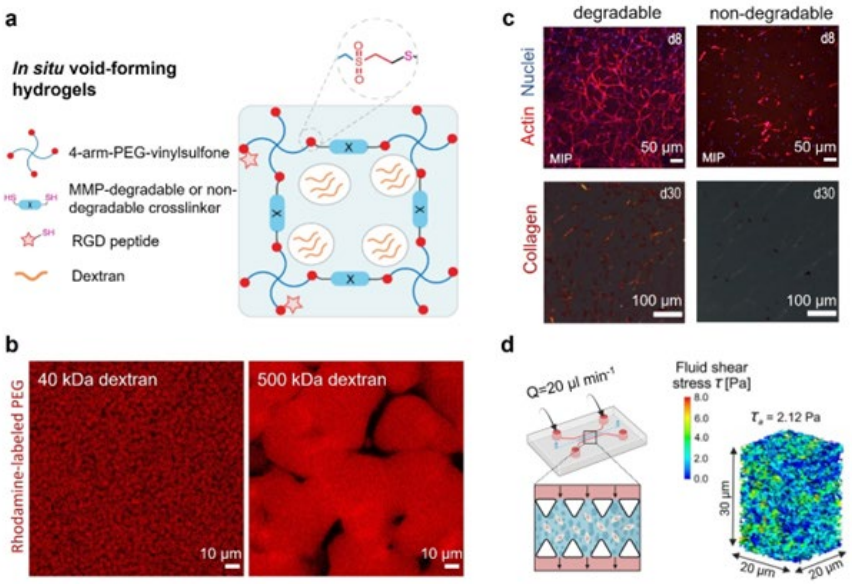


Figure 1. a) *Macroporous PEG hydrogels formed by PIPS.* ***b)*** *Confocal microscopy images of labeled PEG hydrogels with tunable pore size.* ***c)*** *hMSCs embedded in degradable hydrogels showing cell network formation on day 8 and collagen secretion on day 30.* ***d)*** *CFD simulation of local mechanical environment within a macroporous hydrogel under perfusion showing the FSS distribution and average FSS (τ_a).*

[1] N. Broguiere, A. Husch, G. Palazzolo, F. Bradke, S. Madduri, M. Zenobi-Wong, Biomaterials 2019, 200, 56.

PoA.15.29

Spheroid Calcification for Regenerative Medicine Applications

Steven Vermeulen[1], Kèvin Knoops[2], Hans Duimel[2], Denis van Beurden[1], Carmen López Iglesias[2], Stefan Giselbrecht[1], Roman Truckenmüller[1], Pamela Habibović[1], Zeinab Tahmasebi Birgani[1]

[1]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands. [2]Maastricht MultiModal Molecular Imaging Institute - M4i, Microscopy CORE Lab, Maastricht, Netherlands

Abstract

INTRODUCTION: Musculoskeletal disorders place a significant burden on individuals and the healthcare system. In this context, a challenge is to recapitulate the complex, three-dimensional (3D) calcified microenvironment of bone *in vitro* to develop regenerative therapies. On the other hand, tissue calcification, vital for proper bone function, also occurs unwanted in soft tissues. Examples are atherosclerosis and the Stone man disease of which limited knowledge exists concerning disease development and therapies[1,2]. Therefore, there is an urgent need to develop better calcification models to understand the underlying pathophysiology. Here, we discuss the development of spheroid models to study bone regeneration, pathological calcification and to identify compounds that promote or inhibit calcification.

METHODS: Polycarbonate film-based arrays of 500 µm diameter microwells were generated using microthermoforming[3] **(Fig. 1A)**. Human mesenchymal stem cells (hMSCs) were seeded inside the microwells for aggregation into spheroids **(Fig. 1B)**. 24 hours after cell seeding, calcification media (CM; 4 mM Ca^{2+} or 2 mM inorganic phosphate (Pi)) was added to allow spheroid calcification **(Fig. 1C)**. Gene expression was assessed by qPCR. Compounds were added during spheroid calcification at a final concentration of 10 µM. Calcification was quantified using OsteoImage and measuring fluorescence intensity.

RESULTS AND DISCUSSION: A spheroid culture and Ca^{2+}/Pi signaling influenced the osteogenic marker bone morphogenetic protein 2 (BMP2) **(Fig. 2A)**. Ca^{2+}/Pi signaling further enhanced BMP2 levels in the spheroid microenvironment. Next, we assessed if compounds affect calcification using a high-throughput screening approach. **Fig. 2B** represents a hit with the compound dorsomorphin, which increased calcification. These findings suggest that a spheroid environment combined with inorganic signaling is an efficient way to enhance calcification, induce osteogenesis, and allows the identification of compounds that alter calcification for regenerative medicine applications.

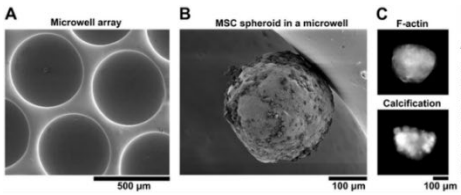


Figure 1: Creation of calcification models in microwells. A) Scanning Electron Microscopy (SEM) image of a 289-microwell array. B) SEM image of an MSC spheroid. C) MSC spheroids cultured in CM exhibit internal calcification after 10 days.

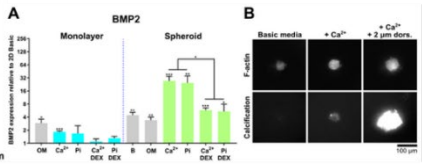


*Figure 2: Calcified spheroids as a model system. A) Spheroid culture and Ca^{2+}/Pi signaling affects BMP2 (B=basic medium; OM=osteogenic medium; DEX=dexamethasone;*P<0.05;**P<0.01;***P<0.001). B) Dorsomorphin promotes calcification.*

REFERENCES

1. Ross, R. Mech. Dis. 340, 115–126
2. Mahboubi, S., et al. Pediatr. Radiol. 31, 307–314
3. Kakni, P. et al. Adv. Biosyst. 2000126

ACKNOWLEDGMENTS

This work received funding from Interreg Vlaanderen-Nederland (grant no. 0433), RegMedXB, the Dutch Province of Limburg (no. SAS-2014-00837, SAS-2018-02477), the Gravitation Program of the NWO (no. 024.003.013).

PoA.15.31

Next-generation tissue engineering: advancing *in vitro* cell culture with human protein-based materials

Catarina A Custódio[1,2], Cátia F Monteiro[1], Sara C. Santos[1], Diogo Correia[1,2], João F. Mano[1]

[1]Department of Chemistry, CICECO - Aveiro Institute of Materials University of Aveiro, Aveiro, Portugal. [2]Metatissue, Ílhavo, Portugal

Abstract

In the past years, there has been a surge of interest in creating materials that closely resemble human tissues for use in tissue engineering, regenerative medicine and drug screening. Human protein-based materials hold immense potential to develop such platforms. We were pioneers in developing human protein-based materials that have controllable mechanical properties, using biological waste materials like expired blood units and placenta. (1,2) In the presented work we have successfully validated the use of human based hydrogels and sponges for engineering a disease model for osteosarcoma and expanding human cells, without the need for any animal-derived supplements. The blood components used in this work were acquired from hospitals and clinical institutions, ensuring that all mandatory consent or authorization requirements were met prior to procurement. Upon receipt of the tissues, we isolated the proteins and use our proprietary technology to chemically modify them. The resulting products are identified as methacrylated platelet lysates (PLMA). 3D *in vitro* models for cancer have the potential to reveal valuable insights into the disease mechanisms. As a proof-of-concept we have developed a osteosarcoma(OS)-on-a-chip model to recreate the early metastatic process of tumor invasion. A non-metastatic or metastatic OS tumor spheroid (MG-63 and 143B, respectively) was embedded in PLMA and co-cultured with human bone marrow mesenchymal stem cells. The model was able to replicate the interactions between tumor and stromal cells, as well as the interaction between cells and the extracellular matrix. The model was also able to demonstrate increased drug resistance in metastatic cells when exposed to doxorubicin, suggesting its potential for drug discovery. In a different approach PLMA porous microsponges were prepared by freeze drying PLMA based hydrogels under controlled conditions. The porous particles based on PLMA provide a revolutionary way to culture cells for cell expansion without the need for animal derived serum supplements. Results show that cells can be easily detached from the particles with a simple enzymatic treatment with high viability. This breakthrough offers novel avenues for clinically-compliant protocols for GMP *in vitro* cell culture.

[1] Santos, S. C., et al. (2018).Adv. Healthc. Mater. 7,1800849

[2] Deus, I.A. et al. (2022).Biomaterials Advances. 134,112574

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC) and also within the scope of European Union's H2020 InterLynk project (grant agreement No 953169).

PoA.15.32

Micro-perfusion of bioengineered tissues with an automated platform to induce vascularization

Stéphanie Boder-Pasche[1], Sarah Heub[1], Ary Marsee[2], Manon Garzuel[1], Charlotte Fonta[1], Diane Ledroit[1], Jonas Goldowsky[1], Réal Ischer[1], H. Baris Atakan[1], Thomas M. Valentin[1], Kerstin Schneeberger[2], Bart Spee[2], Gilles Weder[1]

[1]CSEM SA, -, Switzerland. [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Regenerative Medicine Center Utrecht, Utrecht University, Utrecht, Netherlands

Abstract

Bioengineered constructs such as with bioprinting techniques need to undergo maturation phases before leading to functional, vascularized tissues. By recreating a physiological micro-environment, continuous medium perfusion through the bioconstruct provides oxygen and nutrients to the tissue, thereby promoting the formation of a vascular network. Proposing a disruptive alternative to donor organs for liver regenerative medicine, the EU project ORGANTRANS is developing a liver tissue printing platform from cell source to functional tissue, relying on organoid technology as building block for liver tissue transplantation [1]. A liver bioconstruct was printed by combining spheroids and hydrogel, with sacrificial channels to enhance vascularization. To mature the freshly bioprinted liver construct into functional tissue, a fluidic platform was developed, enabling perfusion of the construct in a standardized and automated way. Compatible with multi-well plates, it relies on a microfluidic "Smart Lid" for perfusion, leading to automated and parallelized medium circulation through the tissue, thereby enabling long-term tissue growth, vascularization and maturation. The perfusion platform consists of six disposable sterile inserts, into which the bioconstruct is printed, and which fit into standard 6-well plates. A sterile lid with integrated microfluidic features is sealed onto the inserts, creating a closed perfusion chamber. Medium flows thanks to a multi-channel peristaltic pump controlled by dedicated electronics. Biocompatible and sterilizable materials are used for all parts being in contact with the tissue and cell culture media.
The perfusion platform enables continuous and unidirectional flow through the six wells in parallel, with tunable flow rates from 10 to 500 µL/min. Perfusion of 2D cell culture for two weeks in an incubator showed cell proliferation with no impact on cell viability. Continuous perfusion through six 1x1x0.5 cm^3 freshly printed spheroid-laden liver bioconstructs containing channels lined with endothelium was tested for up to 13 days, showing sprouting and viability of embedded spheroids.
.The perfusion platform enabled to test and tune the maturation process of liver tissue bioconstructs for several weeks, and to initiate *in vitro* vascularization in sterile and closed environment. The platform can be adapted to other tissues and geometries, thereby enabling standardization of tissue engineering processes for regenerative medicine and drug testing applications.

[1] www.organtrans.eu, EU Horizon 2020, grant agreement No 874586

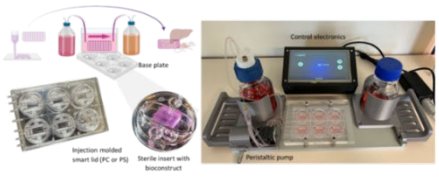


Figure 1. Setup for the automated perfusion platform

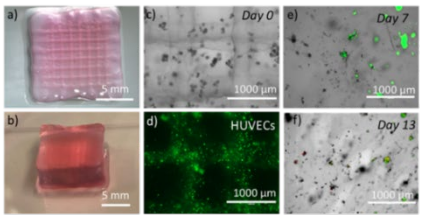


Figure 2. (a,b) Freshly printed spheroid-laden bioconstructs with sacrificial channels; (c-f) microscopy images of the bioconstruct showing viable spheroids in the gel and HUVECs in sacrificial channels

PoA.15.33

Extracellular matrix as a key component in the production of functional andphysiologically stable artificial pancreatic islets using the inkjet method.

Marta Klak[1,2], Dominika Ujazdowska[2], Oliwia Janowska[1], Sylwester Domański[1], Andrzej Berman[1,2,3], Michał Wszoła[1,2,3]

[1]Polbionica Sp. z o.o., Warsaw, Poland. [2]Foundation of Research and Science Development, Warsaw, Poland. [3]Medispace Sp. z o.o., Warsaw, Poland

Abstract

INTRODUCTION: There are clinical trials report using stem cell-derived β-cells as an innovative and future-proof solution for the treatment of T1D. Stem cell-derived β-cells are expected to replace non-functioning pancreatic islets. However, in order to make this possible, it is necessary to create their 3D conformations, which has been proven by subsequent *in vitro* studies. However, apart from functionality, attention should be paid to the possibility of clinical use of beta cells. The process of transplanting claster β-cells even from 3D cultures into the portal vein carries a high risk of damage and lack of functionality, as well as the risk of an undetermined final location of the cells. The aim of this experiment was to evaluate the survival and functionality of β-cells in artificial pancreatic islets, printed with inkjet method. MATERIAL&METHODS: INS-1E cells were used in the study. Two bioinks were used as the encapsulation carrier: 2% HAMA + 20% GELMA (GROUP: H-G_INS); 2% HAMA + 20% GELMA + dECM (GROUP: ECM_INS). The control group was INS-1E in 2D culture. Cell functionality was assessed in the GSIS-test. In addition, FDA/PI vital staining was performed. One test sample contained 3.5 million β-cells. RESULTS: During the 21-day observation, it was shown that the cells encapsulated by the inkjet method show practically 100% viability. However, they accounted for no more than 15% of the examined pool of cells. Cells suspended in the tested variants of hydrogels retained a stable structure and did not disintegrate. On the second day of the experiment, there was no difference in cell activity. Groups of encapsulated cells showed significantly improved functionality from day 7 onwards. Both groups showed over 30% higher functionality compared to the control group. On the 14th day of the experiment, cells suspended in bioink with dECM showed a definite superiority in response to the administered glucose. Compared to the control group, the increase was over 50% (p=0.0005), and with the H-G_INS over 30% (p=0.0073). Day 21 of the experiment also showed a functional advantage in the ECM_INS, almost 30% higher activity compared to the control group (p=0.0040). CONCLUSION: dECM a 3D conformation of cells within a bioprinted islets is a key component for maintaining the proper functionality of insulin-secreting cells. In addition, the developed bioink composition and the method used enable the production of stable 3D structures that can be transplanted in a stable and safe manner without disintegrating in physiological temperature conditions.

PoA.15.35

Generation of three-dimensional hepatic spheroids by ink-jet method for testing the activity and cytotoxicity of drugs.

Małgorzata Popis[1], Oliwia Janowska[1], Anna Papierniak-Wyglądała[1], Tomasz Dobrzański[1], Michał Wszoła[1,2,3], Marta Klak[1,2]

[1]Polbionica Ltd., Warsaw, Poland. [2]Foundation of Research and Science Development, Warsaw, Poland. [3]Medispace Medical Centre, Warsaw, Poland

Abstract

Introduction: The problem of drug toxicity testing is still up to date, as there is a lack of reproducible model mimicking the complexed microenvironment of human liver. Pharmacological research process most usually begins with 2D culture, co-culture or spheroids and then switch to *in vivo* tests on animal models. Such approach fulfills need for testing in complexed environment, however does not address toxicity in human tissue microenvironment. The creation of 3D bioprinted flow tissue models that could solve this problem is conditioned by properties of the biomaterial, such as stimulation of gas exchange and distribution of nutrients, while maintaining printability to create complicated structures such as hepatic lobules. Therefore, the aim of our research was to characterize the biocompatibility of biomaterial and preliminary assessment of its applicability in the generation of biocontructs mimicking human liver tissue.
Materials and methods: The use of ink-jet technology requires the development of a special hydrogel that will be a carrier for suspended cells and at the same time will provide an optimal environment. Two formulations of hydrogels were developed for the purposes of this project. The first consisted of methacrylates: gelatin and hyaluronic acid. The second was enriched with decellularized extracellular matrix. Both variants of the hydrogels were enriched in the LAP photoinitiator, which enabled the cross-linking of three-dimensional structures at 405nm. Both bioink variants were mixed with a hepatocyte and endothelial lines suspended in culture medium. The hydrogel drops were placed on the inserts and cultured for 21 days. At the designated time points, microscopic imaging was performed using live/dead staining (FDA/Pi) and the material was secured for histological analysis. Culture medium was also taken to evaluate cell activity.
Results: In the case of both analyzed hydrogels, a high (over 90%) ratio of living cells was found in the produced bioconstructs. Cell morphology was assessed by direct microscopic observation and H+E staining, demonstrating the uniformly distributed cells and their ability to proliferate to form spheroids. Increased proliferation and thus viability was demonstrated throughout the observation period.
Conclusion: Both bioinks showed biocompatibility with the hepatic cell line and therefore in further stages of work they will be used in the process of 3D bioprinting of tissue models with a flow system. Then, these models will be used in cytotoxicity and activity studies of biologically active substances.

PoA.15.37

Exploring osteoblast-osteoclast interactions in an organ-on-chip model via biomimetic bone-remodeling micro-units

Francisco Conceição[1], Nuno Araújo-Gomes[2], Johanna Husch[2,3], Jeroen van den Beucken[3], Jeroen Leijten[2], Liliana M. Teixeira[1,4]

[1]Department of Advanced Organ Bioengineering and Therapeutics, Technical Medical Centre, University of Twente, Enschede, Netherlands. [2]Department of Developmental Bioengineering, Faculty of Science and Technology, Technical Medical Centre, University of Twente, Enschede, Netherlands. [3]Department of Dentistry-Regenerative Biomaterials, Radboud Institute for Medical Innovation, Radboudumc, Nijmegen, Netherlands. [4]Organ-on-Chip Centre Twente, Technical Medical Centre, University of Twente, Enschede, Netherlands

Abstract

Bone health is maintained through the carefully orchestrated process of bone remodeling. During this process, old and damaged bone is resorbed by specialized cells, known as osteoclasts, and replaced by newly-formed mineralized matrix deposited by bone forming cells known as the osteoblasts [1]. The coupling of resorption and formation occurs in specialized and spatially defined bone-remodeling units found throughout the bone. Imbalanced bone remodeling is the basis of multiple pathologic conditions, including osteoporosis, in which excessive osteoclastic activity leads to increased bone resorption and fracture risk for osteoporotic patients [2].

Recently, organ-on-a-chip (OoC) technology has demonstrated its potential to replace animal testing in drug screening and disease modeling, due to the faithful mimicking of human multicellular architecture and tissue-tissue interfaces. These micro-physiological systems enable the use of human donor derived cells and provide strict control over cellular, molecular, chemical and physical cues within a relevant tissue context. However, the specific application of OoC to study bone tissue has focused mostly on osteoblastic differentiation and mineralization activity [3,4]. To fully replicate the coupling between bone formation and bone resorption that occurs during bone remodeling, the inclusion of human osteoclasts is still lacking in these models.

Herein, we present a novel, perfusable bone-on-chip platform that recapitulates the establishment of bone-remodeling units in a physiologically relevant three-dimensional (3D) matrix (Figure 1). As a proof-of-concept, bone remodeling micro units were established by incorporating RAW264.7 murine osteoclast precursor cells in Dextran-Tyramine (DEX-TA) hollow microgels. Cell laden microgels were then introduced in a 3D mineralized collagen matrix that had been previously seeded with MC3T3-E1 murine osteoblast cells.

Our bone remodeling micro units-on-chip model is amenable to standard immunocytochemistry readouts and genomic/proteomic profiling, allowing versatile monitoring of cell differentiation and bone degradation activity. Furthermore, one of the inherent advantages of OoC platforms is their ability for multi-fluid perfusion with hormones and/or inflammatory cytokines, which is crucial when mimicking the complex bone microenvironment. This is the first step towards the establishment of the first osteoporosis-on-chip model and forms the basis for further fundamental bone research with options for adaption to other diseases such as arthritis or bone cancer.

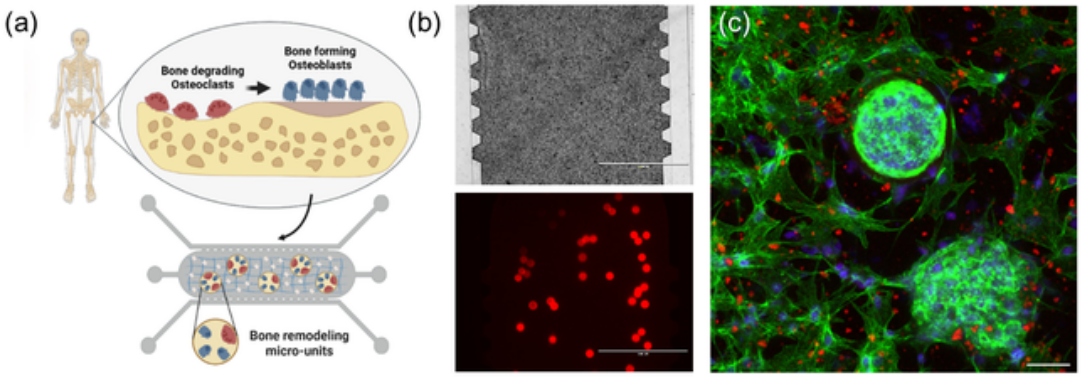


Figure 1. (a) Inclusion of the whole remodeling cycle in a single OoC bone model. (b) Brightfield image (top) and fluorescent images (bottom) of empty DEX-TA microgels on mineralized collagen. Microgels were stained with Ethidium Homodimer. Scale bar 1mm. (c) Bone remodeling micro-units: co-culture of RAW264.7 cell-laden DEX-TA microgels surrounded by MC3T3-E1 cells. Nuclei – Blue; Actin – Green; Hydroxyapatite granules – Red. Scale bar 50μm.

References:

[1] S.Bolamperti et al, *Bone Research*, **10** 1(2022)
[2] S.Song et al, *Phamacol. Ther.*, **237** 108168(2022)
[3] A.R.Atif et al, *Acta Biomaterialia*, **127**(2021)
[4] E.Babaliari et al, *Bioengineering*, **5** 3(2018)

PoA.15.38

On the communication between nuclei and mitochondria in a hydrogel environment

Cathrine Abild Meyer, Brigitte Städler

Interdisciplinary Nanoscience Center, Aarhus, Denmark

Abstract

Mitochondria play a crucial role in the reprogramming of mammalian cells. The mitochondria send a retro signal back to the nuclei in response to mitochondrial dysfunction, which leads to a regulation of the transcription of genes. This regulation can eventually lead to different disease conditions in the cells. The signaling is called mitochondrial retrograde signaling and it is very important for the functional integration of the nuclei and mitochondria in mammalian cells. However, our understanding of the pathways involved in this process, and their downstream effects remains limited.

A major obstacle in the study of mitochondria-nuclei communication is the complex signaling environment inside the living mammalian cells.

Here, the major aim is to eliminate the interference of unrelated signaling by purifying nuclei and mitochondria from donor cells followed by their encapsulation in controlled hydrogel environments with preserved function.

First, nuclei and mitochondria were isolated from HeLa cells after which their retention of function outside the host cells was evaluated. The focus was on the functionality of nuclei pore complexes and mRNA production for the nuclei, while the ATP production was the monitored core property of the mitochondria.

Second, the purified organelles were encapsulated in different types of hydrogel discs including alginate, gelatin methacrylate, and different types of polyethylene glycol diacrylate (PEGDA). Furthermore, the function of the nuclei and mitochondria inside the hydrogel discs was determined and compared for both, the nuclei and mitochondria encapsulated individually and together. In the latter case, the effects of spatial distribution were considered.

Taken together, these efforts are the first steps towards an *in vitro* model that can be used to elucidate pathways involved in mitochondria retrograde signaling.

PoA.15.39

Pharmacological Applications of a Comparative Inflammation-on-a-Chip with Complete 3D Interface

Soohyun Kim, Sung Kyun Lee

Korea Research Institute of Chemical Technology, Daejeon, Korea, Republic of

Abstract

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disorder that poses significant challenges for drug discovery due to the lack of appropriate preclinical models. Addressing this limitation, our study introduces a novel comparative inflammation-on-a-chip (IoC) platform with a complete 3D interface, free from micropillar and phaseguide structures. This IoC model faithfully replicates the crucial characteristic of COPD pathology: chemoattractant-induced neutrophil transendothelial migration. To investigate potential therapeutic interventions, we employed the IoC model to evaluate the pharmacological effects of CXCR2 inhibitors (MK-7123, AZD5069, and SB225002) on neutrophil-like cell migration in poly(I:C)-induced inflammatory conditions and plasma samples from COPD patients. Importantly, our study faithfully emulates the physiological 3D microenvironment, encompassing an endothelial barrier, extracellular compartment, and inflammatory conditions. By successfully bridging the gap between *in vitro* experimentation and clinical relevance, this innovative IoC platform emerges as a valuable tool for advancing our understanding of COPD pathogenesis and facilitating the development of effective therapeutics.

PoA.15.40

Design, Fabrication, and Applications of Soft Microrobots for Thermal Biology

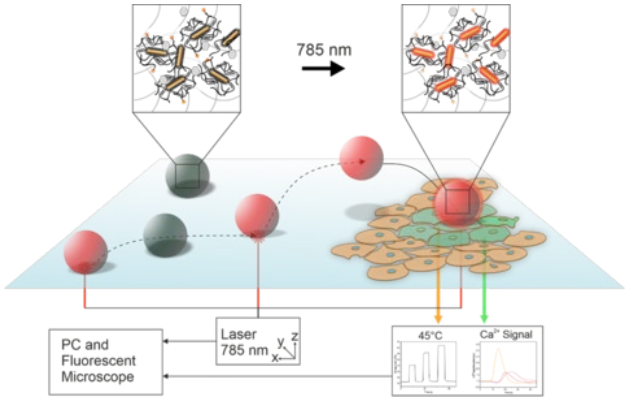
Philipp Harder[1,2,3], Nergishan İyisan[1,2,3], Chen Wang[1,2,3], Fabian Kohler[1,3], Irina Neb[4], Harald Lahm[4], Martina Dreßen[4], Markus Krane[5,6], Hendrik Dietz[1,3], Berna Özkale[1,2,3]

[1]Technische Universität München, Munich, Germany. [2]Munich Institute of Robotics and Machine Intelligence, Munich, Germany. [3]Munich Institute of Biomedical Engineering, Munich, Germany. [4]Institute for Translational Cardiac Surgery, Munich, Germany. [5]Yale Cardiac Surgery, New Haven, USA. [6]German Center for Cardiovascular Research, Munich, Germany

Abstract

A Laser-Driven Microrobot for Thermal Stimulation of Single Cells: https://doi.org/10.1002/adhm.202300904

A crucial challenge for cellular research is adapting *in vitro* conditions to the native 3D environment of cells and tissue. Soft hydrogels have been developed to overcome this challenge, with cell encapsulation and microrobotic manipulation showing promising results in mechanobiology. Building on these advances, we propose our Thermally Activated Cell-Signal Imaging (TACSI) microrobot. These soft, extracellular matrix-like microrobots are fabricated via microfluidics and consist of a biocompatible hydrogel, a thermosensitive sensor, and optically addressable gold nanorods. We use TACSI microrobots for cellular research by monitoring the induced and environmental temperature through the integration of the thermosensitive sensor, Rhodamine-B, and plasmonic heating of gold nanorods, allowing thermophoretic convection for guided locomotion and thermal biology studies. By using a near-infrared laser at 785nm, fast and local thermal actuation is achieved. Next to excellent biocompatibility *in vitro*, our experiments show high spatial control with speeds ranging from 5 to 65 µm s^{-1} of single microrobots in a 3D workspace. Implementing TACSI microrobots on human embryonic kidney cells indicates heat dose-dependent intracellular calcium signaling, precisely heating single- or multiple cells to 57 °C for 1 second. In these experiments, we find the temperature threshold to trigger a relative intracellular calcium change. This platform can help advance thermal biology research in the native, 3D cellular microenvironment.



Schematic representation of TACSI microrobots. Single microrobots are actuated via laser light in 3D workspace, which allows spatiotemporal control over locomotion and heat generation. An integrated thermoresponsive nanosensor provides real-time temperature feedback, while active localized heating leads to thermal actuation of single cells. The system allows measurement of dynamic cellular changes such as intracellular calcium content in parallel.

PoA.15.41

Dynamic 3D culture promotes lymphoid tissue maturation and allows the study of Chronic Lymphocyitc Leukemia (CLL) cells dissemination *in vitro*

Dafne Barozzi[1,2], Fiorella Scagnoli[1], Francesco Mantegazza[2], Federica Barbaglio[1], Davide Ribezzi[3], Silvia Farè[3], Barbara Vergani[2], Valeria Berno[4], Paolo Ghia[1], Cristina Scielzo[1]

[1]Division of Experimental Oncology, Malignant B cells biology and 3D modelling Unit, B cell neoplasia Unit, IRCCS Ospedale San Raffaele, Milano, Italy. [2]Università degli Studi di Milano Bicocca, School of Medicine and Surgery, Milano, Italy. [3]Politecnico di Milano Department of Chemistry, Materials and Chemical Engineering, Milano, Italy. [4]ALEMBIC, advanced microscopy laboratory, IRCCS Ospedale San Raffaele and Università Vita-Salute San Raffaele, Milano, Italy

Abstract

CLL is a dynamic disease characterized by the accumulation of mature B cells in peripheral blood and lymphoid tissues. Circulating malignant cells are mainly resting and tend to home within lymphoid tissues, where they acquire an activated phenotype and start to proliferate. This points out the importance of understanding what happens in the tissue context. Our aim is to establish a macroscale *in vitro* model of lymphoid tissues, in which circulate CLL cells and study their behavior in an *in vivo*-like environment.
We used a fully characterized collagen-based scaffold on which we seeded human bone marrow (BM) stromal cells or lymph node (LN) fibroblasts and endothelial cells. The scaffolds were maintained both in static condition and in a dynamic millifluidic system for at least 15 days, and a CLL cell line (MEC1) was used for circulation experiments. We performed in silico computational studies to define the experimental settings. We used and optimized different techniques to characterize our 3D models as real-timePCR, IHC, IF, SEM to analyze tissue viability and maturation, by comparing static and dynamic cultures. By FC we then evaluated CLL cells immunophenotype in circulation at different time points.
Through the analysis of viability and expression of specific functional markers (Collagen IV, CD31) we observed that the dynamic condition promotes a more viable and compact tissue-like architecture, and stimulates the organization of CD31+ luminal structures, underlining that we can obtain vascularized scaffolds thus reduction the risk of necrotic core (Fig1-2). At the end of the tissue maturation, we recirculate CLL cells into both models separately comparing pre and post-circulation conditions, observing that CLL cells efficiently home in both compartments (Fig2). Deep analysis is ongoing, but we preliminary observed up-regulation of CXCR4 and CD49d in circulating cells compared with 2D ones, resembling the *in vivo* situation.
In conclusion, we here demonstrated the feasibility and the advantages of using a 3D dynamic culture to obtain viable, organized, and vascularized 3D lymphoid tissues to study leukemic cells dissemination *in vitro*. Moreover, this approach opens to the possibility to increase the complexity by adding other relevant cell types to the model, and to interconnect the different tissues to obtain multiorgan system for CLL and other hematological malignancies.

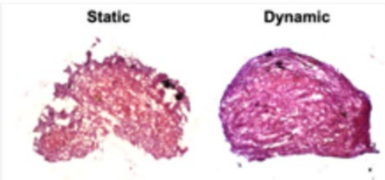


Fig.1 H&E staining of LN model slices comparing static and dynamic conditions.

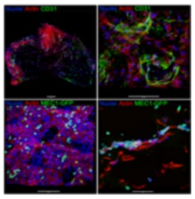


Fig.2 IF staining of LN model with luminal CD31+ structures (first row) and MEC1 circulating cells that entered the scaffold (second row).

PoA.15.42

In vitro evaluation of breast cancer cells behaviour under simulated microgravity

Silvia Strada[1,2], Cristina Volpini[1,2,3], Nora Bloise[1,2,3], Patrik Hollos[4], Livia Visai[1,2,3]

[1]Department of Molecular Medicine, Centre for Health Technologies (CHT), INSTM UdR of Pavia, University of Pavia, 27100 Pavia, Italy, Pavia, Italy. [2]Medicina Clinica-Specialistica, UOR5 Laboratorio di Nanotecnologie, ICS Maugeri, IRCCS, 27100 Pavia, Italy, Pavia, Italy. [3]Interuniversity Center for the promotion of the 3Rs principles in teaching and research (Centro 3R), University of Pavia Unit, Italy, Pavia, Italy. [4]Litegrav (litegrav.AI OU) Tatari 56, 10134 Tallinn, Estonia, Tallinn, Estonia

Abstract

Many astronauts have reported various side effects after long-term space missions in orbit as cardiovascular changes, reduction in bone density, muscle atrophy, and risk of kidney stone formation. The effects of microgravity (μg) on cellular properties can be related to these health issues. Numerous studies have indicated that μg has a great impact on cancer cells by influencing proliferation, survival, and migration, shifting breast cancer cells toward a less aggressive phenotype. Both cancerous and non-cancerous cells behave differently in absence of gravity. Several studies were performed on MCF-7, a human breast cancer cells expressing ER-α receptors, showing that in microgravity these cells product genes that are involved in organization and regulation of the cell shape, in cell tip formation and membrane to membrane docking. The goal of this study was to evaluate both MCF-7 and SKBR-3, a human breast cancer cells with HER-2 overexpression, behavior under simulated microgravity. 3D MSCR (microgravity simulator research cube) supplied by Litegrav as 3D clinostat mode with random path distribution and μ-Slide 8 Well for cells growth were used. Specifically, the evaluation of cancer cell behaviour at different time points (1, 3 and 7 days) was carried out by phase-contrast microscopy, cytoskeleton staining, viability assays (MTT assay) and changes in gene and protein expression by real-time PCR with western blot confirmation. Morphology changes were observed under simulated microgravity on both cell cancer types. Viability assays showed that the cell viability was not impaired. CLSM (Confocal Laser Scanning Microscopy) noticed a different actin filaments organization of cells in microgravity. These data, although preliminary, show how simulated microgravity induces changes in cell morphology and suggest the activation of specific gene programs, that may be involved in the development of the tumor or in the metastatic process. In our fight against cancer, a deeper understanding of the mechanisms involved may lead to the development of new therapeutic strategies. In addition, μg research can be a reliable tumor model to study various processes of cancer progression.

PoA.15.43

ASTROCARDIA: A heart-on-chip platform in space to study cardiac ageing

Agnes Dobos[1], Kevin Tabury[2], Jasper Van Hoorick[1], Aysu Arslan[1], Hilde Stenuit[3], Martijn Reniers[4], Sarah Baatout[2]

[1]BIO INX, Ghent, Belgium. [2]Belgian Nuclear Research Center, Radiobiology Unit (SCK SEN), Mol, Belgium. [3]Space Applications Services, Brussels, Belgium. [4]QbD Group, Antwerpen, Belgium

Abstract

Cardiovascular diseases (CVDs) currently represent one of the leading causes of death worldwide. Ageing induces functional changes in the heart that increase the risk of CVDs and impair functional capacity.[1] However, the causes of age-related CVDs are still poorly understood. As of today, no adequate human-derived model exists for studying cardiac ageing.

It has been reported that exposure to the space environment causes accelerated cardiac ageing, leading to the pre-development of several heart conditions because of radiation- and microgravity-mediated inflammation, DNA damage, and senescence as well as impairment of DNA damage repair. Although vascularized cardiac patches, heart organoids and vascular beds have already been successfully 3D bioprinted here on Earth, progress in the research of 3D bioprinted heart tissue in space is still in its early stages. [2]

In recent years, advances in organ-on-chip technologies have allowed the generation of biological platforms for drug testing and disease modelling that recapitulate human physiology more accurately than traditional 2D cultures and animal models.[3] Furthermore, by applying multiphoton lithography in an organ-on-a-chip platform, macro- and microvasculature can be integrated through addition of endothelial cells and growth factors. In this way, the supply of nutrients and oxygen can be guaranteed.

The present research focuses on the development of a human-derived vascularized cardiac organ-on-chip (OoC) model for testing the influence of cardiac ageing in space. To this end, three-dimensional (3D) *in vitro* models have been 3D bioprinted using a gelatin-based bio-ink (BIO INX), via multiphoton lithography. As a result, hearts-on-chip were bioprinted using cardiomyocyte spheroids as a platform to study cardiac ageing.

[1] Skardal, A., Shupe, T., & Atala, A. (2016). Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling. *Drug discovery today*, 21(9), 1399-1411.

[2] Tabury, K., Rehnberg, E., Baselet, B., Baatout, S., & Moroni, L. (2023). Bioprinting of Cardiac Tissue in Space: Where Are We?. *Advanced Healthcare Materials*, 2203338

[3] Roh, J., Rhee, J., Chaudhari, V., & Rosenzweig, A. (2016). The role of exercise in cardiac aging: from physiology to molecular mechanisms. *Circulation research*, 118(2), 279-295.

PoA.16.01

Evaluation of the biological properties of biopolymer- and gold-/silver-nanoparticles-bioactive glass composites - *in vivo* protocol

Alexandra I Dreanca[1], Emoke Pall[1], Corina Toma[1], Andreea N Astilean[1], Marian Taulescu[1], Klara Magyari[2]

[1]Faculty of Veterinary Medicine, University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Romania. [2]Nanostructured Materials and Bio-Nano-Interfaces Center, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babes-Bolyai University, Cluj-Napoca, Romania

Abstract

The most crucial idea behind employing bone scaffolds is the material's biocompatibility to prevent a local inflammatory reaction and a response similar to that to a foreign body. The following qualities must be present in a candidate: angiogenesis, osteoconductivity, osteopromotion, and mechanical support for cell proliferation. Composites, containing bioactive glasses including gold nanoparticles and/or silver nanoparticles, and bioactive glasses have been employed to improve bone regeneration. These composites were examined utilizing MTT tests, immunofluorescence, scanning electron microscopy analyses, and *in vitro* testing on fibroblast and osteoblast cell lines, as well as *in vivo* testing on Sprague-Dawley rats with an experimental bone deficiency. In terms of cell proliferation, morphology, migration, and attachment, both composites promoted adequate biological effects on human fibroblastic BJ (CRL 2522TM) cell lines and human osteoblastic cells isolated from the human patella. Most importantly, they did not result in pathological apoptosis and necrosis. Both composites encouraged new bone development at 60 days, according to the histology and immunohistochemical findings. Data from this study reveals that neither the *in vitro* nor the *in vivo* results are adversely affected by the little level of silver content. Also, we have obtained precise data demonstrating the validity of *in vitro* bioactivity research by showing that the recovered composites from defect areas have an appetite layer and proteins on their surface.

Keywords: biocompatibility, immunofluorescence, cell attachment, bone regeneration biomarkers

PoA.16.02

Creating an innovative 3D-printable bone substitution ink – β-TCP and Ca-polyphosphate reinforced by *in situ* orthophosphate polymerization

Meik Neufurth, David Molter, Xiaohong Wang, Werner E.G. Müller

ERC Advanced Investigator Grant Research Group at the Institute for Physiological Chemistry, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany

Abstract

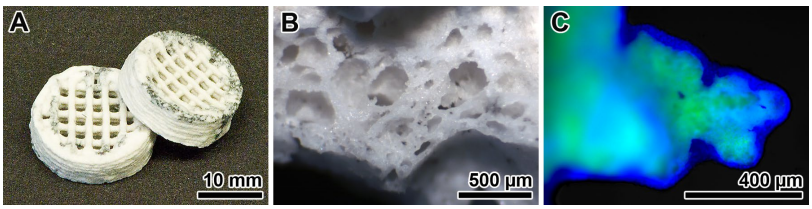
Introduction: Today, autogenous grafts are still the gold standard for bone substitution. However, the use of autografts implicates several challenges including limited availability, donor site morbidity and the need for a second surgery with the accompanying wound healing challenges. Since the population is growing older, the demand for bone substitutes is constantly increasing. An ideal bone replacement should not cause adverse reactions while it integrates into the host bone. It should be resorbable, induce bone formation (osteoinduction) and serve as a framework for the growing bone tissue (osteoconduction). At best, it should show beneficial impact on wound healing.

Objectives: Our aim was the development of a 3D-printable porous bone substitute ("bone-ink") which could be hardened in its final shape. The material should induce mineralization, provide a fully resorbable substrate for the development of new bone and provide an attractive environment for the bone-cells. It should prevent rejection reactions and accelerate wound healing.

Methodology: Our paste-like bone substitute is composed of β-tricalcium-phosphate, NaH_2PO_4, Ca-polyphosphate-microparticles, κ-carrageenan, xanthan-gum and water. The material can be 3D-printed or manually sculptured. After shaping, the material is frozen (-80°C) and freeze-dried to gain the desired porosity. Next, the material is heated (700°C) to induce the *in-situ* polymerization of orthophosphate to Na-polyphosphate and thus to initiate the mechanical reinforcement.

Results: Our bone-ink is easily processable in extrusion-based bioprinters. The porous scaffolds possess a compressive strength (1-2 MPa) comparable to human trabecular bone (A). Porosity (~ 40%) and density (~ 1,7 g/cm³) are also in accordance (B). The scaffolds were tested for *in vitro* cytotoxicity (ISO 10993-5) which revealed no cytotoxic effects. Furthermore, the scaffolds were seeded with osteoblast-like SaOS-2 cells and cultured for 40 days to test their cytocompatibility and their ability to induce biomineralization. It turned out, that the cells did not only accept the material but moreover they were thriving on the material. The results of the mineralization assays showed bone-cell mineralization all over the scaffold (C).

Conclusion: We developed a hardenable and porous 3D-printable bone substitute with mechanical characteristics comparable to human trabecular bone. The hardened material is cytocompatible, non-cytotoxic, induces mineralization and serves as a suitable substrate for osteoblast-like cells. Furthermore, we could show in separate studies, that the incorporated Na-polyphosphate dramatically enhances wound healing *in vivo*. These data suggest, that our material could not only serve as a promising bone substitute but moreover elicits additional beneficial effects in post-surgical wound healing.



PoA.16.03

Manufacturing and assessment of a multi-well osteoblast-osteoclast co-culture *in vitro* model.

Alexander Sieberath[1], Kenny Dalagarno[2], David Eglin[3], Elena Della Bella[4], Christoph Sprecher[4], Jochen Salber[1]

[1]University Hospital Knappschaftskrankenhaus Bochum, Bochum, Germany. [2]Newcastle University, Newcastle upon Tyne, United Kingdom. [3]Ecole des Mines de Saint-Etienne, Saint-Etienne, France. [4]AO Research Institute Davos, Davos, Switzerland

Abstract

Osteoblasts and osteoclasts play key roles in the bone remodeling cycle. Hence, co-culture models comprising these cells hold immense potential in enhancing the drug development process for bone remodelling associated diseases. Moreover, these models offer an alternative to animal testing in the future.
Here, we report the development of a 2D and 3D *in vitro* model of the bone remodelling process containing osteoblast- and osteoclast-like cells with the potential to be used in high throughput applications such as drug screening (Figure 1).

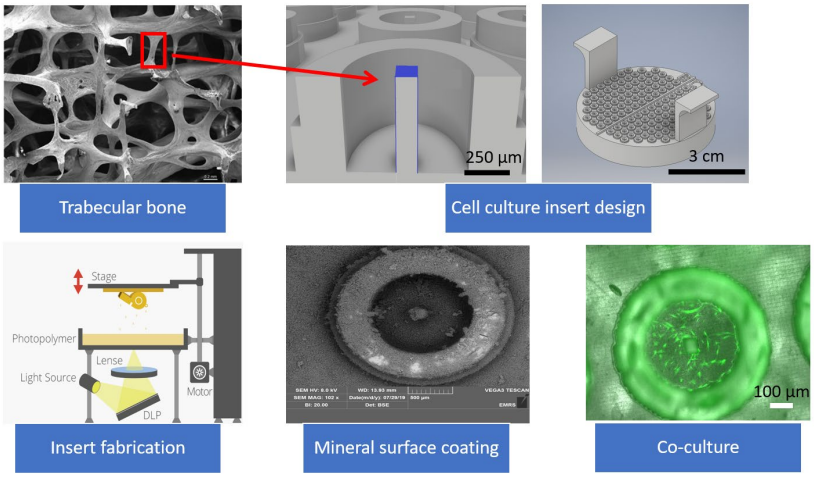
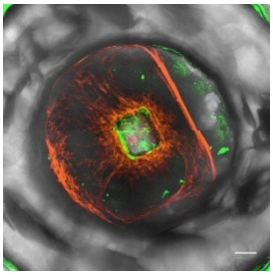
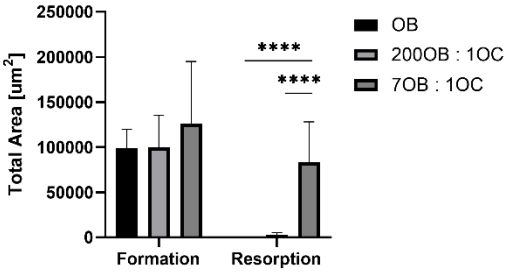


Figure 1: Development of a multi-well osteoblast-osteoclast co-culture model. The trabecular bone structure was mimicked by a central pin with the average size of a human trabecula, which was located in the center of a 3D printed microwell. A bone-like microenvironment consisting of hydroxyapatite and collagen type I was generated on the printed polymer. osteoblasts and osteoclast were co-cultured in the microwells, and their remodeling activity assessed by fluorescent staining of the hydroxyapatite particles.

The trabecular bone environment was mimicked by placing a vertical pin with the average size human trabecula into a stereolithography printed microwell. These wells were located on a cell culture insert which fits into conventional cell culture plates. A bone-like microenvironment consisting of hydroxyapatite and type I collagen was generated on the polymer surfaces using a patented coating technique. Lastly,

osteoblasts and osteoclasts were co-cultured in the microwells and their remodelling activity was assessed.





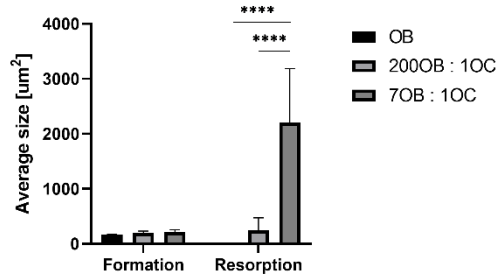


Figure 2: Quantification of the remodeling activity in osteoblast-osteoclast co-cultures. B) Quantification of the total remodeled area. C) Quantification of the average size of remodeling sites.

The co-culture of human osteoblasts and osteoclasts demonstrated cellular interaction and remodeling of the coated surface, which was quantified using ImageJ (Figure 2). Resorptive activity increased in co-cultures with an increased osteoclast number, while mineral formation activity of osteoblasts remained unaffected (Figure 2 B, C).

In conclusion, the developed model offers a quick and efficient method for assessing remodeling activity of osteoblasts and osteoclasts, while also providing a bone-like extracellular environment.

Acknowledgements

This research was funded by (i) the UKEPSRC Centre for 3D Printing (grant number EP/L01534X/1), (ii) the AO Research Institute Davos, Switzerland, and (iii) Newcastle University, UK, (iv) European Union's Horizon 2020 research and innovation program, EvPro project (Grant no: 814495).

PoA.16.04

DYNAMIC IN-VITRO STUDY OF AN ALIGNED POLYMERIC SCAFFOLD FOR TENDON ENGINEERING

Florencia Diaz[1,2], Nicholas R Forsyth[2], Aldo R Boccaccini[1]

[1]Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany. [2]The Guy Hilton Research Laboratories, School of Pharmacy and Bioengineering, Faculty of Medicine and Health Sciences, Keele University, Stoke-on-Trent, United Kingdom

Abstract

Introduction: The rotator cuff is a complex unit of tendon and muscles, maintaining the stability of the shoulder and enabling movement of the arm. When these tendons suffer an injury, either due to trauma, age, or disease, the most common treatments include physical therapy, anti-inflammatory drugs, and in the most severe cases, surgical intervention.
However, re-tear rates following surgery have raised significant concerns, and highlight a need for improved therapeutic alternatives. In this work, we seek to develop a new polymeric scaffold, produced by the unidirectional freeze-drying technique, capable of providing mechanical stability to the injury site, as well as establishing a platform for cells to infiltrate, proliferate and regenerate new tissue [1]. The biomaterial is designed to closely mimic the native structure of the supraspinatus tendon, be biocompatible and biodegradable.
Methodology: Scaffolds were produced through the aligned ice templating technique, by freezing a polymeric solution unidirectionally in an isolated 3D printed mould [2]. The polymer blend includes chitosan, gelatin, cellulose and halloysite nanotubes, which are incorporated to reinforce the materials mechanical properties. Following freeze drying, the scaffolds are crosslinked in a genipin solution, to further improve their mechanical properties and degradation behaviour. The scaffolds are characterized for their biomechanical properties, including tensile testing, hydrophilicity, porosity measurements, and cell culture studies.
Results: The biomaterial achieves superior mechanical properties compared to materials found in literature and appropriate hydrophilicity and degradation behaviour for applications in tendon regeneration. The scaffolds were seeded with human fibroblasts to evaluate cell viability, which was assayed with the WST-8 assay, and revealed no cytotoxicity after 10 days in culture. SEM images reveal the formation of a cell monolayer on the surface of the scaffold after 7 days, indicating that the surface promotes cell adhesion, allowing for cells to align themselves in the direction of the pores.
Acknowledgments: This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Maria Skłodowska-Curie Grant Agreement N° 955685
References:
[1] - Qian S. et al, *A Collagen and Silk Scaffold for Improved Healing of the Tendon and Bone Interface in a Rabbit Mode*l. Med Sci Monit. 2019 Jan 9;25:269-278.
[2] - Joukhdar, H. et al, *Ice Templating Soft Matter: Fundamental Principles and Fabrication Approaches to Tailor Pore Structure and Morphology and Their Biomedical Applications.* Adv. Mater. 2021, 33, 2100091.
Contact author: Florencia Diaz
Email: florencia.diaz@fau.de

PoA.16.05

Ferroelectric $BaTiO_3$ coating on beta-titanium alloy supported stem cell osteogenic differentiation *in vitro* and bone healing *in vivo*

Elena Filova[1], Premysl Vanek[2], Ladislav Cvrcek[3], Elena Buixaderas[2], Jan Drahokoupil[2], Andrej Litvinec[1,4], Rastislav Ballay[1,5], Radka Vrbova[6], Stefan Juhas[7], Jana Juhasova Juhasova[7], Martin Bartos Bartos[6], Lucie Svobodova[1], Radek Sedlacek[8], Kristyna Kubasova[8], Lucie Bacakova Bacakova[1]

[1]Laboratory of Biomaterials and Tissue Engineering, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, Prague, Czech Republic. [2]Institute of Physics of the Czech Academy of Sciences, Prague, Czech Republic, Prague, Czech Republic. [3]Department of Materials Engineering, Faculty of Mechanical Engineering, Czech Technical University in Prague, Czech Republic, Prague, Czech Republic. [4]Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic, Prague, Czech Republic. [5]First Orthopedic Clinic of the First Medical Faculty of Charles University and University Hospital Motol, Prague, Czech Republic. [6]Institute of Dental Medicine, First Faculty of Medicine and General University Hospital, Charles University, Prague, Czech Republic, Prague, Czech Republic. [7]Institute of Animal Physiology and Genetics, CAS, Libechov, Czech Republic, Libechov, Czech Republic. [8]Department of Mechanics, Biomechanics and Mechatronics, Faculty of Mechanical Engineering, Czech Technical University in Prague, Prague, Czech Republic, Prague, Czech Republic

Abstract

Permanent metallic bone implants made of beta-titanium alloy have better mechanical compatibility with bone than the standard titanium alloy Ti6Al4V ELI. A bioactive ferroelectric coating can improve the osseointegration of bone implants made of beta-titanium alloys. We prepared the Ti-39wt%Nb (Ti39Nb) alloy samples coated with a Ti interlayer that was oxidized to TiO_2. The $BaTiO_3$ coating on the sample surface was prepared by a hydrothermal reaction with $BaCl_2$ and NaOH in the autoclave at 300°C for 48h. $BaTiO_3$ shows ferroelectric properties, proven via micro-Raman spectroscopy by the presence of a peak at 300 cm^{-1}, concomitant with its tetragonal phase. We evaluated the bioactivity of $BaTiO_3$ coating *in vitro* by measuring the adhesion and metabolic activity/viability of human bone marrow-derived mesenchymal stromal cells (resazurin assay) and their osteogenic differentiation, i.e. collagen production, alkaline phosphatase activity and calcium deposition of bone marrow stem cells, on smooth samples from Ti39Nb, Ti39Nb/TiO_2 and Ti39Nb/TiO_2/$BaTiO_3$ and glass. The positive effect of the $BaTiO_3$ coating was obtained mainly at longer time intervals. Furthermore, cylindrical sandblasted samples of Ti39Nb, Ti39Nb/TiO_2/$BaTiO_3$, and Ti6Al4V ELI were implanted into the pig femur for 6 and 12 weeks. After bone samples harvesting, histological sections were performed, stained, and analyzed for the percentage of implant surface in direct contact with bone tissue (BIC parameter). The mechanical integration of the implants into bone was measured by pull-out tests. The Ti39Nb/TiO_2/$BaTiO_3$ samples evinced higher BIC value at 12 weeks. Mechanical parameters, e.g. maximal force, displacement at failure, shear strength, did not show significant differences among samples.

Supported by the Czech Science Foundation, grant No. 20-01570S, and by the Academy of Sciences of the Czech Republic, Praemium Academiae grant No. AP2202.

Keywords: bone implants; piezoelectric; osseointegration; alkaline phosphatase; stem cells; porcine model; bone-to-implant contact; pull out test

PoA.16.06

Collagen gel and rhPDGF-BB with collagen gel vehicle improved defect regeneration of alveolar bone in osteoporotic rats

Hyun Ju Kim[1,2], Kyoung-Hwa Kim[2], Yang-Jo Seol[2]

[1]Department of Periodontics, Seoul National University Dental Hospital, Seoul, Korea, Republic of. [2]Department of Periodontology and Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Korea, Republic of

Abstract

Background: The aim of this study was to evaluate the effect of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and collagen gel on the improvement of alveolar bone regeneration in osteoporotic rats.

Materials and methods: Twenty-four female rats were divided into the following groups: a PDGF-BB/Collagen group (n=8), a Collagen group (n=8), and a control group (n=8). After the induction of osteoporosis, a bone defect was created using a customized drill on each side of maxilla and the defects were grafted with rhPDGF-BB/collagen gel or collagen gel. The defects of the control group were left empty. The rats were euthanized at 2 weeks and 4 weeks and histological and microcomputed tomographic (micro-CT) analyses were performed.

Results: According to the micro-CT analysis, both of the PDGF-BB/Collagen group and the Collagen group showed significantly larger amount of new bone fraction (bone volume per tissue volume) compared to the control group at 2 weeks and 4 weeks. Especially, the PDGF-BB/Collagen group exhibited higher bone mineral density compared to the Collagen group and the control group at 4 weeks. Histologically, the PDGF-BB/Collagen group and the Collagen group showed larger amount of bone regeneration within the defect area compared to the control group.

Conclusion: The locally delivered collagen gel and rhPDGF-BB with collagen gel vehicle improved the bone regeneration of alveolar bone defect in osteoporotic rats.

PoA.16.07

Bone regeneration driven by a “green” and sustainable biocomposite scaffold for periodontal tissue application

Mariana Souto-Lopes[1,2,3], Liliana Grenho[4,5], Yaideline Manrique[6,7], Madalena Dias[6,7], José Carlos Lopes[6,7], Maria Helena Fernandes[4,5], Fernando Jorge Monteiro[1,2,3,8], Christiane Salgado[1,2]

[1]i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal. [2]INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [3]Faculty of Engineering of the University of Porto, Porto, Portugal. [4]Faculty of Dental Medicine of the University of Porto, Porto, Portugal. [5]LAQV/REQUIMTE - Laboratório Associado para a Química Verde/ Rede de Química e Tecnologia, Porto, Portugal. [6]LSRE-LCM - Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Faculty of Engineering of the University of Porto, Porto, Portugal. [7]ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering of the University of Porto, Porto, Portugal. [8]Porto Comprehensive Cancer Center (P.CCC), Porto, Portugal

Abstract

Despite bone self-regeneration capability, some periodontal traumatic and infection-derived defects are so large that the tissue repair isn't possible. Recently, periodontal clinical treatment shifted from simply prevention disease progression, to guided tissue/bone neo-formation with bioengineering strategies such as the development of biodegradable scaffold biomaterials that fill the empty spaces, guide and stimulate stem cells populations migration and differentiation [1]. The aim of this work is to evaluate the osteoinduction and osteoconduction of a biocomposite (chitosan and nanohydroxyapatite - nHAp/CS) produced and sterilized by a sustainable scaffolding technique (supercritical CO_2) that allows higher surface area and porosity.

Nanohydroxyapatite/chitosan (nHAp/CS) scaffolds were produced as described in a previous work [2]. Human dental follicle mesenchymal cells (DFMSC) were seeded ($0.3x10^6$ cells) within scaffolds and evaluated cellular morphology, proliferation, ECM production (osteopontin) and differentiation after 7, 14 and 21 days. The *in vivo* study was approved by the Animal based studies Ethical Committee and fulfilled all legal requirements (i3S Animal Ethical Committee and DGAV, Portugal). Two bone defects (4 mm) were performed (scaffold and empty) at the calvaria bone of male Wistar rats (10 weeks-old) and were evaluated after 1, 2, 3 months by microCT. Afterwards, bone explants were processed for histological analysis.

There was no difference on ALP activity and total protein concentration between materials, but a tendency of an increase with nHAp/CS scaffolds was observed. After microscope evaluation, nHA/CS showed higher cellular density after 7 days, but the cellular number in the CS scaffold increased only after 14 days. There was higher osteopontin deposition over nHAp/CS surface after 21 days. *In vivo* evaluation showed increase in bone formation with nHAp presence until 2 months (Figure 1).

Results showed that the DFMSC had favorable conditions for proliferation and differentiation. Optimal bone formation *in vivo* was observed throughout nHAp/CS scaffold along the time. Future studies should address the co-culture *in vitro* into a bioreactor to evaluate mineralization and angiogenesis that shall allow fully periodontal regeneration.

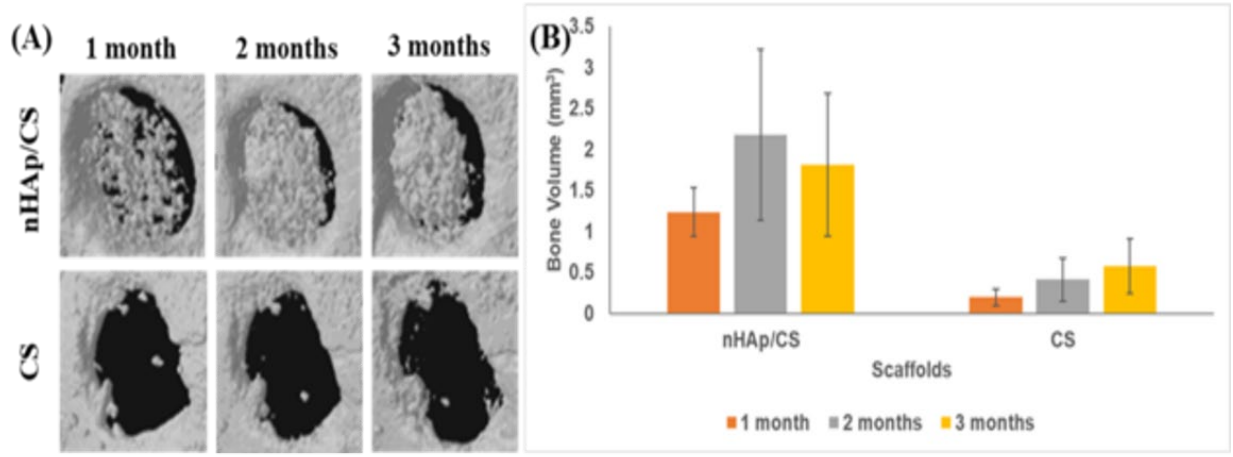


Figure 1: (A) MicroCT models of nHAp/CS and CS implants until 3 months. (B) Bone volume calculation by image analysis (microCT scans after surgery were used as control).

Acknowledgement**:** The authors acknowledge FCT for financial support (CEEC-INST/00091/2018/CP1500/CT0019, UIDB/04293/2020, LA/P/0045/2020, UIDB/50020/2020, PTDC/EME-APL/3058/2021).

References*:*

[1] S. Basyuni, et al*. (2020) Br J Oral Maxillofac Surg. 58 632-642.*

[2] G. Ruphuy, et al*. (2018) J Biomed Mater Res B Appl Biomater. 106.*

PoA.16.08

Development of a new decellularized and lyophilized human amnio-chorionic membrane for bone regeneration

Paul Galvez[1,2], Naïma Ahmed Omar[1], Robin Siadous[1], Samantha Roques[3], Marlène Durand[3,1], Xavier Lafarge[4], Florelle Gindraux[5,6], Loic Sentilhes[7], Nicolas L'Heureux[1], Jean-Christophe Fricain[1,2], Mathilde Fenelon[1,2]

[1]Laboratory BioTis, Bordeaux, France. [2]CHU Bordeaux, Department of Oral Surgery, Bordeaux, France. [3]CHU Bordeaux, CIC 1401, Bordeaux, France. [4]French Blood Establishment, Bordeaux, France. [5]CHU Besançon, Department of Orthopedic, Traumatology and Plastic Surgery, Besançon, France. [6]Nanomedicine Lab, Imagery and Therapeutics, Besançon, France. [7]CHU Bordeaux, Department of Obstetrics and Gynecology, Bordeaux, France

Abstract

INTRODUCTION: Due to their unique biological properties and low immunogenicity, placental membranes (i.e amniotic and chorionic membranes) are widely in the field of tissue engineering. We previously reported the interest of using amniotic membrane (hAM) to regenerate both non-critical and critical bone defects. However, some limitations have been observed due to hAM thinness making it difficult to handle. In this study, we proposed to combine the chorion with the hAM because chorion is three to four times thicker than the amnion and contains growth factors in higher quantities, which can optimize the properties of the membrane for bone tissue engineering applications. The objective of this study was first to develop a new method of preservation of amnio-chorionic membrane (ACM) and to assess its mechanical, biological and osteogenic properties compared to hAM *in vitro* and *in vivo*.

EXPERIMENTAL METHODS: ACM was cut out from placentas recovered after elective cesarean surgery (n=12). ACM was decellularized using an enzymatic followed by a detergent decellularization methods, and was lyophilized and gamma-sterilized (DL-ACM). The effectiveness of the decellularization process was assessed using DAPI staining, DNA quantification and separation by agarose gel electrophoresis. Biomechanical behavior of DL-ACM was characterized. *In vitro* cytotoxicity of DL-ACM was studied by assessing the effects of DL-ACM soluble extracts on human bone marrow mesenchymal stem cells viability and activity. The biocompatibility of DL-ACM was then assessed *in vivo* in a rat subcutaneous model. Finally, its osteogenic properties were assessed in a rat femoral bone defect model and compared to hAM and a widely used commercial membrane for guided bone regeneration.

RESULTS: The effectiveness of the decellularization process was validated. The thickness and the suture retention strength of DL-ACM was significantly increased compared to hAM and DL-ACM was easier to handle. *In vitro*, the preservation method did not confer any indirect cytotoxicity. *In vivo*, histological analysis of explanted rat subcutaneous samples indicated a slightly inflammatory reaction. Micro-CT and histomorphometric analysis of the non-critical bone defect showed that DL-ACM was as effective as hAM and significantly increased early bone regeneration compared to the empty defect and the commercial membrane.

CONCLUSION: In this study, we developed a simple and reproducible method for effective decellularization of a non-cytotoxic and biocompatible DL-ACM. DL-ACM is a new biomaterial which promoted early bone regeneration and has better biomechanical properties than hAM. Its application in bone regeneration will be further assessed in a critical bone defect.

PoA.16.09

Development of two novel polysaccharidic membranes for guided bone regeneration: *in vitro* and *in vivo* studies

Naïma Ahmed Omar[1], Paul Galvez[1,2], Jéssica Roque[1], Robin Siadous[1], Olivier Chassande[1], Sylvain Catros[1,2], Joëlle Amédée[1], Samantha Roques[3], Marlène Durand[3,1], Céline Bergeaut[4], Laurent Bidault[4], Paola April[5], Didier Letourneur[5,4], Jean-Christophe Fricain[1,2,3], Mathilde Fenelon[1,2]

[1]Laboratory BioTis, Bordeaux, France. [2]CHU Bordeaux, Department of Oral Surgery, Bordeaux, France. [3]CHU Bordeaux, CIC 1401, Bordeaux, France. [4]Siltiss, Saint-Viance, France. [5]Laboratory LVTS, Paris, France

Abstract

Introduction: Due to the complexity of new bone formation in orthopedic and maxillofacial surgeries, developing a guided bone regeneration (GBR) membrane to maintain bone in growth remains challenging. Specific requirements are needed to reach clinical outcomes of GBR membranes such as biocompatibility, porosity, or mechanical handling. Natural polymers such as dextran and pullulan have interesting biological and physicochemical properties that can be used for bone tissue engineering. However, these two materials have never been used simultaneously towards this purpose. The present study aimed to develop and characterize two novel polysaccharidic-derived membranes for GBR.

Experimental Methods: Two membrane formulations, made from dextran/pullulan and combined with or without hydroxyapatite (HA), were produced and characterized by scanning electron microscopy and nanoindentation. Their cytotoxicity on human bone marrow mesenchymal stem cells was evaluated according to ISO standard. *In vivo*, subcutaneous implantation in rats was performed to evaluate their biocompatibility and their resorption at 1-, 4-, and 16-weeks post-surgery (n=5 per condition and per time). A non-critical size bone defect was also performed to assess their potential for GBR procedures and to compare them to a commercially and widely used GBR membrane (e.g., Bio-Gide®). Bilateral femoral defects were thus performed and defects were either left empty or covered by one of the two polysaccharidic membranes or with the commercially available one (n=6 per condition and time). After 1, 2, and 4 weeks, bone repair and neovascularization were analyzed radiographically and histologically using Masson-Goldner's trichrome staining.

Results and Discussion: Both pullulan/dextran-based membranes combined or not with HA presented a porous structure and two distinct sides could be identified using scanning electron microscopy. Both membranes showed comparable Young's moduli. *In vitro* and *in vivo* studies showed that they were cytocompatible and biocompatible. Both membranes were still presented up to 16 weeks and the pullulan/dextran-based membrane combined with HA showed the slowest rate of resorption. Micro-computed analysis and histomorphometrical measurements of femoral defects revealed that bone regeneration occurred significantly faster for the pullulan/dextran-based membrane combined with HA compared to the commercially available membrane two weeks after implantation.

Conclusion: Several studies have reported the interest in pullulan and or dextran-based biomaterials used as injectable hydrogels or microbeads to promote bone regeneration. Here, we developed two pullulan/dextran-based membranes for GBR. Incorporating mineral contents such as hydroxyapatite further enhances their potential for GBR procedures by promoting early bone regeneration compared with the commercial membrane.

PoA.16.10

Development of a Bioresorbable Bone Adhesive for Bone Fixation Using Calcium Phosphate Cement, Phosphoserine, and Polydopamine Nanoparticles.

Matthias Schlund, Julien Dartus, Sarah Defrançois, Joël Ferri, Nicolas Blanchemain, Patrice Woisel, Joël Lyskawa, Feng Chai

Univ. Lille, Lille, France

Abstract

Bone fixation is a common surgical procedure used to repair bone fractures or stabilize bone grafts to reconstruct a bone defect. Metallic implants, traditionally used for this, are not suited to any situation (e.g., comminuted, articular or pediatric fractures) and can cause various complications and often require secondary removal surgeries. Therefore, a bioresorbable bone adhesive is needed to overcome these unmet challenges.

This study aims at developing an adhesive using tetracalcium phosphate cement (TTCP), phosphoserine (OPS), and nanoparticles of polydopamine (nPDA). The optimal formulation was determined to be 50%molTTCP/50%molOPS-2%wtnPDA with a liquid-to-powder ratio of 0.21 mL/g by offering a rupture strength at 2.75 ± 0.65 MPa on titanium samples. The sterilizability of the adhesive was investigated and g irradiation was opted as the sterilization method for it.

This adhesive was characterized after 24 hours under simulated physiological conditions for its adhesiveness by traction test: *in vitro* with titanium samples, ex vivo on glued end-to-end bovine cortical bone samples, and *in vivo* on a rat fibula glued to the tibia. Overall, this adhesive demonstrated significantly superior adhesive properties compared to the one without nPDA (Figure 1), a comparable compressive strength and non-cytotoxic. This adhesive was further shown in a novel *in vivo* model (simulating a clinical scenario of autograft fixation under low mechanical load – rat fibula glued to the tibia) to effectively stabilize the graft without displacement and demonstrated fully biocompatible and an osteoinductive property and led to significant coverage of newly formed bone on its surface (Figure 2). Furthermore, the biofunctionalization of the adhesive for offering an antibacterial property through ciprofloxacin (20%) loaded on the nPDA was assessed. Microbiological studies showed a prolonged release profile of ciprofloxacin from functionalized adhesive with an antibacterial activity till 30 days against Staphylococcus aureus.

The results of this study suggest that the TTCP/OPS-nPDA adhesive offers a promising alternative to metallic implants for bone fixation. Thanks to polydopamine nanoparticles, the adhesive's superior adhesive properties and osteoinductive properties make it an attractive option for orthopaedic, maxillofacial or other surgeons. Its simple chemistry, which is free of cytotoxic or mutagenic effects, can form a strong and durable bond directly to living bone and metallic implants. Moreover, the adhesive can be functionalized to offer additional biological activities, such as anti-infection properties, further enhancing its potential clinical applications. Further research and clinical studies are needed to evaluate the long-term safety and efficacy of this adhesive.

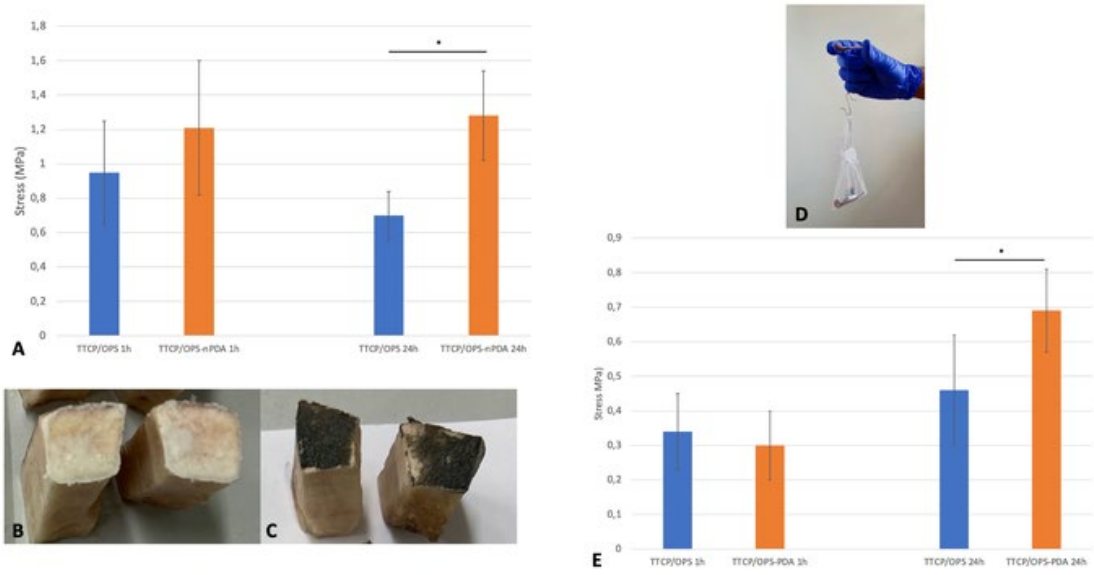


Figure 1: Ex vivo traction test comparing TTCP/OPS glue versus TTCP/OPS-nPDA glue after 1-hour and 24-hour immersions in a 37°C PBS bath: (A) Instrumental traction test using cuboid bovine cortical bone samples; surfaces of bone samples glued with TTCP/OPS (B) with TTCP/OPS-nPDA (C) after rupture, mostly showing a cohesive failure for both; (D) experimental set-up of manual traction test on the glued rat tibia/fibula; (E) maximum traction stress of manual traction test of glued fibular/tibial bone. * stands for a statistically significant difference between two marked groups.

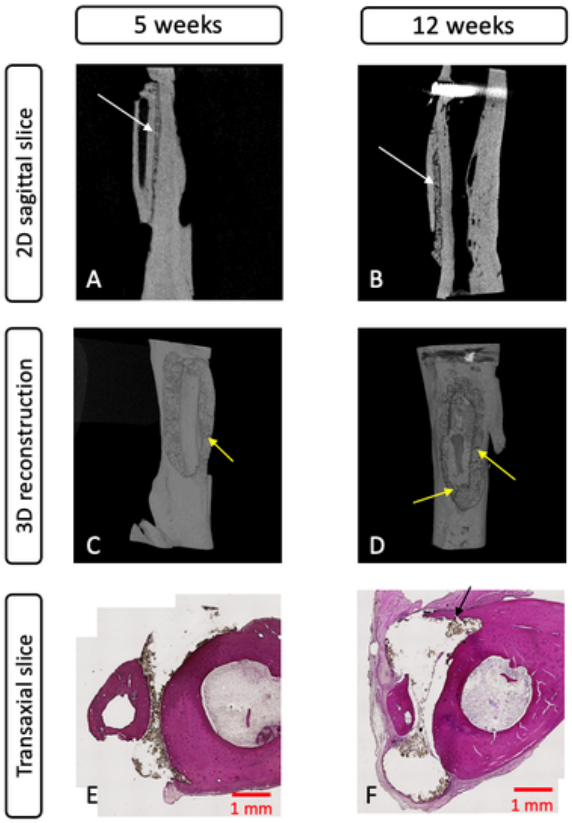


Figure 2: Evaluation in vivo of glued rat fibula/tibia with TTCP/OPS-nPDA glue at 5 or 12 weeks: microCT images of 2D sagittal slices (A, B) and 3D volume reconstruction (C, D) of a stably fixed fibular graft on a rat tibia showing a continuity of interface between the graft, the adhesive and the tibia (white arrows) with some rare fissures in the adhesive indicated by yellow arrows (C, D); non-decalcified histological sections (E, F) of glued fibular autograft to tibia with clinical success stained with hematoxylin and eosin (×10) showing the absence of inflammation and the coverage of newly formed bone over the TTCP/OPS-nPDA glue at 12 weeks (F, black arrow).

PoA.16.11

Evaluation of the neoformation bone using Micro CT analysis in osteoporotic rats following the application of bioactive glass and gold nanoparticles doped composites

Aștilean Andreea Niculina[1], Dreancă Alexandra[1], Gog-Bogdan Sidonia[1], Tăulescu Marian[1], Dindelegan Maximilian[2], Gherman Mădalina Luciana[3], Magyari Klara[4], Oana Liviu Ioan[1]

[1]Faculty of Veterinary Medicine, University of Agricultural Science and Veterinary Medicine, 400372 Cluj-Napoca, Romania, Cluj-Napoca, Romania. [2]Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Victor Babeș 8, 400012 Cluj-Napoca, Romania, Cluj-Napoca, Romania. [3]Experimental Centre of University of Medicine and Pharmacy "Iuliu Hațieganu" Cluj-Napoca, România, Cluj-Napoca, Romania. [4]Nanostructured Materials and Bio-Nano-Interfaces Center, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babes-Bolyai University, 400271 Cluj-Napoca, Romania, Cluj-Napoca, Romania

Abstract

Applications of micro-CT produce functional information by translating *in vivo* experimental protocols to model tissue engineering systems. The images provided by micro-CT analysis can be used to quantitatively represent bone geometry through a series of calculated parameters based on attenuation [(Wu et al., 2015]. The aim of this study was the qualitative and quantitative evaluation of the neoformation bone at the level of a bone defect (3 mm) created in the femural dystal dyaphyses, in 30 osteoporotic Sprague Dawley rats. The biological effects of biomaterials based on alginate-pullulan-bioactive glass composites containing gold nanoparticles (Alg-Pll-BGAuSP) and alginate-pullulan with tricalcium phosphate/hydroxyapatite (Alg-Pll-βTCP/HA) using as the positive control, were evaluated by histomorphometric methods on a two-dimensional section model (2-D), at different time intervals. The study was conducted in the authorized Laboratory Animal Facility (University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca). The entire femur bones were collected from osteoporotic Sprague Dawley rats, and were preserved in 10% formaldehyde for 7 days, then scanned and analyzed by micro-CT. The specific parameters: total bone volume, surface bone, bone volume, and density showed higher values for the group treated with Alg-Pll-BGAuSP in comparison to the controls, osteoporotic and Alg-Pll-βTCP/HA treated. More interesting, the 3D reconstruction of the defect site showed 90% surface gap filling in the same Alg-Pll-BGAuSP treated group at 30 days and 60 days post-implantation. All tested biomaterials had osteopromoting activities, more evidenced in the Alg-Pll-BGAuSP group, thus being considered a good candidate in pathological bone tissue engineering.

Keywords: biomaterials, bioactive glass, gold nanoparticles, micro CT scan, defect 3D reconstruction.

PoA.16.12

Osteoinductivity and immunogenicity of engineered human cartilage grafts in immunocompetent models

Alejandro Garcia Garcia[1,2], Sujeethkumar Prithiviraj[1,2], Deepak B. Raina[3], Steven Dupard[1,2], Magnus Tägil[3], Paul E. Bourgine[1,2]

[1]Cell, Tissue & Organ engineering laboratory, Biomedical Centre (BMC) B11, Department of Clinical Sciences Lund, Stem Cell Centre, Lund University, Lund, Sweden. [2]Wallenberg Centre for Molecular Medicine, Lund University, Lund, Sweden. [3]Clinical and Experimental bone healing, Department of Orthopedics, Lund University, Lund, Sweden

Abstract

Human hypertrophic cartilage matrices (HyC) can be customized in composition and manufactured using dedicated human mesenchymal stromal cells (hMSC) lines[1]. Following devitalization and lyophilization, the resulting HyC grafts exhibit exceptional osteoinductive properties in immunodeficient (ID) mice. However, the relevance of ID mice in preclinical validation is questionable due to their lack of an efficient immune system. Here, we established a protocol for HyC decellularization, in order to evaluate their performance in immuno-competent (IC) models and identify the associated immune response. To this end, HyC generated using the human MSOD-B line were decellularized using a combination of detergent (SDS) and DNase washes. The osteoinductivity of decellularized grafts was first assessed through subcutaneous implantation in ID and IC mice. Recruitment of immune cells was investigated on day 3/7/10 post-implantation in both ID and IC mice. The regenerative potential was further evaluated in a critical-sized femoral defect (5 mm) in Sprague Dawley rats. Bone repair was assessed 6- and 12 weeks post-implantation through histological, micro-computed tomography (µCT), and mechanical analyses.
Ectopically, we observed full bone formation in ID mice while despite an efficient decellularization the HyC poorly remodeled in IC setting. This was associated with a clear M2 macrophages polarization pattern in ID animals which is absent in IC. However, in the IC orthotopic rat model, we report full bridging of the femoral defect in 6 weeks, and complete bone repair 12 weeks post-implantation with restored mechanical properties.
Altogether, our results indicate a retained immunogenicity of human decellularized HyC despite removal of DNA and cellular debris. We also highlights the poor relevance of ectopic models in predicting the performance at orthotopic sites. Our findings point at a clear site-specific immune response, decisive in the pro-regenerative versus rejection outcome of the grafts. Finally, the remarkable orthotopic performance of decellularized MSOD-B HyC warrants further assessment in clinically relevant large animal models.

1. Pigeot, S. et al. Manufacturing of Human Tissues as off-the-Shelf Grafts Programmed to Induce Regeneration. Adv. Mater. 2103737 (2021) doi:10.1002/ADMA.202103737.

PoA.16.13

3D Printed pectin constructs for the regulation of mesenchymal stem cell differentiation in an endochondral ossification *in vitro* model

Dalila Petta[1,2], Anna Lapomarda[3,4], Chiara Arrigoni[1,2,5], Maria Vittoria Colombo[1,2], Mike A Geven[6], Elena Pulidori[7], Celia Duce[7], Christian Candrian[2,5], Giovanni Vozzi[3,4], Matteo Moretti[1,2,5], Carmelo De Maria[3,4]

[1]RMT Lab Ente OSpedaliero Cantonale, Bellinzona, Switzerland. [2]Servizio di Ortopedia e Traumatologia Ente Ospedaliero Cantonale, Lugano, Switzerland. [3]Research Center 'E. Piaggio', University of Pisa, Pisa, Italy. [4]Department of Ingegneria dell'Informazione, University of Pisa, Pisa, Italy. [5]Facoltà di Scienze Biomediche, Università della Svizzera Italiana, Lugano, Switzerland. [6]Laboratory of Polymers and Biomaterials, Istituto Italiano di Tecnologia, Genovaq, Italy. [7]Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa, Italy

Abstract

Endochondral ossification (ECO) is the developmental process underlying long bone formation. It starts from the formation of a cartilage template, which undergoes hypertrophy, finally resulting in vascularization and neo-bone formation. Approaches to mimic ECO mainly rely on the initial seeding and chondrogenic differentiation of progenitor cells on a scaffold, then inducing hypertrophy *in vitro* using specific growth factors. The final step of vascularization and bone formation is generally achieved by means of *in vivo* implantation. In this study we aimed at investigating how scaffold physicochemical properties influences the first ECO stages (i.e., the sequential chondrogenic and hypertrophic differentiation). To this end, we used extrusion bioprinted scaffolds made of pectin crosslinked with (3-glycidyloxypropyl) trimethoxysilane (GPTMS), previously developed [1]. We tuned their physicochemical properties by modifying GPTMS content and/or by adding hydroxyapatite (HA). Human bone marrow mesenchymal stromal cells (hBMSCs) were seeded on the scaffolds, previously coated with fibrin to overcome the lack of adhesive sites on pectin backbone. We cultured hBMSCs in chondrogenic medium for two weeks, followed by two further weeks in hypertrophic medium. At the end of culture, we evaluated chondrogenic and hypertrophic differentiation through RT-PCR and immunostaining for collagen II and collagen X, respectively. We found that 3D printed pectin scaffolds presented a microporous structure in all conditions (Figure 1A), and that increase in GPTMS but not in HA content led to increased compressive modulus of the scaffolds. Cells were viable in all the conditions (Figure 1B), although the presence of fibrin significantly increased cell viability at day 7 as compared to non-coated scaffolds. Regarding chondrogenic differentiation we found that hBMSCs seeded on the scaffold with the lowest GPTMS content and with no HA produced more GAGs, showed a stronger staining for collagen II and expressed higher levels of ACAN, COL2 and SOX-9 chondrogenic genes (Figure 1C), overall suggesting a better chondrogenic differentiation. Surprisingly, also the staining for collagen X and the expression of hypertrophic/osteogenic genes such as COLX, MMP13 and RUNX-2 was higher in the scaffold with the lowest GPTMS content and with no HA. In conclusion, our results suggest that for a better replication of the hypertrophic stage, it is key to obtain a more differentiated chondrogenic template, which is best achieved with lower compressive modulus and no osteogenic cues in the scaffold.

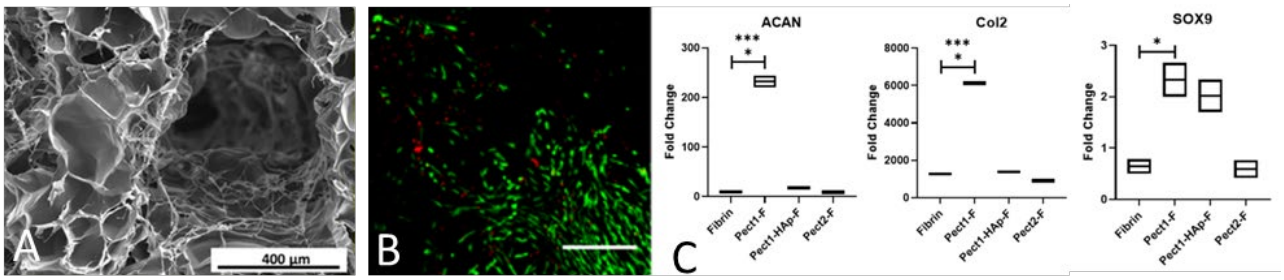


Figure 1 A) SEM of pectin scaffolds, B) Live&dead staining C) chondrogenic gene expression

[1] Lapomarda et al, Biomacromolecules 2020

PoA.16.14

AUTOLOGOUS TISSUE ENGINEERED GRAFT FOR PHALANX CONSTRUCTION IN CHILDREN WITH SYMBRACHYDACTYLY, A PROOF OF CONCEPT.

Romain Schaller[1], Masoor Chaaban[2], Gangyu Zhang[2], Dirk Schaefer[1], Alexandre Kaempfen[1], Ivan Martin[2], Scherberich Arnaud[2,1], Adrien Moya[2]

[1]Department of Plastic, Reconstructive, Aesthetic, and Hand Surgery, University Hospital Basel, Basel, Switzerland.
[2]Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland

Abstract

Symbrachydactyly is a rare congenital upper limb anomaly, that occurs in 1/30,000- 1/40,000 live births resulting in children born with short boneless fingers. Nowadays, these pediatric patients are treated with phalangeal bone transfer from the foot. However, morbidities are occurring at the donor site which result in unstable toes with significant disfigurations that worsen with the child growth. In this project, we used adipose-derived stromal cells (ASC) from adult and pediatric donors in a developmentally-inspired strategy to engineer osteogenic grafts for phalanx reconstruction via endochondral ossification (ECO).
To that aim, adult human ASCs isolated from the stromal vascular fraction (SVF) were seeded into collagen sponges and exposed to chondrogenic and hypertrophic factors *in vitro* to generate clinically-pertinent osteogenic grafts in the form of hypertrophic cartilage templates (HCTs) shaped as a phalanx. Then, the bone forming capacity of these HCTs was assessed by implantation in an ectopic immunocompromised mouse model, reflecting the clinical scenario of phalangeal soft tissue pocket, for up to 12 weeks.
In vitro, we were able to show that (i) cell expansion, (ii) cell density and (iii) duration of exposure to chondrogenic and hypertrophic factors had an impact on the degree maturation of the HCTs (i.e., amount of cartilage and hypertrophic cartilage present in the graft).
In vivo, we could observe that the bone formation and homing of bone marrow were correlated with the degree of maturation of the HCTs. Surprisingly, even the HCTs that had very limited cartilage at the time of implantation were capable of generating bone once implanted. In addition, HCTs obtained from pediatric ASCs (donor 15-month-old) were also capable of remodeling into bone tissue *in vivo*. Most interestingly, upon implantation chronologically-primed pediatric ASCs were able to produce growing cartilage mimicking some features of the growth plate, evidenced by the presence of proliferating chondrocytes (PThRP+, coll II+, Ki67+), expression of Indian Hedgehog (IHH) and increase glycosaminoglycan (GAG) content in the extra cellular matrix observed after 12 weeks of implantation.
Taken together, these results demonstrate the feasibility of an autologous approach using ASCs to generate osteogenic phalanx grafts of pertinent clinical size for children born with symbrachydactyly despite the limited amount of tissue available in pediatric patients.

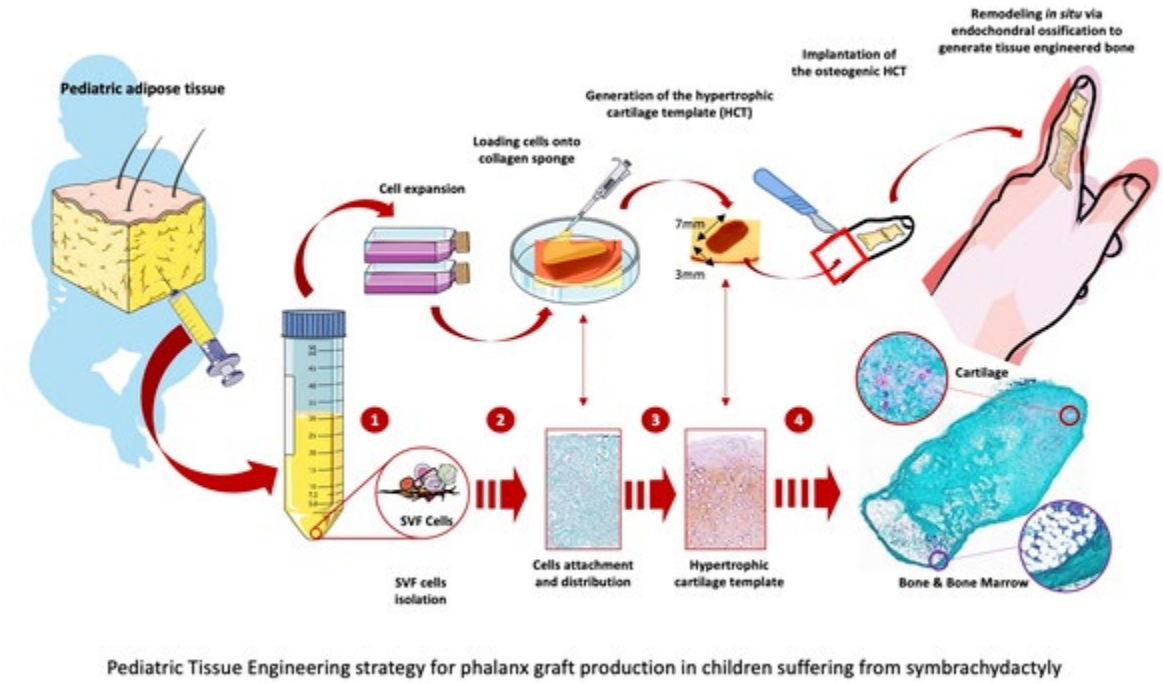


Figure 1: Pediatric Tissue Engineering strategy for phalanx graft production in children suffering from symbrachydactyly.

PoA.16.15

New advanced nanostructured coatings from mollusks for orthopaedics and dentistry

Gabriela Graziani[1,2], Enrico Sassoni[3], Giuseppe Falini[3], Fabio Nudelman[4], Daniele Ghezzi[3], Martina Cappelletti[3], Matteo Montesissa[3], Giorgia Borciani[3], Tiziana Fischetti[1], Nicola Baldini[3,1]

[1]Rizzoli Orthopaedic Institute, Bologna, Italy. [2]Polytechnic University of Milan, Milano, Italy. [3]University of Bologna, Bologna, Italy. [4]University of Edinburgh, Edinburgh, United Kingdom

Abstract

Bone regeneration and infection are important challenges in orthopedics and dentistry. To address them, functionalization of implants surface by biomimetic coatings is a promising approach. Among biomimetic materials, green sources permit higher sustainability. In addition, calcium carbonate from waste marine sources outperforms the synthetic one since it is naturally multi-doped (Mg, Na, etc). For these reasons, here, we propose the use of new, green, mollusks-based, bone-like and enamel-like coatings, obtained by Ionized Jet Deposition (IJD). We explore two routes: for bioactive coatings, we convert seashells into apatite, by a newly developed synthesis route at room temperature. For the antibacterial coatings, we use *Lingula Anatina* (LA) seashell, a natural zinc-doped fluorapatite, left untreated. To convert marine calcium carbonate (mussel-shells, cuttlefish bone) into hydroxyapatite, CaCO3 is reacted with diammonium hydrogen phosphate (DAP, (NH4)2HPO4). Different treating solutions are tested, varying the DAP concentration and using ethanol and isopropanol to boost hydroxyapatite formation (Graziani et al., 2016). Mollusks powders (before/after conversion) and LA are characterised, before and after IJD deposition (FT-IR, ICP, FEG-SEM/EDS, FIB, AFM). Coatings dissolution profile (immersion in pH 7.4 medium, FEG-SEM) and biocompatibility (osteoblast-like SAOS-2 cells, Alamar Blue) are studied. Then, antibacterial activity of lingula coatings is studied on S. aureus and E. coli from culture collections (inhibition of planktonic cells growth and of bacterial adhesion). Results show that mollusks can be converted into hydroxyapatite at room temperature, and they maintain ion-doping. The efficiency of the conversion is strongly enhanced by the addition of ethanol and isopropanol, and the optimal treating solution is substrate-dependent. Upon deposition, the target composition (converted mussels, converted cuttlefish, LA) is maintained. Coatings are nanostructured and biocompatible. FIB shows that thickness is 700 nm and that interlayers form between the coating and the substrate. When in medium, coatings progressively dissolve, but remain up to 21 days. For the first time, we show that LA has a significant antibacterial activity, owing to both the organic and mineral part, the latter being a carbonated fluorine-doped apatite, also containing zinc. The effect is dose-dependent (delay/impairment of bacterial growth at low concentration, toxic/killing effect at high concentration). Coatings cause a high reduction of viability of S. aureus (-90%), and a moderate effect on E. coli (-20%), indicating that the antibacterial activity of the inorganic part is preserved. Thanks to their biomimicry and tunable properties, mollusks shells-based biomimetic and antibacterial coatings appear promising for orthopedics and dentistry.

PoA.16.16

Silicate and silicate bioactive glasses restore bisphosphonate-inhibited bone formation

Maria Florez-Martin, Weijia Huang, Mohammad Aghamir, Joel Turner, Azadeh Rezaei, Kaveh Shakib, Gavin Jell

Division of Surgery and Interventional Sciences, University College London, London, United Kingdom

Abstract

Introduction Silicate (Si) may play a crucial role in maintaining bone and connective tissue health[1]. *In vitro* studies have demonstrated that Si-containing bioactive glasses (SBGs) can enhance bone regeneration, making it a potential candidate for the treatment of medication-related osteonecrosis of the jaw (MRONJ)[2]. MRONJ is a severe bone disease most commonly observed as a side effect of bisphosphonate (BP)-containing medications. The prevalence of MRONJ has been reported to be ~0.1% in osteoporosis patients and 1-5% in those with metastatic diseases[3,4], with an incidence rate of up to 1,231.7 per 100,000 person-years for patients treated with BPs[5]. In the UK, these patients account for up to 7% of referrals to oral and maxillofacial surgery[6]. Therefore, novel treatments focusing on restoring bone formation are necessary to improve patient care. **Methods** This study explored the potential of Si (sodium metasilicate, Na2SiO3) ions and SiBGs (45S5 Bioglass®, 45 SiO2, 24.5 Na2O, 24.5 CaO and 6 wt% P2O5) to restore bone formation in MRONJ using an in-vitro rat osteoblast model and a quantitative multidisciplinary characterization approach (morphological, biochemical, and ultrastructural analysis). **Results and discussion** Si ions alone at a concentration of 0.5mM showed a significant increase in bone formation (primary rat osteoblasts) as determined by nodule size (Fig. 1). BPs inhibited bone formation in a concentration dependent manner. The addition of 0.5mM Si partially restored bone nodule formation in zoledronate treated osteoblasts (0.067 and 0.2µM) (Fig. 2). Initial findings demonstrated that the restoration of bone formation may be via a reduction of ROS production induced by BPs. This presentation will also show the effect of Si on BP-treated osteoblasts on proliferation, differentiation and angiogenic capabilities. This study demonstrates the potential of Si therapy for the treatment (or prevention) of MRONJ.

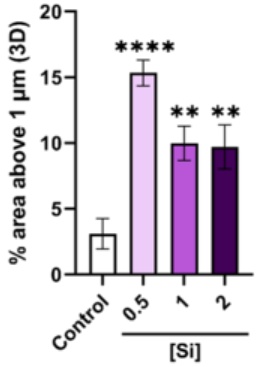


Figure 1. 3D morphological analysis. Percentage area of sample above 1 µm, calculated by interferometry using thresholding on Mx software (Zygo), n=3

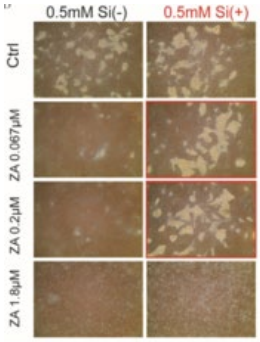


Figure 2. Optical microscopy representative images of zoledronate (ZA) and ZA+Si treated osteoblasts on day 7 of exposure.

Acknowledgements

Financial support for this project was provided by the Royal Free Charity.

References

1. Nielsen, F. H., J. Trace Elem. Med. Biol. Organ Soc. Miner. Trace Elem. GMS 28, 379–382 (2014). 2. Hoppe, A. & Boccaccini, A. R., Biomater. Oral Craniomaxillofacial Appl. 17, 22–32 (2015). 3. Hallmer, F. et al., Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 126, 477–485 (2018). 4. Srivastava, A. et al., Support. Care Cancer 29, 2305–2317 (2021). 5. Ishimaru, M. et al., J. Oral Maxillofac. Surg. 80, 714–727 (2022). 6. Clark, A. & Pretty, I. BDJ Open 8, 1–5 (2022).

PoA.16.17

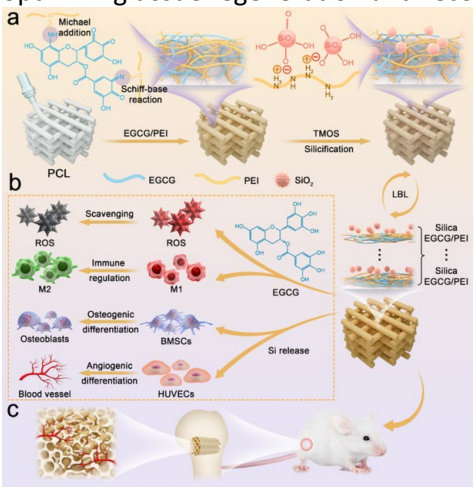
A multifunctional coating strategy for promotion of immunomodulatory and osteo/angio-genic activity

Jidong Li, Shiqi Xiao, Yubao Li

The Research Center for Nano-Biomaterials, Analytical and Testing Center, Sichuan University, Chengdu, China

Abstract

Building of multifunctional coatings in a more effective way is crucial for meeting the multilevel requirements of regenerative medicine. Herein, inspired by diatom and mussel, a specific but universal approach is proposed for building multifunctional coatings on slow-degradable and fast-degradable scaffolds or various substrates by using epigallocatechin gallate (EGCG) and polyethyleneimine (PEI) as bridges of silicon coupling. The results reveal that the polyphenol EGCG facilitates silica precipitation and coating topological morphology in synergy with PEI, and realizes antioxidant and immunomodulatory effects. The introduction of EGCG and the release of silicon ions present effective modulation of the immune microenvironment and remarkable promotion of angiogenesis and osteogenesis. The EGCG/silica coating strategy demonstrates a promising perspective for designing multifunctional coatings and optimizing tissue regeneration and reconstruction.



Schematic of: (a) the preparation process of silica coating on 3D printed PCL scaffolds induced by EGCG/PEI layers. (b) EGCG incorporated in coatings exhibits anti-oxidant and immune regulation activity and the release of Si ions facilitates the osteogenic and angiogenic differentiation of bone mesenchymal stem cells (BMSCs) and human umbilical endothelial cells (HUVECs), respectively. (c) Implantation of the silica-coated scaffolds enhances bone regeneration.

Shiqi Xiao; Jiawei Wei; Shue Jin; Xue Xia; Li Yuan; Qin Zou; Yi Zuo; Jidong Li; Yubao Li . Advanced Functional Materials, 2022, 2023(33): 2208968

PoA.16.18

Comparative study of novel 3D printed bioceramic b-TCP implants and off-the-shelf b-TCP implants

Sif S Dahl[1], Nanna B Madsen[2], Martin B Jensen[2]

[1]Ossiform Aps, Odense, Denmark. [2]Ossiform, Odense, Denmark

Abstract

In the past 40 years, bioceramic materials have proven very attractive bone graft substitutes, with beta-tricalcium phosphate (b-TCP) and hydroxyapatite (HA) being frequently used due to their osteoconductive and osteoinductive properties. While b-TCP and HA have many similarities, b-TCP has been proven to have superior properties compared to HA in terms of cell-mediated resorption and osteoconductivity. Due to these desirable features, b-TCP has been widely used within the dental and orthopedic fields in different shapes such as wedges, blocks, granules, and even cement. At Ossiform, we have developed a technology which enables us to 3D print micro- and macroporous bioceramic implants consisting of b-TCP. The 3D printability allows us to create patient specific, polymer-free, bioceramic implants with cortical and trabecular structures. Such implants are, to the best of our knowledge, currently not commercially available for orthopedic surgery. To study the effect of the 3D printed design of the Ossiform bone graft substitute, a timeline study of the Ossiform bone graft substitute compared to a commercially available porous b-TCP bone graft substitute (chronOS, Depuy Synthes) was setup using 42 male New Zealand White rabbits. A critical sized femoral condyle defect was made in each side, with one side left untreated and the other side implanted with either Ossiform bone graft substitute or chronOS. The implant material, tissue reaction, new bone formation and remaining material was evaluated 4 weeks, 8 weeks, and 13 weeks after surgery. Here, it was found that there was no significant difference between the Ossiform bone graft substitute and chronOS with respect to the formation of new bone. Furthermore, no differences in tissue reaction or neovascularization were found. There was, however, a clear difference between the resorption rates of the two implants, with the rate of material degradation after 13 weeks being 34.40% for chronOS and 15.61% for the Ossiform bone graft substitute. As both ChronOS and Ossiform bone graft substitutes are based on b-TCP with similar sized microporosities, one might hypothesize that the lower resorption rate observed in Ossiform's bone graft substitute could be a result of the trabecular and cortical structures produced using Ossiform's proprietary technology. Altogether, the data suggests that Ossiform's technology can be used to alter the resorption rate of b-TCP implants. This results in more time for the defect to be filled with native bone before the implant is fully resorbed, thereby ensuring structural integrity throughout the healing process.

PoA.16.19

Unravelling the role of biomaterial properties in orchestrating osteoclastogenic events during biomaterials-driven bone regeneration

L. Fermin[1], D. de Melo Pereira[1,2], H. Yuan[3], N. Davison[1,2], E. R. Balmayor[4], P. Habibovic[1], Z. Tahmasebi Birgani[1]

[1]Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands. [2]Instructure Labs, The Hague, Netherlands. [3]Kuros Biosciences BV, Bilthoven, Netherlands. [4]Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany

Abstract

Introduction: Biomaterial-driven osteoinduction refers to the ability of a biomaterial to form bone in a non-bone environment, often assessed by ectopic bone formation *in vivo*. Some, yet not all, calcium phosphates (CaPs) have been shown to be osteoinductive. CaPs with similar chemical composition, and different topographies and microstructures led to different osteoinductive properties.[1] Specifically, CaPs with smaller grains and pores simultaneously favored ectopic bone formation, and osteoclast attachment and resorption.[2,3] However, modulating osteoclastic response with biomaterial properties and consequent effects on osteoinduction has yet to be investigated thoroughly. Hence, this research aims to decouple CaPs' microstructure and topography from their chemical composition, and to investigate the osteoclastogenic events during biomaterial-driven osteoinduction. **Materials and Methods**: Grained topographies of known osteoinductive and non-osteoinductive beta-tricalcium phosphate ceramics (TCP-S and TCP-B) were inversely replicated onto shims using nickel galvanoforming. The shims were used to replicate the topographies onto thermoplastic polyurethane (TPU) films with different hardness grades (80A, 55D, 80D) using a nanoimprinting technique. TPU replicas were analyzed with profilometry, scanning electron microscopy (SEM), contact angle measurement and Fourier transform infrared spectroscopy (FTIR). Buffy coat-derived peripheral blood mononuclear cells (PBMCs) were seeded onto TPUs with and without the topographies, and control bone slices at a density of $3x10^6$ cells/cm^2 in osteoclast differentiation medium. Osteoclast markers and cell phenotype will be analyzed at multiple time points. **Results and Discussion:** Nanoimprinting showed the ability to recreate TCPs' three-dimensional microstructures and topographies on TPUs with great fidelity compared to the original TCPs (Figure 1A). Profilometry confirmed that the surface roughness of imprints on TPUs were also similar to that of TCP (Figure 1B). **Conclusion**: Here, we have shown a fabrication method for high-fidelity replication of TCPs topographies onto polymer films, decoupling chemical composition and topographical properties of TCPs. TCPs polymeric replica will be used to analyze potential modulatory roles of their topographies in morphological and phenotypical changes in osteoclasts. TPU replicas supported the osteoclastic differentiation of PBMCs. Expression of osteoclastic markers and phenotypical analysis of the osteoclasts will be performed next.

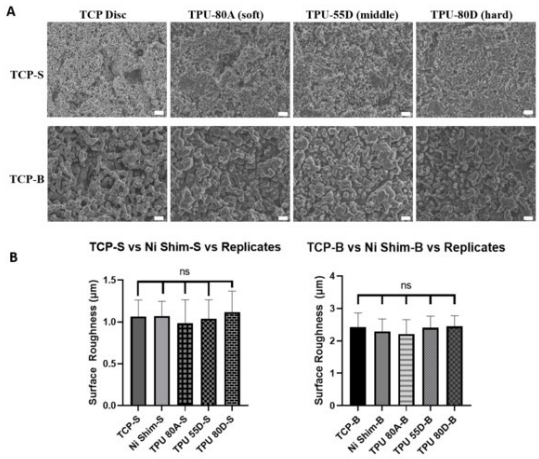


Figure 1. A. SEM images (scale bar: 10µm) and B. surface roughness of TCPs and TPU replicas.

References

1. J. Zhang et al., 2014, Acta Biomaterialia 10(7).
2. N.L. Davison et al., 2015, European Cells and Materials 29.
3. N.L. Davison et al., 2014, Biomaterials 35.

Acknowledgements: This research has been made possible by the Materials-Driven Regeneration Gravitation Program of the Dutch Research Council.

PoA.16.20

A biomimetic *in vitro* bone model based on osteoclasts-osteoblasts spheroidal co-culture

L. Fermin[1], N. Davison[1,2], E. R. Balmayor[3], P. Habibovic[1], Z. Tahmasebi Birgani[1]

[1]Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands. [2]Instructure Labs, The Hague, Netherlands. [3]Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany

Abstract

Introduction: Bone is a complex tissue with a specialized extracellular matrix (ECM), providing unique biochemical cues and biomechanical functions. Bone is multicellular in nature, containing bone-forming osteoblasts, bone-resorbing osteoclasts, osteocytes, bone lining, and immune cells.[1] The *in vitro* bone-mimicking models for studying bone physiological or pathological states and screening new drugs or bone graft substitutes, often do not adequately represent this complexity. They commonly use only osteoblasts or two-dimensional osteoclast-osteoblast co-cultures.[1] Three-dimensional (3D) co-cultures provide physiologically relevant alternative *in vitro* models, more closely mimicking the structure and cellular content.[2] This research aims to develop a 3D spheroidal model based on osteoclasts-osteoblasts co-culture and bone ECM-mimicking tricalcium phosphate (TCP) microparticles. **Materials and Methods:** TCP microparticles were placed in non-adherent tissue culture plates and sterilized with 70% ethanol. Human peripheral blood mononuclear cells (PBMCs) were seeded onto microparticles in osteoclast differentiation medium, and analyzed for attachment and osteoclastogenesis. After osteoclastic differentiation, human mesenchymal stromal cells (hMSCs) will be added to osteoclast-carrying TCP microparticles to form a co-culture. hMSCs attachment and differentiation will be investigated with immunocytochemical analysis. **Results and Discussion:** Preliminary results showed that PBMCs attached to TCP microparticles and differentiated towards osteoclasts, forming multinucleated cells positive for tartrate-resistant acid phosphatase (TRAP) osteoclastic marker.[3] hMSCs also attached to TCP microparticles forming a co-culture, in which osteoclast differentiation was continued (Figure 1).

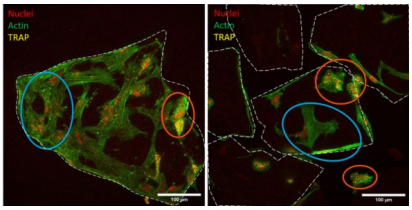


Figure 1. Immunofluorescent image of differentiating PBMCs and hMSCs on TCP microparticles; hMSCs and osteoclasts circled in blue and orange, respectively.

We are currently optimizing the cell densities in the co-culture to generate 3D spheroids. We expect that TCP microparticles allow hMSC differentiation into osteoblasts and osteoclastic resorption, as they provide a bone-mimicking matrix.[3,4]

Conclusion: Here, we have shown the first steps towards establishing a spheroidal co-culture model of osteoclasts and osteoblasts, aiming to mimic the 3D structure and multicellularity of bone. Next steps include optimization of the cellular content and characterization of the cell phenotypes and matrix remodeling in spheroids.

References:

1. I. Yuste et al., 2021, Pharmacological Research 169.
2. A. Bernhardt et al., 2021, International Journal of Molecular Sciences 22(14).
3. N.L. Davison et al., 2014, Biomaterials 35.
4. N.L. Davison et al., 2014, European Cells and Materials 27.

Acknowledgement: We thank Dr. Huipin Yuan for kindly providing the TCP particles. This research was supported by the Materials-Driven Regeneration Gravitation Program of the Dutch Research Council (NWO).

PoA.16.22

3D printed nanocomposite scaffolds with the potential to enhance osteogenesis-angiogenesis coupling

Athanasios Xanthopoulos[1], Eleni Mourkogianni[1], Artemis Tsirogianni[2], Lykourgos Kontaxis[3], Constantinos Athanassopoulos[2], Vassilis Kostopoulos[3], Despoina Deligianni[3], Evangelia Papadimitriou[1]

[1]University of Patras, Dept of Pharmacy, Patras, Greece. [2]University of Patras, Dept of Chemistry, Patras, Greece. [3]University of Patras, Dept of Mechanical Engineering & Aeronautics, Patras, Greece

Abstract

Defects of bone tissue are major orthopedic problems that compromise the patient's lifestyle. Although the use of autologous or heterologous bone transplantations has been suggested, its clinical development is limited because of several severe problems, and efforts have been and are being focused on the development of scaffolds that will have the composition, structure, mechanical properties, and biocompatibility to enhance osteogenesis and angiogenesis to accelerate bone formation. In the present work, we used nanocomposite 3D printed scaffolds, with 500 μm sized pores, consisting of polycaprolactone (PCL), chitosan (CS), and multiwalled carbon nanotubes (MWCNTs) and studied their effect on osteoblasts and endothelial cells' viability, proliferation, migration, and differentiation into osteocytes and tubes, respectively. Two groups of specimens were studied: PCL as a reference material and PCL reinforced with MWCNTs, functionalized with CS. Osteoblasts were isolated from C57BL/6 mice calvaria and endothelial cells either from the lungs of C57BL/6 mice or the vein of human umbilical cords. Scaffolds were loaded with sustained-release growth factors and peptides. All scaffolds used were biocompatible, did not compromise the viability of either osteoblasts or endothelial cells, and promoted their growth and differentiation within the scaffold. The effect of the factors released by the scaffolds on osteoblasts and endothelial cell functions will be discussed, together with their potential to provide an effective platform for bone tissue engineering.

Acknowledgment: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T2EDK-03681).

PoA.16.23

Degradable thiol-ene composites for bone repair

Jorge San Jacinto, Daniel J Hutchinson, Natalia Sanz del Olmo, Michael Malkoch

KTH Royal Institute of Technology, Stockholm, Sweden

Abstract

Open reduction internal fixation (ORIF) metal plates provide exceptional support for unstable bone fractures; however, they often cause soft tissue adhesions which can lead to a reduction in flexibility of nearby joints after healing. In addition, their rigid shape limits the extent to which they can be customized by the surgeon.Our strategy for overcoming these issues is to replace the metal plate with a composite patch fixated to the bone either with metal screws or by priming the bone surface. This composite is a mixture of allyl- and thiol-containing monomers with a high percentage of hydroxyapatite that is rapidly cured on demand via high-energy visible-light-induced thiol–ene coupling chemistry.[1,2] *In vivo* studies showed that this bone-like composite patch did not induce soft-tissue adhesions. The customization, bone-like nature and soft-tissue repelling properties of the composite fixation patch approach are highly compelling features that current traditional metal plating fixators lack. Moreover, the composite is applied topologically over the bone fracture as opposed to being inserted in the cross-section of the bone, which means that it doesn't interfere with the bone healing process.[1] Thus far, the composites that we have developed have not shown degradability over time. However, it is our hypothesis that degradability of the composites can be tuned through the addition of degradable polymers that exhibit hydrolysable groups in their backbone. Aliphatic polycarbonates and polyester are good examples of such degradable polymers that provide controlled degradation rates and are therefore widely used in the medical field as degradable agents.[3,4] In this work, we will present the development of novel composites systems that include polycaprolactones and polycarbonates. The impact of these polymers with respect to degradation and mechanical properties of the final composites will be described thoroughly in order to introduce degradable composite-based fixator for bone fracture repair.

1. Hutchinson, D.J; Granskog, V.; Kieseritzky, J.; Alfort, H.; Stenlund, P.; Zhang, Y.; Malkoch, M. Avd. Funct. Mater. 2021, 2105187.
2. Arseneault, M.; Granskog, V.; Khosravi, S.; Heckler I.M.; Mesa-Antunez, P.; Hult, D.; Zhang, Y; Malkoch, M. Adv. Mater. 2018, 30, 1804966.
3. Hult, D.; García-Gallego, S.; Ingverud, T.; Andren, O.; Malkoch, M. Polym. Chem., 2018, 9, 2238-2246.
4. Juan, P.-K.; Fan, F.-Y.; Lin, W.-C.; Liao, P.-B.; Huang, C.-F.; Shen, Y.-K.; Ruslin, M.; Lee, C.-H. Polymers, 2021, 13, 2718.

PoA.16.24

A clinical translation path for human engineered & decellularized, osteoinductive extracellular matrices.

Sebastien Pigeot[1], Alejandro Garcia-Garcia[2,3], Thibaut Klein[1], Anke Wixmerten[1], Sylvie Miot[1], Andrea Montali[4], Tim Buchholz[5], Lena Gens[5], Dominic Gehweiler[5], Dirk Nehrbass[5], Deepak B. Raina[6], Magnus Tägil[6], Stephan Zeiter[5], Paul E. Bourgine[2,3], Ivan Martin[1]

[1]Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland. [2]Laboratory for Cell, Tissue and Organ engineering, Lund stem cell center, Lund, Sweden. [3]Wallenberg Centre for Molecular Medicine at Lund University, Lund, Sweden. [4]Technology Transfer, AO Foundation, Davos, Switzerland. [5]Preclinical Team, AO Foundation, Davos, Switzerland. [6]Clinical and Experimental bone healing, Department of Orthopedics, Lund University, Lund, Switzerland

Abstract

Bone repair is a robust process, though in critical cases, it requires augmentation through bone substitute materials. In order to overcome the limitations of currently available therapeutic solutions, we have recently developed a novel off-the-shelf strategy to engineer cell-based extracellular matrices (ECM) enriched in bone morphogenic protein 2 (BMP-2) content[1]. Constructs are generated using a cell line derived from human bone marrow mesenchymal stromal cells, engineered to overexpress BMP-2. Upon seeding on collagen scaffolds and induction with chondrogenic cues, cells consistently generate cartilaginous tissues, which upon devitalization retain strong osteoinductive properties and outperform alternative bone tissue engineering approaches[1]. Here, we describe the ongoing and envisioned translational path for clinical use of such materials in bone regeneration. We first generated, in a GMP clean room, a master cell bank (MCB) for standardized production of large ECM batches. Concomitantly, we developed a protocol for efficient decellularization and DNA removal, in order to maintain ECM osteoinductivity while avoiding immune rejection. This was validated by complete bone healing after 6 weeks in a femur bone defect in an immunocompetent rat model. Currently, we are assessing the ECM dosage for safe and efficient bone repair in a sheep femur and tibia drill hole model (5mm diameter and 15mm deep). Preliminary results from the *in vivo* computed tomography (CT) scans, taken at three weeks intervals along the twelve weeks' time course of the dosage study, suggest increased mineralization in holes filled with a mixture of autologous blood and ECM compared to holes filled with autologous blood alone or autologous blood mixed with collagen scaffolds alone (negative controls). Successful healing assessed by micro-CT and histological analysis will validate the approach and pave the way for a Proof-of-Concept study for the envisioned clinical indication, namely intervertebral spinal fusion. Data from further safety animal studies conducted under GLP and production within a GMP environment will complete the pre-clinical data required for the submission of a phase I Clinical Trial application. More broadly, our work will define a regulatory compliant translational pathway for engineered and decellularized, cell-line based ECMs as bioactive materials in regenerative medicine.

1. Pigeot, S. et al. Manufacturing of Human Tissues as off-the-Shelf Grafts Programmed to Induce Regeneration. Adv. Mater. 2103737 (2021) doi:10.1002/ADMA.202103737.

PoA.16.25

Bioactivity of pyomelanin and pyomelanin-modified polymer/nano-hydroxyapatite composites in the process of targeted bone tissue regeneration

Mateusz M. Urbaniak[1,2], Przemysław Płociński[1], Marcin Włodarczyk[1], Monika Biernat[3], Paulina Tymowicz-Grzyb[3], Agnieszka Antosik[3], Anna Woźniak[3], Adrian Najmrodzki[3], Monika Nowak[1], Justyna Sawicka[4], Sylwia Rodziewicz-Motowidło[4], Małgorzata Gazińska[5], Karolina Rudnicka[1]

[1]Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland. [2]Bio-Med-Chem Doctoral School, University of Lodz and Lodz Institutes of the Polish Academy of Sciences, Łódź, Poland. [3]Biomaterials Research Group, Łukasiewicz Research Network-Institute of Ceramics and Building Materials, Kraków, Poland. [4]Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland. [5]Department of Engineering and Technology of Polymers, Faculty of Chemistry, Wrocław University of Science and Technology (WUST), Wrocław, Poland

Abstract

Each year, approximately 1.5 million people suffer from bone fractures and biocomposite implantation. The leading trends in targeted regeneration of bone tissue is the modification of their surface or components with biologically active substances supporting the osteoinduction and osteointegration processes. One of the biological sources of biomaterial modifiers are bacteria. Pyomelanin (PyoM) isolated from *Pseudomonas aeruginosa* is an extracellular polymer chemically similar to alkaptomelanin (ALKM) produced by humans. The formation and accumulation of ALKM deposits in patients with alkaptonuria leads to pathological ossification of the joints. Thus, our strategy was to invert pathological processes that appear in alkaptonuria as osteoinductive agent in controlled bone regeneration. We have optimized the production of PyoM by *P. aeruginosa* on the proprietary Pyomelanin Minimal Medium II (PL patent application no. P.438865), achieving high production yields, reducing the level of impurities and maintaining the bioactivity of this bacterial polymer. In addition, we developed a method for the isolation of the primary, water-soluble form of PyoM and characterized it physiochemically using FT-IR, DSC, TGA and SEM techniques. We have shown that in contrast to synthetic version, bacterial PyoM remain cytocompatible in a wide range of concentrations (1-1024 µg/ml) towards various cells (human monocytes, osteoblasts, chondrocytes) and *Galleria mellonella in vivo* model. We have also demonstrated the immunomodulatory activity of PyoM (1 µg/ml) by activating the NF-kB pathway (A600: 0.71 vs 0.10) and promoting phagocytosis of bacteria that are a risk factor in post-implantation infections (Phagocytic Index: 2.4 vs 1.0). PyoM supported the process of *in vitro* bone tissue regeneration (% of wound healing: 93 vs 85) and stimulated the osteoinduction of osteoblasts by increasing the biosynthesis of osteocalcin (2750 vs 849 pg/mL), interleukin (IL) 6 (263 vs 49 pg/mL), IL-10 (576 vs 150 pg/mL), alkaline phosphatase (6.5 vs 4.8 U/mL) and tumor necrosis factor (TNF)-α (366 vs 55 pg/mL) after 35 days of culture. The elastomeric composites based on polymer and hydroxyapatites containing PyoM (*in situ* and covalently linked by APTES) were developed and physicochemical (TGA, DSC, PyoM secretion profile) and biological (biocompatibility, immunomodulation, osteoinduction) characterization were performed for further implantation studies in animal models.

This research was funded by the Foundation for Polish Science through the European Union under the European Regional Development Fund, within the TEAM-NET program entitled “Multifunctional biologically active composite for application in bone regenerative medicine.” (POIR.04.04.00-00-16D7/18-00).

PoA.16.26

Development of a low-intensity pulsed ultrasound print-head to drive the differentiation of 3D bioprinted skeletal stem cells

Martina Marcotulli[1,2], Chiara Scognamiglio[1], Andrea Barbetta[3], Giancarlo Ruocco[1], Dario Carugo[4], Gianluca Cidonio[1,5]

[1]3D Microfluidic Bioprinting Lab, Center for Life Nano- & Neuro-Science (CLN2S), Italian Institute of Technology (IIT), Rome, Italy. [2]Department of Biochemical Sciences, University of Rome "La Sapienza", Rome, Italy. [3]Department of Chemistry, University of Rome "La Sapienza", Rome, Italy. [4]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS), University of Oxford, Oxford, United Kingdom. [5]Bone and Joint Research Group, Centre for Human Development, Stem Cells and Regeneration, Institute of Developmental Sciences, University of Southampton, Southampton, United Kingdom

Abstract

Clinical approaches are failing to provide readily functional implants for active bone repair. To solve this, an innovative biomaterial system combined with novel microfluidic 3D bioprinting strategy is proposed towards the engineering of next-generation skeletal substitutes. In this work, we demonstrate the engineering of a microfluidic 3D bioprinting platform capable of triggering the differentiation of human bone marrow stromal cells (HBMSCs) by exploiting low-intensity pulsed ultrasound (LIPUS). Over the past two decades, LIPUS has been applied as an innovative technology for the repair of bone defects, accelerating bone repair with associated transmission of mechanical stimuli and activation of bone-specific cellular pathways. We designed an ultrasonic stimulation chamber comprised in the microfluidic print-head for the purpose of stimulating HBMSCs prior to printing and subsequent crosslinking of the scaffold. LIPUS stimulation was carried out at a frequency of 2.25 MHz, intensity of 100 mW/cm2, 20% duty cycle, 5-min pre-print exposure. Following LIPUS stimulation, functional HBMSCs were encapsulated in a porous gelatin methacryloyl (GelMA) and alginate-based hydrogel. To enhance the LIPUS-driven differentiation, the biomaterial carrier was supplied with bone morphogenetic protein 2 (BMP-2) loaded-microbubbles (MBs). The new smart biomaterial ink was able to locally release BMP-2 by ultrasonic activation, augmenting the cellular response. Indeed, LIPUS were found to drive osteogenic differentiation by increasing gene expression at day 7 of osterix (OSX), osteocalcin (OCL) and BMP-2 compared to unstimulated controls. The porosity and charged surface of the proposed biomaterial had a strong impact on the mineralisation examined by Alizarin Red staining, with the LIPUS-stimulated scaffolds characterised by increased growth of calcium phosphate deposits at day 14 compared to controls. The expression of alkaline phosphatase (ALP) was significantly greater in LIPUS-stimulated constructs at day 7 in comparison with controls. Furthermore, quantitative ALP-assay analysis confirmed a significantly ($p < 0.005$) higher increase in ALP expression in LIPUS-stimulated scaffolds than in controls (Fig. 1). Finally, the Live/Dead assay performed on stimulated and non-stimulated HBMSCs within the hydrogel was investigated up to day 21, showing sustained viability in response to ultrasonic stimulation. In conclusion, the results here reported demonstrate that the engineered microfluidic 3D bioprinting system can harness LIPUS stimulation to promote osteogenic differentiation of printed HBMSCs. The active stemness remodelling triggered by LIPUS might influence a rapid differentiation of HBMSCs, allowing the fabrication of a readily implantable skeletal substitutes, to reduce hospitalisation time and worldwide economic burden.

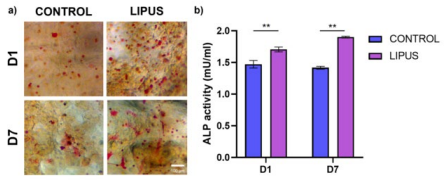


Figure 1. ALP-staining (a) and ALP-assay (b)

PoA.16.28

LOOKING AT SCAFFOLD-BASED STRATEGY FOR BONE DISEASE MANAGEMENT: FROM DRUG LOADING TO ALTERNATIVE ANTIMICROBIAL AGENTS

Elisabetta Campodoni[1], Sara Bernardoni[1], Valentina Possetti[2,3], Antonio Inforzato[2,3], Cristina Sobacchi[4,2], Monica Sandri[1]

[1]Institute of Science, Technology and Sustainability for Ceramics (ISSMC-CNR), Faenza, Italy. [2]IRCCS Humanitas Research Hospital, Rozzano, Italy. [3]Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy. [4]Institute for Genetic and Biomedical Research, (CNR-IRGB), Rozzano, Italy

Abstract

Bone tissue can regenerate itself completely and continuously; however, the self-healing process cannot take place alone for large-scale bone defects caused by trauma, infection or tumour resection. Among bone diseases, Osteomyelitis is a bacterial inflammatory condition of bone tissue due to the microorganism *Staphylococcus aureus* that causes immunological response leading to bone necrosis. Furthermore, S*taphylococcus aureus* with the production of biofilms becomes resistant to antibacterial agents; for these reasons, traditional antibiotic administration is not effective and the local delivery through scaffold or the availability of specific antimicrobial agents become the only promising therapeutic strategy. Choosing the adequate biomaterials and fabrication technology it is possible to create a device which fulfils the biological, mechanical, and physical requirements for bone regeneration providing structural support and favouring cell and nutrient diffusion for the new bone tissue growth. In particular, hybrid bone-mimetic scaffolds composed of hydroxyapatite nanocrystals nucleated and grown on collagen fibres (HA/Coll) are excellent candidates for bone regeneration since displayed chemical-physical and biological properties very close to those of the natural bone, promoting cell adhesion and differentiation. Their highly porous structure and hydrophilicity allows them to absorb different kind of drug solution. Furthermore, HA crystals are endowed with the possibility to host different foreign ions into the lattice conferring distinct features and functionality. For example, the introduction of Zn(II) and Mg(II) ions induces antimicrobial properties useful to counteract potential bacterial infections during tissue regeneration. In the present study, two different strategies have been evaluated to develop hybrid scaffolds made of doped-hydroxyapatite and collagen and suitable to contrast bacterial infections simultaneously to stimulate bone regeneration. The first strategy involves the absorption of drug solution (vancomycin or gentamicin) into the HA/Coll scaffold thanks to its high swelling obtaining a local drug release. In the second strategy, several foreign ions (Fe, Cu, Zn and Mg), well-known for their antimicrobial potential, were introduced into the HA during the biomineralization process consisting in the direct growth of HA particles on Coll fibers. This allows conferring to the hybrid scaffold an intrinsic antimicrobial activity lasting throughout the bone tissue regeneration process. Both scaffolds have been completely characterized in terms of morphology, porosity, swelling and stability. Biological tests demonstrate the cell adhesion and proliferation into the scaffolds. Moreover, microbiological tests demonstrated the effective ability of the scaffolds to inhibit the proliferation of Staphylococcus aureus conferring them as promising candidates for the management of bone infections.

PoA.16.29

Engineered 3Dprinted vascularized hydrogels for efficient presentation of growth factors

Oana Dobre[1], Xie Ruoxiao[2], Liliang Ouyang[3], Molly Stevens[4], Manuel Salmeron-Sanchez[1]

[1]Centre for the Cellular Microenvironment, Advanced Research Centre, University of Glasgow, Glasgow, United Kingdom. [2]Department of Materials, Department of Bioengineering, Institute of Biomedical Engineering, Imperial College London, London, United Kingdom. [3]Departament of Mechanical Engineering, Tsinghua University, Beijing, China. [4]I Department of Materials, Department of Bioengineering, Institute of Biomedical Engineering, Imperial College London, London, United Kingdom

Abstract

Purpose: Bone is the second most transplanted tissue and can regenerate by itself to a certain degree, but in cases of critical size defect, this capacity of selfregeneration is severely impaired. This type of lesion requires additional treatment with bone graft materials. There is an urgent unmet clinical need for personalised scaffolds that promote the regeneration of large bone defects. We aim to develop a bioink system by incorporating PEGylated fulllength laminin (LM) into gelatin methacryloyl (GelMA). Methods: 3D LMbased bioinks containing different concentrations of PEGylated human LM, GelMA and a photoinitiator were printed using a 3D Bioprinter. Then the printed structures were crosslinked via photopolymerization. The mechanical properties of the bioinks were characterized by shear bulk rheology. hMSCs and HUVECs with the addition of specific growth factors were incorporated into the LM bioinks to evaluate cell cytotoxicity, and study their release kinetics and potential to drive vasculogenesis and osteogenesis. Results: We investigated the possibility of 3D LM/GelMA to promote bone regeneration by delivering growth factors in a controlled manner. To do this, 3D LM/GelMA with tuneable stiffness and different concentrations of LM 332 were printed to mimic the native bone microenvironment. Protein and gene expression results of coculturing hMSCs and HUVECs encapsulated in 3D LM/GelMA bioinks loaded with BMP2 and VEGFA165 showed upregulated expression of osteopontin (OPN) and endothelial cell adhesion marker CD31 after 14 days. Conclusion: We report on the development and characterization of 3D LM/GelMA hydrogels with tuneable mechanical and highly efficient growth factor presentation for potential applications such as regeneration of bone defects with an adequate vascularisation.

Acknowledgments: This work was completed with support from the Engineering and Physical Sciences Research Council (EPSRC) Grant EP/P001114/1.

PoA.16.30

pMHMGCL melt electrowritten scaffolds covalently grafted onto silk fibroin methacryloyl hydrogels to improve the mechanical properties of cartilage constructs.

Martina Viola[1,2], Madison Ainsworth[1], Marko Mihajlovic[2], Miguel Castilho[3,4,1], Jos Malda[1,5], Tina Vermonden[2]

[1]Department of Orthopaedics, University Medical Centre Utrecht, Utrecht, Netherlands. [2]Division of Pharmaceutics, Department of Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, Netherlands. [3]Department of Biomedical Engineering, Technical University of Eindhoven, Eindhoven, Netherlands. [4]Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, Netherlands. [5]Department Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands

Abstract

Hydrogels, as materials mostly composed of water, are ideal for providing a suitable environment for the development of encapsulated cells, making them suitable for applications in regenerative medicine. However, hydrogels must often be coupled with reinforcement structures as they do not possess the adequate mechanical strength in order to functionally replace human tissues (like cartilage or bone tissues). The relevance of interactions at the interface of materials is often overlooked, even though it is now known that these interfaces are important drivers of the mechanical and biological performance. In this work, we explored the variation in mechanical properties of silk fibroin methacryloyl hydrogels reinforced with three-dimensional melt electrowritten scaffolds with and without the presence of covalent interfacial interactions. A thermoplastic polymer blend of poly(hydroxymethylglycolide-co-e-caprolactone)/poly(e-caprolactone) (pHMGCL/pCL) was compared to its respective methacrylate polymer blend pMHMGCL/PCL. Both blends were printed using melt electrowriting technique and used as reinforcing structures for silk fibroin methacryloyl hydrogels (silkMA). We compared the effect of the interface grafting between the fibres and the hydrogels on mechanical properties of the construct and the cartilage-specific matrix production *in vitro*. We show through creep experiments the presence of covalent bonds between the pMHMGCL/PCL blend and silk fibroin methacryloyl hydrogels, resulted in an elastic response to the application of a torque. We observed an improved resistance to compression and traction in the scaffolds with covalent links at the interface compared to those without interactions. We further observing no significant differences in the cytotoxicity of both mixtures (pHMGCL/PCL and pMHMGCL/PCL) compared to PCL alone. Moreover, chondrocytes embedded within the reinforced silkMA hydrogel were able to form cartilage-specific matrix *in vitro*. Thus, we showed that by grafting different materials at the interface, hybrid cartilage constructs can be engineered.

PoA.16.31

Polyhydroxyalkanoate/bioactive glass 3D printing composite scaffolds with antimicrobial properties for bone tissue engineering applications

Andrea Mele[1], Ipsita Roy[1], Aldo Roberto Boccaccini[2], Atiq Ur Rehman Muhammad[3]

[1]University of Sheffield, Sheffield, United Kingdom. [2]University of Erlangen-Nuremberg, Erlangen, Germany. [3]Institute of Space Technology, Islamabad, Pakistan

Abstract

Bone tumour removal, traumas with large defects or infections, and degenerative diseases are the main catastrophic events impeding complete bone healing. Autologous and allogenic bone grafting and biologically inert metallic devices have limitations such as non-availability of autogenous bone, risk of infectious disease transmission, subsequent surgical removal, and bacterial infections. Therefore, to overcome the limitations, we created 3D printed composite scaffolds based on a combination of Poly(3-hydroxybutyrate) [P(3HB)], a natural biocompatible and bioresorbable polymer of bacterial origin, and a Borosilicate-based bioactive glass doped with Zinc to support bone tissue regeneration and provide antibacterial activity.P(3HB) was produced by bacterial fermentation of B.sacchari. Solvent casting has been used to produce composite films with different bioactive glass concentrations (v/v%) to select the best composition to be used for the development of 3D printing scaffolds with different geometry using Fused Deposition Modelling (FDM). MG63 human osteoblast cell line and primary osteoblasts were cultured on the 3D printed structures to assess biocompatibility and proliferative activity through Resazurin and Live/Dead assays. Primary Osteoblasts were used to investigate the mineralisation activity induced by the scaffolds. To evaluate the antimicrobial activity, MIC and MBC, Halo Test, and biofilm formation assay were performed against E. coli 8739, S. aureus 2569 and S. aureus 6538P for the scaffolds.The chemical and thermal analysis confirmed that the polymer produced was Poly(3-hydroxybutyrate) with very similar properties to previous studies and commercially available products. The addition of bioglasses induced a nanostructured topography on the surface of the composites and the production of hydroxyapatite, as visible by SEM. Moreover, X-ray CT confirmed the homogeneous distribution of the bioglass filler in the polymeric matrix. The resazurin assay demonstrated the biocompatibility of P(3HB) and the related composites. 3D printed composite scaffolds with high v/v% of bioglass were successfully developed with high reproducibility, showing a high level of mineralisation towards primary osteoblasts cells. Antimicrobial assays confirmed the activity of the composites against the three pathogens bacteria species used in the study.The results demonstrated the development of a promising composite material with suitable physicochemical properties and antimicrobial activity for bone tissue engineering applications.

PoA.16.32

Assessment of cell migration into fibrin hydrogels in an *ex-vivo* murine bone model

Miruna Chipara, Richard Moakes, Melissa Finlay, Amy Naylor, Liam Grover

University of Birmingham, Birmingham, United Kingdom

Abstract

INTRODUCTION Bone damage following blast or ballistic wounding results in the mechanic destruction and death of tissue at the site of injury. Surgeons debride the tissue, removing any necrotic regions but bone continues to turn gradually necrotic, thus the wound remains open, increasing the risk of infection. Currently there are no methods that enable full characterisation of such fragments in anatomically relevant positions within the fracture. We have worked on the use of a structured or fluid-gel materials as support matrices in which cell bearing gels can be immobilised, allowing for the construction of large and complex tissues. We have recently explored the possibility of using these materials to support and process tissue fragments such that they could be maintained in an anatomically relevant configuration ex-vivo. EXPERIMENTAL METHODS Ex-vivo model: Fresh rat and mouse fractured bones were added to a polyethylene glycol (PEG) fluid gel support medium. A fibrin gel was injected in between the fracture site. Additionally, to understand better cell migration and mineralisation within the fibrin gels, bone chips in fibrin constructs were analysed separately. RESULTS AND DISCUSSION The support medium plays a vital role in maintaining the viability of the tissue ex-vivo, therefore, to test the capability of PEG gels to deliver nutrients to the encapsulated bones, as well as to eliminate metabolic waste, we analyzed the rheological and diffusion properties. A fluid gel has the property to self-heal, thereby providing support to another gelling material to be printed by dispersing it into the interstices of the supporting fluid gel particles. This enables relatively complex structuring, whilst providing sufficient support to prevent the structure from collapsing under its own weight and preserving the bony ends in a relevant anatomical configuration. Fibrin gel was selected as the bridging material due to its structural and biochemical similarities to the microenvironment of the callus formed early in fracture healing. Cells were able to migrate into the fibrin gel within a few days and displayed an early osteocyte marker, podoplanin as early as one week, to three weeks in culture. Moreover, after 25 days, extracellular matrix surrounding an organised cell network resembled mineral deposition in bone and it is currently being analysed further. CONCLUSION In conclusion, traumatic injury field could highly benefit from the usage of biomaterials, for both a deeper understanding of injury profile through more accessible models and improvement of early intervention practices.

Fig. 1

Fig. 2

PoA.16.33

BIOMIMETIC BIOREACTORS AS A TOOL FOR MORE RELEVANT BIOMATERIAL ASSESSMENT

Jovana Zvicer[1], Jasmina Stojkovska[1], Djordje Veljovic[1], Mia Milosevic[2], Bojana Obradovic[1]

[1]University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia. [2]Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia

Abstract

Development of novel biomaterials for use in biomedical applications requires careful assessment due to the intended interactions with cells and tissues. Understanding biocompatibility, non-toxicity, and capability of promoting desired biological responses requires thorough characterization of biomaterial, including its chemical composition, surface properties, mechanical strength, degradation rate, etc. Traditional *in vitro* methods for evaluating biomaterials in cell monolayers are convenient but limited by the lack of specific biophysical signals found *in vivo*, which can lead to unreliable results. This *in vitro-in vivo* gap can result in the unnecessary sacrifice of a large number of animals for testing purposes. Therefore, there is a need for alternative approaches that better mimic the *in vivo* environment and accurately predict the behavior of the biomaterial after implantation. Biomimetic bioreactors are primarily developed for tissue engineering to provide the key biochemical (e.g., nutrients, gases, growth factors) and biophysical signals (e.g., shear stress, hydrostatic pressure, mechanical strains) found *in vivo* and thus could be indispensable tools in physiologically relevant biomaterial assessment. Our group introduced the application of two biomimetic bioreactors for the physiologically relevant characterization of two types of composite biomaterials aimed for bone and osteochondral tissue engineering. In specific, macroporous composite scaffolds were produced using two natural polymers (gellan gum and alginate) as matrices imitating organic phase of bone tissue with incorporated particulate bioactive glass (BAG) and β-tricalcium phosphate (β-TCP) as hydroxyapatite (HAp) precursors. In addition, in osteochondral scaffolds, gellan gum hydrogel served as a cartilaginous layer on top of the porous composite base. Integrity and mechanical properties of all prepared scaffolds were monitored for 14 days under physiological levels of mechanical compression (up to 10% strain, compression rate 337.5 µm s-1) in a bioreactor with dynamic compression and medium perfusion. Bioactivity and HAp formation within the scaffolds were investigated in a perfusion bioreactor under the flow of simulated body fluid for up to 28 days. The scaffolds were assessed by SEM, EDS, and XRD analyses indicating a significant increase in HAp formation under bioreactor conditions as compared to static controls in all investigated samples. Moreover, the formed HAp crystals were more uniformly distributed throughout the scaffolds showing a more cauliflower-like morphology and thus, indicating potentials for bone/osteochondral tissue engineering applications. The obtained results confirm the high influence of experimental conditions on the outcomes of biomaterial characterization and importance of closely mimicking physiological conditions, thus putting forward biomimetic bioreactors as a means in this direction.

PoA.16.34

Biomechanical analysis of a novel osteosynthesis device with standard and physiological testing

Peter Schwarzenberg[1], Thomas Colding-Rasmussen[2], Daniel J Hutchinson[3], Jorge San Jacinto Garcia[3], Stine Jacobsen[4], Peter Horstmann[5], Michael Mørk Petersen[6,4], Dominic Mischler[1], Viktor Granskog[7], Tatjana Pastor[1], Tine Weis[2], Michael Malkoch[3], Christian Wong[2,4], Peter Varga[1]

[1]AO Research Institute, Davos, Switzerland. [2]Hvidovre University Hospital, Copenhagen, Denmark. [3]KTH Royal Institue of Technology, Stockholm, Sweden. [4]University of Copenhagen, Copenhagen, Denmark. [5]Herlev and Gentofte Hospital, Hellerup, Denmark. [6]Rigshospitalet, Copenhagen, Denmark. [7]Biomedical Bonding AB, Stockholm, Sweden

Abstract

To overcome certain limitations in the customizability of traditional metal osteosynthesis hardware, a new osteosynthesis method, AdhFix, has been developed using a light-curable polymer composite for highly customizable fixation solutions1. With the advanced capabilities of new biomaterials such as these, the biomechanical evaluation should not be limited to the existing status quo but should also include approaches optimized to best fit specific applications. In an initial study, AdhFix was compared to traditional metal hardware using an ex vivo ovine phalanx model with both well reduced fractures and a gap model simulating comminution. This study showed that AdhFix had superior stiffness in both torsion and well reduced fractures in bending. Additionally, AdhFix was able to support equivalent max torques as the traditional metal solution. However, it fell short in comminuted fractures in four-point bending with the maximum bending moment being 1220 Nmm and the metal plate-based fixation being one order of magnitude stronger (Figure 1). However, it remained unclear what level of bending moment a fixation solution would need to withstand under physiological conditions and if the AdhFix solution would exceed this limit. To answer this question, a follow up study was conducted to determine the loading environment during clinical rehabilitation exercises in the proximal phalanx. By implementing a custom PEEK (Polyether ether ketone) plate, measuring deformation with stereographic motion tracking, and using specimen specific finite element (FE) modeling, the internal forces in the proximal phalanx were calculated during standard clinical rehabilitation exercises of flexing each digit until the fingertip reached the palm. This study showed that the average bending moment across the proximal phalanx during this exercise was 6.78 ± 1.62 Nmm. These results, taken together from both studies, indicate that even though AdhFix may not be able to sustain the same maximum bending moment to failure as metal plates, it far exceeds the strength required for an osteosynthesis device when used clinically in the proximal phalanx during rehabilitation exercises. Furthermore, these studies highlight the importance to design novel osteosynthesis devices to the requirements of the intended application. Further studies are needed to better understand different loading modes, physiological movements, and anatomical sites.

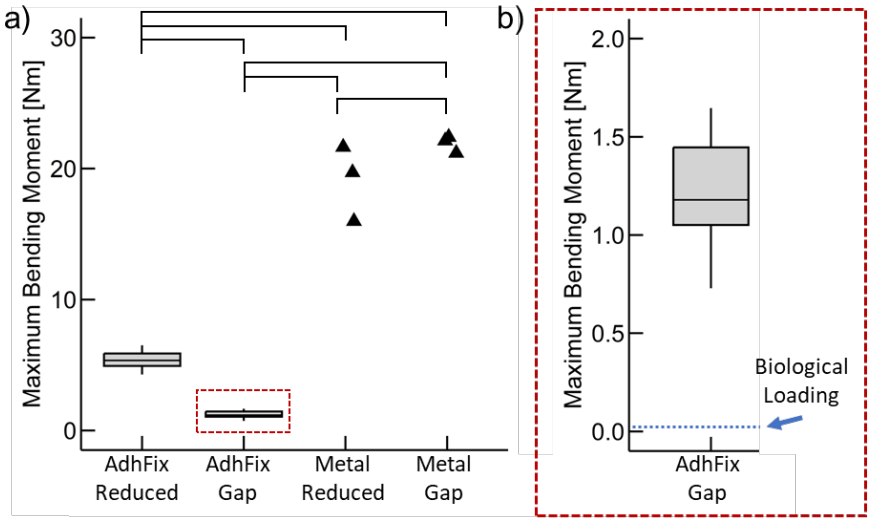


Figure 1: a) Box plots of the maximum bending moment results from the four-point bending testing. Significance is denoted by bars ($p < 0.05$). b) Zoomed view of weakest AdhFix maximum bending moment results compared to biological loading during rehabilitation exercises shown by the blue dotted line.

PoA.16.35

Phosphoserine-enhanced alpha-TCP adhesive for improved bone regeneration with novel mechanical and handling properties

Antzela Tzagiollari[1], Gerard Insley[2], Philip Procter[2], Benjamin Pippenger[3], Ole Zoffmann Andersen[3], Helen O. McCarthy[4], Tanya J. Levingstone[1], Nicholas J. Dunne[1]

[1]Dublin City University, Dublin, Ireland. [2]Biomimetic Innovations, Shannon, Ireland. [3]University of Bern, Bern, Switzerland. [4]Queen's University Belfast, Belfast, United Kingdom

Abstract

The annual increase in bone fractures, and the lack of effectiveness of current treatments, is a challenge for orthopaedic surgeons. This study aimed to develop phosphoserine-modified calcium phosphates (PM-CPC), which incorporate phosphoserine associated with human bone cell adhesion mechanisms, with alpha-tricalcium phosphate (alpha-TCP) and calcium silicate for bone stabilisation and regeneration. The PM-CPC formulation was optimised using a Design of Experiment (DoE) approach, assessing key inputs such as alpha-TCP particle size, liquid-powder ratio and phosphoserine loading. Further characterisation of the optimal PM–CPC included assessment of physico-chemical, setting and rheological properties, as well as the adhesive potential to the bone under pseudophysiological conditions. *In vivo* assessment was completed using fresh and artificial extraction sockets (bundle and native trabecular bone) in female Göttingen mini pigs over an 8-week period. The primary implant stability quotient (ISQ) values, adhesive mechanical properties and osseointegration were investigated. Additionally, pre-mixed formulations of paste-based systems and a custom-designed double syringe mixing system were developed and assessed. The optimal PM-CPC provided a setting time of 2–3 min, compressive strength of 29.2±4.9 MPa and bone-to-bone shear adhesion of 100 N under wet environment (Fig.1a,b). The fast setting indicates accelerated hydroxyapatite nucleation in the presence of phosphoserine, providing suitable properties for surgeons to apply and stabilise bone fractures. During the initial stages of natural bone healing, effective support and stability of the bone fragments can be achieved due to the slow degradation (Fig.1c) and the high force obtained. *In vivo* experiments demonstrated the ability of PM-CPC to stabilise the implant within the trabecular bone, with primary ISQ values of 65–85 after 15 min. The PM-CPC–bone–implant interface was sufficiently mature enough to have a measurable mechanical effect (torque strength of ~80 Ncm) at 8 weeks post-surgery. The high torque strength indicates that the implant can remain securely attached over time, withstanding forces that are generated in the mouth during biting and chewing. The *in vitro* high biocompatibility (Fig.1d) combined with the effective *in vivo* osteoconduction and bone remodelling (Fig.1e), demonstrated the potential of the PM-CPC to promote osteogenesis. The paste–based systems developed in order to achieve minimal invasive on-demand mixing and delivery were homogeneously mixed with significantly higher mechanical properties (Fig.1f) than hand–mixed adhesive. The proposed PM-CPC adhesive represents a critical step forward in the treatment of complex bone injuries and has the potential to improve patient outcomes with precise delivery within a surgical environment.

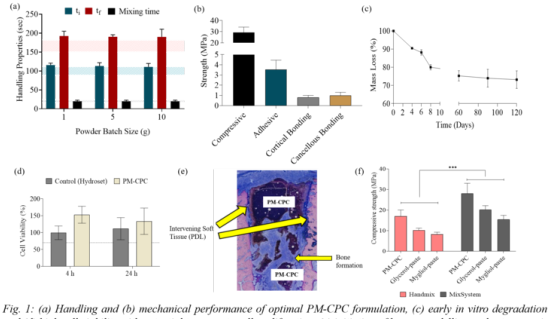


*Fig. 1: (a) Handling and (b) mechanical performance of optimal PM-CPC formulation, (c) early in vitro degradation and (d) high cell viability with potential to promote cell proliferation, (e) initiation of bone remodelling and osteogenesis by week 2 and (f) comparison of mechanical properties between MixSystem and Handmix.***p-value=0.001*

PoA.16.36

Pleiotrophin-loaded mesoporous silica nanoparticles as a possible treatment for osteoporosis

Daniel Lozano[1,2], Beatriz Leiva[1], Inés S Gómez-Escalonilla[1], Sergio Portal-Núñez[3], Arancha R de Górtazar[3], María Vallet-Regí[1,2], Miguel Mazano[1,2]

[1]Departamento de Química en Ciencias Farmacéuticas, Facultad de Farmacia, Universidad Complutense de Madrid, Instituto de Investigación Hospital 12 de Octubre (i+12), Plaza de Ramón y Cajal s/n, 28040, Madrid, Spain. [2]ntro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), 28029, Madrid, Spain. [3]Grupo de Fisiopatología Ósea, Departamento de Ciencias Médicas Básicas, Instituto de Medicina Aplicada de la Universidad San Pablo-CEU, Facultad de Medicina, Universidad San Pablo CEU, CEU Universities, Urbanización Montepríncipe s/n, 28925, Madrid, Spain

Abstract

Introduction: Osteoporosis is the most common type of bone disease. Conventional treatments are based on the use of antiresorptive drugs and/or anabolic agents. However, these treatments have certain limitations, such as a lack of bioavailability or toxicity in non-specific tissues. In this regard, pleiotrophin (PTN) is a protein with potent mitogenic, angiogenic, and chemotactic activity, with implications in tissue repair. On the other hand, mesoporous silica nanoparticles (MSNs) have proven to be an effective inorganic drug-delivery system for biomedical applications. In addition, the surface anchoring of cationic polymers allows for greater cell internalization, and, therefore, increasing treatment efficacy. **Matherial and methods**: The synthesis of MSNs was carried out by a modified Stöber method. Subsequently, MSN were coated with polyethylenimine and recombinant human PTN protein was observed on their surface. These nanoplaftorms were characterized by different microscopy, chemical and surface characterization techniques. Cellular assays were carried out on MC3T3-E1 mouse pre-osteoblastic cells and human mesenchymal stem cells (hMSCs). Viability (Alamar Blue), cell internalization (flow cytometry) and osteoblast differentiation (alizarin red staining and gene expression) studies were performed in the presence of different concentrations of MSNs@PEI loaded or unloaded with PTN. **Results**: In order to load and release the PTN to improve its effectiveness, MSNs were successfully internalized in MC3T3-E1 mouse pre-osteoblastic cells and human mesenchymal stem cells. PTN-loaded MSNs significantly increased the viability, mineralization, and gene expression of alkaline phosphatase and Runx2 in comparison with the PTN alone in both cell lines, evidencing its positive effect on osteogenesis and osteoblast differentiation. **Conclusion**: This proof of concept demonstrates, for the first time, that MSN can take up and release PTN, developing a potent osteogenic and differentiating action *in vitro* in the absence of an osteogenic differentiation-promoting medium, presenting itself as a possible treatment to improve bone-regeneration and osteoporosis scenarios.

PoA.16.37

Delivery of osteoanabolic genetic cargo using a peptide-based delivery system for healing of large bone defects

Ahmed Elkashif[1], Monika Ziminska[1], Jordan Wilson[1], John Redmond[2], Antzela Tzagiollari[2], Auden Balouch[3], Seth Donahue[3], Nicholas J Dunne[2,1], Helen O McCarthy[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Dublin City University, Dublin, Ireland. [3]University of Massachusetts Amherst, Amherst, USA

Abstract

Introduction Bone injuries are among the leading causes of morbidity worldwide. Nanotechnology-based approaches are increasing in popularity with the goal of delivering cargo intracellularly, in a minimally invasive fashion. We have used RALA, a peptide-based nanoparticle (NP) to deliver microRNA 26a (miR-26a) to mesenchymal stem cells (MSCs). The aim of this study was to deliver miR-26a *in vitro* to interrogate its effect on osteogenic signalling and *in vivo* to assess its potential as a bone-regenerative therapeutic. Methods NPs were formulated via electrostatic interaction by incubating negatively charged nucleic acids with the positively charged RALA peptide for 30 min at room temperature. Particle size and charge was measured using a Nano ZS Zetasizer. Cells were transfected with NPs for 4 h in serum free media. Gene expression was measured by quantitative real time polymerase chain reaction (RT-PCR). Critical-size calvarial defects with a diameter of 6.8 mm were made in the calvaria of Wistar male rats. RALA/miR-26a was delivered via athermoresponsive chitosan-graft-poly(N-isopropylacrylamide) injectable hydrogel. Results /Discussion RALA/miR-26a NPs were formulated at various NP ratios, with N:P 6 and above showing optimal characteristics for intracellular delivery (size < 100 nm, charge > 10 mV) (Fig. 1A). Transfection of human MSCs resulted in a greater than 10-fold upregulation in miR-26a and increases in the osteogenic markers COL1, RUNX2 and OCN (Fig. 1B/C). *In vivo* analysis showed that treatment with RALA/miR-26a NPs resulted in increased bone volume and bone mineral density at 8 weeks following treatment compared to empty defect (Fig. 2). Conclusion This work shows for the first time that RALA can be used to condense miR-26a into nanoscale particles for delivery into MSCs. Transfection led to increased osteogenic signalling by MSCs. RALA was also used to treat critical size calvarial defects in rats, with increased rate and quality of bone healing shown 8 weeks following treatment.

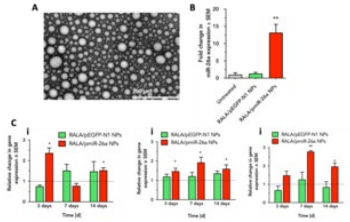


Figure 1 (A) TEM image showing monodispersed, spherical RALA/miR-26a NPs at N:P 6. (B) qRT-PCR analysis of miR-26a expression 48 h post-transfection with RALA/pEGFP-N1 NPs and RALA/miR-26a NPs in hBMSCs. (C) qRT-PCR analysis of (i) COL1, (ii) RUNX2 and (iii) OCN 3-, 7- and 14-days following transfection with RALA/miR-26a NPs.

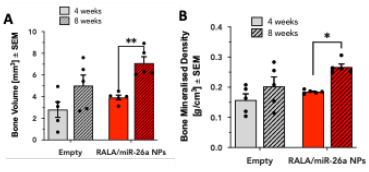


Figure 2 (A) Volume of bone within region of interest of untreated and RALA/miR-26a treated rat calvarial defects at 4 and 8 weeks. (B) Bone mineral density shown as average density of all tissues within the region of interest at 4 and 8 weeks.

PoA.16.39

Tailored polyelectrolyte multilayer systems by variation of polyelectrolyte composition and EDC/NHS cross-linking: Controlled drug release vs. drug reservoir capabilities and cellular response for improved osseointegration

Uwe Schirmer[1], Johanna Ludolph[1], Klaus Liefeith[1], Christina Behrens[2], Henning Schliephake[2]

[1]Institute for Bioprocessing and Analytical Measurement Techniques e.V, Heiligenstadt, Germany. [2]Dept. of Oral and Maxillofacial Surgery, Georg-August-University, Göttingen, Germany

Abstract

Polyelectrolyte multilayers are versatile tools to investigate fundamental interactions between material related parameters and the resulting performance in stem cell differentiation respectively in bone tissue engineering. In the present study, we established a PEM-system that allows a precise adjustment of the physicochemical properties of the selected film architecture regarding the desired applications. In this study for the first time, different collagen/heparin films have been prepared and cross-linked with EDC/NHS chemistry and deeply characterized with several methods like Quartz-crystal-microbalance, Zeta-potential-analyzer, diffuse reflectance Fourier transform infrared spectroscopy, atomic force microscopy and ellipsometry. To prove the functionality of the system the cell response as well as the suitability of the PEMs for use as drug carriers for human bone morphogenetic protein 2 (rhBMP-2) have been investigated. For a specific modification of cell response, loading capacity and release kinetics, the PEMs were stepwise cross-linked before loading with cytokine. The analysis reveals that the cell response correlates not only with the collagen amount but also with the EDC concentration. For drug release it was demonstrated that it's possible to immobilize significant amounts of rhBMP-2 in all multilayer systems and to specifically tune its release via cross-linking. Furthermore, we prove that the drug release of rhBMP-2 plays only a minor role in the differentiation of osteoprogenitor cells. We find a significant higher influence of the immobilized rhBMP-2 within the collagen-rich coatings that obviously represent an excellent mimicry of the native extracellular matrix. The cytokine immobilized in its bioactive form was able to achieve an increase of orders of magnitude both in early stages of differentiation and in late calcification compared to the unloaded layers.

PoA.16.43

Gallic acid-functionalized, MgO nanoparticle-loaded hydrogels for the treatment of rheumatoid arthritis

Yashika Thakur, Yashveer Singh

Indian Institute of Technology Ropar, Rupnagar, India

Abstract

INTRODUCTION Rheumatoid arthritis (RA), an autoimmune disorder, is characterized by the chronic inflammation of joints, leading to their destruction[1]. Due to an excessive influx of inflammatory cytokines, hyperplasticity of fibroblast-like synoviocytes, increased levels of reactive oxygen species (ROS), matrix metalloproteinases are overexpressed, creating an acidic microenvironment, which leads to the irreversible destruction of cartilage and bone[2]. Methotrexate is used for the treatment of RA but it is associated with adverse effects, and very few drug-free strategies targeting disease progression and tissue regeneration are known. Gallic acid induces apoptosis in fibroblast-like synoviocytes and magnesium oxide (MgO) nanoparticles are known for their ability to promote bone tissue regeneration. Therefore, we have developed a drug-free, oxidized chondroitin sulfate-dextran hydrogel loaded with gallic acid-functionalized MgO nanoparticles to alleviate RA and promote bone tissue regeneration. EXPERIMENTAL METHODS MgO nanoparticles were fabricated using $MgCl_2.6H_2O$ and functionalized with APTES. The amine groups in APTES were used to functionalize the nanoparticles with gallic acid, and these were characterized using DLS, Zeta, PXRD, TGA, EDX, and FESEM. Chondroitin sulfate and dextran were oxidized using NaIO4 and characterized by FT-IR, ^{1}H NMR, and GPC. Hydrazone linkage-based hydrogels were fabricated from oxidized polymers, using adipic acid dihydrazide as the crosslinking agent, and their viscoelastic and self-healing properties were determined using rheology. The ROS scavenging ability of the material was studied using ABTS assay along with its cell viability, bone mineralization ability, and cellular uptake characteristics. RESULTS AND DISCUSSION Gallic acid-functionalized MgO nanoparticles (MgO-GA) were loaded into hydrazone linkage-based hydrogel responsive to the low pH (~6) present in the RA microenvironment. It showed excellent viscoelastic properties and around 90% radical scavenging activity, as determined by the ABTS assay. It was found non-toxic to mesenchymal stem cells (MC3T3) and RAW 264.7 (macrophages) and demonstrated bone mineralization properties, as investigated by the alkaline phosphatase and alizarin red assays.

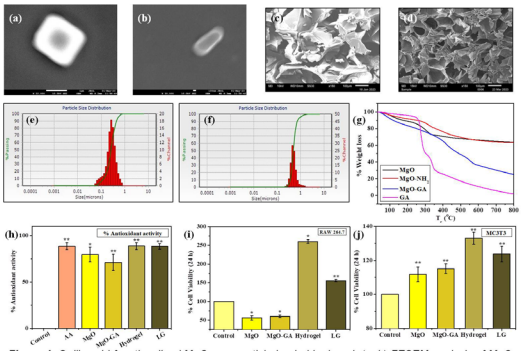


***Figure 1**. Gallic acid-functionalized MgO nanoparticle-loaded hydrogel. (a, b) FESEM analysis of MgO and MgO-GA nanoparticles. Scale bar: 1 µm (c, d) SEM analysis of oxidized chondroitin sulphate-dextran hydrogel (unloaded and loaded with MgO-GA). Scale bar: 100 µm (e, f). Particle size analysis. (g) TGA profiles. (h) Percentage antioxidant activity. (i, j) Percentage cell viability. n = 3, mean ± SD; *p < 0.05, **p < 0.5. AA: ascorbic acid, and LG: MgO-GA loaded hydrogel.*

CONCLUSIONS We have created a drug-free, gallic acid-functionalized nanoparticle-loaded hydrogel system to provide the release of nanoparticles at a low pH and relieve inflammation in RA-affected joints. It can reduce the oxidative stress and induce cell proliferation and bone mineralization. It shows a potential for bone tissue regeneration in the affected joints.

REFERENCES

1. Smolen et al., The Lancet 388 (10055), 2023–2038, 2016.
2. Li et al., Nano Today 42, 101367, 2022.

PoA.16.44

Novel device for vacuum-assisted loading of hydrogel into a PCL-TCP scaffold for bone tissue engineering applications

Jolene Quek[1,2], Yen Choo[2], Kee Woei Ng[3]

[1]HealthTech, Interdisciplinary Graduate Programme, Nanyang Technological University, Singapore, Singapore. [2]Stem Cell Science and Regenerative Medicine, Lee Kong Chian School of Medicine (LKC Medicine), Novena Campus, Clinical Sciences Building, Singapore, Singapore. [3]School of Material Science and Engineering, Nanyang Technological University, Singapore, Singapore

Abstract

Introduction: To date. there is no standardized method of cell loading into a porous scaffold and are generally loaded via manual pipetting. This procedure of manual loading can vary based on individuals and are highly subjected to human error. Cell loading can also be highly influenced by other factors, such as hydrophobicity of scaffolds, viscosity of hydrogel and the presence of air bubbles. Method To develop a standardized and systematic procedure for cell loading into the scaffold, a 3D-printed device was designed and customized to snugly fit the scaffold, with an inlet and outlet with specific luer connection design, to induce a vacuum chamber for vacuum-assisted cell loading. The device containing the cell-loaded scaffold was crosslinked with blue light oven at 405nm. PCL-TCP scaffolds were tested for cell loading efficiency and *in vitro* performance of viability, proliferation and osteogenic differentiation.

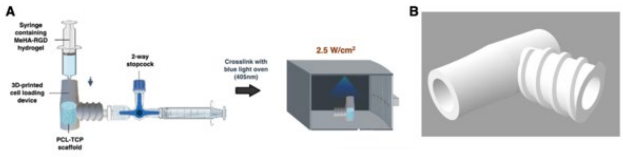


Figure: (A) Schematic diagram of 3D cell loading device set-up. (B) Prototype of 3D-printed device

Results The hydrogel loaded with the device was able to penetrate the scaffold and fill the entirety of the scaffold. Cells were able to be efficiently loaded into the scaffold with the device, which was demonstrated by a directly proportional increase of alamarBlue readout with increasing cell density. Hydrogel loading with the device provided a more homogenous loading throughout the scaffold compared to manual loading via manual pipetting. Cells in the scaffold, loaded with the device, were able to remain viable and proliferate throughout the 14 days. Furthermore, cells loaded in the scaffold, with the device, was able to undergo osteogenic differentiation with an increasing expression of osteogenic markers (ALP, OCN, OPN and BSP) from day 7 to 21. Discussion and Conclusion We demonstrated that the novel device for vacuum-assisted cell loading was able efficiently load cells and support cell viability, proliferation and differentiation thereafter. This method for cell loading is a promising strategy to standardize cell loading into the scaffold for bone tissue engineering applications and can be potentially applied for further applications.

PoA.17.01

Polydopamine nanoparticles as a photoacoustic contrast agent

Matteo Battaglini[1], Alessio Carmignani[1,2], Paolo Armanetti[3], Luca Menichetti[3], Margherita Montorsi[1,2], Gianni Ciofani[1]

[1]Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera, Italy. [2]The Biorobotics Institute, Scuola Superiore Sant'Anna, Pontedera, Italy. [3]Institute of Clinical Physiology (IFC) National Research Council, Pisa, Italy

Abstract

Polydopamine nanoparticles (PDNPs) are nanostructures derived from the polymerization of dopamine. PDNPs present several properties that make them a promising tool for biomedical applications,[1,2] including high biocompatibility, antioxidant features derived from polydopamine surface functional groups, and the ability to act as a photothermal conversion agent by converting near infra-red (NIR) radiation into heating.[1,2] Moreover, PDNPs are biodegradable, overcoming some of the limitations connected to the use of inorganic nanostructures. In this work, we investigated another promising characteristic of PDNPs, that is still largely underused, namely their ability to act as photoacoustic (PA) contrast agents. In particular, we analyzed how the diameter of PDNPs used as contrast agents in PA imaging affects the relative PA signal. Our analysis involved a preliminary phase where we investigated the physical and morphological properties of PDNPs at various sizes through scanning electron microscopy (SEM), atomic force microscopy (AFM), and dynamic light scattering (DLS). Afterward, we analyzed and compared the PA signal derived from these nanostructures. PA imaging was carried out in solution, on an ex-vivo animal tissue model, and on a spheroid model derived from colorectal cancer cells. Our analysis confirmed that PDNPs can act as excellent PA imaging contrast agents in all three conditions. We also observed a correlation between the diameter of the PDNPs (from 150 nm to 1000 nm) and the PA signal obtained following irradiation with a pulsed laser at various wavelengths (from 675 nm to 975 nm). In particular, we observed how “bigger” nanostructures behaved as better contrast agents compared to “smaller” ones: we found a 4-fold increment of the PA signal between PDNPs at 145 nm and PDNPs at 710 nm. A representative image of PDNPs in an ex-vivo model is shown in Figure-1. Finally, we developed a computational model describing the PA properties of PDNPs. In conclusion, our work shows that PDNPs can be used as organic contrast agents, enabling stain-free imaging of nanostructures and biological structures.

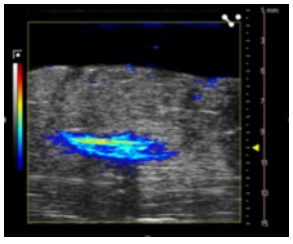


Figure 1: Representative PA imaging of PDNPs in an ex-vivo model acquired by Vevo LAZRX (Fujifilm Visualsonics INC, Toronto), in gray the ultrasound signal, rainbow scale for photoacoustic signal

The authors acknowledge Euro-BioImaging (www.eurobioimaging.eu) for providing access to imaging technologies and services via the Molecular Imaging Italian Node (IFC CNR, Pisa, Italy)

1 M. Battaglini, [...], G. Ciofani, ACS Appl. Mater. Interfaces, 2020, 12, 35782.

2 A. Carmignani, [...], G. Ciofani, ACS Appl. Nano Mater., 2022, 5, 1702.

PoA.17.02

NIR-II activated photothermal/NO combination therapy from plasmonic heterostructured nanotherapeutics against triple-negative breast cancer

HSIN-CHENG CHIU, Reesha Kakkadavath Vayalakkara

National Tsing Hua University, HSINCHU, Taiwan

Abstract

A plasmon-semiconductor nanotheranostic system comprising Au nanostars/graphene quantum dots (AuS/QD) hybrid nanoparticles loaded with BNN6 and surface modified with PEG-pyrene was developed in this work for the photothermal effect and NO production as the dual modality treatment against orthotopic triple-negative breast cancer. The structure and morphology of the hybrid nanodevice was characterized and the NIR-II induced thermal response and NO production was determined. The hybrid nanotherapeutics has shown enhanced plasmonic energy transfer from localized surface plasmonic resonance of Au nanostars to QD semiconductor that activates the BNN6 species loaded on QD surfaces, leading to the effective NO production and gas therapy in addition to the photothermal response. The prominent therapeutic efficacy of the photothermal/NO combination therapy from the BNN6-loaded AuS@QD nanodevice with the NIR-II laser irradiation at 1064 nm against 4T1 breast cancer was observed both *in vitro* and *in vivo*. The NO therapy for the cancer treatment was evidenced with the increased cellular nitrosative and oxidative stress, nitration of tyrosine residues of mitochondrial proteins, vessel eradiation and cell apoptosis. The efficacy of the photothermal treatment was corroborated directly by severe tissue thermal ablation and tumor growth inhibition. The NIR-II triggered thermal/NO combination therapy along with the photoacoustic imaging-guided therapeutic accumulation in tumor shows prominent effect to fully inhibit tumor growth and validates the promising strategy developed in this study.

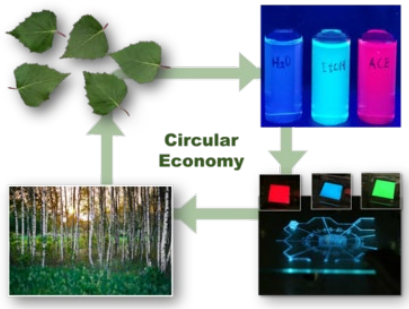
PoA.17.03

Synthesis of biobased emitters for application in sustainable optoelectronics

Jia Wang, Yongfeng Liu, Shi Tang, Henry Opoku, Ludvig Edman

Physics Dept., Umeå University, Umeå, Sweden

Abstract



Organic semiconductors are widely utilized in advanced optoelectronic applications, such as organic light-emitting diodes (OLEDs) and solar cells, due to their high-energy-utilization efficiency and tailorable functions. However, the reliance of organic semiconductors (including conjugated polymers and rare-metal complexes) used in optoelectronic applications on critical raw materials and fossil-based substances, presents a sustainability challenge. At the end of their lifespan, these materials contribute to the growing issue of electronic waste. In this context, carbon dots (CDs) have emerged as a promising alternative to conventional organic semiconductors due to their tunable band gap over a broad range, high photoluminescence quantum yield, and resistance to photo-bleaching. Most important, CDs can be synthesized with facile and cost-efficient routes, using abundant and benign bio-based starting materials, making them an attractive option for future resource-efficient and eco-friendly optoelectronic technologies. Recently, we reported on the synthesis and characterization of emissive and soluble CDs from both bio-derived precursors and biomass and explored their application as emitters in functional light-emitting devices. The first type of CDs was synthesized with a catalyst-free solvothermal reaction using naturally occurring phloroglucinol as the sole starting material. The second type of CD was synthesized with a one-step solvothermal process, using plant leaves as the sole starting material. The phloroglucinol-derived CD in ethanol solution features a narrow cyan emission (peak wavelength = 485 nm) with a high photoluminescence quantum yield of 77%, while the leaf-derived CD features deep red emission (peak wavelength = 670 nm) with a photoluminescence quantum yield of 27%. We further explored these CDs as bio-based emitters used in light-emitting devices, achieving peak luminance of over 110 cd/m2 for both phloroglucinol-derived CD light-emitting devices and leaf-derived CD light-emitting devices. Our results demonstrate the feasibility of developing CDs as a bio-based emitter for resource-efficient and eco-friendly optoelectronic technologies.

PoA.17.05

Anti-CD44 antibody decorated gold nanoparticles for endometriosis photothermal therapy

Cristina Volpini[1,2,3], Nora Bloise[1,2,3], Mattia Dominoni[4], Fabio Barra[5,6], Valerio Gaetano Vellone[7,8], Barbara Gardella[4,9], Simone Ferrero[5,6], Livia Visai[1,2,3]

[1]Molecular Medicine Department (DMM), Centre for Health Technologies (CHT), UdR INSTM, University of Pavia, Pavia, Italy. [2]Medicina Clinica-Specialistica, UOR5 Laboratorio di Nanotecnologie, ICS Maugeri, IRCCS, Pavia, Italy. [3]Interuniversity Center for the promotion of the 3Rs principles in teaching and research (Centro 3R), University of Pavia Unit, Pavia, Italy. [4]Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Pavia, Italy. [5]Academic Unit of Obstetrics and Gynecology, IRCCS Ospedale Policlinico San Martino, Genova, Italy. [6]DINOGMI, University of Genova, Genova, Italy. [7]Anatomia Patologica Universitaria, IRCCS Ospedale Policlinico San Martino, Genova, Italy. [8]Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate (DISC), Università di Genova, Genova, Italy. [9]Department of Obstetrics and Gynecology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Abstract

INTRODUCTION Endometriosis is an estrogen-dependent inflammatory condition defined as the presence of endometrial glands and stroma outside the uterine cavity, predominantly, but not exclusively, in the pelvis [1]. Since endometriosis and cancer share many pathophysiological features, some fundamental principles of cancer nanomedicine can be adapted to develop novel nanoparticle-based strategies for the treatment and imaging of endometriosis [2]. The study aims to evaluate an active targeting of CD44 (transmembrane glycoproteins) overexpressing endometriosis cells by using gold nanoparticles conjugated with anti-CD44 antibody (Au@antiCD44) with and without photothermal therapy (PPT). EXPERIMENTAL METHODS Gold nanostars (GNS) and gold nanospheres (GNP) were synthesized, pegylated (PEG), and conjugated to anti-CD44 antibody using the Maleimide chemistry [3]. The conjugated nanoparticles were characterized using UV-visible spectroscopy (UV-Vis), DLS, Bicinchoninic Acid (BCA), and dot blot analyses. The *in vitro* tests were performed to verify the biocompatibility (MTT assay), the receptor recognition and the internalization (confocal microscopy (CLSM) and inductively coupled plasma-mass spectrometry (ICP)) of the nanoconjugate against three different cell lines: CD44 overexpressing cells (Z12) and compared to CD44 low expressing cells (Thesc) and normal fibroblast cell line (NIH-3T3). Viability studies with/without laser treatment were performed to verify the efficiency of nanosystems. RESULTS AND DISCUSSION DLS and Zeta potential showed that the conjugation increased particle size reduced negative zeta potential. The UV-Vis analysis evidenced a spectrum shifted with respect to PEG-GNSs and PEG-GNPs. The antibody's presence on the surface of the conjugated nanoparticles was also assessed by dot blot and BCA studies. The viability assays showed the biocompatibility of the obtained conjugated to all the three cell lines. CLSM and ICP data revealed that nanoconjugate better recognized the receptor on Z12 cells (the one overexpressed CD44), in which the internalization was higher compared to the other cell lines. The photothermal effect of the nanosystem was observed by *in vitro* studies only on Z12 cells. CONCLUSION Au@antiCD44 could be a new therapeutic approach in endometriosis disorder exploiting the nanoparticles mediated photothermal activity on the endometriotic cells. Further investigations on 3D *in vitro* models and *in vivo* studies are required.

ACKNOWLEDGMENTS

The authors would like to thank the Italian Ministry of Health, BANDO RICERCA ENDOMETRIOSI (Grant no: ENDO-2021-12371975) for providing financial support to this project. The authors acknowledge the grant of the Italian Ministry of Education, University and Research (MIUR) to the Department of Molecular Medicine of the University of Pavia under the initiative “Dipartimenti di Eccellenza (2023-2027).

PoA.17.06

Dipeptide mediated biosynthesis of zinc (hydro)oxide nanoparticles on biohybrid nanofibers; a wound healing material

Shahin Homaeigohar[1], Mhd Adel Assad[2], Amirhossein Azari[2], Mady Elbahri[2]

[1]University of Dundee, Dundee, United Kingdom. [2]Aalto University, Espoo, Finland

Abstract

Introduction

Biosynthesis of nanohybrid materials assures low cost, eco-friendly preparation of biomaterials for various biomedical applications including wound healing. In this study, L-carnosine (CAR), a dipeptide of β-alanyl-L-histidine, was loaded on hydrolysed polyacrylonitrile (PAN) nanofibers (NFs) to act as a mediator for biosynthesis of ZnO and/or $Zn(OH)_2$ nanoparticles (NPs) on the NFs surface. CAR per se can offer a wound healing effect thanks to its promising antioxidant, anti-inflammatory, and anti-neoplastic properties. On the other hand, due to its immunomodulatory activity, the release of Zn^{2+} ions from ZnO NPs contributes to wound healing. **Results and discussion** Thanks to homogenous loading of CAR ligands, the biosynthesized ZnO/$Zn(OH)_2$ NPs (23 ± 7 nm) were uniformly distributed on the surface of CAR/PAN NFs (Fig. 1a&b). EDX (Fig. 1c) and XRD analysis (Fig. 2a) validated that the NPs were composed of ZnO and/or $Zn(OH)_2$ (possibly as a core-shell NP). The co-existence of CAR and ZnO NPs led to a superhydrophilicity effect and promoted the elastic modulus and tensile strength of ZnO-CAR/PAN NFs (Fig. 2b-d). The PAN NFs become stiffer and stronger after hydrolysis, due to intermolecular bonding between the PAN chains functionalized with oxygen bearing functional groups. This effect is intensified after biofunctionalization of PAN NFs with CAR. The CAR ligands act as the cross-linkers connecting the NFs, thereby raising stiffness and strength of the mat comprising thereof. As a result, inter/intrafiber bonding brings about a considerably higher elastic modulus/tensile strength as 462%/44% (cPAN), 1036%/315% (CAR/PAN), and 314%/118% (Zn-CAR/PAN) increase, when comparing with that of PAN NFs.

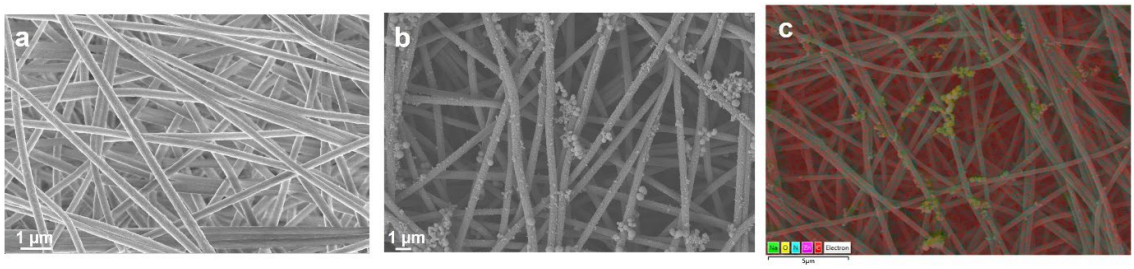


Figure 1. SEM images of CAR/PAN (a) and ZnO-CAR/PAN NFs (b). The uniform distribution of biosynthesized ZnO NPs across the NF mat is evident. C) EDX elemental map confirms the formation of Zn rich NPs.

Conclusion

The ZnO-CAR/PAN bionanohybrid NFs were produced through biosynthesis of ZnO NPs in the presence of CAR. The release of Zn^{2+} ion, an essential trace element involved in the chemistry of different transcription factors or enzymes, can potentially enhance wound healing effect.

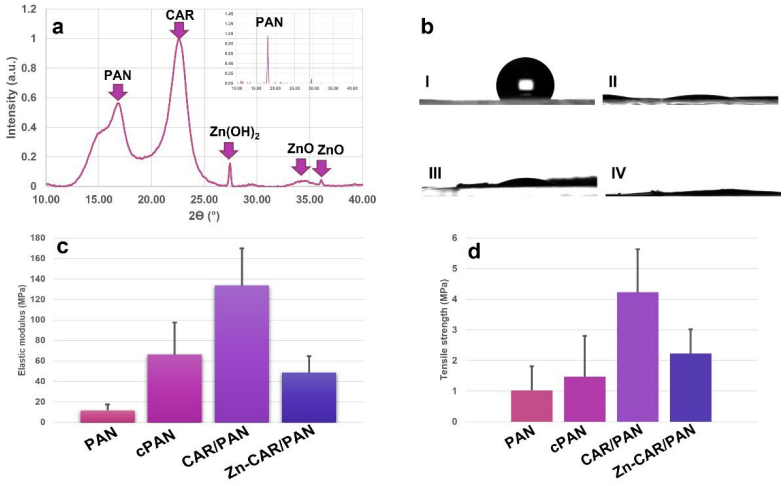


Figure 2. a) XRD analysis verifies the formation of ZnO/Zn(OH)$_2$ NPs on the NFs surface, b) Superhydrophilicity effect induced by CAR ligands and ZnO/Zn(OH)$_2$ NPs (I-IV: PAN, hydrolysed PAN, CAR/PAN, and ZnO-CAR/PAN NFs, respectively), c) Elastic modulus, and d) Tensile strength of ZnO-CAR/PAN NFs.

PoA.17.07

The end of orthopedic device-related infections using dhvar5-chitosan nanogels fabricated by microfluidic

Bruna Costa[1,2,3], Pedro Alves[1,2,3], Diana Fonseca[1,2,3], Filipa Campos[4,2], Ana C. Monteiro[4,2], Rúben Pereira[4,2,5], Paula Gomes[6], Guillermo Martínez-de-Tejada[7,8], Cláudia Monteiro[4,2], M.C.L Martins[4,2,5]

[1]Instituto de Investigação e Inovação em Saúde, Universidade do Porto, porto, Portugal. [2]Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [3]FEUP–Faculdade de Engenharia, Universidade do Porto, Porto, Portugal. [4]Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [5]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal. [6]CIQ-UP e Centro de Investigação em Química, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal. [7]Department of Microbiology and Parasitology, University of Navarra, Pamplone, Spain. [8]Navarra Institute for Health Research (IdiSNA), Pamplone, Spain

Abstract

Orthopedic Device-Related Infections (ODRIs) are a major medical challenge, particularly due to the involvement of biofilm-encased and multidrug-resistant bacteria [1]. Current treatments, based on antibiotic administration, have proven to be ineffective [2]. Consequently, there is a need for antibiotic-free alternatives [3]. Antimicrobial peptides (AMPs) are a promising solution due to their broad-spectrum of activity, high efficacy at very low concentrations, and low propensity to induce resistance [4]. We aim to develop a new AMP-based chitosan nanogel coating to prevent ODRIs. Chitosan was functionalized with norbornenes (NorChit) through the reaction with carbic anhydride and then, a cysteine-modified AMP Dhvar5 was covalently conjugated to NorChit (NorChit-Dhvar5), through a thiol-norbornene photoclick chemistry, under UV-photoactivation [5]. Characterization was done by Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance spectroscopy (NMR) analyses, and a successful functionalization of chitosan with norbornenes and posterior Dhvar5 immobilization was proved. For NorChit-Dhvar5 nanogels production, the NorChit-Dhvar5 solution (0.15% w/v) and Milli-Q water were injected separately into a microfluidic system. The nanogels were characterized regarding size, concentration, shape, and charge, using Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA) and Dynamic light scattering (DLS). The nanogels antibacterial properties were assessed in Phosphate Buffer (PBS) for 6 h, against four relevant microorganisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. aureus* methicillin-resistant (MRSA)), and in Muller-Hinton Broth (MHB), 50% (v/v) in PBS, supplemented with human plasma (1% (v/v)), for 6 and 24 h against MRSA. The obtained NorChit-Dhvar5 nanogels, presented a round-shaped and ~100 nm. NorChit-Dhvar5 nanogels in a concentration of 10^{10} nanogels/mL in PBS were capable of reducing the initial inoculum of *E. coli* by 90%, *P. aeruginosa* by 99%, *S. aureus* by 99%, and *S. aureus* MRSA by 90%. These results were corroborated by a 99% S. aureus MRSA reduction, after 24 h in medium. Furthermore, NorChit-Dhvar5 nanogels do not demonstrate signs of cytotoxicity against osteoblastic MC3T3-E1 cells (a pre-osteoblast cell line) after 14 days, having high potential to prevent antibiotic-resistant infection in the context of ODRIs.

[1] G. Muthukrishnan *et al*. Curr. Osteoporos. Rep., 2019, 17, 395–404.
[2] M.P. Siljander *et al*. J. Arthroplasty, 2018, 33,185-194.
[3] R. Sinha & P. Shukla. Protein Pept. Lett., 2019, 26,79–87
[4] Y. Huan *et al*. Front. Microbiol., 2020, 11, 582779.
[5] P.M. Alves *et al*. ACS Appl. Polym. Mater. 2022, 4, 5012−5026.

PoA.17.08

Nanodiamonds for sequestration of fibroblast growth factor-2

Stepan Stehlik

New Technologies–Research Centre, University of West Bohemia, Pilsen, Czech Republic

Abstract

Diamond is a multifunctional material that excels in mechanical and chemical stability, biocompatibility, and provides unique optical and surface properties (10.1021/acsami.7b14436). In particular, hydrogenation of the diamond makes the diamond surface electrically conductive and reduces an energetic barrier for electron emission. Diamond nanoparticles, nanodiamonds (NDs), bring the outstanding properties of diamonds to the nanoscale. In addition, the hydrogenation of the ND surface leads to the formation of NDs with a strongly positive zeta potential and high isoelectric points (pH ≥ 10). Recently, detonation nanodiamonds (DNDs) with positive zeta potential were identified as highly effective and selective fibroblast growth factor (FGFs) binders, sequestering FGF molecules and preventing their interaction with receptors under physiological conditions, thus potentially eliminating the effect of FGF overexpression (10.1016/j.biomaterials.2018.05.030). Blocking the FGF interactions with their receptors at the cell membrane is one of the promising strategies for the treatment of diseases related to dysregulated FGF signaling, such as cancer, metabolic syndromes, and developmental disorders. Here we investigate the influence of key ND parameters on the sequestration ability and binding affinity of FGF2, a representative member of the FGF family (10.1016/j.carbon.2022.04.017). We employ NDs of different origins (detonation vs. high-pressure high-temperature), surface chemistry (oxidized vs. hydrogenated), and size down to a few nm only. The ND properties are characterized by Raman and infrared spectroscopy, transmission electron microscopy, and dynamical light scattering techniques. We show that all the NDs with the positive zeta potential, given by the partially or fully hydrogenated ND surface, sequestered FGF2 at its physiologically relevant concentrations. Using Western blot and ELISA, we quantify the strength of the interaction between selected DNDs and FGF2 and show that size-reduced fully hydrogenated DNDs have the highest sequestration ability. Using the Langmuir model, we estimated the apparent dissociation constant between FGF2 and fully hydrogenated DND to be in the nanomolar range in full fetal bovine serum. Because such tight interaction between a protein and a solid nanoparticle occurred in ~10^5-fold molar excess of serum proteins, we propose that hydrogenated NDs can potentially be used *in vivo* as selective FGF2 traps to regulate disorders caused by aberrant FGF2 signaling.

PoA.17.09

Multifunctional Eu^{3+}-tannic acid nanocomplexes for targeted bone regeneration under oxidative and inflammatory microenvironments

Daniel Fernández-Villa[1,2], María Rosa Aguilar[1,2], Luis Rojo[1,2]

[1]Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC), Madrid, Spain. [2]Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain

Abstract

INTRODUCTION Osteopenia is a bone-affecting condition characterized by a reduced mineral density, leading to an increased risk of bone fractures, with its subsequent social and economic consequences.[1] Moreover, certain osteopenic diseases (e.g., rheumatoid arthritis or osteoporosis), present oxidative and inflammatory microenvironments too, ultimately hindering strategies envisioned to promote bone regeneration.[2] In this work, we rationally devised metal-organic nanocomplexes based on tannic acid (TA) and europium(III) (EuTA NCs) to simultaneously target those different mechanisms by combining the properties of each moiety. EXPERIMENTAL METHODS EuTA NCs were prepared by dropwisely adding $EuCl_3$ to a TA solution in HEPES pH 7.3 under intense sonication. Different Eu:TA molar ratios were employed to obtain NCs with different degrees of complexation (D.C.). Physicochemical characterization was carried out by ICP and UV-VIS measurements, DLS, ATR-FTIR, TGA and XRD. The bioactivity of EuTA NCs was evaluated in cultures of fetal human osteoblasts (fHOBs) and RAW264.7 macrophages. Cytocompatibility was checked by AlamarBlue. Their osteogenic potential was evaluated by measuring the ALP activity and matrix mineralization degree in fHOBs cultures. Anti-inflammatory properties were characterized by measuring the nitric oxide (NO) production by LPS-stimulated macrophages. Finally, antioxidant properties were analyzed by different methods: DPPH, H_2O_2 scavenging and Fe^{2+} removal. RESULTS AND DISCUSSION EuTA NCs with different D.C.s were obtained as shown in Figure 1. EuTA NCs were stable in dH_2O, and presented a controllable size (80 – 160 nm) and negative surface charge (ζ ≈ -25 mV).

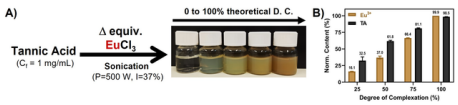


Figure 1. A) Synthesis procedure of EuTA NCs; B) Contents of Eu^{3+} and TA of each formulation.

Eu(III) complexation reduced the TA toxicity (Figure 2A), improving its antioxidant (↑13.3% radical scavenging activity vs. TA) and anti-inflammatory properties (↓17.6% NO production vs. TA). Moreover, NCs increased ALP activity of fHOBs and enhanced the matrix mineralization degree following a 7-days incubation (Figure 2B).

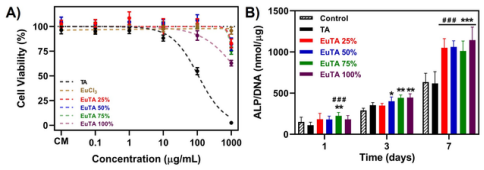


Figure 2. A) Cytotoxicity in fHOBs and B) ALP activity of fHOBs treated with 10 μg/mL TA/EuTA NCs.

CONCLUSION This work presents for the first time EuTA NCs with tunable characteristics as well as antioxidant, anti-inflammatory, and osteogenic properties as a promising platform for bone regeneration by simultaneously targeting multiple pathophysiological mechanisms of disease.

REFERENCES

1. Marini. et al., Nat. Rev. Dis. Prim. 3, 1–19, 2017
2. Gibon et al., J. Orthop. Transl., 10, 28–35, 2017

ACKNOWLEDGMENTS

Spanish Ministry of Science, Innovation and Universities (PID2020-114086RB-I00; FPU18/04683), Comunidad Autónoma de Madrid (S2022/BMD-7406, RADIOPROTECT-CM) and CSIC (PTI-SusPlast+ and Nanomedicine-HUB ref.202180E048).

PoA.17.10

Impact of physicochemical properties of biofilms on nanoparticle-biofilm interactions: toward development of high-efficacy nanodrugs against multidrug-resistant biofilms

Mihyun Lee, Qun Ren Zulian

Empa, St. Gallen, Switzerland

Abstract

Antimicrobial resistance-associated diseases pose an increasing burden to public health and economy worldwide. Bacteria have a number of resistance mechanisms against antibiotics such as enzymatic destruction of antibiotics and antibiotic efflux. Furthermore, when bacteria form a biofilm, a community of bacteria embedded in a self-produced polymeric matrix, they activate antibiotic-resistant genes, and the antibiotic diffusion is severely hindered by the dense biofilm matrix, which makes the bacteria 10-1000 times more resistant than planktonic bacteria. A myriad of functionalized nanoparticles (NPs) have been developed as potential antimicrobial drugs alternative to traditional antibiotics against multidrug-resistant bacteria and/or biofilms. However, in most nanomaterials studies, the differential biological and physicochemical properties between different growth states of biofilms are rarely considered to design the materials and/or interpret the resultant antimicrobial/antibiofilm efficacy. In the current study, NPs with varying size and surface charges are prepared using LUDOX silica NPs. Biofilms of E. coli, gram-negative bacteria, at different maturation states are prepared by varying the cultivation periods from 1 to 14 days. The physicochemical properties of the biofilms are thoroughly studied by various techniques. Subsequently, the penetration and distribution of NPs within the biofilm of each growth stage are analyzed by treating each biofilm with fluorescein-labeled NP followed by fluorescence imaging, and correlated with the biofilm properties. This study will not only provide crucial insights into a novel design principle(s) for high-efficacy nanodrugs targeting biofilms at different physiological states but also suggest new considerations for adequate testing conditions for antimicrobial NPs.

PoA.17.11

Influence of the different structures of cerium (IV)-oxide nanoparticles on the applicability in biological systems

Zsejke Réka Tóth[1,2], Alexandra Dreancă[3], Emma Bobu[4], Alexandra Feraru[1,2], Lilla Nánai[5], Kata Saszet[1,2], Lucian Baia[1,4], Klara Magyari[1]

[1]Nanostructured Materials and Bio-Nano-Interfaces Center, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babeș-Bolyai University, Cluj-Napoca, Romania. [2]Doctoral School of Physics, Faculty of Physics, Babeș-Bolyai University, Cluj-Napoca, Romania. [3]Faculty of Veterinary Medicine, University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Romania. [4]Faculty of Physics, Babeș-Bolyai University, Cluj-Napoca, Romania. [5]Faculty of Materials and Chemical Engineering, Institute of Physical Metallurgy, Metal Forming and Nanotechnology, University of Miskolc, Miskolc, Hungary

Abstract

Cerium (IV)-oxide (CeO_2) application in biological systems (such as wound healing, drug delivery, and cancer therapy) became very widespread. One of its most important biological applications is in wound healing. Wound healing has four steps: hemostasis (1), inflammation (2), repair/proliferation (3), and remodeling (4). The cerium (IV)-oxide particles can be applied in steps 2-4. But what can happen to a person, who has several problems with hemorrhage? Hemorrhage given by traumatic injury, surgery, and bleeding, as well as its side effects (such as anemia, organ failure, and hypothermia), have a well-known role in determining human morbidity and death. It would be useful to develop materials that could be applied in hemostasis. Cerium is the most present rare-earth element on the Earth and switches its oxidative state depending on the environment from which is applied in different areas. The use of CeO_2 is also shape-dependent. Therefore, the main focus of this research is the different structural effects on the applicability of CeO_2 in hemostasis. CeO_2 nanoparticles were synthesized with 3 different shapes (nanosphere, nanorod, and nanocubes) and without a well-defined form (polyhedral). The obtained particles were analyzed by X-ray diffractometer, UV-Vis spectrophotometer, transmission electron microscope, and Fourier-transform infrared spectrometer. To be applied in the hemostasis the materials should have an antioxidant character, which as a new approach was analyzed by photocatalytic degradation since an antioxidant are free-radical scavengers, which are the opposite of the photocatalytic character. Also, the possible applicability in hemostasis was analyzed by understanding the relationship between the hemoglobin and the particles. The obtained measurement shows that the crystallinity of the particles depends on the structure. In the case of methyl-orange, no degradation or only adsorption was observed, while using paracetamol as a model pollutant no degradation was observed. These results predicted good antioxidant character. The hemoglobin test showed that the hemoglobin is adsorbed on the surface of the particles. This work was supported by a grant of the Ministry of Research, Innovation and Digitalization, CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2021-2176, within PNCDI III. This work was supported by the project "The Development of Advanced and Applicative Research Competencies in the Logic of STEAM + Health”/POCU/993/6/13/153310, project co-financed by the European Social Fund through The Romanian Operational Programme Human Capital 2014-2020. Zs.-R. Toth acknowledges the financial support of MTA Domus 153/10/2022/HTMT.

PoA.17.12

Assessment of antioxidant activity and biocompatibility of the graphene oxide-based nanocomposite material for tissue engineering applications

Bansod Sneha Bharat[1], Thirumalai Deepak[2], Ramakrishna Prasad Are[2], Anju R Babu[2]

[1]National Institute of Technology, Rourkela, Rourkela, India. [2]National Institute of Technology Rourkela, Rourkela, India

Abstract

This research study aims to synthesize graphene oxide (GO)-based nanocomposite material and analyze the material properties, radical scavenging activity, biocompatibility, and toxicity level detection for biomedical applications. The sol-gel method was utilized to synthesize titanium dioxide (TiO_2) nanoparticles; GO was prepared by the improved hummer's method and reduced graphene oxide (rGO) by the thermal reduction process. TiO_2 and GO (GO@TiO_2) and TiO_2 and rGO (rGO@TiO_2) nanocomposites were synthesized by a single-step hydrothermal method. The physicochemical properties of synthesized nanocomposites were characterized. The X-ray diffraction graph confirmed the hexagonal structure for GO and rGO. Also, the nanocrystalline anatase form of TiO_2 nanoparticles is obtained. The Fourier transforms infrared spectrum shows the characteristics peak of Ti-O-Ti bonds between 500 to 850 cm-1. The Raman spectrum peak of GO was obtained at 1349 cm-1 for bands D and 1594 cm-1 for the G band, respectively. The antimicrobial properties of GO@TiO_2 and rGO@TiO_2 nanocomposites were examined against two positive and negative bacteria by agar well diffusion technique, and free radical scavenging assays were studied to determine the antioxidant properties of the GO@TiO_2 and rGO@TiO_2 nanocomposites. Both the nanocomposite showed doses dependent cytotoxicity. The results show that the nanocomposites had remarkable cytotoxic properties and significantly improved antibacterial efficiency. The antibacterial and antioxidant activities of the nanocomposite were improved by the synergistic interactions between rGO and TiO_2 nanoparticles.

PoA.17.13

Characterization of novel biomaterials to enable mechanobiology studies of living cells

Maximilian Seuss, Dimitar R Stamov, Tanja Neumann, André Körnig, Torsten Müller, Heiko Haschke

JPK BioAFM, Bruker Nano GmbH, Berlin, Germany

Abstract

Development of novel biomaterials for tissue engineering is driven by the biomechanical and molecular cues provided to cells by their environment. The topography and mechanical properties of biomaterials are crucial parameters that influence motility, behavior, and the fate of progenitor cells. Thus, processes such as the self-assembly of single matrix molecules, and adhesion-induced structural changes in living cells must be further explored. We studied the assembly kinetics and structural hierarchy of collagen I, as one of the most used proteins in tissue engineering. Furthermore, we were interested in understanding how cell behavior is driven by the cytoskeletal dynamics and cell mechanics in typical cell culture scaffold scenarios. We applied high-speed imaging atomic force microscopy (AFM), with a temporal resolution on the second to millisecond scale to resolve dynamic processes such as the collagen fibrillogenesis and cytoskeletal dynamics in living cells. As a tool for analyzing the complex cellular mechanobiology, we went beyond purely elastic models, and performed sine oscillations (up to 500 Hz, amplitude 5-60 nm) in Z while in contact with the surface to probe the frequency-dependent response of living fibroblasts. We will provide insight into the structural formation of collagen type I, emphasizing the intermediate steps in the process. We will demonstrate how cell spreading and migration in living KPG-7 fibroblasts and CHO cells, can be associated with spatially resolved cytoskeletal reorganization events. We will further discuss how to calculate the viscoelastic properties, characterized by the dynamic storage and loss modulus (E', E'') distribution in living fibroblast cells. AFM can be successfully applied to study the mechanobiology of living cells during tissue engineering, and to evaluate the structure of and the interaction with their cell culture substrates.

PoA.17.14

Designing novel photo-crosslinked metacryloyl gelatin (GelMA)-based nanogels for drug delivery applications

Roberta Pappalardo[1,2], Monica Boffito[1], Valeria Chiono[1], Gianluca Ciardelli[1]

[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy. [2]Department of Surgical Sciences, Università degli Studi di Torino, Turin, Italy

Abstract

Recently, drug delivery systems (DDSs) have received high interest in the scientific community and have been widely investigated to engineer therapeutic platforms for controlled and site-specific release. Nanocarriers as DDSs could be exploited to protect drugs from degradation, increase their bioavailability and prolong the release, thus minimizing side effects and improving their therapeutic efficacy. In this scenario, nanogels, hydrogels with a three-dimensional (3D) porous structure and submicrometer size, represent promising delivery systems thanks to their highly hydrated nature and the ability to encapsulate hydrophobic or hydrophilic drugs in their internal network. Starting from these premises, in this contribution we reported the design of new photo-crosslinked nanogels based on gelatin methacryloyl (GelMA). Moreover, to evaluate their potential application as drug delivery systems, the encapsulation and release of a model molecule was investigated. GelMA was synthesized by reacting gelatin (type A, from porcine skin) with a variable amount of methacrylic anhydride (MA, 0.1-1 mL/$g_{gelatin}$), thus obtaining GelMAs with different degrees of methacryloylation (DoM) ranging between 50% and 100%. GelMAs were characterized by infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopies, and the Ninhydrin assay. GelMA-based nanogels were prepared by an optimized desolvation method, using acetone as non-solvent. Then, they were crosslinked through UV-light exposure (365 nm, 10-12 mW-cm^2), by adding phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as photo-initiator. Dynamic light scattering analysis was performed to measure nanogel size, size distribution and their stability over time at different temperatures. The hydrodynamic diameter of the nanogels obtained from GelMA with medium DoM resulted to be in the 200-250 nm range, with a polydispersity index (PDI) of 0.3. In addition, the nanogels showed good stability in suspension over time and by increasing temperature from 25 to 45°C. Moreover, the possibility to resuspend the freeze-dried nanogels, with no changes in their size or aggregate formation, made their storage and practical use easier. Lastly, as proof of concept of their use for drug delivery applications, a model molecule (i.e., rhodamine B) was encapsulated. The molecule was progressively released in a controlled manner up to one week. In this contribution, we reported for the first time the preparation of photo-crosslinked GelMA-based nanogels with favorable characteristics to design promising drug delivery systems.

PoA.17.15

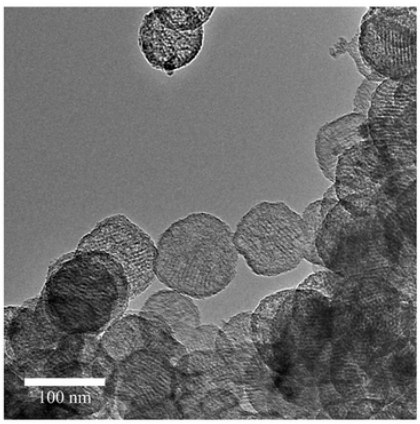
Evaluation of the antibacterial properties of Ce and Ca co-doped mesoporous silicate nanoparticles produced by the sol gel method

Anastasia Beketova[1], Georgia K. Pouroutzidou[1], Veronica S. Giourieva[2], Konstantinos Tsachouridis[3], Rigini M. Papi[2], Ioannis Tsamesidis[1], Antonios D. Anastasiou[3], Petros Koidis[1], Eleana Kontonasaki[1]

[1]School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece. [2]School of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece. [3]Department of Chemical Engineering and Analytical Science, University of Manchester, Manchester, United Kingdom

Abstract

Introduction Recently, the increase of antibiotic resistance in patients with severe periodontitis was reported [1]. Application of inorganic nanoparticles (NPs) with antibacterial effect seems to have great potential for elimination of perio-pathogenic microorganisms, because of their ultra small dimensions and increased solubility, and non-specific action against bacterial membrane [2]. The aim of this research was synthesis of novel compositions of Ce and Ca co-doped mesoporous silicate nanoparticles and evaluation of their antibacterial activity against anaerobic bacteria of periodontal pockets. **Experimental methods** Mesoporous and calcium/cerium-doped silicate nanopowders in the composition of 60SiO2-35CaO-5CeO (in % mol) (Ce-SiCa1, Ce-SiCa2, Ce-SiCa3,) were synthesized in alkaline environment (pH 12) via a surfactant-assisted cooperative self-assembly process and calcinated at at 550 °C, for 6 h. Their properties were evaluated by XRD, BET/BJH and TEM. Antibacterial activity of the materials was assessed at concentrations (0,125, 0,25 0,5, 1, 2 mg/ml) with the anaerobic strains *Prevotella intermedia* (DSM 20706) and *Porphyromonas gingivalis* (DSM 20709). A 10% (v/v) of each bacterium suspension (corresponding to 10^8 CFU/ml) was treated with the NPs for 3 days for each bacterium. Bacterial growth was evaluated spectrophotometrically (600nm). **Results and discussion** The obtained mesoporous nanoparticles had spherical morphology, particle size ranging from 50 to 75nm, and high surface area (448-784 m^2/g) (Figure 1).



Material	CTAB (g)	TEOS (ml)	pH	Surface area (m^2/g)	Average particle size (nm)
Ce-SiCa1	1.0	5.0	12	681	70
Ce-SiCa2	1.5	5.0	12	783	50
Ce-SiCa3	1.0	7.5	12	448	75

Figure 1. TEM micrograph of Ce-SiCa3 and physico-chemical and morphological characteristics of the obtained NPs

The best antibacterial activity was observed in Ce-SiCa2 material reaching up to 80% for *P. gingivalis* and 43% for *P. intermedia* at the highest concentrations (2mg/ml). The other two materials exhibited moderate antibacterial activity. These differences might be attributed to the increased surface area and smaller particles size of Ce-MSSiCa3, as compared to the other two materials.

Conclusions

Mesoporous and calcium/cerium-doped silicate nanoparticles presented excellent morphological and physicochemical characteristics and antimicrobial activity against

anaerobic pathogens and could be potentially applied for local treatment of severe periodontitis and peri-implantitis. They can also serve as fillers in hydrogels or other polymeric scaffolds and matrices in the surgical management of both bacteria related pathogenic conditions.

References

1. Jepsen K, et al. 2022, https://doi: 10.3390/antibiotics11030385.
2. Mercado N, et al. 2019, https://doi.org/10.1007/978-3-030-06115-9_24.

Acknowledgements. This work is supported by European Union's Horizon 2020 research and innovation programme under grant agreement No 953128, project: Smart, Multifunctional Dental Implants: A solution for peri-implantitis and bone loss.

PoA.17.16

Manganese-loaded CMCht-PAMAM nanoparticles for magnetic resonance Imaging in biomedical applications

Eduarda P. Oliveira[1,2], F. Raquel Maia[1,2], Lorena Colás[3], Rui L. Reis[1,2], Pedro Ramos-Cabrer*[3,4], J. Miguel Oliveira*[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciencia e Tecnologia, Barco, Guimarães, Portugal. [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal. [3]CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), San Sebastian, Spain. [4]Basque Foundation for Science, Ikerbasque, Bilbao, Spain

Abstract

Introduction Contrast agents enhance MRI signal intensity differences between regions of interest, by shortening the longitudinal (T1) or transverse (T2) relaxation times of surrounding water molecules, originating the so-called T1- or T2-weighted images[1]. Manganese-enhanced magnetic resonance has unique applications in biomedicine, particularly for tracking neuronal processes (behaves as a Ca2+ analogue). However, manganese ions have a short life span and can diffuse rapidly from tissues, biomaterials, or cells, limiting its efficiency[2]. We propose a novel manganese-based contrast agent that could overcome this problematic, with interesting applications in biomedicine. Methods CMCht-PAMAM-NPs (1.5G) were dissolved in water (1% w/v), and the manganese was dissolved in water at 0.4 mM. Equal amounts of both solutions were mixed, left stirring for 24h, and dialyzed for 24 hours to obtain CMCht-PAMAM-Mn. Manganese incorporation into the particles was analyzed by 1H-NMR, XPS, SEM-EDS, and ICP-MS, and particles were characterized by DLS, FIB-SEM, and AFM. Metabolic activity, proliferation, and internalization in cell cultures were evaluated using human Adipose Stem Cells (hASCs), and haemolytic properties using human erythrocytes from whole blood. Longitudinal (r1) and transverse (r2) magnetic relaxivities were characterized *in vitro* at 1.5T, and CMCht-PAMAM-Mn phantoms were imaged at 7T and 11.7T MRI systems. Results/Discussion We have confirmed the successful attachment of manganese to the CMCht-PAMAM-NPs by 1H-NMR, ICP-MS, XPS, and SEM-EDS, and DLS studies revealed a non-significant alteration of particles' size in this process. Coupling of Manganese ions to the surface of the particles yield magnetic relaxivities of r1: 17.5 mM-1s-1 (1.5 T), 9.7 mM-1s-1 (7T), and 6.8 mM-1s-1 (11.7 T); r2: 136.1 mM-1s-1 (1.5 T), 80.9 mM-1s-1 (7T) and 75.0 mM-1s-1 (11.7 T) and r2/r1 ratios of: 2.1, 8.4 and 11.1. These values show a typical T1-contrast agent behavior at low fields, acting as a dual T1-T2 agent at higher magnetic fields. MRI parametric maps (figure 1) of nanoparticle solutions at different dilutions show a clear concentration-dependent drop in both T1 and T2 relaxation times. Furthermore, nanoparticles presented non-cytotoxic and non-hemolytic behaviors and are easily internalized by hASCs, displaying promising features for biomedical imaging applications. Conclusions We have developed and characterized a novel non-cytotoxic and biocompatible manganese-based contrast agent with exciting potential for biomedical applications.

1. Alzola-Aldamizetxebarria, S., et al., 10.1021/acsomega.2c03549.
2. Yang, J., et al., 10.3389/fneur.2020.00143.

Acknowledgments E. P. Oliveira PhD scholarship (SFRH/BD/37726/2018/J621340zkMFP) by Portuguese Foundation for Science and Technology supported this work.

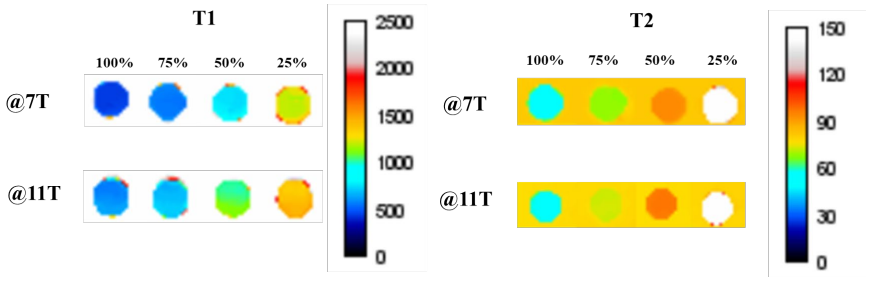


Figure 1- Analysis of the MRI phantoms.

PoA.17.17

Insights into the effects of magnetic nanoparticles and their heating mechanisms on healthy and tumor cell lines

Joana Santos[1], Catarina Rolo[1], João Paulo Borges[1], Tânia Vieira[2], Paula I.P. Soares[1]

[1]i3N/CENIMAT, Department of Materials Science, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal. [2]i3N/CENIMAT, Department of Physics, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal

Abstract

Cancer diagnostics and treatment have significantly improved in recent years, however, current treatment limitations and the increased incidence, mortality, and heterogeneity of cancer diseases lead to growing concern and expectation about the development of new and improved cancer diagnostic and/or treatment [1]. In recent years, nanotechnology developments towards biomedical applications enable the design of devices with a high level of complexity to significantly improve cancer theranostics outcome [1, 2]. Magnetic nanoparticles (MNPs) demonstrate a considerable potential to substantially improve theranostics platforms for cancer. Their unique properties, particularly their ability to respond to an external magnetic field, enable them to be used as imaging probes in the diagnostic feature and as magnetic fluid hyperthermia (MFH) agents in the treatment feature [1, 3].The main objective of this work is to understand the interaction and internalization mechanisms of superparamagnetic iron oxide nanoparticles (SPIONs) with normal and tumor cells, and macrophages. Additionally, the effect of the application of an external alternating magnetic field was assessed. SPIONs were produced by chemical precipitation according to well established protocols [4]. To ensure stability in physiological environment, SPIONs were coated with positive and negatively charged molecules. Additionally, a post-synthesis hydrothermal treatment was used to enhance the magnetic properties of the nanoparticles. Structural, physicochemical, and morphological properties (XRD, FTIR, SEM, TEM) of SPIONs was assessed. SPIONs aggregation in biological environment was assessed by dynamic light scattering and zeta potential measurements. Despite the type of coating, SPIONs demonstrated excellent stability in water during storage (average hydrodynamic size ranging from 90 to 200 nm, depending on the coating molecule), while in PBS and DMEM significant aggregates were formed. Additionally, the heating ability of SPIONs was successfully maintained during storage in water. Internalization studies are being performed in fibroblasts, melanoma cells and macrophages to evaluate by which cell lines are SPIONs preferably internalized, and if their aggregation state and stability in physiological environment significantly impacts the internalization pathways. Finally, cell death mechanism induced by SPIONs are being studied in the presence and absence of an alternating magnetic field.

1. P. Soares, et al., Progress in Materials Science, 116: 100742, 2021
2. Chen, X. S.T.C. Wong, Chapter 1 - Cancer Theranostics: An Introduction, in Cancer Theranostics, X. Chen and S. Wong, Editors. 2014, Academic Press: Oxford. p. 3-8.
3. Hu, Y., et al., Chem Soc Rev, 2018. 47(5): p. 1874-1900.
4. P. Soares, et al, Appl Surf Sci, 2016, 383: 240-247.

PoA.17.18

Biocompatibility and osteogenic potential of cerium oxide nanoparticles

Iason Chatzimentor, Ioannis Tsamesidis, Maria Bousnaki, Maria-Eleni Ioannou, Georgia K. Pouroutzidou, Eleana Kontonasaki

School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

INTRODUCTION Tissue engineering (TE) constitutes a science that investigates the interaction of cells with scaffolds and bioactive factors to achieve cell-based therapies[1]. Nanoparticles (NPs), offer a strongly positive ally in the task of TE. Cerium (Ce) belongs to the rare earth metals[2]. Due to its two distinct redox states (Ce^{3+} and Ce^{4+}), it can provide antioxidant, catalytic and ROS scavenging properties[3]. In this study the cytotoxicity and osteogenic differentiation capacity of CeO_2 NPs on human periodontal ligament cells (hPDLCs) was studied. **EXPERIMENTAL METHODS** For MTT cell viability assay 10^3 cells/well were placed in 96-well plates. Measurements were performed for cerium oxide samples (synthesized with 1 to 5g of cerium nitrate hexahydrate)[4] at different concentrations (C1=0,125mg/ml, C2=0,25mg/ml and C3=0,5mg/ml), after 1, 3 and 5 days. Osteogenic differentiation were measured using Alizarine staining method (ARS) and Alkaline phosphatase levels. ALP levels of hPDLCs were evaluated at 14 and 21 days for 2 concentrations (C1=0,125mg/ml and C2=0.5mg/ml) of CeO_2 5g. Both experiments were analyzed spectrophotometrically. **RESULTS AND DISCUSSION** MTT assay analysis (Figure 1) showed that although initially the NPs inhibited cell proliferation, in the course of time they increased the viability of hPDLCs, which verifies the time-dependent cell viability of CeO_2 NPs and in general the absence of cytotoxicity.

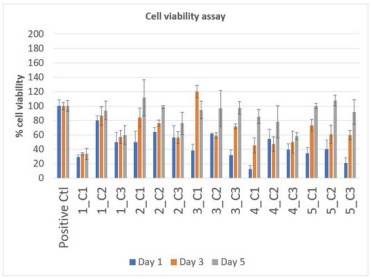


Figure 1. MTT results of all tested CeO_2 NPs. 1 to 5 CeO_2 NPs with 1-5 g of cerium nitrate hexahydrate.

Minimal mineralized activity was observed at 14 days. At 21 days, a massive increase in the expression of the mineralization marker was observed. In the treated with NPs cells, there was a statistically significant increase in the expression of mineralization marker in conventional (CCM) and osteogenic (OM) at 14 and 21 days, respectively (Figure 2). Increase of ALP values for both concentrations of NPs, after incubation for 14 day was observed. At day 21, the ALP levels of hPDLCs incubated with 5g CeO_2 were decreased as expected.

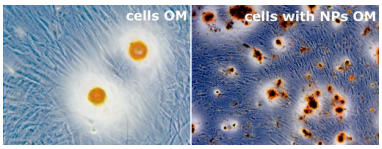


Figure 2. Photomicrographs of in vitro biomineralization of hPDLCs cultured with CeO_2 NPs for 21 days at OM.

CONCLUSIONS CeO_2 NPs successfully promoted the proliferation of hPDLCs and appeared to be non-cytotoxic. The osteogenic properties of the NPs were also highlighted, and the outcome seems very promising for further investigation and application in regenerative medicine.

REFERENCES

1. Hosseini et al., 2020. doi:10.3390/ma13143072
2. Scirè S. et al., 2020. doi:10.1016/b978-0-12-815661-2.00001-3
3. Chen et al., 2018. doi:10.1080/07388551.2018.1426555
4. Ioannou et al., 2023. doi:10.3390/app13042676

PoA.17.20

Tailoring PLA surface with anchor lipids to fabricate transmembrane protein-loaded nanometric bioresorbable platform.

Ahammed HM Mohammed-Sadhakathullah[1], Paria Pashazadeh-Panahi[2], Sofia Paulo-Mirasol[1], Elaine Armelin[1], Slawomir Sek[2], Juan Torras[1]

[1]Universitat Politècnica de Catalunya, Barcelona, Spain. [2]University of Warsaw, Warsaw, Poland

Abstract

Bioresorbable sensors have enormous potential in biomedicine because of their propensity to degrade and resorb after a certain period of time. This would eliminate the requirement for an expensive and risky follow-up medical procedure to remove non-bioresorbable sensors. However, the design and build-up of safe body-degradable sensors are still challenging. The primary goal of the present investigation is to create a bioresorbable polymeric platform that can be used for biosensing purposes and is capable of supporting artificial lipid bilayers and transmembrane proteins, like ion channels, as biomimetic models. The platform's development included three phases: self-assembly, alkaline hydrolysis, and surface activation. Firstly, self-assembling monolayers (SAMs) of a mixture of thiolated-poly(lactic) acid (T-PLA) and thioglucose on gold electrodes were prepared/obtained. Then, the monolayers were pre-treated with NaOH to introduce reactive functional groups on the surface. Surface activation of the hydrolyzed monolayers was performed using amine-reactive coupling agents (ethyl carbodiimide and N-hydroxysuccinimide (EDC and NHS)). An anchor lipid, poly(ethylene glycol)-cholesterol (Chol-PEG-NH2), was used to secure the lipid layers over the polymeric framework. Finally, Gramicidin A (GA), a peptide that has been shown to generate channels, was used to investigate the efficacy of the polymeric platform by electrochemical impedance spectroscopy (EIS) to detect the transport of monovalent cations. Nowadays, such biomimetic systems, based on lipids and transmembrane proteins, have an enormous potential for biofiltration and biosensing applications.[1] and this study proposes a novel bioinspired technique for producing such a platform that is totally bioresorbable and biocompatible.

1) Mohammed-Sadhakathullah *et al.* Advances in Functionalization of Bioresorbable Nanomembranes and Nanoparticles for Their Use in Biomedicine. Int. J. Mol. Sci. 2023, 24, 10312.

Acknowledgments: The authors thank the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement BioInspireSensing No 955643 for supporting this work.

PoA.17.21

Topical skin delivery of tyrosinase using functionalized carbon nanotubes

Woojin Jeong, Min Jeong Lee, Junghyeon Ko, Nathaniel S Hwang, Seon-Yeong Kwak

Seoul National University, Seoul, Korea, Republic of

Abstract

Tyrosinase is an essential enzyme for skin protection from ultraviolet(UV) radiation. If melanocytes stop producing functional tyrosinases, skin diseases such as vitiligo or albinism can occur. Although direct delivery of tyrosinase to the skin can effectively alleviate these symptoms, delivering proteins has been challenging due to the large molecular weight of protein and tight skin barrier. Here we demonstrate topical skin delivery of tyrosinase using single-walled carbon nanotubes (SWNTs) since topical skin delivery has the advantages that it is non-invasive, painless and capable of self-administration. SWNT is suitable for facilitating delivery proteins to the skin because of its high aspect ratio, large surface area, and excellent mechanical strength. Therefore, we fabricated functionalized single-walled carbon nanotube (SWNT) as a carrier for tyrosinase and applied reverse electrodialysis (RED) battery to give electric repulsive force for accelerating tyrosinase-SWNT complex (TYR-SWNT) transport. The characteristic properties of the TYR-SWNTs suitable for topical skin delivery were investigated, and the enzymatic activity of the immobilized tyrosinase on SWNT was comparable to free tyrosinase. The penetration efficiency of TYR-SWNTs in *ex vivo* porcine skin and *in vivo* mouse skin was analyzed by confocal Raman microscopy. Finally, we demonstrated UV protection ability of TYR-SWNT *in vivo* mouse skin through topical administration. We believe that this SWNT-guided topical protein delivery paves the way for an efficient protein drug delivery.

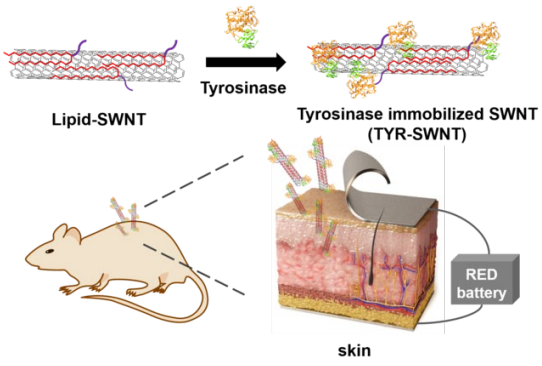


Figure. Schematic illustration of the topical skin delivery of TYR-SWNT with RED battery to in vivo mouse skin.

PoA.17.22

Stabilizing polymer coatings alter the protein corona of DNA origami and can be engineered to bias the cellular uptake

Hugo J. Rodríguez-Franco[1], Jorieke Weiden[1], Pauline Hendrickx[1,2], Maartje M.C. Bastings[1]

[1]École Polythecnique Fédérale de Lausanne, Lausanne, Switzerland. [2]Université Catholique de Louvain, Brussels, Belgium

Abstract

With DNA-based nanomaterials being designed for applications in cellular contexts, it is crucial to accurately understand their surface interactions with biological targets. When exposed to protein-rich media, a protein corona will establish around DNA nanoparticles, potentially altering the a-priori intended particle functionality. Furthermore, the challenging nuclease activities and low salt concentrations posed by these intricate milieus emphasize the criticality of safeguarding the structural integrity of DNA nanomaterials within them. In this study, we stabilized DNA origami nanoparticles with the widely used oligolysine-PEG polymeric coatings, initially assessing and comparing the role of the PEG block length in their protective performance. We then set out to identify the protein corona adsorbed around DNA origami nanomaterials when exposed to standard cell culture conditions, evaluating the impact of the different oligolysine-PEG stabilizing coatings. By implementing a label-free methodology, the distinctly coated nanoparticles show unique protein profiles, predominantly defined by differences in the molecular weight and isoelectric point of the adsorbed proteins. We eventually bias the protein corona through pre-incubation with selected proteins and show significant changes in the particle cellular internalization. Our study contributes to a deeper understanding of the complex interplay between stabilized DNA nanomaterials, proteins, and cells at the bio-interface, providing valuable insights for rational decisions on particle stabilization strategies.

PoA.17.23

Engineering DNA-based nanoparticles with lipid coating for cytoplasmic drug delivery

Pauline Hendrickx[1,2], Sarina Kopf[1], Maartje Bastings[1]

[1]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. [2]Université Catholique de Louvain, Brussels, Belgium

Abstract

Efforts in advancing therapeutic interventions are propelled by the development of precise and efficient drug delivery systems. In this study, we investigate the engineering of a DNA-based nanoparticle with a lipid coating, aimed at facilitating cytoplasmic drug delivery. The research encompasses the investigation of fundamental interactions between lipids and double-stranded DNA, with a focus on optimizing DNA-lipid interactions and minimizing DNA-DNA interactions to prevent aggregation. Parameters such as lipid composition, DNA concentration, and ion composition were examined to understand their impact on stability and binding affinity. These findings were utilized to develop a lipid-coated DNA-based nanoparticle, aimed at maximizing the coating efficiency and characterized for physicochemical properties. Preliminary cell uptake experiments demonstrated the potential of the particle for efficient drug delivery. This study provides insights for optimizing DNA-lipid interactions and lays the groundwork for further development of this novel drug delivery system.

PoA.18.01

Transplantation of neuron-inducing grafts embedding positively charged gold nanoparticles for the treatment of spinal cord injury

Seil Sohn

CHA University, Gyeonggi-do, Korea, Republic of

Abstract

In this study, we aimed to investigate the recovery after traumatic spinal cord injury (SCI) by inducing ellular differentiation of transplanted neural stem cells (NSCs) into neurons. We dissociated NSCs from he spinal cords of Fisher 344 rat embryos. An injectable gel crosslinked with glycol chitosan and oxidized hyaluronate was used as a vehicle for NSC transplantation. The gel graft containing the NSC and positively charged gold nanoparticles (pGNP) was implanted into spinal cord lesions in Sprague–Dawley rats (NSC-pGNP gel group). Cellular differentiation of grafted NSCs into neurons (stained with β-tubulin III [also called Tuj1]) was significantly increased in the NSC-pGNP gel group (***$p < 0.001$) compared to those of two control groups (NSC and NSC gel groups) in the SCI conditions. The NSC-pGNP gel group showed the lowest differentiation into astrocytes (stained with glial fibrillary acidic protein). Regeneration of damaged axons (stained with biotinylated dextran amines) within the lesion was two-fold higher in the NSC-pGNP gel group than that in the NSC gel group. The highest locomotor scores were also found in the NSC-pGNP gel group. These outcomes suggest that neuron-inducing pGNP gel graft embedding embryonic spinal cord-derived NSCs can be a useful type of stem cell therapy after SCI.

PoA.18.02

Functionalization of soft contact lens with copolymer modification

Tadashi Nakaji-Hirabayashi[1,2], Jun Isobe[3], Chizuko Ishihara[3], Shusaku Mandai[3]

[1]University of Toyama, Toyama, Japan. [2]National Institute for Materials Science, Ibaraki, Japan. [3]Mitsubishi Chemical Corporation, Osaka, Japan

Abstract

It is estimated that there are around 340 million contact lens (CL) users [1]. Approximately 30 % of CL users have developed ocular diseases, and this trend is increasing year by year [2]. Non-specific adsorption of proteins and bacteria to CLs is mainly caused by the ocular diseases. From the background, we aimed to develop CL enable to prevent and cure the ocular diseases while wearing it. Specifically, we developed soft CL materials modified with copolymers which can suppress the non-specific adsorption of proteins and bacteria and selectively release DNA drugs for ocular disease therapy. Copolymers composed of carboboxymethyl betaine methacrylate (CMBMA) [3,4] and 3-(methacryloylamino)propyl trimethylammonium chloride (MAPTAC) were introduced into the CL (Figure 1A). The CL modified with CMBMA-MAPTAC copolymers were evaluated for the loading and release of model DNA drug, as well as protein adsorption and bacterial adhesion. The DNA with a sequence of 20 nucleotides and 40% GC content was used as a model drug. Moreover, PBS containing proteins in tears (albumin, lysozyme, mucin, lactoferrin and immunoglobulin G) was used as an artificial tear solution (ATS). The major results are shown in Figure 1B and 1C. It was revealed that the copolymers and loading model DNA drugs were present in the interior of CL. Moreover, it was indicated that DNA release from the CL modified with $CMBMA_5$-$MAPTAC_5$ copolymers was almost suppressed in PBS, whereas DNA drugs were released by the drastic change of the salt concentration or by exchanging with proteins in ATS. The results demonstrate that DNA can be selectively released in the response to the changes of salt and protein concentration of tears caused by dry eye in wearing CL.

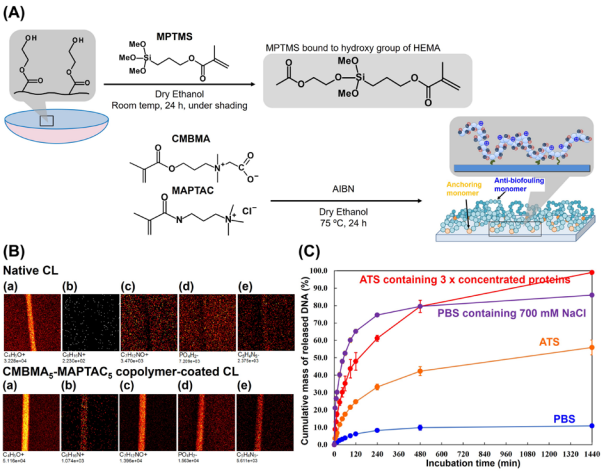


Figure 1. (A) Schematic illustration of strategy for coating CMBMA-MAPTAC copolymer to CL. (B) The distribution and localization of model DNA and $CMBMA_5$-$MAPTAC_5$ copolymer in CL. The distribution and localization of (a) HEMA, (b) CMBMA, (c) MAPTAC, (d) phosphoryl group of DNA, and (e) adenine of DNA were analyzed by TOF-SIMS measurement. (C) The release curve of DNA drugs from CMBMA5-MAPTAC5 copolymer-coated CL (binding amount of DNA = 1540 ± 86 μg) in PBS, PBS containing 700 mM NaCl, ATS, and ATS containing 3 times concentrated protein.

[1] Nature Commun., **2022**, 13, Article number: 5518. [2] Invest. Ophthalmol. Visual Sci., **2018**, 59, DES102-DES108. [3] J. Biomed. Mater. Res. A, **2016**, 104A, 2029-2036. [4] Colloids Surf. B, **2020**, 189, 110859.

PoA.18.04

Poly(sebacic-co-ethylene glycol anhydride) microparticles loaded with azithromycin as drug delivery systems to the lungs

Konrad Kwiecień[1], Karolina Knap[1], Rick Heida[2], Dorota Ochońska[3], Joanna Płonka[4], Daria Niewolik[5], Alicja Kazek-Kęsik[4], Katarzyna Reczyńska-Kolman[1], Monika Brzychczy-Włoch[3], Katarzyna Jaszcz[5], Peter Olinga[2], Elżbieta Pamuła[1]

[1]Department of Biomaterials and Composites, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Kraków, Poland. [2]Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Groningen, Netherlands. [3]Department of Molecular Medical Microbiology, Chair of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland. [4]Department of Inorganic Chemistry, Analytical Chemistry and Electrochemistry, Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland. [5]Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland

Abstract

Introduction: Oral or intravenous administration of antibiotics is not sufficiently potent to cure bacterial lung infections due to limited bioavailability. Therefore, inhalable dry powders are designed to administer drugs directly to the lungs. Among others, poly(sebacic-co-ethylene glycol anhydride)s (PSAEG)s are considered for such purposes owing to their fast degradation. This study aimed to develop PSAEG microparticles (MPs) loaded with azithromycin (AZM), and to characterize the physicochemical, aerodynamic, and biological properties of the powders. Experimental: PSAEG250 and PSAEG600 were obtained from sebacic acid and carboxyl-terminated PEG250 or PEG600 via polycondensation. MPs were manufactured using solid-in-oil-in-water emulsification, freeze-dried, and investigated with scanning electron microscopy (SEM), laser diffraction, and zeta potential measurement. AZM loading efficiency was assessed by HPLC. Carr's index (IC), Hausner ratio (HR), and median aerodynamic diameters were calculated based on tapped and untapped densities. The cytocompatibility of the particles was evaluated *in vitro* on A549 and BEAS-2B human lung epithelial cells and ex vivo on rat lung tissue slices after 24 hours of incubation with MPs dispersed in a culture medium. The bactericidal properties were evaluated with the Kirby-Bauer test on two reference and two clinical strains of Staphylococcus aureus. Results and discussion: Obtained powders consisted of spherical MPs. Particle size measurements indicated that the MPs smaller than 5 µm were 82-90% for PSAEG600 and 63-77% for PSAEG250, respectively. As MPs sizes should be within 1-5 µm, PSAEG600 showed superior properties. On the other hand, due to low tapped density, aerodynamic diameters were smaller than volumetric ones. IC and HR values indicated fair to good flowability of the powders with the best performance for PSAEG600 with higher AZM content. HPLC measurements showed almost complete encapsulation efficiency, and the MPs surface was charged negatively. Both *in vitro* and ex vivo tests showed that PSAEG powders were cytocompatible up to 100 µg/ml. Kirby-Bauer tests showed bactericidal effects from 50 µg/ml concentration. Conclusion: This study describes novel polyanhydride-based microparticle formulations for the pulmonary delivery of azithromycin. Physicochemical, aerodynamic, and biological evaluation of PSAEG600 with a high azithromycin content was chosen as the most promising material to treat lung bacterial infections in cytocompatible doses. However, more studies on the behavior of the powders in commercial inhalers are required.

Acknowledgment:

This study was supported by the National Science Centre Poland (project No 2019/35/B/ST5/01103) and by the Program 'Excellence Initiative – Research University' for the AGH University of Science and Technology.

PoA.18.05

Encapsulation of hydrophobized tobramycin in polyanhydride microparticles to treat lower respiratory tract infections

Karolina Knap[1], Konrad Kwiecień[1], Gabriela Markowicz[1], Daria Niewolik[2], Dorota Ochońska[3], Katarzyna Jaszcz[2], Monika Brzychczy-Włoch[3], Elżbieta Pamuła[1]

[1]AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Biomaterials and Composites, Krakow, Poland. [2]Silesian University of Technology, Faculty of Chemistry, Department of Physical Chemistry and Technology of Polymers, Krakow, Poland. [3]Jagiellonian University Medical College, Faculty of Medicine, Chair of Microbiology, Department of Molecular Medical Microbiology, Krakow, Poland

Abstract

INTRODUCTION Currently, many patients are struggling with bacterial resistance to antibiotics. One way to prevent it is to deliver antibiotics encapsulated into degradable polymer microparticles (MPs) directly to the infection site. Tobramycin (Tob) is often used in the treatment of bacterial lung infections. However, Tob is hydrophilic and it is difficult to encapsulate it in hydrophobic polymers, e.g., poly(sebacic anhydride) (PSA). The aim of this study was to: 1) modify Tob with dioctylsulfosuccinate (AOT) [1]; 2) encapsulate TobAOT in PSA microparticles (MPs) in different feed ratios and 3) characterize their properties from the point of view of pulmonary delivery. METHODS PSA MPs loaded with TobAOT were manufactured using oil in water emulsification. Morphology and size of MPs were characterized by scanning electron microscopy (SEM). The theoretical aerodynamic diameter was calculated based on the geometric diameter and tapped density of the MPs. The flowing character of MPs was evaluated by the Carr index. The encapsulation efficiency and drug loading were evaluated using an OPA assay. Cytotocompatibility with BEAS-2B lung epithelial cells was evaluated by the AlamarBlue assay and live/dead staining. Antibacterial activity was tested in contact with Staphylococcus aureus using the Kirby-Bauer method. RESULTS AND DISCUSSION We successfully obtained spherical MPs as shown by SEM analysis. The theoretical aerodynamic diameter was in the range of 1 – 5 µm. This size is optimal for deposition in the lower respiratory tract. Depending on drug loading, the flow character was changing. The encapsulation efficiency for all the samples was higher than 90%. For all samples, the inhibition zone of S. aureus growth was observed. The MPs were not cytotoxic for BEAS-2B at concentrations lower than 100 µg/ml. CONCLUSION TobAOT-loaded PSA MPs are promising carriers for pulmonary delivery for the treatment of lower respiratory lung infections. However, prior to clinical trials, it would be necessary to perform more advanced tests on their aerodynamic properties followed by *in vivo* tests.

REFERENCES

[1] M. Hill, R. N. Cunningham, R. M. Hathout, C. Johnston, J. G. Hardy, and M. E. Migaud, “Formulation of Antimicrobial Tobramycin Loaded PLGA Nanoparticles via Complexation with AOT,” J. Funct. Biomater., vol. 10, no. 2, Art. no. 2, Jun. 2019, doi: 10.3390/jfb10020026.

ACKNOWLEDGEMENTS

This study was supported by the National Science Centre Poland (project No 2019/35/B/ST5/01103) and by the Program ‘Excellence Initiative – Research University’ for the AGH University of Science and Technology.

PoA.18.06

Engineering of a glomerular filtration unit of the kidney using polyhydroxyalkanoates

Syed Mohammad Daniel Syed Mohamed[1], Jack Tuffin[2], Elbaraa Elghazy[3], Judy Watson[2], Caroline Sarah Taylor[1], Hamta Majd[4], Mohan Edirisinghe[4], Gavin Welsh[2], Ipsita Roy[1]

[1]Department of Materials Science and Engineering, University of Sheffield, Sheffield, United Kingdom. [2]Bristol Renal, Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom. [3]Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, United Kingdom. [4]Mechanical Engineering Department, University College London, London, United Kingdom

Abstract

Abstract Kidney failure happens due to acute kidney injury (AKI) and chronic kidney disease (CKD). Haemodialysis cannot replace the physiological activities of a normal kidney, and kidneys are scarce for transplantation. Hence, developing a bioartificial filtration barrier is of great interest, especially utilising live kidney cells. This research explores bacteria-derived polymers, Polyhydroxyalkanoates (PHAs), as a potential biomaterial to engineer a bioartificial glomerular filtration unit. It is known to be incredibly biocompatible and widely utilised in biomedical applications. Two types of PHAs have been selected, and their processability and cytocompatibility with glomerular cells have been investigated. The PHAs have been subjected to processing techniques to form fibres and woodpile structures. **Methodology** PHAs produced *via* fermentation, utilising a selected bacterial strain fed with fatty acids to produce a medium chain-length PHA (mcl-PHA), an elastomeric PHA, and glucose to produce a short chain-length PHA (scl-PHA), a stiff PHA. Conditionally immortalised human podocytes (CIHP) and conditionally immortalised glomerular endothelial cells (ciGEnC) were used to test the biocompatibility of PHAs in monocultures and co-cultures.[4, 5] The PHAs were subjected to electrospinning, core-sheath PHA-PLA gyrospinning, and 3D-printing by Fused Deposition Modelling (FDM). **Results and Discussion** Cytocompatibility studies showed for the first time that the PHAs were highly biocompatible with glomerular cells. Hence, PHAs are a great sustainable material for kidney tissue engineering applications. The PHAs were produced with a high yield of 40-80% dry cell weight. Tensile testing confirmed the elastomeric nature of mcl-PHA and the stiff nature of the scl-PHA. We have successfully electrospun the scl-mcl-PHA blend, gyrospun in the form of core-sheath fibres with PLA and printed the PHAs with good definition using a temperature-controlled printhead by FDM. These techniques resulted in structures that supported the growth of CIHP and ciGEnC, further confirming the suitability of the PHAs for the 3D culture.

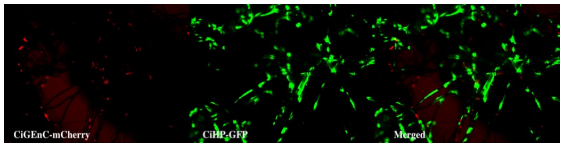


Figure 1: CIHP (green) and ciGEnC (red) co-culture on gyrospun PHA, scale bar is 500 µm.

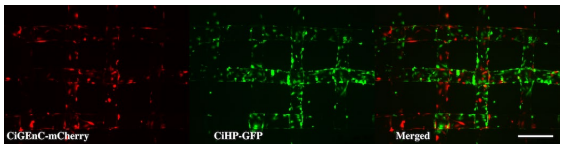


Figure 2: CIHP (green) and ciGEnC (red) co-culture on printed PHA struts, scale bar is 500 µm.

Conclusion We have conclusively shown that the different PHA fabrication techniques resulted in fibres/structures supporting the kidney cells' adherence and growth. This strategy should lead to the development of a highly functional bioartificial kidney filtration barrier. In future, the bioartificial filtration barrier developed will be assessed for its potential as an *in vitro* model of the kidney glomerulus.

PoA.18.08

High throughput biofabrication of *in vitro* connective tissue and fibrotic model representative of glaucoma

Hannah C Lamont[1], Michael Jones[2], Showan N Nazhat[3], Liam M Grover[1], Alicia J El Haj[1], Anthony D Metcalfe[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Cell Guidance Systems, Cambridge, United Kingdom. [3]McGill University, Montreal, Canada

Abstract

Introduction Glaucoma is a leading cause of irreversible blindness worldwide, with cases estimated to increase by 74% by 2040. Glaucoma occurs due to dysregulation in intraocular pressure (IOP), with IOP maintenance being mediated by the trabecular meshwork (TM), a piece of connective tissue that becomes fibrotic within patients. Nevertheless, the drivers of TM function and dysfunction during fibrosis are still unknown. Consequentially, current treatments for tackling the fundamental issues associated with glaucoma are limited, with low clinical trial success rates. Therefore, there is a clear need for more high-throughput, reproducible, TM *in vitro* models to help tackle these issues. Thus, this study aims to create the first biomimetic TM *in vitro* model with tunable collagen properties, aimed towards high-throughput testing. **Methods** Initially, type I collagenous constructs were formed by an automated gel-aspiration-ejection (GAE) method that was optimised for the processing of 96 well plates. Human trabecular meshwork cells (TMC) were embedded within collagen hydrogels before further GAE processing, with viability initially assessed within varying collagen fibre parameters. Thereafter, modulation of TMC phenotype (endothelial-mesenchymal transition) and expression of *in vivo* markers (α-β-crystallin, elastin, and myocillin) was analysed. Once characterised, transforming growth factor-beta2 (TGF-β2) was introduced utilising the PODS® technology to induce an increase in fibrotic protein markers (alpha-SMA, fibronectin and intracellular TGF-β2). **Results** Collagen constructs were designed during GAE process with a high degree of anisotropy and modulation of collagen fibre density/elastic moduli depending on gauge needle applied. Once optimised, TMC expressed increased *in vivo* markers, extracellular-matrix deposition (fibronectin, α-β-crystallin, elastin, myocillin), phenotypic alterations, and cellular alignment 3 days post incubation. Metabolic activity of GAE constructs was sustained for 2 weeks, with limited cell death in comparison to collagen controls. Once characterised, a fibrotic response was estimated by quantifying increased pro-fibrotic protein expression and alterations in elastic modulus.

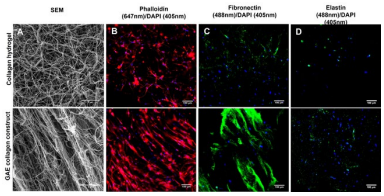


Figure 1: Difference in collagen architecture and protein deposition; (A) SEM images present heightened anisotropic properties following the GAE method. 3 days post incubation, cell shape was analysed (B), with altered fibronectin (C), and elastin deposition (D) observed in GAE (5µm/100µm scale bar).

Discussion/Conclusions These results demonstrated that design and optimisation of such biomimetic TM models was produced in a high-throughput manner for further translation of the field into disease modelling and drug testing platforms. Furthermore, such results highlighted the importance of collagen fibre properties and mechanics in the production *in vitro* models that emulate the *in vivo* connective tissue, such as the TM.

PoA.18.09

GENERATION AND CHARACTERIZATION OF SCAFFOLDS FOR PERIPHERAL NERVE TISSUE ENGINEERING THROUGH 3D BIOPRINTING TECHNOLOGY

Miguel Etayo-Escanilla[1,2], Óscar Darío García-García[1,2], José Manuel Baena[3,4], David Sánchez-Porras[1,2], Noelia Campillo[3,4], Víctor Carriel[1,2]

[1]Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain. [2]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain. [3]REGEMAT 3D SL, Granada, Spain. [4]BRECA Health Care SL, Granada, Spain

Abstract

Introduction: Peripheral nerves injuries (PNI) are a heterogeneous and distinct group of disorders that often leads to significant impairment and permanent disability. In short nerve defects, surgery is the preferred treatment, and nerve autograft is the gold standard for the repair of critical PNI (1). However, the drawbacks associated to autograft push researchers to find novel therapeutic alternatives such as the use of bioartificial substitutes. The innovative 3D bioprinting technology has enabled great advances in tissue engineering by making possible the generation of these substitutes with highly reproducibility and repeatability (2). The objective of this work was to make a screening of most suitable biomaterials to generate scaffolds through extrusion-based 3D printing for applications in the treatment of PNI. Methods: Polycaprolactone (PCL), polylactic acid (PLA), a flexible and electroconductive thermoplastic polyurethane (FF), a thermoplastic elastomer (FD) and gelatine methacrylate (GelMA) were employed to generate 3D printed scaffolds by using the REG4Life 3D bioprinter (REGEMAT 3D). They were subjected to scanning electron microscopy (SEM) and tensile tests (Instron, Model 5943). For the *in vitro* assays, human neuroblastoma cells (SK-N-AS cell line) were cultured on the printed scaffolds and the cell viability was determined by using WST-1 and Live/Dead assays after 7 days of culture. Results: Thermoplastic materials exhibited superior printing results in terms of resolution and shape-fidelity and higher stress at fracture and Young modulus values based on scanning electron microscopy (SEM) and tensile tests, respectively. Although GelMA exhibited inferior mechanical properties, it offered several advantages over thermoplastics. It can be polymerized at room temperature, making it suitable for 3D bioprinting with cells. Additionally, GelMA has tuneable structure and biomechanical properties, and has shown to be considerably more biocompatible than the thermoplastics in terms of cell viability and metabolic activity. Discussion: All materials showed promising structural, biomechanical and printing properties. Even though thermoplastic scaffolds demonstrated higher biomechanical properties and were easier to handle, GelMA scaffolds exhibited considerably better biocompatibility *in vitro* and supported adequate growth of neural cell lines, thus making GelMA a promising candidate for neural tissue engineering applications. Future *in vivo* studies will determine the therapeutic efficacy of these biomaterials in peripheral nerve repair.

Funding: Study financed by Grant CPP2021-009070 and FIS P20-0318. N.Campillo is granted by PTQ2019-010731.

1. Carriel V. et al. Expert Rev Neurother. 2014;14(3):301-18.

2. Cui H. et al. Adv Healthc Mater. 2017;6(1).

PoA.18.10

Decellularized porcine liver: Improved scaffold preparation and its characterization including initial cell-scaffold interaction

Vladimira Moulisova[1], Lenka Cervenkova[1], Maria Stefania Massaro[1], Richard Palek[1,2], Jachym Rosendorf[1,2], Roshan Kumar Singh[1], Miroslav Jirik[1,3], Lukas Bolek[4], Shashank Pandey[1], Magdalena Chottova-Dvorakova[1], Claudia Schindler[5,6], Janine Arlt[6], Sandor Nietzsche[7], Uta Dahmen[6], Vaclav Liska[1,2]

[1]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Plzen, Czech Republic. [2]Department of Surgery, Faculty of Medicine in Pilsen, Charles Universityry,, Plzen, Czech Republic. [3]Department of Cybernetics, University of West Bohemia, Plzen, Czech Republic. [4]Institute of Biophysics, Faculty of Medicine in Pilsen, Charles University, Plzen, Czech Republic. [5]Institute of Occupational, Social and Environmental Medicine, University Hospital Jena, Jena, Germany. [6]Department of General, Visceral and Vascular Surgery, University Hospital Jena, Jena, Germany. [7]Center of Electron Microscopy, University Hospital Jena, Jena, Germany

Abstract

Decellularized liver scaffold represents highly promising biomaterial for liver tissue engineering. Maintaining the extracellular matrix as intact as possible to keep natural cues for successful cell repopulation is considered crucial. Thus we decided to compare a standard decellularization technique, the use of frozen tissue, with a new technique when a fresh organ is processed immediatelly after explantation utilizing porcine model. We were further interested in protein composition of different liver scaffold compartments that could have an impact on recellularization. We also had a closer look at initial interaction of the scaffold with cells *in vitro*. Both decellularization methods produced scaffolds of high quality, nevertheless the method of fresh liver decellularization appeared to be more gentle in preservation detailed scaffold morphology. Both types of scaffolds supported cell adhesion, however, whether the difference in morphology can have an effect on longer term culture it still has to be investigated. Comparing protein composition of interlobular and sinusoidal parts of pig liver scaffolds separated by laser microdissection and identified by proteomic analysis revealed differences particularly in levels of several collagen types as well as some proteins of focal adhesions. Finally, during cell-scaffold interactions we identified colocalizations of adhesion molecules such are integrins with their extracellular matrix targets. In conclusion, optimizing liver scaffold preparation in combination with building up the knowledge about scaffold protein composition and cell-scaffold interaction can help to elucidate natural liver cell microenvironment, and in a longer term it can contribute to successful liver tissue preparation *in vitro*.

PoA.18.11

Development and characterisation of commercial ophthalmic biomaterials for contact lens applications

Gregor Mack[1], Adyl-Michaël El Guamra[2], Paul Demian[2], Mouad Lamrani[2], Julie Gough[1], Michael Read[1], Philip Morgan[1]

[1]The University of Manchester, Manchester, United Kingdom. [2]Menicon Co., Ltd, Geneva, Switzerland

Abstract

Introduction *In vitro* assessment of the surface properties and dynamic changes of hydrophilic materials is indicative of *in situ* characteristics such as tear film compatibility, lipid and protein deposition, and subjective comfort [1]. In particular, wettability, swelling characteristics, and fouling properties, each play vital roles in influencing the functionality and biocompatibility of ophthalmic biomaterials. Understanding such characteristics is important when assessing the ocular compatibility of candidate materials for vision correction and other ophthalmic uses [2].This work explored these parameters for combinations of four acrylate monomers currently used in commercial contact lenses. **Methods** Co-polymer films of 20 wt% increments were fabricated by a custom-made template using variations of: 2-hydroxyethyl methacrylate (HEMA), glycerol monomethacrylate (GMA), N,N-dimethylacrylamide (DMA), and methyl methacrylate (MMA). Fourier-transform infrared (FTIR) spectroscopy was performed to first validate the films had inherited both monomers of interest following polymerisation. For wettability, water contact angle using the sessile drop technique was measured on each film (n=3). For swelling, films were submerged in saline solution and evaluated gravimetrically at timepoints over a 24-hour period. For protein deposition, immunohistochemistry was performed on films following 24-hour rotational incubation at 36.5°C with either IgE or MMP-9 (n=3). **Results** FTIR data presented characteristic peaks of the monomers in each co-polymer matrix, with the intensity of the vibrational peaks reducing with decreasing monomer concentrations. Contact angles ranged from 35°-79°, with clear trends expressed for the various co-polymer mixtures. Films containing high amounts of MMA were significantly more hydrophobic, and wettability also found dependent on the paired monomer. For swelling, between approx. 1-400% mass increases were found, the latter of those with a large DMA content showing the highest degrees of swelling. Protein deposition of both MMP-9 and IgE was greatest on films constituted of HEMA and DMA. **Discussion and Conclusions** FTIR confirmed each co-polymer was constituted of both respective acrylate monomers, and an expected linear relationship between concentration and bond presence seen. The wettability influence of the four selected monomers is broadly in agreement with literature, and discrepancies likely due to greater surface presence of a specific monomer. Co-polymers containing DMA had a greater tendency to swell, corroborated by the highest degrees of protein fouling. Overall, the four selected monomers show a variation in their suitability for ophthalmic applications.

[1] Lorentz, H., *et al. Molecular Vision* **17**, 3392-3405 (2011).

[2] Willcox, M., *et al. Contact Lens and Anterior Eye* **44**, 157-191 (2021).

PoA.18.12

Improved treatment of bladder disease through hydrogel augmentation

Paige J LeValley[1,2], Alexandre H. C. Anthis[2,1], Thomas Rduch[1,3], Inge K Herrmann[1,2]

[1]Empa, Saint Gallen, Switzerland. [2]ETH Zuirch, Zurich, Switzerland. [3]Kantonsspital Saint Gallen, Saint Gallen, Switzerland

Abstract

Each year millions of people worldwide suffer from diseases affecting the bladder such as bladder cancer, bladder pain syndrome (BPS), and overactive bladder (OAB). Specifically, BPS is estimated to affect more than 1 in 100 women, while OAB effects 1 in 10 people and bladder cancer is the fifth leading malignancy among men. Treatment of bladder diseases remains a challenge due to a lack of specific therapeutics leading to inefficient treatments with off-target side-effects or a reliance on invasive treatments that must be repeated often for efficacy. For BPS particularly, the most effective treatment, glycosaminoglycans (GAGs) replacement therapy, requires multiple intra-bladder injections over six months for symptom relief. Further, OAB commonly presents as a comorbidity of BPS, for which the treatment is botulinum toxin A (BTA) injections. There is a need to develop new treatment regimens for patients suffering from bladder conditions to improve therapeutic efficacy and reduce the burden on patients. Herein, we engineered crosslinked hydrogels to augment diseased bladder tissue and deliver therapeutics locally. Extensible hydrogels were designed to mimic the natural properties of the bladder and remain stable in simulated urine highlighting their potential value for bladder augmentation. The hydrogels were applied to porcine bladder tissue ex vivo and *in vivo*. Raman spectrometry and histology were utilized to investigate the integration of the hydrogels into the urothelium before and after repeated extension and cell response to the addition of the hydrogel layer, respectively. Further, tensile testing was performed on the hydrogel augmented bladder tissue to ensure the hydrogel would not inhibit natural bladder function when applied as a therapeutic method. Finally, the release of GAGs and a protein mimic for BTA from the hydrogel networks was investigated to demonstrate the platforms potential in acting as a therapeutic delivery vehicle. The easily modifiable hydrogel platform presented has the potential to not only provide new opportunities in the delivery of therapeutics to the bladder but also act as a protective layer to aid in reducing pain associated with many of these conditions improving the long-term livelihood of patients.

PoA.18.13

Replacement of decellularized tissues by synthetic 3D fibrous polymer scaffolds: full thickness equivalents of the small intestine and airways

Niklas Pallmann[1], Sabrina Herrmann[1], Tobias Weigel[2], Christina Fey[2], Jeanne Mérignac-Lacombe[1], Rinu Sivarajan[1], Totta Ehret-Kasemo[3], Agmal Scherzad[3], Stephan Hackenberg[4], Maria Steinke[2], Daniela Zdzieblo[2], Jörn Probst[2], Florian Groeber-Becker[2]

[1]University Hospital Würzburg, Chair of Tissue Engineering and Regenerative Medicine, Wuerzburg, Germany. [2]Translational Center Regenerative Therapies (TLC-RT), Fraunhofer Institute for Silicate Research (ISC), Wuerzburg, Germany. [3]University Hospital Würzburg, Department of Oto-Rhino-Laryngology, Plastic, Aesthetic & Reconstructive Head and Neck Surgery, Wuerzburg, Germany. [4]RWTH Aachen University Hospital, Department of Otorhinolaryngology – Head and Neck Surgery, Aachen, Germany

Abstract

INTRODUCTION Regulatory developments, ethical aspects and differences in drug response between species shift life science more and more away from animal studies towards human based tissue models. Often, a vital part of these models to increase the predictivity of is the stroma. Unfortunately, most current tissue equivalents are assembled using animal based stromal scaffolds such as collagen, gelatin or decellularized tissues. Due to ethical concerns, batch-to-batch variances and costs, synthetic alternatives are needed. Previously, a porous electrospun 3D-scaffold was developed, which allowed cellular migration, followed by the formation of a stromal equivalent via secretion of human collagen. Applicable for epithelial attachment, it successfully replaced animal derived collagen in full thickness skin equivalents[1]. To demonstrate the broad applicability of the material platform, we performed a comparative study of small intestine and airway tissue models based on the synthetic 3D-scaffolds compared to decellularized porcine small intestinal submucosa (SIS) as current standard. METHODS Biological and synthetic fiber-based scaffolds were seeded with human primary airway or intestinal fibroblasts as well as epithelial cells and were comparatively analyzed. Small intestinal: biologization was characterized by histology, quantitative hydroxyproline assay and nano indentation. The subsequent small intestinal tissue models were analyzed concerning their barrier integrity, viability and their cellular polarization. Furthermore, mucin secretion and cell-type specific marker were evaluated by histology. Airway: differentiated models were characterized concerning their epithelial barrier, mucociliary phenotype (microscopy and histology) and cytokine response (LPS treatment). RESULTS Both primary tissue specific fibroblasts were able to migrate into both, synthetic and decellularized, scaffolds. In the synthetic variant, the biologization process was monitored by increasing hydroxyproline content, raising mechanical properties and a homogenous distribution of collagen. Comparing differentiated models of airway and small intestine on both scaffold types, high similarities were detected for barrier integrity, vitality and polarization. Regarding the intestinal tissue models we observed positive impacts on cell proliferation and differentiation, due to enhanced cell-cell communication in the synthetic scaffolds. On the airway mucosa models, LPS treatment showed a trend of increased IL-6 and IL-8 secretion on the synthetic scaffolds, indicating higher sensitivities to external stimuli. CONCLUSION Comparison of both scaffolds demonstrated a feasible replacement of animal derived structures. Adverse for the synthetic variant was the additional time in fibroblast-related biologization, but enabled the analysis of extra cellular matrix expression without interferences by the presence of animal structural proteins. REFERENCES

1. Weigel T. et al., Adv. Mater. 2106780, 2022

PoA.18.14

Investigating the effects of local delivery of glial cell-derived neurotrophic factor on neurite growth *in vitro* and *ex vivo.*

Rebecca Lomax, Fred Claeyssens

University of Sheffield, Sheffield, United Kingdom

Abstract

Research indicates that peripheral nerve regeneration is a complex cellular and molecular process. Schwann cells produce neurotrophic factors (NF) to promote axonal outgrowth from transected nerve and in turn regenerating axons promote the survival of Schwann cells via growth factor secretion. Large peripheral nerve gap injuries (>5 mm) are difficult to repair and affected nerves usually do not recover full function. Evidence suggests gaps >5 mm lead to unsuccessful nerve bridge formation and consequently prevents the migration of nerve growth-promoting Schwann cells from proximal and distal ends. The current “gold standard” autologous nerve grafts (autografts) come with limitations such as second surgical site and donor site morbidity. Presently, preclinical outcomes of alternatives such as nerve guide conduits (NGC) and cell-based therapies do not compare with autografts. Advances in nerve guide design such as intraluminal structures e.g. scaffolds or surface chemistry modification, has led to improved regeneration outcomes compared to empty NGCs. However, further development of design and manufacturing methods is required. To improve the nerve regeneration capability of our NGCs, we are investigating the effects of local delivery of glial cell-derived neurotrophic factor (GDNF) *in vitro* (NG108-15 neuronal cell line and chick dorsal root ganglion (DRG) models) and *in vivo* (thy1-YFP mice). Various concentrations of GDNF were immobilised to heparin sulphate bound PCL films using allylamine plasma polymerisation or aminosilane incubation following air plasma treatment to introduce amine groups at the nano-surface layer. The effect of the different concentrations of immobilised GDNF on neurite outgrowth from neuronal cells was assessed through neurite differentiation assays and immunolabeling. To determine the biocompatibility of the films post surface modification, the metabolic activity of neuronal cells was measured *via* Alamar blue assay.We found that neurite length of neuronal cells cultured on GDNF-immobilised films were longer than neurites of cells cultured in media supplemented with corresponding GDNF concentrations and controls. Cell metabolic activity was higher in cultures grown on GDNF-immobilised films compared to cultures grown on PCL with GDNF supplemented media. 100 pg/mL immobilised GDNF gave longest neurite growth and highest cell metabolic activity compared to controls. Our data suggests that immobilisation of bioactive factors can lead to longer neurite growth and higher metabolic activity compared to exogenous delivery. Based on *in vitro* results we have selected two concentrations of GDNF to investigate in chick DRG models. Lastly, our data indicates the potential of GDNF to improve axon regeneration *in vivo*.

PoA.18.15

Natural polymer based nerve guidance conduit design to increase vascularization in peripheral nerve tissue regeneration

Dila Hatun SAL, Caroline S. Taylor, Emmanuel Asare, John W. Haycock, Ipsita Roy

Department of Materials Science & Engineering, Kroto Research Institute, The University of Sheffield, Sheffield, United Kingdom

Abstract

Increasing vascularization in damaged tissues is vital for the delivery of oxygen, metabolites and nutrients required for regeneration. Studies have shown that vascular endothelial growth factor (VEGF) promotes proliferation of Schwann cells (SCs), neuronal survival, neuronal migration, and proliferation of neurons in peripheral nervous tissue. Therefore, the incorporation of VEGF in the framework of nerve guidance conduits (NGCs) is a viable option to potentially increase vascularization in the repair of peripheral nerve tissue injuries. Biocompatible and sustainable polyhydroxyalkanoates (PHAs) produced by bacterial fermentation support peripheral nerve tissue regeneration and are suitable biomaterials for NGC design. It has also been reported that silane modification with air plasma is a good surface modification method for peripheral nerve tissue regeneration. In this study, blends of 50:50 poly(3-hydroxybutyrate) (P(3HB): medium chain length PHA, obtained by 2D solvent casting were used. The effects of 8 different VEGF concentrations on the growth and viability of NG108-15 neuronal cells, Schwan cells (SC) and Human dermal microvascular endothelial cells (HDMECs) cells were initially investigated by resazurin assay, following which the two best performing VEGF concentrations were selected for each cell type. On the PHA scaffolds, VEGF immobilization was carried out at two different concentrations using heparin, air plasma and silane. VEGF immobilizations were confirmed using XPS analysis. The results obtained were compared with the effects of different VEGF concentrations on cells seeded on unmodified polymer scaffolds. Resazurin assay and live dead assays were used for cellular metabolism and growth analyses respectively. NG108-15 neuronal cells were labelled with βIII tubulin to assess the length of neurite outgrowth and primary Schwann cells were labelled with S100β to assess Schwann cell phenotype, length, and aspect ratio. HDMECs were labelled with CD-31 for their cellular analysis. VEGF immobilization onto 50:50 P(3HB): MCL-PHA blend films was confirmed to be a highly suitable option for peripheral nerve tissue regeneration and to increase peripheral nerve tissue vascularization. In future it is planned to evaluate the vascularization of 3D NGC constructs.

Acknowledgments

We acknowledge the Turkey Ministry of National Education and The University of Sheffield for providing funding and support for this project.

PoA.18.16

Skin models for the prevention of pressure injuries in newborns

Tino A. Jucker[1,2], Simon Annaheim[1], René M. Rossi[1,2]

[1]Empa, St. Gallen, Switzerland. [2]ETH, Zürich, Switzerland

Abstract

Pressure injuries (PI) are highly prevalent among neonates in intensive care units due to their immature and vulnerable skin. Stratum corneum is about to be formed after birth only. Prevalence rates of up to 75% have been reported, and studies indicate a high clinical impact on mortality rates, which are estimated to be two times higher for PI patients. Understanding the pathophysiology of PI formation in neonates is crucial for developing effective preventive measures. PIs in newborns are primarily caused by direct compression-related cell damage, reperfusion injury, and ischemia. Therefore, the aim of this PhD project is to develop mechano-biological skin models to better understand how mechanical force is transducted through the skin layers and how this relates to cytokine release. The release of pro-inflammatory cytokines due to mechanical stress can be a valuable indicator for detecting the onset of PI in human skin. This mechanism is replicated in a biological skin model by combining keratinocytes and fibroblasts with a hydrogel, mimicking the dermis and epidermis. A reinforcement with an electrospun nanofiber membrane for mechanical stability will be investigated. To characterize cytokine release, the skin model will be exposed to mechanical stress, such as compression and shear. Upon pressure levels of 16 to 33 mmHg, an elevated release of pro-inflammatory cytokines, especially interleukin-1α, is expected. The vertical force transmission through the skin layers shall be investigated using a fully mechanical skin model by incorporating polymer optical fibers into 3D knitted textiles. These optical fibers sense pressure on different depth levels representing different skin layers. The combination with hydrogels will mimic the viscoelastic properties of human skin. Both skin models will be combined into one to become a mechano-biological skin model, serving as a valuable testing platform and offering significant new insights into the onset of PI. Based on the new insights gained from the skin models, new pressure-relieving support surfaces will be developed to reduce the risk of developing PI. Preliminary research revealed that conventional foam mattresses can result in high pressure peaks upon compression. In contrast, air mattresses allow for deformation without material stiffening, leading to improved immersion and envelopment, thus potentially reducing the risk of PI. In conclusion, this project aims to better understand PI formation in neonates and to develop an effective preventive measure.

PoA.18.17

Development of tribological skin models for the investigation of material - skin interaction

Magda I. Plaga[1,2], Fabian Itel[1], Nicola Migliore[1], Rolf Stämpfli[1], Simon Annaheim[1], René M. Rossi[1,2]

[1]Empa, St. Gallen, Switzerland. [2]ETH, Zürich, Switzerland

Abstract

The skin serves as a protective barrier between humans and the external environment, and is simultaneously the primary organ for man-machine interfaces. The utilization of wearable devices and soft robotics has facilitated the preservation of independence among patients and the elderly, potentially enhancing occupational safety. However, prolonged direct contact of these devices with the skin can often result in irritations or injuries. Therefore, it is imperative to further enhance development of materials and design of systems. At present, *in vivo* testing on human skin is conducted to demonstrate the safety of devices applied to the skin. Nonetheless, a systematic and reliable investigation of the interactions occurring at the skin-device interface, and in the particular skin-material junction cannot be fully comprehended through short-term tests performed on living tissue. Consequently, there is a necessity for novel skin models that can reduce the reliance on *in vivo* testing. The existing physical skin models, which mainly rely on metals or textiles merely enable the investigation of specific aspects of skin and consequently possess limited significance. To comprehensively evaluate complex interactions between skin and materials, a model that replicates multiple properties of human skin simultaneously is required. Hydrogels are a promising alternative for the basic mono-parametric skin models. By incorporating various additives (such as nanoparticles, textiles, fibers, and others), hydrogels allow for easy adjustment of numerous properties to emulate various physical parameters of human skin. Furthermore, hydrogels exhibit water-responsive behavior, a characteristic also observed in the outer layers of human skin. This poster primarily focuses on presenting research pertaining to the development of tribological skin models based on gelatin. To replicate the swelling behavior of the human stratum corneum following exposure to water, and thus the consequent increase in friction coefficient under wet conditions, materials with different degrees of crosslinking have been obtained and tested. Manipulating crosslinking strategies has facilitated the development of mechanically robust and abrasion-resistant models that accurately replicate the mechanical properties of human skin. The establishment of such models will facilitate investigations into the forces exerted on the skin by various devices under different conditions. As a result, further advancements in products such as medical textiles, breast pumps, soft electrodes, and others will become attainable. By improving their functionality and comfort use, the quality of life for patients will be significantly enhanced.

PoA.18.18

Evaluation of the performance of a ZnO-nanoparticle-coated hydrocolloid patch in wound healing

Le Thi Van Anh[1], Trinh Xuan Tung[1], Pham Ngoc Chien[1], Nguyen Ngan Giang[1], Zhang Xin Rui[2,1], Nam Sun Young[1], Heo Chan Yeong[2,1]

[1]Seoul National University Bundang Hospital, Seongnam, Korea, Republic of. [2]Seoul National University, Seoul, Korea, Republic of

Abstract

Wound healing is a complex and highly regulated process, consisting of four phases that include hemostasis, inflammation, proliferation, and remodeling. The application of wound dressings aims to enhance the process of natural healing and work to counter many aspects that plague poorly healing wounds, including excessive inflammation, ischemia, scarring, and wound infection. A combination of a hydrocolloid and nanoparticles (NPs), such as gold (Au), improves the wound healing rate, but Au-NPs are expensive and unable to block ultraviolet (UV) light. Herein, we combined zinc oxide nanoparticles (ZnO-NPs) with hydrocolloids for a less expensive and more effective UV-blocking treatment of wounds. Using Sprague–Dawley rat models, we showed that, during 10-day treatment, a hydrocolloid patch covered with ZnO-NPs (ZnO-NPs-HC) macroscopically and microscopically stimulated the wound healing rate and improved wound healing in the inflammation phase as shown by reducing of pro-inflammatory cytokines (CD68, IL-8, TNF-α, MCP-1, IL-6, IL-1β, and M1) up to 50%. The results from the *in vitro* models (RAW264.7 cells) also supported these *in vivo* results: ZnO-NPs-HCs improved wound healing in the inflammation phase by expressing a similar level of pro-inflammatory mediators (TNF-α and IL-6) as the negative control group. ZnO-NPs-HCs also encouraged the proliferation phase of the healing process, which was displayed by increasing expression of fibroblast biomarkers (α-SMA, TGF-β3, vimentin, collagen, and M2) up to 60%. This study provides a comprehensive analysis of wound healing by measuring the biomarkers in each phase and suggests a cheaper method for wound dressing.

PoA.18.19

A regenerative bioadhesive for the treatment of infected corneal ulcers

Yashoda Chandorkar[1], Annina Mittelholzer[1], Kongchang Wei[1], Kuldeep Dole[2], Shilpa Joshi[2], Simon Pot[3], Markus Rottmar[1]

[1]Empa Swiss Federal Laboratories for Materials Science and Technology, St Gallen, Switzerland. [2]H. V. Desai Eye Hospital, Pune, India. [3]Ophthalmology Section, Equine Department, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Abstract

Corneal infections constitute a silent epidemic with >800'000 new cases per year worldwide, often resulting in blindness, which is preventable with timely expert treatment. Surgical treatment options, such as corneal transplantation, are associated with high costs, and require specialist infrastructure and expertise, which are often unavailable in developing countries, leaving such cases untreated. Alternative standard of care procedures for the stabilization of corneal ulcers include cyanoacrylate-based or hydrogel (e.g. fibrin) based glues, which either exhibit a mismatched elastic modulus and exothermic gelation process, or are mechanically weak, rendering them unsuitable for certain types of defects. To overcome these problems, we developed an adhesive inspired by dopamine based mussel chemistry [1], [2], able to seal a corneal ulcer and serve as drug release system, as well as facilitate cell infiltration from the surrounding micro-environment. The adhesive is biodegradable and should support the regeneration of new transparent corneal tissue, and restoration of sight. For this, we mimic crosslinking aspects of catechol chemistry using a two-component glue based on chemically modified cold fish gelatin and hydrogen peroxide (H2O2) as an oxidizing agent to achieve a gelation temperature ≤ 25 °C. To achieve a fast (≤ 2 min) gelation time, as assessed via rheology and tube inversion techniques, that is compatible with the sensitive corneal ulcer environment, we evaluated different H2O2 concentrations. Tissue adhesion under wet conditions was assessed by applying the adhesive to a corneal defect in an ex vivo porcine cornea model, where we observed retention of the glue even in the presence of excess water. Finally, we demonstrated that human fibroblast cells could attach to and spread on the surface of the bioadhesive. Taken together, our results show that the adhesive developed holds promise for corneal application and warrants further *in vivo* testing.

[1] Wei et al, ACS Applied Materials and Interfaces, 2019

[2] Xu et al, Biomaterials Science, 2016

PoA.18.20

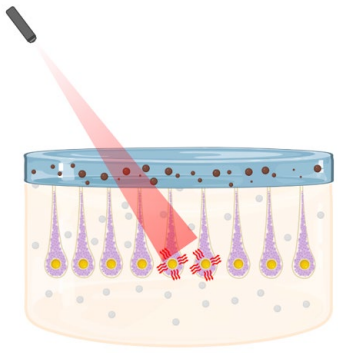
Development of a 3D-printed bio-hybrid skin model for photothermal therapy applications

Dardan Bajrami[1,2,3], Fabrizio Spano[2], Kongchang Wei[1], Mathias Bonmarin[2], René Rossi[1,3]

[1]Empa, St.Gallen, Switzerland. [2]ZHAW, Winterthur, Switzerland. [3]ETHZ, Zürich, Switzerland

Abstract

Skin models or phantoms are valuable tools used in biomaterial research to mimic the structure and function of human skin. Optical skin phantoms, in particular, have emerged as promising solutions for studying light interaction with human skin in various biomedical applications, including imaging and laser treatments. However, current optical skin models have limitations in achieving accurate representations of the real skin. These models often fail to consider important factors such as skin color, skin thickness, integration of appendages (e.g., hair follicles, sweat glands, sebaceous glands), and biological response. In this study, we present a novel approach to overcome the limitations of existing artificial skin phantoms by developing a 3D-printed bio-hybrid skin phantom. Our proposed model aims to replicate the optical and thermal properties of human skin and the complex geometry of hair follicles. To achieve this, we combine a skin model mimicking the optical and thermal properties of human skin with the embedding of biological skin cells. The basis of our bio-hybrid skin model is a 3D-printed tissue phantom material that allows for the fabrication of hair follicle like structures. Subsequently, we incorporate biological cells, such as hair follicle cells, into the skin model and engineer induced hair follicle cells to simulate the effects of photothermal therapy on specific cell populations. This enables a comprehensive study of the optical and thermal mechanisms involved in the complex interaction between skin tissue, hair, and the light source. Additionally, we investigate the influence of skin structures, including skin thickness and color, on the outcomes of photothermal therapy. The knowledge gained from our bio-hybrid skin model extends beyond photothermal therapy applications. It can also contribute to the development and testing of other optical treatments, such photodynamic therapy or photobiomodulation. The novel approach provides a platform to study the optical and thermal interactions in biomedical applications. The insights gained from our bio-hybrid skin model have the potential to enhance future advancements in the field of biomaterials.



PoA.18.21

Bioactive bacterial nanocellulose loaded with bromelain as a promising strategy for the enzymatic debridement of burns

Urška Jančič[1], Janja Trček[2], Selestina Gorgieva[1]

[1]University of Maribor, Faculty of Mechanical Engineering, Institute of Engineering Materials and Design, Smetanova ulica 17, 2000 Maribor, Slovenia. [2]University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, Koroška cesta 160, 2000 Maribor, Slovenia

Abstract

Early and efficient eschar removal is crucial in treating deep partial thickness, full-thickness and mixed-depth burns to promote wound healing, reduce infection risk, minimize scarring and treatment time. While tangental excision remains the most widely used method for eschar removal, enzymatic debridement offers benefits such as reduced blood loss, decreased need for subsequent skin grafting, preserving viable dermis and aiding wound healing, especially in challenging anatomical regions [1]. In the field of burns, bacterial nanocellulose (BnC) is a remarkable material due to its high water-holding capacity, biocompatibility, ultra-high purity, non-toxicity and mechanical stability [2]. Its lack of bioactivity, specifically in terms of proteolytic and antimicrobial properties, can be overcome by incorporating specific bioactive components. In this study, the proteolytic enzyme bromelain (BR) was immobilized into a BnC matrix to develop a bioactive wound-dressing material for the enzymatic debridement of burns. The BnC membranes were produced using a novel bacterial strain *Komagataeibacter melomenusus* AV436^T under static fermentation conditions and incubation at 30 °C for 4 days in 50 mL of RAE medium. The culture medium was modified *in situ* with carboxymethyl cellulose (CMC) to add a carboxyl group to the BnC matrix. Furthermore, bromelain was *ex situ* immobilized on a (modified) wet BnC membrane applying 2 methods: adsorption and covalent binding using a water-soluble crosslinker 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC) in the presence of N-hydroxysuccinimide (NHS). Response surface methodology (RSM) was used to design the experiment and optimize the enzyme immobilization process toward the highest specific proteolytic activity by investigating 3 independent variables: concentration of BR, CMC and crosslinking agent (Fig. 1). The optimal conditions were defined as c_{CMC}=8.8 mg/mL, c_{EDC}=0 mg/mL and c_{BR}=10 mg/mL. Finally, the physicochemical, morphological and mechanical properties of optimized BnC-BR composite were evaluated to analyze its suitability for debridement of burns.

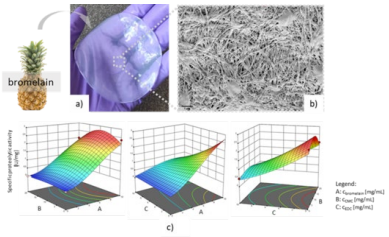


Fig. 1: a) BnC-CMC membrane harvested from static fermentation, b) SEM image of BnC-CMC-BR membrane, c) 3D RSM plots for specific proteolytic activity of bromelain as a function of BR/CMC/EDC concentration.

Acknowledgements The authors would like to acknowledge the financial support received in the frame of young researcher program P2-0118/0795 and project J2-2487, both financed by the Slovenian Research Agency (ARRS).

References

[1] A. A. Alekseev et. al., Life, vol. 13, no. 2, pp. 1–11, 2023.
[2] S. Gorgieva, Processes, vol. 8, no. 5, 2020.

PoA.19.03

Encapsulation phenomena of PLA-PCL blending

Fabiana Amiri[1,2], Pierpaolo Fucile[2], Gianmarco Nuzzo[1], Massimiliano Zingales[3], Lorenzo Moroni[2]

[1]Università degli studi di Palermo, Dipartimento di Ingegneria, Palermo, Italy. [2]MERLN Institute for Technology Inspired Regenerative Medicine, Complex Tissue Regeneration department, Maastricht University, Maastricht, Netherlands. [3]Bio/NanoMechanics for Medical Sciences Laboratory (BNM 2 -Lab),Advanced Technology Network (ATeN)-Center, Palermo, Italy

Abstract

“Viscous Encapsulation” is a rheological phenomenon in which the fluid with higher viscosity usually encapsulates the less viscous one, influencing fibers structure and, consequently, the entire structure. This leads to the formation of interesting core-shell structures that can be exploited to modulate the final mechanical properties of the devices. However, only few works have been published about this topic, since in polymer science it is often a phenomenon that is to be avoided. In this work, we studied the viscous encapsulation that occurs into polymeric blends of Poly(lactide-ϵ-caprolactone)(PLC) and Poly(L-lactide)(PLA) in different ratios, which were extruded through Additive Manufacturing (AM) techniques with the ultimate goal to exploit, instead of avoiding, this phenomenon for the fabrication of multi-functional multi-material 3D printed scaffolds. The integrity of the blends, as well as their rheological properties, were assessed through DSC analysis. Inter-phases of PLA and PCL were further investigated through SEM analysis on both normal and transverse direction of the scaffolds, in addition to the TEM analysis on the cross section of the single fibers. To further validate the structure, we also performed dissolution tests in Acetic Acid to selectively dissolve PCL within the scaffolds. Moreover, we have investigated the influence on the phase-separation phenomenon of many process parameters, such as temperature, shear stress, pressure, nozzle length/diameter ratio, and screw and extrusion speed. Consequently, knowing the influence of these factors, we were able to create an interesting bi-phasic circular structure, using only one material into the cartridge of the 3D-printer. The resulting scaffolds could be used into several biomedical applications where a structure with tunable mechanical and physico-chemical properties are required, such as for prosthetic implants, scaffolds for regenerative medicine, anti-adhesive surfaces, and controlled release devices. Moreover, we investigated PLA-PCL blends, which are widely used in biomedical and biomaterials engineering, but this phenomenon could also be extended to blends of different immiscible polymers.

PoA.19.04

Crystallinity index and mechanical properties of explanted polyethylene cup

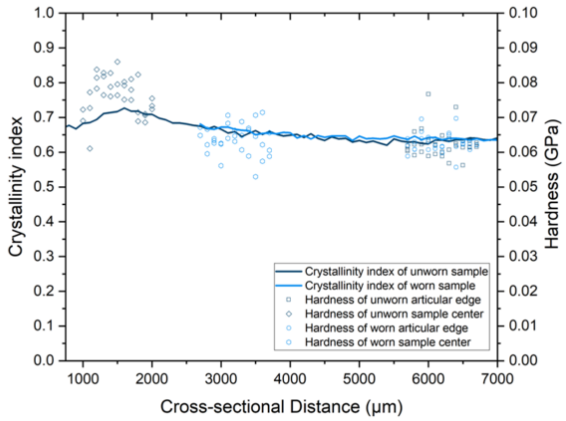
Jaroslav Lukes[1], Vlastimil Kralik[2], Miroslav Slouf[3]

[1]Bruker Nano Surfaces & Metrology, Prague, Czech Republic. [2]Czech Technical University in Prague, Prague, Czech Republic. [3]Institute of Macromolecular Chemistry of the CAS, v. v. i., Prague, Czech Republic

Abstract

Explanted UHMWPE acetabular cup was scanned for volumetric wear calculation over the articular surface. Worn region was compared to unworn area in terms of local molecular structure and mechanical properties. Oxidative degradation causes polymer chain scissions and additional crystallization which consequently alters mechanical properties. We focused on correlation of crystallinity index with hardness and elastic modulus. A cross-section from worn and unworn region of acetabular cup was mapped by IR microspectroscopy. Additionally, areas with the highest and lowest crystallinity index defined by analysis of IR spectra were mapped by nanoindentation coupled with Raman spectroscopy. Colocalized crystallinity index by means of Raman spectra analysis and hardness and elastic modulus measured by nanoindentation provide direct correlation of molecular structure and mechanical performance at microscale. Such localized measurements have been shown to correspond with more global picture of molecular structure given by IR spectroscopy (fig. 1).

Figure 1: Nanoindentation hardness follows the trend line of crystallinity index across the cross-section of worn and unworn sample.



PoA.19.05

In vivo release kinetics and histopathological evaluation of a PDMS-based balloon-type drug delivery device

Muhammad Tausif[1], Jin-Kyu Park[2], Sohee Kim[1]

[1]Daegu Gyeongbuk Institute of Science and Technology, Daegu, Korea, Republic of. [2]Kyungpook National University, Daegu, Korea, Republic of

Abstract

Statement of purpose: When creating implantable drug delivery devices, *in vitro* and *in vivo* pharmacokinetic evaluations are needed because drug release properties can be influenced by environmental changes [1]. Previously, we proposed and successfully fabricated a soft polydimethylsiloxane (PDMS)-based balloon-type implantable drug delivery device for a long-term controlled release [EMBC2021]. In addition, based on the PDMS membrane thickness and composition, the release kinetics profiles were successfully demonstrated. As the PDMS membrane thickness decreased, the release rate substantially increased. Similarly, when the amount of curing agent of PDMS increased, the release rate significantly decreased [2]. In this study, we further investigated the device performance in *in vivo* environment and analyzed foreign body responses histologically. **Materials and method:** The developed devices were implanted for 28 days subcutaneously in male Sprague-Dawley (SD) rats. For *in vivo* evaluation, devices were explanted at scheduled intervals (n = 3), and the residual amount of Rhodamine B (R.B) in the device was measured at 551 nm using UV-vis Spectrophotometer (Agilent Technologies). Finally, the released R.B amount was calculated compared to baseline non-implanted devices (n = 5). For histopathological evaluation, rats were sacrificed at 35 days. The tissues surrounding the devices were explanted, fixed, paraffinized, and sliced into 5µm thick slices and stained with hematoxylin and eosin (H&E). The stained tissues were investigated using an upright motorized microscope (Eclipse Ni-E, Nikon, Japan) at 200x magnification to assess the degree of inflammation and capsule thickness. **Results:** In the *in vivo* environment, devices exhibited a zero-order release pattern up to 28 days (R^2 = 0.995), releasing 56.5% (186.41 µg) of the total amount with a release rate of 5.4 µg/day (Fig.1). These findings clearly demonstrate that the *in vivo* release kinetics were consistent with the *in vitro* release characteristics, and a zero-order release was achieved using the suggested device. Histopathological evaluation revealed that the groups of animals implanted didn't show any complications and that the degree of inflammation was mild (Fig. 2), attributed to the biocompatibility and soft mechanical properties of PDMS and the device design. Noticeably, the fibroblast capsule thickness was less than the previously developed drug delivery devices[5][6].

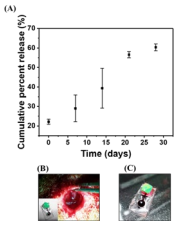


Fig. 1. (A) In vivo Rhodamine B release characteristics of the device in living rats, (B-C) device before, and after implantation.

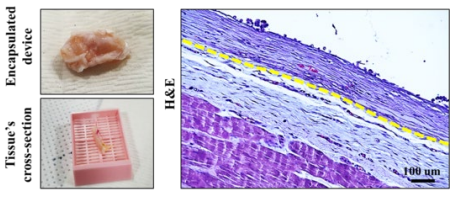


Fig. 2. Representative stained histopathological images of tissues surrounding the device after 35 days of implantation.

PoA.19.07

Cryopreservation by intracellular permeation of zwitterionic polymers

Ryota Yamasaki, Kazuaki Matsumura

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa, Japan

Abstract

Cryoprotectants are used to protect cells from cryoinjury during cryopreservation. Previous studies have reported the cryoprotective effect of poly sulfobetaine (poly(SPB)), a zwitterionic polymer, but its cryoprotective effect is not high. Recently, it has been reported that some poly(SPB) can permeate into cells without going through endocytosis. If polymers can permeate into cells, osmotic shock during freezing can be alleviated. In this study, we synthesized copolymers of poly(SPB) and 2-(methylsulfinyl)ethyl methacrylate (MSEMA), which have dimethyl sulfoxide (DMSO)-like structures, and attempted to improve the cryoprotective effect by allowing these polymers to permeate into cells. All polymers were synthesized by Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization. For intracellular permeation of the polymers, the polymers were first dissolved in PBS or 0.5 M NaCl solution to a concentration of 10 % (w/w). Next, 1 ml of polymer solution was added to 35 mm dishes seeded with a cell density of 1.0 x 10^6 cells/ml and incubated at 4 °C for the prescribed time. The cells were then washed three times with PBS and trypsinized to detach from the dish. Cells were collected and suspended in 10 % (w/w) poly(SPB) solution containing 0.5 M NaCl at a cell density of 1.0 x 10^6 cells/ml and frozen at -80 °C for 24 hours. The suspension was then thawed at 37 °C and subjected to trypan blue assay to assess cell viability. Intracellular permeation of the polymers was observed by confocal microscopy. Intracellular penetration of the polymer was confirmed using confocal microscopy (Fig. 1).

Fig. 1 Confocal microscopy image of fluorescence modified poly(SPB) in L929 cells.

Cryopreservation results showed that cell recovery was as low as 10 % in the absence of polymer permeabilization treatment. However, when cells were incubated with 10 % (w/w) poly(SPB) solution containing 0.5 M NaCl, cell recovery improved at all incubation times and was over 60 % at both 10 or 40 minutes (Fig. 2).

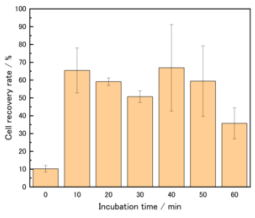


Fig. 2 Cryoprotective effect of poly(SPB) permeation into L929 cells.

Results on cell growth and poly(SPB/MSEMA) permeabilized systems will be reported at the conference.

PoA.19.08

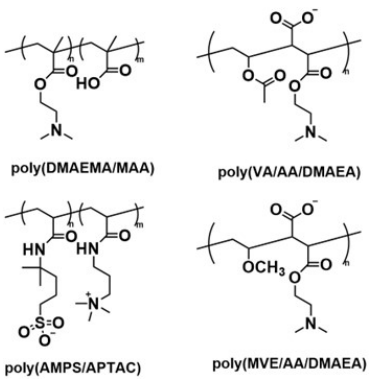
Relationship between structure and cryoprotective effect of synthesized polyampholytes using variable-temperature solid-state NMR

Ryota Yamasaki, Robin Rajan, Kazuaki Matsumura

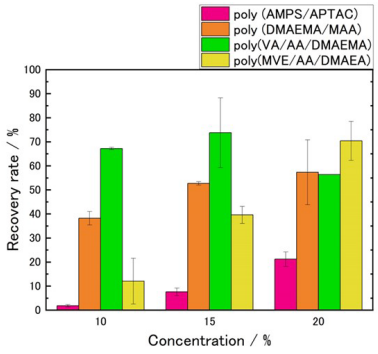
School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa, Japan

Abstract

Cryopreservation techniques are important for the long-term storage and transport of cells and tissues. Cryoprotectants are used to protect cells from cryoinjury during cryopreservation. Previous studies have reported low cytotoxicity and high cryoprotective efficacy of carboxylated poly-L-Lysins. In addition, temperature-variable solid-state NMR has shown that a reduction in the mobility of the polymer itself is important for cryoprotection. Other cryoprotective polymers, poly(2-(dimethylamino)ethyl methacrylate (DMAEMA)/methacrylic acid (MAA)), have been reported, but the cryoprotective mechanism is not clear. In this study, we synthesize four polymers with different side-chain structures and correlate the polymer structure with the cryoprotective effect (Fig. 1). *Fig. 1 Structure of polyampholytes.*



Poly(DMAEMA/MAA) and poly(2-acrylamido-2-methylpropanesulfonic acid (AMPS)/(3-acrylamidopropyl)trimethylammonium chloride (APTAC)) were Reversible Addition-Fragmentation Chain Transfer (RAFT)-polymerized at 70 °C. Poly(vinyl acetate (VA)/acrylic acid (AA)/2-(dimethylamino)ethyl acrylate (DMAEA)) and poly(methyl vinyl ether (MVE)/AA/DMAEA) were prepared by dissolving poly(VA/maleic anhydride (MA)) or poly(MVE/MA) in 50 ml of THF at 50 °C, adding 2-dimethylamino-ethanol and allowing the reaction to proceed overnight. The cryoprotective effect of the synthesized polymers was confirmed by freezing at -80 °C for 24 hours. The correlation between the polymer structure and the cryoprotective effect was investigated by evaluating the ice recrystallization inhibition and performing temperature-variable solid-state NMR measurements. Cell recovery after cryopreservation for poly(DMAEMA/MAA) was calculated to be up to 60% at 20 w/v%. For poly(VA/AA/DMAEA) and poly(MVE/AA/DMAEA), which have the same degree of dissociation, the recovery rates were found to be up to 80% at 10 w/v% and 20 w/v%, respectively, indicating that the cryoprotective effect is similarly high. However, recovery rate for poly(AMPS/APTAC), which has a high degree of dissociation, was found to be up to a maximum of 20 %, indicating a poor cryoprotective effect (Fig. 2). *Fig. 2 Recovery rate of the polymer agents*



Detailed discussions on the evaluation of ice recrystallization inhibition and molecular motion of each cryoprotective polymer solution at low temperatures will be reported the day of the conference.

PoA.19.10

Spinning of high-strength Poly (vinyl alcohol) fiber with high orientation - Approach by addition of salt-

Yusuke Taoka, Riza Asmaa Saari, Takumitsu Kida, Masayuki Yamaguchi, Kazuaki Matsumura

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai Nomi Ishikawa, Japan

Abstract

Poly (vinyl alcohol) (PVA) is an inexpensive, biodegradable synthetic polymer with low environmental impact. PVA fiber is used in many fields, including infrastructure, because of its high mechanical strength. However, there is a limit to the high strength of PVA fiber. It is a crystalline polymer, and strong hydrogen bonds between hydroxyl groups inhibit stretching, which increases molecular orientation and strength, causing orientation defects. In our research, this hydrogen bonding was temporarily suppressed by the addition of lithium iodide (LiI) to reduce orientation defects during stretching and to further enhance strength. PVA fiber was spun by wet spinning method with a primary drawing ratio of 4x. After washing, the fibers were heat-stretched 5 to 7 times with varying stretching conditions. The properties of the heat-treated samples were evaluated. About half of the fibers without LiI broke at about 5 times increase in stretch ratio. However, the stretchability of the LiI-added PVA fiber improved significantly, and the fiber did not fracture even after being heat stretched more than 7 times (Figure 1). This indicates that the addition of LiI suppresses hydrogen bonding and improves ductility.

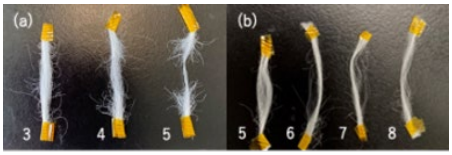


Figure 1 Heat-stretched PVA fiber. (a) PVA, (b) LiI-added PVA

Fibers were evaluated by tensile testing. The final drawing ratios were DR (Draw ratio) 20, DR24, and DR28. The fiber with DR28 had the highest values of 1.4 GPa for tensile strength and 18.9 GPa for Young's modulus when drawn at 180 ℃ and 1.5 mm/s and heat-treated at 150 ℃ for 1 hour. However, the fiber heat-treated at 120 ℃ for 1 night had a lower tensile strength of 1.0 GPa and Young's modulus of 1.6 GPa. In addition, when the drawing speed was increased at 120 ºC, the fibers broke. These results show that the strength and modulus of the fiber increased significantly with stretching (Figure2). However, they also suggest that heat treatment conditions and stretching speed have a strong influence on the fiber properties as well. The orientation of the fiber increased with increasing drawing ratio: for DR28, the orientation was about 93 %, a significant increase compared to 73 % for the fiber that was not heat-stretched. Although the target values could not be achieved in this study, it is expected that further improvements in drawing methods will enable the fabrication of even stronger PVA fibers with the addition of LiI.

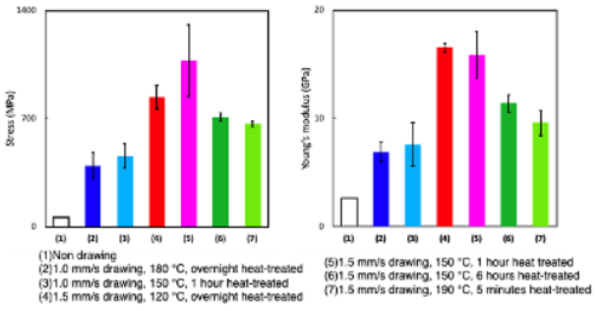


Figure 2 Tensile strength (Left) and Young's modulus (Right) of heat-stretched fibers.

PoA.19.11

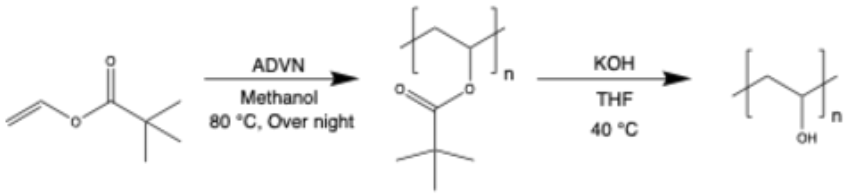
Facile preparation of hydrogel with high mechanical strength by syndiotactic rich Poly (vinyl alcohol)

Yusuke Taoka, Miyu Nakazawa, Kazuaki Matsumura

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai Nomi Ishikawa, Japan

Abstract

Poly (vinyl alcohol) (PVA) is a highly biocompatible, crystalline polymer that forms a physical cross-linked gel called PVA hydrogel (PVA-H), which is used in research on artificial cartilage and joints, and as a biomodels. When used for artificial joints, PVA hydrogel must have excellent strength and abrasion resistance. However, the commonly used atactic PVA (aPVA) has irregularly arranged hydroxyl groups on the main chain, which limits its strength. In this study, we aimed to produce high-strength PVA-H by using syndiotactic PVA (sPVA), which has excellent stereo-regularity. sPVA is not widely used because it is less-soluble in water and most organic solvents. The hot press (HP) method is a technique for producing high concentrations of PVA-H that does not require dissolution in water or organic solvents. sPVA was polymerized at 80 ºC using vinyl pivalate (VPi) with 2,2'-azobis(2,4-dimethylvaleronitrile) (ADVN) as an initiator. The resulting polymer was saponified with KOH to form PVA (Scheme 1). Distilled water and sPVA were mixed well in a 6:4 ratio, placed in a mold, and compressed at 130 ℃ for 2 MPa, 5 min, 10 MPa, 10 min, and 20 MPa, 15 min using a compressor. The gel was then allowed to stand at room temperature for 2 days to gel. It was heat treated under several conditions and compared with aPVA-H.



Scheme 1 Synthesis of sPVA

Water content was measured by swelling the completely dried gel with water for 2 days after heat treatment. sPVA showed lower water content than aPVA, even though the heat treatment conditions were similar. This is thought to be because sPVA has high stereo-regularity and is easily crystallized, resulting in many cross-linking points by micro-crystals. The elastic moduli of aPVA-H and sPVA-H with similar water content were evaluated by tensile testing. While there was no significant difference in elastic modulus at a water content of about 45 %, there was a significant difference at a low water content of about 25 %: a-PVA-H heat-treated at 200 ℃ : 31.5 MPa, s-PVA-H heat-treated at 180 ℃ : 58.8 MPa (Figure 1). This is related to the uniformity of crystal size of PVA, etc. It is highly likely that crystals of uniform size are formed in sPVA. These detailed results will be presented at the conference.

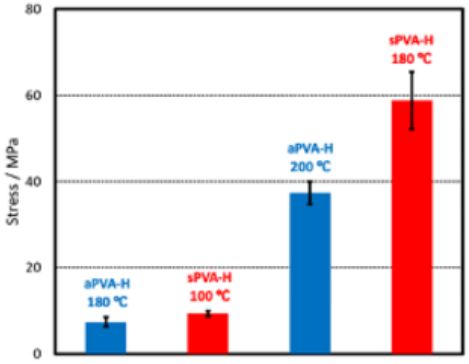


Figure 1 Comparison of elastic modulus of PVA-H

PoA.19.12

Design and fabrication of a fully biodegradable oral device for GI delivery of macromolecules

Reece McCabe, Lasse Højlund Eklund Thamdrup, Mahdi Ghavami, Anja Boisen

Technical University of Denmark, Kongens Lyngby, Denmark

Abstract

Recent developments in the field of oral devices have shown an ability to interface with the epithelium in the stomach or small intestine, which can offer the opportunity to deliver clinically relevant doses of macromolecules. These devices are often fabricated in materials which are bioinert or non-biodegradable, thus impacting the commercialisation potential. Previous studies in our research group have revolved around conceptualizing and producing elastomeric self-unfolding devices, that are capable of carrying substantial amounts of powder drug formulations. Through utilizing proximity within the small intestine, our devices seek to increase local concentration of drug formulations, to enable higher oral bioavailability. The materials for these devices need to be safe for oral dosing, whilst providing the elastic recovery to enable unfolding at the target location in the small intestine. The work presents the synthesis of a polyester-based biodegradable elastomer, and the subsequent fabrication of an oral device form factor.

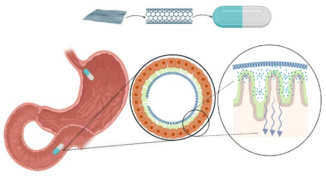


Figure 1 Upper row: Schematic showing loading, rolling, and filling of capsule with foil device. Lower row: Dosing and unfolding of foil-device, with subsequent unidirectional release into the intestinal epithelium. Schematic created using biorender.com

The polyester-based elastomer requires thermal crosslinking to be applied to the pre-polymer to create the final crosslinked polymer network. Subsequently, the moulding and casting of the pre-polymer was optimised to ensure the oral device form factor was achieved, whilst the degree of crosslinking was controlled to achieve the desired mechanical properties. The relationship between these crosslinking parameters and the mechanical properties, can be further modified by altering the thickness of the device. This allows for tailoring the force the device will exert on the epithelial tissue of the small intestine. The device activation was assessed using an ex vivo setup, in which a capsule loaded with the device, was subject to disintegration followed by the elastic recovery of the device which effectively establishes an intimate contact between the device and the epithelium. The mechanical properties of the biodegradable elastomer-based devices are demonstrated to be comparable to devices fabricated from typical non-degradable elastomers such as PDMS. These devices could be loaded with powder drug cargoes, possibly including permeation enhancers, to improve the oral bioavailability of model drugs.

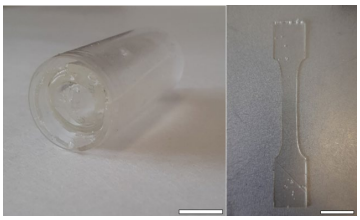


Figure 2 Left image: Prototype foil device placed into size 00 capsule (scale bar 4mm) Right image: CO2 laser cut" dogbone" sample of cast polyester elastomer for tensile testing (scale bar 13mm)

PoA.19.13

Stabilization of polyampholytes coacervates by PEGylation during phase separation behavior

Tomoka HIROSE, Kazuaki MATSUMURA

Japan Advanced Institute of Science and Technology · School of Materials Science, 1-1 Asahidai , Nomi, Ishikawa, Japan

Abstract

Polyampholytes are polymers with anionic and cationic groups in the same molecule. Their properties allow them to exhibit phase-separation behavior in response to temperature and pH, and these same properties have also been studied and applied to protein models. In particular, polyampholytes that exhibit liquid-liquid phase separation (LLPS) behavior have significant implications for understanding how RNA and other substances regulate various cellular functions *in vivo*. However, the dynamic nature of the LLPS behavior makes it difficult to quantitatively evaluate the droplet size of the coacervate. Controlling and stabilizing of droplet size of these polymers with such properties will contribute to various fields of application including biomaterials. In this report, we describe the stabilization of particle size during phase separation behavior by decorating some of the functional groups of temperature-responsive polyampholytes. A 25% ε-poly-L-lysine (PLL) solution was stirred with phthalic anhydride (PA) to carboxylate the amino groups of PLL. (Fig. 1)

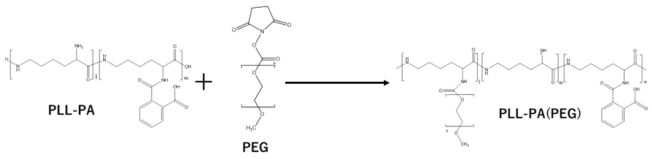


Figure 1. Structural of PLL-PA(PEG)

The polymer (PLL-PA(PEG)) was synthesized by further modifying the synthesized carboxylated phthalic anhydride-added poly(lysine) (PLL-PA) with polyethylene glycol (PEG). Sample solutions of the synthesized polymer were prepared by varying the polymer concentration and the solvents of pure water or phosphate buffered saline (PBS), respectively. The polymer composition was confirmed by 1HNMR measurement. The particle size during phase separation was evaluated by dynamic light scattering (DLS) at variable temperatures.The synthesized PLL-PA (PEG) shows lower critical solution temperature (LCST) type temperature response, which is a characteristic of PLL-PA, and the phase separation behavior depends on the polymer composition, concentration, and salt concentration in the solvent. The particle size during the phase separation was found to be stable at the same temperature. (Fig. 2)

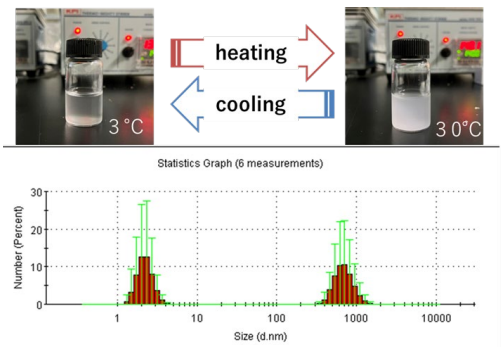


Figure 2. Polymer solution at various temperatures and particle size

PEG modification preserved the same characteristics while stabilizing the particle size. Because of its stable particle size and micelle-like structure, it is expected to be applied to protein adsorbents and other biomaterials.

PoA.19.14

Hybrid composites of polyampholytes and liquid metals as next-generation temperature-responsive DDS carriers

Tomoka HIROSE, Eijiro MIYAKO, Kazuaki MATSUMURA

Japan Advanced Institute of Science and Technology · School of Materials Science, 1-1 Asahidai, Nomi, Ishikawa, Japan

Abstract

Drug delivery systems (DDS), which are technologies to deliver drugs, including anticancer drugs, at the right time and place in the required amount, are one of the most important ideas in the field of biotechnology and pharmaceutical sciences in recent years. Thermoresponsive polymers, one of the materials used as carriers for DDS, are designed to change their structure, such as phase separation, in response to external stimuli, such as temperature, and to act on the drug. Since it is difficult to maintain a stable reaction environment for a long period of time due to the homeostatic function of the body, it is an important issue how sensitive and instantaneous the response to thermal stimuli can be. Therefore, we have designed a carrier that is independent of external thermal stimuli in terms of temperature response to drug action by preparing a composite of a thermoresponsive polyampholyte and a liquid metal with photothermal properties. In this report, we report on the preparation and evaluation of a new DDS carrier that uses the heat generated by a near-infrared laser beam to induce drug action through phase separation. A 25% ε-poly-L-lysine (PLL) solution was stirred with phthalic anhydride (PA) to carboxylate the amino groups of PLL. To further increase the affinity with liquid metal, 2-iminothiolane hydrochloride was added to thiolate a portion. The resulting polymer (PLL-PA(-SH)) was lyophilized. The composite was then dissolved in PBS solution with doxorubicin hydrochloride (DOX), an anticancer drug, and sonicated with liquid metal. The temperature response of the polymers was evaluated by measuring absorbance. The photothermal effects of the composites were evaluated using laser microscopy and thermography while irradiating with a near-infrared laser. The prepared composite was confirmed to have the ability to act on the drug by heating when triggered by near-infrared laser light irradiation and phase separating the polymers with the heat. (Fig. 1・2)

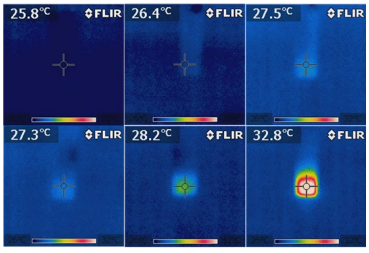


Figure. 1 Thermographic results during laser irradiation (upper: PBS solution / lower: prepared composite)

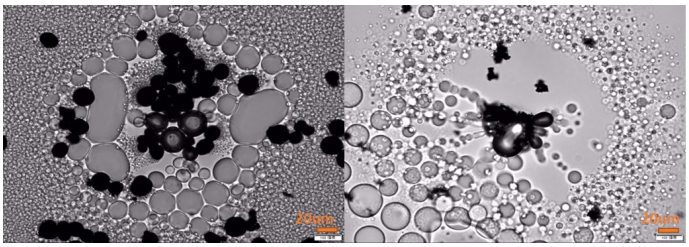


Figure 2. Characterization of composites by laser microscopy

The phase separation temperature can be controlled by the concentration of the polymer. It was also found that the temperature increase can be controlled by the output of the laser. Unlike general temperature-responsive DDS carriers, it was found to have the property of concentrating the drug. We have confirmed that the complex can effectively concentrate and act on DOX at low concentrations that are not toxic to cells.

PoA.19.15

Mechanical characterization of thermoformed and non thermoformed polyethylene terephthalate glycol and 3D printed shape memory resin used for orthodontic aligners production

Sara Drago, Marco Migliorati, Virginia Astio, Alberto Lagazzo

Università degli Studi di Genova, Genova, Italy

Abstract

Introduction and aim: The orthodontic therapy with clear aligners has encountered an increasing interest in the last decade thanks to the improvement of the applied materials and the related biomechanics and it has become a valid alternative for patients who require an invisible treatment. The conventional production technique is based on vacuum thermoforming with thermoplastic material molding on physical models of the dental casts. Recently, a novel biocompatible photopolymer material has been introduced to overcome the current limitation of thermoforming and direct 3D printing of aligners has been made possible. The aim of the present study is to provide a characterization of the mechanical properties of a conventional material and the novel resin at a given temperature setting. Materials and Method: A study was designed in which three different materials were examined: thermoformed and non thermoformed polyethylene terephthalate glycol (PETG) and Tera Harz TC-85 resin. In order to carry out the tests, two types of dog-bone were prepared: overall width 10 mm, gage width 5 mm and overall length 40 mm for the small samples; overall width 20 mm, gage width 10 mm and overall length 90 mm for the large samples; thickness: 2 mm for TC-85 samples, 1 mm for PETG samples, for a total of 32 dog-bone samples of the given materials. The Zwick/Roell Z0.5 machine (full scale range 500N) was used to apply a tensile test on the samples until rupture at room temperature (18°C). A blinded statistical analysis was performed. Data were tested for normality, and the Levene's test was used for the homogeneity of variances assessment. Differences among groups for wires were evaluated using the Kruskal-Wallis rank sum test, and multiple post-hoc comparisons were carried out with the Bonferroni method. Results are expressed as median and interquartile ranges, and differences with a p-value <0.05 were selected as significant. Data were acquired and analyzed using the R v4.2.2 software environment. Results: The elastic moduli of non thermoformed PETG, thermoformed PETG and TC-85 were 2301.20 (IR = [2267.05, 2381.53]) MPa, 2364.63 (IR = [2264.86, 2454.33]) and 1455.70 (IR = [1278.25, 1589.22]) MPa, respectively, and the stiffness of PETG was significantly higher ($p<0.001$). Further, PETG and TC-85 fractured at approximately 236% and 37% elongation, respectively. Conclusion: at room temperature TC-85 resulted as more brittle.

PoA.19.16

Silk sericin nanomaterials as carriers for therapeutic substances in cancer therapy

Catalin Zaharia[1], Ionut-Cristian Radu[1], Bianca Galateanu[2], Madalina Necolau[1], Ariana Hudita[2], Eugenia Tanasa[3], Cosmin Catalin Mustaciosu[4], Roxana Popescu[5]

[1]University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, Romania. [2]Department of Biochemistry and Molecular Biology, University of Bucharest, Bucharest, Romania. [3]University Politehnica of Bucharest, Bucharest, Romania. [4]Horia Hulubei National Institute for Physics and Nuclear Engineering IFIN-HH, Magurele-Ilfov, Romania. [5]University Politehnica of Bucharest, Faculty of Medical Engineering, Bucharest, Romania

Abstract

INTRODUCTION The modern drug delivery systems in the nanoscale range could be used to deliver therapeutic agents to specific targeted sites in a controlled manner. The use of nanoparticles in drug delivery comes with many advantages like easy incorporation of drugs and biological molecules, effective transport and release, good biocompatibility and biodegradability, accumulation in human organs without too much side effects, slow-release ability to reduce the drug toxicity, ability to pass body barriers, possibility to be used in cancer diagnosis and therapy [1]. EXPERIMENTAL METHODS Sericin nanoparticles have been developed based on the grafting functionalization of silk sericin with synthetic side chains (Poly(2-dimethylamino ethyl methacrylate)). The grafted silk protein was used to prepare small size nanocarriers by acetone nanoprecipitation. Drug loading with temozolomide and 5-fluorocuracil and release tests were performed by drug dissolution into the grafted sericin media followed self-assembling (various pH conditions and enzymatic medium). SEM and DLS were used to evaluate the nanocarriers' size, size distribution and shape. Pristine and drug-loaded nanoformulations were further tested for their *in vitro* biocompatibility and cytotoxic potential effect on human glioblastoma cells cultured in microfluidic devices. *In vivo* tests were employed on mice to evaluate the specimens' biocompatibility and organ distribution. RESULTS AND DISCUSSION Silk sericin-based particles have shown narrow size distribution in nanometric range. Temozolomide and 5-fluorouracil release tests revealed an expected behavior considering the pH conditions and enzymatic medium. The biological assessment revealed that the pristine nanoparticles did not display cytotoxic effects on the cells, while the drug loaded nanoformulations exerted similar toxic effects as the drugs alone. *In vivo* test showed good biocompatibility for the silk sericin nanocarriers with a nice organ distribution. CONCLUSION In conclusion, we reported here the preparation of novel silk sericin nanoformulations able to deliver temozolomide and 5-fluorouracil drugs with potential benefits for brain cancer (glioblastoma). The nanocarriers were successfully investigated in terms of morphology, size distribution, drug release behavior and biological assessment (both *in vitro* and *in vivo*).

REFERENCES 1. Cheng, Z., Li, M., Dey, R. et al, Nanomaterials for cancer therapy: current progress and perspectives. J Hematol Oncol, 14, 85 (2021). https://doi.org/10.1186/s13045-021-01096-0.ACKNOWLEDGMENTS This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PN-III-P4-ID-PCE-2020-1448, within PNCDI III.

PoA.19.17

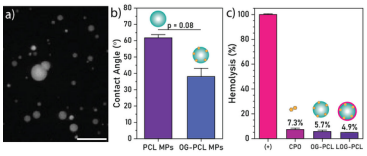
Polymeric oxygen-generating microparticles as enhanced synthetic erythrocytes

Francisca L. Gomes[1,2,3], Roger Mora[2], Jasper van Weerd[3], Pascal Jonkheijm[2], Jeroen Leijten[1]

[1]Leijten Lab, Dept. of Developmental BioEngineering, Faculty of Science and Technology, Technical Medical Centre, University of Twente, Enschede, Netherlands. [2]Dept. of Molecules and Materials, Laboratory of Biointerface Chemistry, Faculty of Science and Technology, MESA+ Institute for Nanotechnology, University of Twente, Enschede, Netherlands. [3]LipoCoat BV, Enschede, Netherlands

Abstract

Introduction Blood is a life-saving resource in patient care and research. However, since blood banks depend entirely on donations to obtain blood, supply is often not enough to meet demand. To prevent shortages, synthetic blood substitutes are explored.[1] Recently, our group produced hydrophobic oxygen-generating particles based on calcium peroxides (CaO_2, or CPO), a compound which releases H_2O_2 - and subsequently O_2 - through enzymatic action in aqueous media. Particles sustainably released O_2 over 12 days and maintained cell viability[2], showing promising results as potential erythrocyte substitutes. However, their hemocompatibility remains largely unexplored. In this work, we produced oxygen-generating polycaprolactone microparticles at sub-5 µm sizes with an erythrocyte membrane-mimicking lipid coating. We determined their blood response *in vitro* to assess their potential as synthetic blood cells. **Methods** Oxygen-generating CPO was dispersed in a Pluronic® F68 solution and incorporated into polycaprolactone microparticles (PCL MPs) through a double emulsion solvent evaporation method, producing OG-PCL MPs. An erythrocyte membrane-inspired lipid coating was further applied to these particles using a solvent gradient-based lipid coating method developed in-house, producing LOG-PCL MPs. Particle characterization was performed through dynamic light scattering, water contact angle measurements, and fluorescence and holotomography microscopies. Hemolysis assays were conducted by incubating particles with human erythrocytes at 37°C (4 h) and quantifying hemoglobin release. **Results and Discussion** Oxygen-generating CPO suspensions of average hydrodynamic sizes below 2 µm were prepared. After incorporation into OG-PCL MPs, results revealed non-hollow particles (Fig.1a) with reduced contact angle values (Fig.1b), suggesting CPO assembles at the surface of the hydrophobic particles. The lipid coating on LOG-PCL MPs was confirmed through fluorescence microscopy. Preliminary hemocompatibility results indicate both polymer and lipid coating slightly reduce CPO-induced hemolysis, indicating a double hemoprotective effect (Fig.1c). Figure 1. *a) Reconstructed holotomography image of OG-PCL MPs. Scale bar equals 5 µm. b) Contact angle measurements of air-dried films of PCL and OG-PCL MPs. c) Hemolysis of CPO, OG-PCL, and LOG-PCL MPs.*



Conclusions We describe a novel, bio-inspired and self-oxygenating synthetic erythrocyte, with promising preliminary data on blood response *in vitro*. Ongoing and following work will focus on coagulation, platelet activation, immune marker analysis and injectability studies for *in vivo* testing. **Acknowledgements** F.L.G. acknowledges the TechMed Donor Service of the University of Twente and its donors. Authors acknowledge financial support from Health~Holland (Project LSHM19074).

References

1 Charbe, N. B. *et al. Blood Reviews* 54, 100927 (2022).
2 Farzin, A. *et al. Advanced Functional Materials* 31, 2100850 (2021).

PoA.19.19

A modular microfluidic platform for development of biomedical fiber materials

Kongchang Wei[1,2], Wuchao Wang[1], Khushdeep Sharma[1], René M. Rossi[1], Luciano F. Boesel[1]

[1]Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biomimetic Membranes and Textiles, St. Gallen, Switzerland. [2]Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biointerfaces, St. Gallen, Switzerland

Abstract

Fiber materials play an important role in modern healthcare. Compared to other fiber production methods, such as melt spinning and electrospinning, microfluidic wet spinning (MWS) represents a unique fiber processing technology, featuring milder conditions (e.g., low temperature, no voltage) for preservation of bio-derived structures and bioactive components.(1) In addition, MWS offers excellent control over the compositional and structural properties of fibers, owing to the high-precision fluid manipulation enabled by modern microfluidic pumping systems.(2) The "wet" nature and mild processing conditions make MWS particularly attractive for in situ encapsulation of therapeutic cargoes (e.g. proteins and living cells) and biosensing elements (e.g. enzymes and nanoparticles) in fiber materials for various biomedical applications.(3,4) For this purpose, different types of microfluidic devices have been developed for manipulating fluid flows in microscale, which is a prerequisite for the production of microfibers via MWS.5 However, microfluidic devices require complicated and tedious design and fabrication procedures, or rely on costly instrument such as 3D printers and clean-room facilities; or are prone to damage, clotting, and contamination, hence difficult to be re-used. To address these limitations of existing microfluidic devices for MWS, we developed a modular microfluidic platform via facile assembly of capillaries and nozzles in PDMS elastomers. In this contribution, we will introduce the development of such a modular microfluidic platform (Figure 1A-B), and its precise control over the laminar flows of polymer solutions (Figure 1C), thereafter examples of polymer fibers produced with such a MWS platform and their potential biomedical applications will be given.

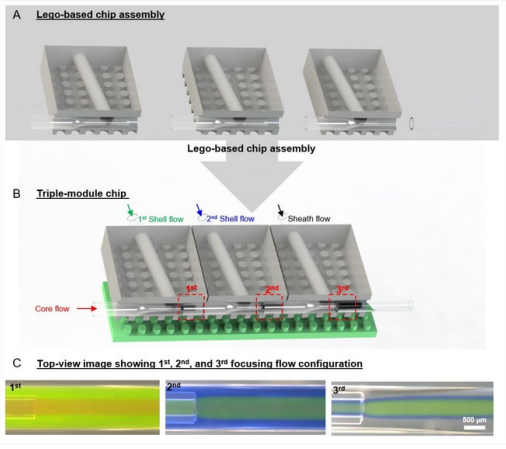


Figure 1. The assembly of a tri-module microfluidic chip (A) with triple coaxial focusing flow configurations (B-C).

Acknowledgements The authors acknowledge funding by the Swiss National Science Foundation (SNSF) and Innosuisse BRIDGE Discovery funding opportunity (project number 40B2-0_180983) and by the SNSF Spark grant (CRSK-2_190493).

References (1) Cheng, J.; Jun, Y.; Qin, J.; Lee, S. H. *Biomaterials* **2017**, 114, 121–143. (2) Wei, K.; Toncelli, C.; Rossi, R. M.; Boesel L. F. (2022) Hydrogel Fibers Produced via Microfluidics. In Ricardo A. Pires, Iva Pashkuleva, Rui L. Reis (Eds.) Multifunctional Hydrogels for Biomedical Applications, pp. 233-274. Wiley-VCH GmbH. (3) Filippi, M.; Buchner, T.; Yasa, O.; Weirich, S.; Katzschmann, R. K. *Adv. Mater.* **2022**, 34, 2108427. (4) Jun, Y.; Kang, E.; Chae, S.; Lee, S. H. *Lab Chip* **2014**, 14 (13), 2145–2160. (5) Du, X.; Li, Q.; Wu, G.; Chen, S. *Adv. Mater.* **2019**, 31 (52), 1903733.

PoA.19.20

Doxorubicin-loaded chitosan-copper microspheres for the treatment of bone tumor

Andrea Lončarević[1], Sandra Clara Trujillo[2,3], Leonard Bauer[1], Gloria Gallego Ferrer[2,3], Anamarija Rogina[1]

[1]University of Zagreb, Faculty of Chemical Engineering and Technology, 10000 Zagreb, Croatia. [2]Centre for Biomaterials and Tissue Engineering (CBIT) Polytechnic university of València, 46022 Valencia, Spain. [3]Biomedical Research Networking Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), 46022 Valencia, Spain

Abstract

The development of less invasive smart materials as drug delivery systems has emerged from the need for advanced procedures of tumor treatment. The targeted drug delivery ensures localized treatment of the tumor's tissue minimizing the exposure of the whole body [1]. Biocompatible and biodegradable materials such as pH-sensitive chitosan-based carriers are exceedingly promising systems for the encapsulation and simultaneous release of the antitumor drug and therapeutic metal ions. This work focuses on the development of metal-ion assisted drug carriers loaded with a lower dosage of the antitumor drug. The incorporation of copper (II) ions into the chitosan matrix can be achieved through complexation reactions with functional amino- and hydroxyl- groups of chitosan, resulting in physically crosslinked chitosan microgels [2, 3]. Chitosan-copper complex microspheres with a size between 20 and 60 µm were obtained by simple complexation chemistry from emulsion. The amount of copper (II) ions in chitosan microspheres was chosen to be slightly cytotoxic. The efficient loading of doxorubicin (Dox) was achieved in phosphate buffer solution at pH 6, while the Dox release in cell culture medium was measured using fluorescence analysis. Different concentrations of Dox-loaded microspheres (0.5 – 1.0 mg/mL) indicated quick drug release during the first 24 h followed by a slow release up to 72 h. The cytocompatibility assay on human mesenchymal stem cells (hMSCs) and MG-63 osteosarcoma cells indicated a cytotoxic effect of unloaded microspheres at higher concentrations. Furthermore, Dox-loaded chitosan-copper microspheres showed increased cytotoxicity towards the osteosarcoma cells with respect to free Dox and unloaded microspheres, while decreased toxic effect was observed on hMSCs during 3 days of culture. The synergy of chitosan-copper complex microspheres and doxorubicin can provide potential drug delivery systems in less invasive bone tumor treatment.

[1] H. Wang, D. J. Mooney, Nature Mater., 2018, 17, 761–772.

[2] M. Rhazi, J. Desbrieres, A. Tolaimate, M. Rinaudo, P. Vottero, A. Alagui, M. El Meray, Eur. Polym. J., 2002, 38, 1523–1530.

[3] J.R.B. Gomes, M. Jorge, P. Gomes, J. Chem. Thermodyn., 2014, 73, 121–129.

PoA.19.21

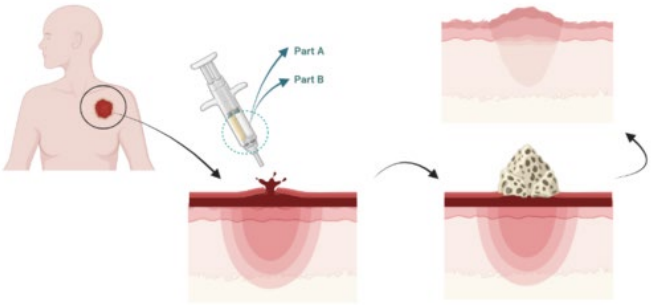
Antimicrobial self-conforming silicone-based sponge for rapid hemostatic treatment

Pritha Sarkar, Kausik Mukhopadhyay

University of Central Florida, Orlando, USA

Abstract

Hemorrhage remains the main cause of preventable death on the battlefield. According to a statement by the US Army Medical Research and Development Command in 2022, nearly 50% of combat deaths have been due to exsanguinating hemorrhage. Of those, about half could have been saved if timely, appropriate care had been available. This underscores the need to develop appropriate FDA-approved hemostatic treatments that can effectively stanch blood loss while being easily applicable at the point-of-care, before professional medical care arrives. While external wound injury can be treated mostly by visual inspection, internal hemorrhages are often much more intractable, causing regular hemostatic dressings to fall short because of deep wounds and obscure points of injury. The need to treat trauma wounds accompanied by severe bleeding requires an immediate solution that can be applied by individual soldiers in the field swiftly and efficiently amidst operations. Although there has been considerable development in recent years in engineering novel hemostats, developing an effective hemostatic material that is biocompatible, fast-acting, durable, with hassle-free application and removal, all while remaining an economically viable option remains a challenge. In our current study, we report a silicone-based hemostatic bandage system that is both antibacterial and self-expanding. The two-component mixture of said hemostatic system chemically reacts in situ to form a stretchable sponge that generates autogenous pressure on the wound to control and arrest bleeding. The hemostatic sponge acts as a 'tamponade' by expanding rapidly and arresting bleeding within a matter of minutes and can be removed without any signs of tissue, muscular or vascular damage. ***Fig. 1:*** *Schematic representation of hemostatic sponge action*



Further, the silicone-polyethylene oxide block copolymer component in the matrix serves to modify the hydrophobicity of the sponge, making it hydrophilic in an aqueous environment. Support data include characterization of the polymers and the hemostatic sponge formed upon reaction. To further assess the efficacy of the sponge, studies on rheological properties, adhesion, hydrophobicity/hydrophilicity, antimicrobial assays, and *in vitro* assays will also be presented. The objective of this novel hemostatic agent is to provide the injured with a means to rapidly stagnate bleeding from external and internal wounds in a manner superior to those currently available. This unique formulation presents an easy and economical approach to a bandage system with spontaneous self-expanding properties that can also remain functional in inclement weather conditions.

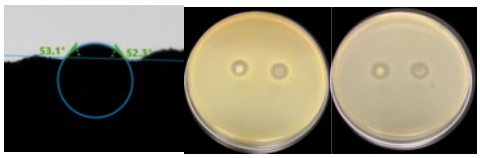


Fig. 2(a): *Sponge hydrophilicity;* ***(b)*** *Antibacterial activity against gram-positive and gram-negative bacteria respectively.*

PoA.19.22

In vivo biocompatibility and tissue response of poly(glycerol sebacate urethane) scaffolds

Andreas Samourides[1], Zacharoula Xenou[1], Despoina Kokkinidou[1], Kyriakos Spanoudes[2], Konstantinos Kapnisis[1], Andreas Anayiotos[1]

[1]Cyprus University of Technology, Limassol, Cyprus. [2]University of Nicosia School of Veterinary Medicine, Nicosia, Cyprus

Abstract

INTRODUCTION Tendor repair scaffolds should be highly tunable, and exhibit properties such as interconnected porous structure, be biocompatible, biodegradable and ideally to degrade linearly at the same rate as the tissue develops 1. Poly(glycerol sebacate urethane) (PGSU) was recently developed and fabricated as a porous scaffold, and it was found to be angiogenic and promote tissue ingrowth *in vitro*, demonstrating high potential in the field of soft tissue engineering. In this study, three PGSU scaffolds, with different mechanical and microstructure properties were fabricated and investigated for *in vivo* for their microstructure, biocompatibility and their ability to degrade at the same rate as tissue develops into the scaffold. MATERIALS AND METHODS PGSU scaffolds were fabricated with hexamethylene diisocyanate (HDI) ratios of 0.8 and 1.0 and polymer concentrations (w/v%) equal to 10% and 15%. Briefly, the PGS pre-polymer was dissolved in 1,4-dioxane at the required w/v concentration and HDI was added at 0.8 or 1.0 ratio to glycerol. The solution was left to react for five hours at 55oC. The solution was then frozen and freeze dried for 16 hours. The scaffolds were characterized for their microstructure using scanning electron microscopy. *In vivo* biocompatibility of the scaffold was investigated by subcutaneous implantation in CD1 albino mice for six weeks. Sixteen mice in total had scaffolds implanted subcutaneously, 12 of which were implanted with four scaffolds of each sample group and the rest of them with Ethilon® Nylon suture 4-0 spheres (positive control). RESULTS AND DISCUSSION It was found that the previously open pore microstructure was filled with new tissue, demonstrating uniform tissue ingrowth. Despite the tissue ingrowth the mass of the scaffolds did not change, but the density increased significantly demonstrating that the scaffolds became less porous (denser) while remaining at the same mass, which demonstrates *in vivo* biodegradation. No inflammatory response was observed during the *in vivo* imaging, which shows that the scaffolds are biocompatible and their degradation by-products do not cause any additional immune response.

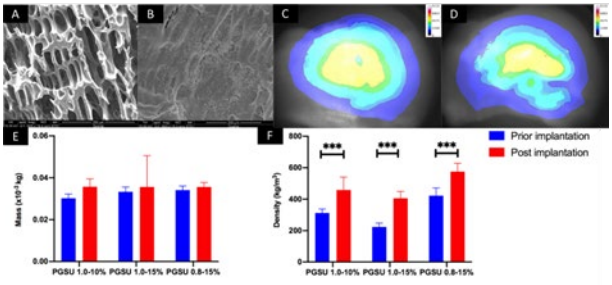


*Figure 1: (A) Representative cross section microstructure of a PGSU scaffold prior implantation, (B) and 42 days post implantation; (C) whole body imaging of the mouse with the positive control, (D) and of the mouse with PGSU scaffold 42 days post implantation; (E) mass and (F) density of the PGSU scaffolds prior and post implantation. *** when p < 0.001.*

PoA.19.23

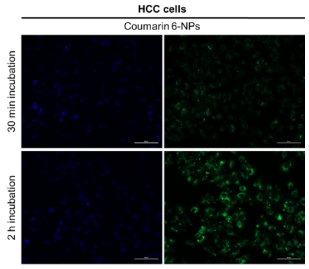
Development of multi-kinase inhibitor encapsulated polymeric nanoparticles for potential treatment of hepatocellular carcinoma

Mattika Thaweesuvannasak, Chatchawan Srisawat, Vorapan Sirivatanauksorn, Nathachit Limjunyawong, Primana Punnakitikashem

Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract

Hepatocellular carcinoma (HCC) is the most prevalent form of liver cancer, being the third leading cause of cancer-related mortality worldwide. Lenvatinib is an orally administered, multi-targeted tyrosine kinase inhibitor (TKI) that has recently been approved as a first-line chemotherapeutic agent for HCC treatment, demonstrating significant advancements over sorafenib in terms of overall survival. However, the clinical use of lenvatinib has been significantly constrained due to its limited solubility and low bioavailability. Also, maintaining a consistent concentration of the drug within tumor tissue at levels sufficient to inhibit tumor growth remains challenging. Consequently, the development of nanocarriers is crucial for the sustained delivery of therapeutics to HCC cells. Polymeric nanoparticles have shown promise in drug delivery, particularly those comprising biodegradable, biocompatible, and non-toxic polymers. Hence, our study aims to develop and optimize a drug delivery system utilizing lenvatinib encapsulated polymeric nanoparticles. We hypothesized that this system can sustained release and inhibit HCC progression. Lenvatinib encapsulated polymeric nanoparticles were synthesized by emulsification solvent evaporation technique, optimized the drug encapsulation efficiency by varying the ratio of nanoparticles and drug. Nanoparticles were characterized for size and zeta potential through dynamic light scattering. To investigate cellular uptake of nanoparticles, fluorescent marker coumarin 6 was encapsulated in polymeric nanoparticles for visualization. CellTiter-Blue® cell viability assay was performed to determine the cytotoxic effect of lenvatinib encapsulated polymeric nanoparticles in HCC cells *in vitro*. This finding showed that different ratio of nanoparticles and drug resulted in different nanoparticle size and drug encapsulation efficiency. In addition, zeta potential results were not significantly different in all ratios. The optimized ratio between nanoparticles and drug was 10:1 (w/w). Average hydrodynamic diameters of unencapsulated and lenvatinib encapsulated polymeric nanoparticles were 203.00±22.90 and 215.00±22.80 nm, respectively. Polymeric nanoparticles encapsulated lenvatinib with efficiency of 56.12±2.48%. *In vitro* cellular uptake studies revealed that polymeric nanoparticles encapsulated with coumarin 6 demonstrated efficient uptake by HCC cells in a time-dependent manner (Figure 1). Furthermore, lenvatinib encapsulated polymeric nanoparticles showed an anticancer effect on the human HCC cell line with significantly lower cell viability compared to that of control. In summary, our findings presented a promising strategy using TKI-encapsulated polymeric nanoparticles as a drug delivery system for the treatment of HCC.



***Keywords*—**Drug delivery system, hepatocellular carcinoma, polymeric nanoparticles, tyrosine kinase inhibitor.

Figure 1. *In vitro cellular uptake of coumarin 6 encapsulated nanoparticles (coumarin 6-NPs) by HCC cells after 30 min and 2 h incubation.*

PoA.20.02

Annealing high aspect ratio microgels into macroporous 3D scaffolds allows for higher porosities and effective cell migration

Alisa Celina Suturin[1,2], Andreas Josef Dietrich Krüger[1,3], Kathrin Neidig[1], Nina Klos[1], Michelle Bund[1], Nina Dolfen[1], Till Gronemann[1], Rebecca Sebers[1], Anna Manukanc[1], Ghazaleh Yazdani[1], Yonca Kittel[1,3], Dirk Rommel[1,3], Tamas Haraszti[1,3], Jens Köhler[1,3], Laura De Laporte[1,3,4]

[1]DWI – Leibniz-Institute for Interactive Materials, Aachen, Germany. [2]Institute of Technical and Macromolecular Chemistry (ITMC) Polymeric Biomaterials, Aachen, Germany. [3]Institute of Technical and Macromolecular Chemistry (ITMC)Polymeric Biomaterials, Aachen, Germany. [4]Advanced Materials for Biomedicine (AMB), Institute of Applied Medical Engineering (AME), Aachen, Germany

Abstract

Growing millimeter-scaled functional tissue remains a major challenge in the field of tissue engineering. To achieve this, injectable hydrogels are designed with the goal to support cells that work together in an orchestrated manner to form new tissue and replacing the hydrogel matrix with their own extracellular matrix. As conventional hydrogels usually have submicron pore sizes, cell migration and in-construct cell-cell contact is often restricted. Therefore, microporous annealed particles (MAPs) are emerging as promising porous biomaterials that are formed by the assembly of microgel building blocks. To further control the pore size and increase the overall MAP porosity of mechanically stable scaffolds, we have developed highly porous scaffolds by chemically interlinking rod-shaped microgels with high aspect ratios of up to 20. Polyethylene glycol (PEG) based microgels (width 10 µm, length 50 to 200 µm) are produced via in-mold polymerization and covalently interlinked into stable 3D scaffolds via epoxy-amine chemistry using an external interlinking agent. For the first time, MAP porosities can be enhanced by increasing the microgel aspect ratio from 5 to 20, with increased porosities from 65 up to 90% and mean pore sizes from 39 to 82 µm, respectively. These overall porosities are significantly higher compared to constructs made from spherical or lower aspect ratio rod-shaped microgels. Cells rapidly migrate and fill these scaffolds as shown with murine and human primary fibroblasts. Overall, this study shows that highly porous, stable macroporous hydrogels can be created, resulting in large empty volumes for facile cell invasion and cell-cell interactions. The significantly smaller partial volume of synthetic material will leave smaller voids when degraded, thus maintaining scaffold stability while promoting tissue formation. Therefore, we believe that these MAPs made from high-aspect-ratio microgels present a promising bottom-up material system for future application in tissue regeneration.

PoA.20.03

3D-Printed nanoporous scaffolds for engineering T cell activation and transduction

Morteza Aramesh, Lotte Duijn, Cecilia Persson

Uppsala University, Uppsala, Sweden

Abstract

Immunotherapy is a promising approach for treating cancer by manipulating the immune system to recognize and attack cancer cells [1]. To improve the effectiveness of immunotherapy, researchers are exploring the use of engineered materials that interact with cells at the molecular and cellular level. Recent studies have shown that the porosity of a material surface can affect the behavior of cells that come into contact with it. Specifically, inducing surface porosity can lead to the formation of microvilli, thin protrusions from the cell surface that can influence gene expression patterns and enhance T cell activation [2]. Building on these findings, we have developed 3D nanoporous scaffolds using polymer-based materials that are biocompatible and biodegradable (e.g. polycaprolactone). The scaffolds have controlled micro-/nanoporous surface topography, achieved through controlled formation of porogens in the bioink prior to printing. The induced porosity in the structure contributes significantly in enhancing T cell activation and induction yields by increasing the surface area available for cell-material interactions as well as confinement of the cell's microvilli. Direct ink printing enables the printing of 3D structures directly inside well-plates, allowing for more accurate comparison with well-plate-based activation and induction assays. We cultured T cells on the nanoporous scaffolds and found that they exhibited increased gene expression of key T cell activation markers (CD69 and CD25), improved cytokine production (IL-2), and enhanced cellular uptake (up to 10 times higher). The enhanced lentiviral uptake by the cells, induced by the surface topography of the material, leads to a better efficiency in CAR-T cell production. This finding suggests that 3D printed nanoporous scaffolds have the potential to be used for developing personalized treatments *in vitro*. The use of nanoporous scaffolds in immunotherapy holds great promise for the future of cancer treatment. The 3D microenvironment provided by these scaffolds can improve the activation and induction yields of T cells, allowing for more effective immunotherapy. Furthermore, the biocompatibility and biodegradability of the polymer materials used in the scaffolds make them suitable for use as implants in cancer therapy. By tailoring the structures to an individual's immune system, researchers could potentially develop personalized treatments that are more effective and have fewer side effects.

[1] Fesnak AD, June CH, Levine BL. Engineered T cells: the promise and challenges of cancer immunotherapy. Nat.Rev.Cancer.2016,16(9):566.doi:10.1038/nrc.2016.97.

[2] Aramesh M, et al. Nanoconfinement of microvilli alters gene expression and boosts T cell activation. PNAS.2021,118(40):e2107535118.doi:10.1073/pnas.2107535118.

PoA.20.04

The comparison of the effect of fetal bovine serum, human platelet lysate and hyperacute serum on growth and differentiation of human adiposed tissue-derived mesenchymal stem cells

Eva Filová[1], Věra Hedvičáková[1], Veronika Hefka Blahnová[1], Aiva Simaite[2], Monika Šupová[3], Håvard Jostein Haugen[4], Olga Kuteń Pella[5]

[1]Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic. [2]InoCURE s.r.o., Prague, Czech Republic. [3]Institute of Rock Structure and Mechanics of the Czech Academy of Sciences, Prague, Czech Republic. [4]Corticalis AS, Oslo, Norway. [5]OrthoSERA GmbH, Krems, Austria

Abstract

Biomaterials for bone regeneration should support growth and osteogenic differentation of mesenchymal stem cells (MSCs). Physico-chemical properties of the scaffold and bioactive compounds affect cell behaviour. We have tested the effect of medium supplements - foetal bovine serum (FBS), platelet lysate and hyperacute serum on osteogenic differentation of adiposed tissue-derived MSCs (AT-MSCs) cultured on fibrous scaffolds from polycaprolactone/polyethylene oxide (PCLPEO) and bioapatite as the first step before the preparation of bioactive composite scaffold. PCLPEO, ratio 3:1 (w/w) and PCLPEOBioapatite nano/microfibre scaffolds containing 2.2wt% bioapatite from porcine bones, were prepared using InoSpin modular electrospinning device (Inocure, Czech Republic). Elemental analysis was performed using EDS, bioapatite was characterized using AT-FTIR analysis. Adiposed tissue derived mesenchymal stem cells (AT-MSCs) (64,500 cells/cm^2) were seeded on the PCLPEOBioapatite scaffolds and cultured in different culture media. Growth medium contained 10% FBS (GF), or 5% platelet lysate with 14 µL/mL heparin (GL, 5000 IU/mL) or 10% hyperacute serum (obtained from OrthoSera, Austria, GH). Osteogenic supplements (10 mM beta-glycerophosphate, 50 µg/mL ascorbate-2-phosphate and 100 mM dexamethasone) were added into osteogenic medium containing FBS (OF), platelet lysate and heparin (OL) or hyperacute serum (OH). Cells were visualized using $DiOC_6$ and propidium iodide staining and confocal microscopy on day 1 and 14. We tested metabolic activity, dsDNA content, and osteogenic differentiation by alkaline phosphatase activity (ALP) on days 7, 14, 21, 38, by alizarin red staining on days 21 and 28 and by collagen type I immunostaining and subsequent confocal microscopy visualization on day 35. Both metabolic activity and DNA quantification showed significantly increased cell proliferation in osteogenic medium in most groups compared to growth medium. ALP was significantly increased in osteogenic medium with OL and OH. Slight improvement was seen on PCLPEOBioapatite compared to control PCLPEO. Mineralization in alizarin red staining was significantly improved by OL and OH medium on PCLPEO scaffolds. Collagen type I synthesis was stimulated mainly on PCLPEOBioapatite scaffolds in growth medium in GL and GH groups. Both platelet lysate and hyperacute serum supported osteogenic differentiation of human AT-MSCs on both PCLPEO and PCLPEOBioapatite scaffolds.

The project was supported by EU Horizon 2020 MSCARISE-2018-Research and Innovation Staff Exchange programme project “iP-OSTEO” under grant agreement 824007.

PoA.20.06

Investigating novel multi-functional coatings via LbL assembly for their use in bone tissue scaffold development to improve the mechanical integrity of open cell structures and enhance bone defect reparation through the incorporation lof therapeutic agents.

Aoife McFerran, Patrick Lemoine, Brian J. Meenan, Jonathan G. Acheson

Ulster University, Belfast, United Kingdom

Abstract

With over 2.2 million grafting procedures performed annually worldwide and the incidence increasing by 13% each year[1], bone defect reparation is rapidly becoming one of the most common procedures in bone regenerative medicine. Although autografts are the current gold standard[2] the limiting factors surrounding this technique highlight the need for an 'off-the-shelf' tissue engineered solution. Tissue engineered (TE) scaffolds must match the physico-mechanical properties exhibited by bone to achieve a biomimetic system; these properties are often sacrificed to meet other structural demands. Through the deposition of a thin film coating via Layer-by-Layer assembly (LbL), it is possible to advance and functionalise scaffold properties. Single function coatings have been studied previously for their use in bone tissue scaffolds, providing improved mechanical integrity or allowing for the incorporation of various reagents. Combining these functions to create a novel multifunctional coating has not been studied in detail and the successful creation of such a coating could transform TE scaffolds for bone defect reparation. Polyethylenimine (PEI), polyacrylic acid (PAA) and cloisite Na+ nanoclay (NC) have been utilised for mechanical reinforcement of porous structures[3]. However, PEI has shown to exhibit cytotoxic properties[4] so finding a biocompatible alternative is essential. Incorporating a therapeutic agent, like an antibiotic, into a coating for bone tissue repair would substantially reduce the risk of infection at the wound site and enhance the healing process. This work investigates the use of multiple LbL functionalised coatings to improve the mechanical integrity of open cell structures and produce a system with substantially lower cytotoxicity than a PEI-containing coating. A model drug was utilised to examine the incorporation and release from the multilayers simulating a potential therapeutic. The demonstration of this 'brick-by-brick' hypothesis by successfully building these multilayers to create a coating with dual functionality proves that it is possible to deposit and interchange layers to produce tailored systems for a wide variety of biomedical applications.

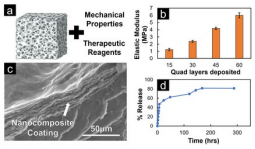


Figure 1 *a) Schematic diagram of project aim, b) elastic modulus of coating as number of quad layers increase, c) SEM of a 60 quad layer coated sample and d) the release profile of methylene blue from a 30 quad layer sample.*

References

1. Archunan, M. W. et al. Cureus 13, e17705 (2021).
2. Wang, W. et al. Bioactive Materials 2, 224–247 (2017).
3. Acheson, J. G. et al. Surface & Coatings Technology 458, 129335 (2023).
4. Khansarizadeh, M. et al. Human & Experimental Toxicology 35, 377–387 (2016).

PoA.20.07

Biopolymeric cryogels based on alginate-gum arabic polysaccharides as biodegradable macroporous scaffolds

Alexandra Feraru[1,2], Zsejke Réka Tóth[1,2], Marieta Mureșan-Pop[2], Monica Baia[3,4], Tamás Gyulavári[5], Emőke Páll[6], Klára Magyari[2], Lucian Baia[2,3,4]

[1]Doctoral School of Physics, Babes-Bolyai University, M. Kogălniceanu 1, 400084, Cluj-Napoca, Romania. [2]Nanostructured Materials and Bio-Nano-Interfaces Center, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babes-Bolyai University, T. Laurian 42, 400271, Cluj-Napoca, Romania. [3]Faculty of Physics, Babes-Bolyai University, M. Kogălniceanu 1, 400084, Cluj-Napoca, Romania. [4]Institute for Research-Development-Innovation in Applied Natural Sciences, Babes-Bolyai University, Fântânele 30, 400294, Cluj-Napoca, Romania. [5]Department of Applied and Environmental Chemistry, University of Szeged, Rerrich B. sqr. 1, 6720, Szeged, Hungary. [6]Faculty of Veterinary Medicine, University of Agricultural Science and Veterinary Medicine, 400372, Cluj-Napoca, Romania

Abstract

Although it is of great interest due to its biodegradability, biocompatibility, and non-toxic effect, the physicochemical properties obtained from the interaction between anionic polysaccharides are still under investigation. Our goal is to optimize the physical and biological properties of biocomposites based on the alginate-gum arabic targeting the development of innovative cryogels that can be successfully involved in the regeneration of soft tissues, wound management, or controlled drug release systems. The study was conducted in the form of a series of experiments and investigations, one of the focuses being the *in vitro* assessment of alginate-gum arabic composites. The cryogel scaffolds based on alginate-gum arabic (Alg-xGA, x=0; 10; 16; 26 wt%) were carried out by the ionic cross-linking method with CaCl2 solution followed by two lyophilization cycles, and their properties were investigated. The cross-linking was demonstrated by FT-IR, Raman, and nuclear magnetic resonance (NMR) spectroscopy. Porosity, swelling ratios, water content, degradation rate, and cell viability (using human fibroblasts) investigation were also performed. Moreover, *in vitro* bioactivity was assessed 42 days after introducing the samples into the simulated body fluid (pH=7.4) at 37 °C, where the apatite formation was demonstrated by using FT-IR and X-ray diffraction measurements. In FT-IR spectra we notice a difference in the shapes and relative intensity of the bands, as the gum arabic content increases, a change that can be associated with the cross-linking. The bands with a gradual decrease in relative intensity in the saccharide region show the effect of the gum arabic on the alginate structure. The cryogels present a porous and interconnected structure that significantly influenced the development of hydroxyapatite. Also, with the addition of GA, improvements in water uptake and biodegradation of the composites are evidenced. The viability of the human fibroblast cell line indicates that the composites are not cytotoxic, proving cellular biocompatibility. The study described here constitutes progress in the development of cryogels based on anionic polysaccharides that have proven an improvement *in vitro* bioactivity.

Acknowledgments

This work was supported by a grant of the Ministry of Research, Innovation and Digitalization, CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2021-2176, within PNCDI III. A.F. acknowledges the financial support of the Doctoral School of Physics, Babeș-Bolyai University by awarding the doctoral grant.

PoA.20.08

Combining phase separation and particulate leaching with 3D printing to fabricate porous scaffolds for bone tissue engineering applications.

Mehmet Serhat Aydin, Theo Jean Luciani, Samih Mohamed-Ahmed, Kamal Mustafa, Ahmad Rashad Elsebahy

University of Bergen, BERGEN, Norway

Abstract

Introduction 3D printing has attracted great attention in recent years to fabricate complex geometries at low cost and high speed. However, conventional 3D printing by itself is incapable to induce micropores within the deposited polymer filament which may affect cell responses. The aim of this study is to print 3D polycaprolactone (PCL) scaffolds with internal multiscale porosity at low temperature (LT) combined with salt leaching to improve cellular activity of printed scaffolds. **Methods** Briefly, 30% PCL solutions were prepared by dissolving PCL pellets (Mw: 80 kDa, Sigma Aldrich) in acetone. Sodium chloride (NaCl) particles (40-90 µm) were added into the polymer solution. Prepared solutions with and without NaCl were printed in a coagulation bath of absolute ethanol at 30 ºC. Next, scaffolds were washed in deionized water (DI) to leach out the salt. Scaffolds were imaged with stereomicroscopy to assess printability and shrinkage. Micro-Computed tomography (Micro-CT) and scanning electron microscope (SEM) were performed for morphological analysis. The effect of the porosity on the mechanical properties was evaluated by a tensile test. To evaluate cellular responses, human bone marrow-derived mesenchymal stem/stromal cells (hBMSCs) were cultured on the scaffolds and their viability, attachment, morphology, and proliferation. **Results** Micro-CT and SEM images showed that the porosity induced by the salt leaching step increased with increasing the salt content. Structure thickness reduced with elevating NaCl content whereas structure separation (pore size) did not change significantly with respect to the salt concentration. Mechanically, the dog bone-shaped specimens exhibited high ductility under tension. Toughness, ductility, strength, and stiffness decreased with increasing porosity/leached salt content. Regardless of the salt concentration, all scaffolds showed excellent cytocompatibility. Cells were able to attach on the surface of the scaffolds and grow up to 14 days. SEM images of the seeded scaffolds showed substantial increase in the formation of extracellular matrix (ECM) network and elongation of the cells. **Discussion and Conclusion** The study demonstrated the ability of combining 3D printing and solvent casting and particulate leaching methods together to fabricate multi-porous PCL scaffolds. The scaffolds were successfully printed in ethanol with various salt content without negatively affecting cell responses. Printing multi-porous thermoplastic polymer at low temperature could be of great importance for drug/growth factor and cell delivery for bone tissue engineering.

PoA.20.10

Natural polymer-based hemostatic dressings: A promising approach for achieving effective hemostasis in deep wounds

Kaushal Shakya, Vivek Verma

Indian Institute of Technology Kanpur, Kanpur, India

Abstract

Major traumatic injuries in military and civilian life usually lead to uncontrolled bleeding and deaths. In cases of severe bleeding, the human body's natural coagulation mechanism may be insufficient to achieve timely hemostasis. As a result, external hemostatic dressings are necessary to induce a blood coagulation cascade and control bleeding. These dressings have become a part of emergency medical care due to their portability, ease of use, and quickness to halt bleeding. In this study, we have developed natural, biodegradable, polymer-based cryogels as hemostatic dressings and demonstrated their hemostatic performance. We used a red seaweed-derived polysaccharide, agar, to fabricate cryogels with a macro-porous structure and superabsorbent properties. The properties of agar cryogels were enhanced by incorporating microbial cellulose nanofiber into the matrix. The composite cryogel showed improved mechanical properties, with the compressive modulus increasing from 27 kPa to 697.2 kPa and a good swelling ratio of over 4000%. The *in vitro* blood clotting experiment showed significantly reduced blood clotting times, with complete coagulation occurring in less than 2 minutes. The composite cryogel samples showed better results than commercialized hemostat sponges made up of chitosan (Axiostat) and gelatin (Surgispon). We also studied the hemocompatibility of the composite cryogels by *in vitro* hemolysis test, proving their compatibility with blood. Finally, *in vitro* cytotoxicity test was performed on mammalian cell lines (Fibroblast NIH3T3) and validated them as safe for hemostatic dressing application.

PoA.20.11

Chitosan functionalized MWCNTs as reinforcement of bioresorbable polymers designed for bone tissue engineering: Preparation of 3D-printed scaffolds, mechanical characterization, and cell response

Lykourgos C Kontaxis[1], Artemis Tsirogianni[2], Dafni Graikioti[2], Athanasios Kotrotsos[3], Athanasios Xanthopoulos[4], Constantinos M Athanassopoulos[2], Vassilis Kostopoulos[3], Evangelia Papadimitriou[4], Despina Deligianni[1]

[1]Laboratory of Biomechanics and Biomedical Engineering, Department of Mechanical Engineering & Aeronautics, University of Patras, Patras, Greece. [2]Synthetic Organic Chemistry Laboratory, Department of Chemistry, University of Patras, Patras, Greece. [3]Laboratory of Applied Mechanics and Vibrations, Department of Mechanical Engineering & Aeronautics, University of Patras, Patras, Greece. [4]Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras, Greece

Abstract

Bone defects constitute a serious clinical concern and can occur as a result of trauma, cancer, or congenital diseases. Synthetic bone grafts that can stimulate bone regeneration and replace current treatments are getting a lot of attention as a bone tissue scaffold component in recent years. A major focus concerns reinforced bioresorbable polymeric composite scaffolds. In the present study, 3D-printed nanocomposite scaffolds were fabricated with a rectilinear infill pattern and interconnected pores with a size of 500μm, to support revascularization and optimal soft-tissue ingrowth. The bioresorbable thermoplastic polymers chosen as matrices were polylactic acid (PLA) and polycaprolactone (PCL), blended with chitosan (CS), in order to enhance biological performance and host tissue integration. As piezoelectricity and high mechanical strength are two of the most important characteristics of the bone tissue, Multiwalled Carbon Nanotubes (MWCNTs) were used as reinforcement. To achieve optimum dispersion of MWCNTs, they were functionalized with CS. The MWCNTs-CS hybrids prepared had three different molecular weights (MW) of CS in order to study the effect of the MW on the scaffold's performance, thus CS with low MW, medium MW, and ultralow MW was utilized. The latter was prepared by treatment of CS with activated MWCNTs (with an outside diameter of 48-78 nm), and the desired products were isolated after 48 hours using vacuum filtration. The isolated products were then dried under a high vacuum at 80°C for 24 hours, resulting in MWCNTs with a CS content of 35% w/w in these conjugates. The six manufactured nanocomposites, along with pure PLA and PCL, were characterized through tensile, compression, and dynamic mechanical analysis experiments, as well as biodegradability experiments. In general, the PLA materials, both pure and reinforced, performed better than their PCL counterparts, presenting higher values in both moduli and strength, while in both cases (i.e., PCL and PLA) the reinforced materials' moduli were higher when compared to their respective pure material. Growth of both osteoblasts and endothelial cells was favored in PCL-containing scaffolds compared to those containing PLA at 30 and 45%, respectively, while MWCNTs had no effect on cell growth.

Acknowledgment: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T2EDK-03681).

PoA.20.12

Printing speed as a key factor in finite element analysis prediction of mechanical properties of melt electrowritten scaffolds

Piotr Stanisław Zieliński[1], Zhaohang Zhang[2], Ilaria Squillante[3], Marcus Koch[4], Giuseppe Portale[3], Anastasiia Krushynska[2], Marleen Kamperman[1], Małgorzata Katarzyna Włodarczyk-Biegun[1,5]

[1]Polymer Science - Zernike Institute for Advanced Materials, University of Groningen, Groningen, Netherlands. [2]Computational Mechanical and Materials Engineering - Engineering and Technology Institute Groningen, University of Groningen, Groningen, Netherlands. [3]Physical Chemistry of Polymeric and Nanostructured Materials - Zernike Institute for Advanced Materials, University of Groningen, Groningen, Netherlands. [4]INM- Leibniz Institute for New Materials, Saarbrücken, Germany. [5]Biofabrication and Bio-Instructive Materials, Biotechnology Center, The Silesian University of Technology, Gliwice, Poland

Abstract

Tissue regeneration is a complex process that involves growing new cells and tissues to replace damaged or lost ones. One approach to facilitating the process is using 3D-printed scaffolds, which provide a supportive structure for cells. However, choosing a scaffold with appropriate mechanical properties can be challenging and often relies on trial-and-error methods. In this project, a prediction model was developed to accurately forecast the mechanical properties of melt electrowritten (MEW) scaffolds, which will facilitate the process of tissue engineering by providing a more efficient way to obtain scaffolds with desired mechanical properties. The model also reveals the importance of printing speed as a scaffold designing parameter. The Solid Mechanics Module with the stationary study in COMSOL Multiphysics® was used to build a prediction model (Fig.1). The model considers various parameters such as the fiber configuration, depth of the fiber connection, number of layers, and polycaprolactone (PCL) properties. SEM was used to visualize the connection between fibers and WAXS to analyze the effect of printing speed on fiber crystallinity and crystallite orientation.

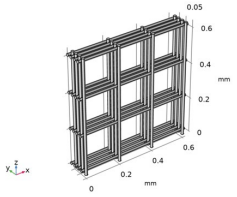


Figure 1. COMSOL model.

To validate the model, various PCL scaffolds were printed using MEW printer. The mechanical properties of the scaffolds were analyzed using a tensile testing machine and compared with computed results. The model based on finite element analysis was successfully proposed and allows good prediction for the mechanical properties of scaffolds with various designs. The depth of the fiber connection was chosen based on SEM results. The model indicated that Young's modulus of PCL should be included depending on the printing speed. WAXs studies confirmed that printing speed influences material properties. By increasing the printing speed from 10mm/s to 80mm/s, the fiber crystallinity decreases from 36.6% to 23.6%, respectively. Numerical simulation revealed that the difference in Young's modulus for scaffolds printed at different speeds can change by 30%. The developed prediction model enables checking the mechanical properties of the scaffold with a given design without printing, accelerating the production of MEW scaffolds with properties specific to the targeted tissue. Importantly, we showed the clear dependence of fiber crystallinity on the printing speed, and therefore, the influence of printing speed on the mechanical properties of the scaffolds with the same architecture (overall design and fiber size). This significant and strong effect was not considered before in the experimental studies using MEW. We conclude that printing speed should not be ignored as a design parameter.

PoA.20.13

Porous iron-based 3D systems as biodegradable implants

Gabriela Gasior[1], Marlena Grodzicka[1], Tomasz Jedrzejewski[1], Jaroslaw W. Drelich[2], Aleksandra Radtke[1]

[1]Nicolaus Copernicus University in Torun, Torun, Poland. [2]Michigan Technological University, Houghton, USA

Abstract

Biodegradable metal systems are one of the futures in interventional surgical treatments. This poster describes the fabrication of novel porous iron-based structures through a replica method in which a porous polymeric template is saturated with a metal powder and thermally processed under a controlled environment. In this presentation, two examples of iron-based materials with different porosity and pore dimensions are presented and characterized for potential applications as biodegradable implants. The fabricated metal structures were imaged using a scanning electron microscope (SEM) and characterized by X-ray spectrometry (EDX). The immersion and electrochemical testing were conducted in Hank's solution. *In vitro* cytotoxicity studies were performed using cell lines: mouse L929 fibroblasts, human aortic smooth muscle cells (HAMSC), and human umbilical vein endothelial cells (HUVEC). Acknowledgments: Completed as part of an internship, "Preludium BIS 1" funded by the Polish National Agency for Academic Exchange

PoA.20.14

Curvature-induced cell suturing controls tissue formation in small tissue defects with implications for biomaterial design

Aaron Herrera[1], Georg N Duda[1], Isabel Orellano[1], Erik Brauer[1], Alicia Serrano[2], Rose Behncke[3], Hans Leemhuis[4], Ansgar Petersen[1]

[1]BIH at Charité Universitätsmedizin, Berlin, Germany. [2]Julius Wolff Institute at Charité Universitätsmedizin, Berlin, Germany. [3]Charité Universitätsmedizin, Berlin, Germany. [4]Matricel GmbH, Aachen, Germany

Abstract

Healing of tissue defects was previously shown to be controlled by the local curvature of the substrate and is generally regarded to follow a centripetal tissue deposition process driven by an inner ring composed of contractile and proliferative cells [1]. Aiming to better understand the final step of defect closure where the curvature increases rapidly, we revealed the onset of a distinct curvature-driven cell organization process with relevance for the treatment of tissue defects and for the design of porous biomaterials. Using topographic cell culture chips (PDMS), we identified a cell type-specific response to curvature. When placed on low curvature concave cylindrical surfaces, stromal cells preferentially aligned in the high-curvature direction of the perimeter in agreement with literature [1]. With increasing cylinder diameter, however, cells responded in two distinct ways. While a fraction of the cells changed their alignment towards the zero-curvature direction (i.e., cylinder axis) the other fraction lifted from the substrate reducing their exposure to curvature. Through the modulation of cell tension via cytoskeletal activators and inhibitors, we showed that the occurrence of cell lifting is controlled by the spatial distribution of focal adhesions across the cell. When studying other cell types (pre-osteoblastic cell line and endothelial cells) focal adhesion distribution again predicted cell response to curvature. Subsequently, we investigated the consequences of the cell-specific curvature-response in an *in vitro* tissue defect model (defect size: 200µm to 1000µm) and in a collagen-based biomaterial with channel-like pores (diameters: 150µm to 900µm). In both systems, we observed a significantly faster closure of the voids through fibroblasts featuring pronounced cell lifting. Furthermore, when cells lifted from the surface, a highly porous, interconnected cell network formed through a process here termed “cell suturing”. The resulting tissue showed a higher structural remodeling capacity and progressed deeper into channel-like pores compared to the denser and less motile tissue produced by pre-osteoblasts with limited lifting capacity. We conclude that addressing the fast formation and high structural remodeling of tissue formed via cell suturing can be advantageous for tissue regeneration strategies by preventing the formation of dense, scar-like tissue with unfavorable structural organization, e.g. in bone healing [2]. This might be achieved by implementing pores with appropriate diameters and associated curvatures into porous biomaterials that promote cell suturing for the cell type of interest.

References

1. Bidan,C.M., et al., Adv. Healthc. Mater. 2, 186-194(2013).

2. Petersen,A., et al., Nat. Commun. 9, 4430(2018).

PoA.20.16

Tunability of scaffolds for bone regeneration by core-shell design and additive manufacturing of bioresorbable polymers

Chiara Pasini, Stefano Pandini, Matteo Ferroni, Federica Re, Domenico Russo, Luciana Sartore

University of Brescia, Brescia, Italy

Abstract

Scaffolds for bone regeneration should simultaneously comply with many mechanical and functional requirements, ensuring both structural stability and cell adhesion and proliferation. This may be challenging, since materials that present excellent biocompatibility, biodegradability, interconnected porosity and osteoinductivity, such as bioactive hydrogels, typically lack to provide temporary mechanical support while new bone tissue grows. In this work, innovative porous scaffolds with core-shell structure were realized, grafting and freeze-drying a bioactive gelatin-chitosan hydrogel shell onto a PLA lattice-structured core obtained by additive manufacturing. Their combination allows to easily tune the scaffold properties according to the specific target application, by varying the lattice geometry and the core/shell ratio. In particular the void volume fraction of the core is expected to play a relevant role in governing the mechanical behavior and in the incorporation of the desired hydrogel amount. The hydrogel shows highly interconnected porosity and thoroughly permeates the core lattice (Figure 1), while maintaining its ability to support cell osteogenic differentiation, as confirmed by SEM observation of calcium phosphate deposits on scaffolds seeded with mesenchymal stem cells (MSCs) in osteogenic medium.

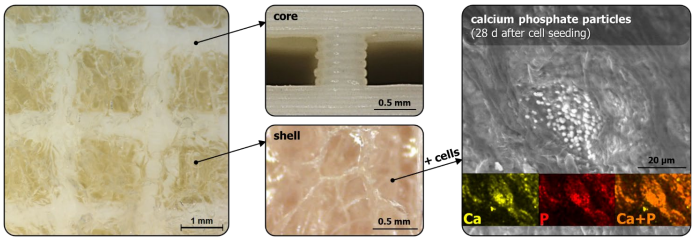
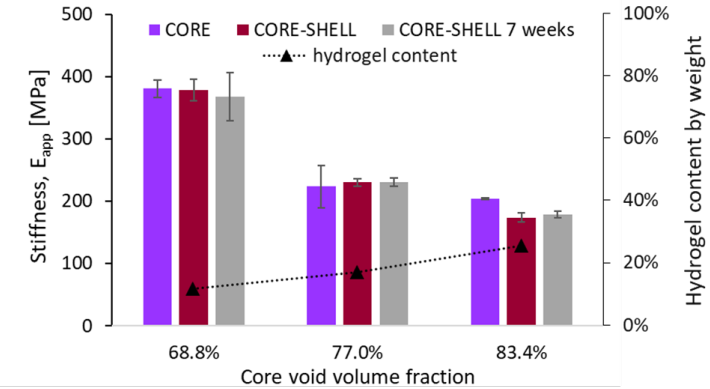


Figure 1: Macro- and microscopic appearance of a core-shell scaffold, with details of calcium phosphate deposits observed by SEM-EDX 28 days after seeding the scaffold with MSCs in osteogenic medium.

The scaffold mechanical properties, investigated under compression, appear to be governed by the core structure, and to decrease with its void volume fraction (Figure 2). Interestingly, stiffness and strength are comparable with those of trabecular bone tissue. In addition, prolonged immersion experiments in water at body temperature revealed that the mechanical properties are stable for several weeks, while the hydrogel shell is gradually hydrolyzed (about 80% mass loss after 7 weeks).

Figure 2: Stiffness and hydrogel content of scaffolds with different values of void volume fraction in the core.



In conclusion, the PLA core may ensure temporary mechanical support and long-term functionality of scaffolds for bone regeneration, while the bioactive shell is expected to be faster replaced by new extracellular matrix deposited by MSCs. The overall mechanical and degradation performance of the scaffolds can be tailored by easy modulation of the core geometry and void volume fraction thanks to additive manufacturing. For this reason, novel approaches to optimize the core material distribution are currently under analysis based on neural networks and on a vaster experimental campaign, aimed at better defining the correlation between mechanical properties and the lattice structure.

PoA.20.20

3D PRINTED PLGA SCAFFOLDS IMPREGNATED WITH ADENOVIRAL CONSTRUCTS FOR EFFECTIVE GENE DELIVERY

Maria A Khvorostina[1,2], Victoria O Mokrousova[2,3], Irina A Nedorubova[2], Tatiana B Bukharova[2], Vladimir K Popov[1], Anatoly A Kulakov[3]

[1]Institute of Photon Technologies of FSRC "Crystallography and Photonics" RAS, Moscow, Russian Federation. [2]Research Centre for Medical Genetics, Moscow, Russian Federation. [3]Central Research Institute of Dental and Maxillofacial Surgery, Moscow, Russian Federation

Abstract

There is an increased request for the development of new materials and fabrication techniques to form highly effective and safe biomedical products for replacing damaged tissues and organs. One of the promising approaches to solving this problem is the use of various bioactive constructs based on porous bioresorbable polymer scaffolds that exactly match the geometry of the defect to be replaced while ensuring effective native tissue regeneration. In this study, PLGA-based scaffolds impregnated with viral particles carrying the *GFP* gene are formed via original antisolvent three-dimensional (3D) printing technique. PLGA has shown great promise in the field of regenerative medicine due to its biodegradability and versatility in various applications. Adenoviral constructs can efficiently deliver genes to a wide range of cells in a prolonged and safe way. Adenoviruses with the *GFP* gene (Ad-GFP) serve as a universal model system where *GFP* can be easily replaced with the therapeutic gene to promote target tissue regeneration. Our antisolvent 3D printing allows to precisely define the architectonics of the bioactive scaffolds and produce it at room or even lower (ca 4°C) temperatures to preserve the original components and maintain the migration and adhesion of progenitor cells, as well as efficient cell transduction in the implantation area. In the present work, PLGA solution in tetraglycol was extruded during antisolvent 3D printing into the water-containing medium in accordance with the computer model to form meshlike disks with 190 and 350 μm fiber diameters. Different concentrations of adenoviral particles (300-3000 TCID50/ml) were used to determine the optimal viral load for effective and non-toxic gene delivery. Ad-GFP release kinetics from 3D printed scaffolds was investigated using quantitative PCR and plaque assay. The transduction efficacy of released adenoviruses was evaluated *in vitro* on the mesenchymal stem cell cultures. After 14 days of incubation with MSCs gene-activated scaffolds were shown to ensure cell viability (>80% of living cells) and effective *GFP* gene expression followed by GFP production (>50% of transduced cells). Moreover, the increase in fiber diameter of the scaffold resulted in the corresponding Ad-GFP release rate decrease. Thus, the original 3D printing technique of the gene-activated PLGA scaffolds was developed and the ability to vary the adenovirus release and transduction rate by changing the scaffold architectonics was demonstrated for the first time. This research was funded by the Russian Science Foundation (Project No. 22-15-00425) in part of the design and *in vitro* characterization of adenovirus-loaded PLGA scaffolds.

PoA.20.21

Design and fabrication of calcium phosphate scaffolds with concave surfaces by direct ink writing

Irene Lodoso-Torrecilla[1,2], Ethan Segura[1], Oriol Romeu[1], Maria-Pau Ginebra[1,2]

[1]Department of Materials Science and Engineering, Group of Biomaterials, Biomechanics and Tissue Engineering, Universitat Politècnica de Catalunya. BarcelonaTech (UPC), Barcelona, Spain. [2]Barcelona Research Centre for Multiscale Science and Engineering, UPC, Barcelona, Spain

Abstract

Osteoinductive biomaterials are those capable to induce the osteogenesis process, by stimulating pluripotent stem cells to differentiate into bone-forming cells. Such biomaterials hold great potential in bone regeneration applications. Recent studies have shown that osteoinduction is highly benefited from the presence of concave surfaces [1]. This represents a challenge for the application of extrusion-based 3D printing techniques in the fabrication of synthetic bone grafts, due to the convex surface of the extruded filaments. The aim of this project was to overcome this limitation by developing a novel method that relies on the infiltration of sacrificial polymeric moulds obtained by direct ink writing with self-setting calcium phosphate slurries, and the subsequent dissolution of the moulds. The synchronization of the two processes, the setting reaction and the progressive dissolution of the polymer, is a crucial aspect in order to obtain ceramic scaffolds with concave porosity. Poly(vinyl alcohol) (PVA) was used to print the sacrificial moulds. In spite of the close imbrication in the ceramic matrix, PVA fully dissolved in one week after immersion in water at 37ºC, and this occurred in parallel to the hardening of the scaffold by transformation from α-tricalcium phosphate to calcium deficient hydroxyapatite. Further, the pores of the resulting scaffold faithfully reproduced the shape of the mould, although the diameter was slightly increased, which was correlated to a small swelling of the polymer before dissolution. Printing parameters such as printing pattern, nozzle diameter, infill density and layer height were modified and their effect on scaffold porosity and mechanical properties was analysed. Three printing patterns were studied, perpendicular, gyroid and crossed (Figure 1), the perpendicular showing the highest compressive strength for the same porosity values. As expected, increasing infill density and layer height increased porosity and decreased the compressive strength. *In vitro* studies with preosteoblastic cells are being performed to analyze the effects of pore geometry and surface curvature of the different scaffolds on cell migration, proliferation and differentiation.

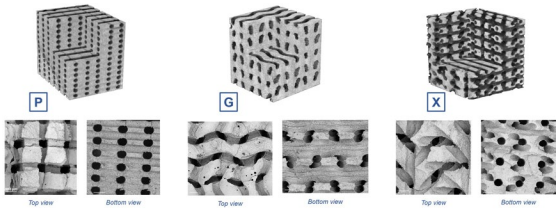


Figure 1: MicroCT (top) and SEM (bottom) images of the three infill patterns studied: perpendicular (P), gyroid (G) and crossed (X)

References [1] Barba, A., Diez-Escudero, A., Maazouz, Y., et al. ACS applied materials & interfaces, 9, 48 (2017) 41722-41736

Acknowledgments: The authors acknowledge the Spanish Government for funding through projects PID2019-103892RB- I00/AEI/10.13039/501100011033 and PCI2021-122079-2B, financed by MCIN/AEI/10.13039/501100011033 and the EU "NextGenerationEU"/PRTR

PoA.20.22

Biofunctionalised 3D-printed gellan gum scaffolds for bone tissue regeneration

Alessandra Soriente[1], Ugo D'Amora[1], Alfredo Ronca[1], Stefania Scialla[1], Paola Manini[2,3], Alessandro Pezzella[1,3,4], Giovanna Calabrese[5], Maria Grazia Raucci[1], Luigi Ambrosio[1]

[1]Institute of Polymers, Composites and Biomaterials, Naples, Italy. [2]Department of Chemical Sciences, Naples, Italy. [3]Bioelectronics Task Force, University of Naples Federico II, Naples, Italy. [4]Department of Physics "E. Pancini", University of Naples Federico II, Naples, Italy. [5]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

Abstract

Gellan gum (GG) was chemically modified with methacrylic moieties to produce a photocrosslinkable biomaterial ink, hereinafter called methacrylated GG (GGMA), with improved physico-chemical properties, mechanical behavior and stability under physiological conditions. Afterwards, GGMA was functionalized by incorporating two different bioactive compounds, a naturally derived eumelanin extracted from the black soldier fly (BSF-Eumel), or hydroxyapatite nanoparticles (HAp), synthesized by the sol–gel method. Different ink formulations based on GGMA (2 and 4% (w/v)), BSF-Eumel, at a selected concentration (0.3125 mg/mL), or HAp (10 and 30% wHAp/wGGMA) were developed and processed by three-dimensional (3D) printing. All the functionalized GGMA-based ink formulations allowed obtaining 3D-printed GGMA-based scaffolds with a well-organized structure. For both bioactive signals, the scaffolds with the highest GGMA concentration (4% (w/v)) and the highest percentage of infill (45%) showed the best performances in terms of morphological and mechanical properties. Indeed, these scaffolds showed a good structural integrity over 28 days. Given the presence of negatively charged groups along the eumelanin back bone, scaffolds consisting of GGMA/BSF-Eumel demonstrated a higher stability. From a mechanical point of view, GGMA/BSF-Eumel scaffolds exhibited values of storage modulus similar to those of GGMA ones, while the inclusion of HAp at 30% (wHAp/wGGMA) led to a storage modulus of 32.5 kPa, 3.5-fold greater than neat GGMA. *In vitro* studies proved the capability of the bioactivated 3D-printed scaffolds to support 7F2 osteoblast cell growth and differentiation. BSF-Eumel and HAp triggered a different time-dependent physiological response in the osteoblasts. Specifically, while the ink with BSF-Eumel acted as a stimulus towards cell proliferation, reaching the highest value at 14 days, a higher expression of alkaline phosphatase activity was detected for scaffolds consisting of GGMA and HAp. In fact, the proposed scaffolds proved their capability of supporting 7F2 osteoblast growth and differentiation processes. While GGMA4/BSF-Eumel induced a higher cell proliferation, reaching a maximum at 14 days, GGMA4/HAp30 led to a higher expression of ALP activity. The overall findings provide interesting hints towards the use of these 3D-printed scaffolds for bone tissue engineering applications.

ACKNOWLEDGMENT

The authors would like to thank PRIN 2017, "SAPIENT – A system approach for identifying connective tissue degeneration in diabetic analogues", Grant N° 2017CBHCWF for financial support (Prot. N. 2017CBHCWF).

PoA.20.23

Manufacturing of dense biphasic (HA/ß-TCP) scaffolds with macroporous architecture by stereolithography

Stéphane Hocquet[1], Danielle Madufo Tchuyassi[1], Florian Jean[2], Marie Lasgorceix[2], Jean-Marie Raquez[3]

[1]BCRC, Mons, Belgium. [2]CERAMATHS-DMT UPHF, Maubeuge, France. [3]SMPC - UMONS, Mons, Belgium

Abstract

Hydroxyapatite (HAp) and beta-phase tricalcium phosphate (ß-TCP) are two materials with excellent biological properties for bone tissue regeneration applications. The combination of these two materials would make it possible to construct a scaffold with controlled resorbability and optimised osteo-induction and -conduction properties. Moreover, the ß -TCP will be replaced by natural bone as HAp will give the scaffold its residual mechanical structure, maximizing then the ration natural/synthetic materials (as expected by surgeons). Ceramic stereolithography (SLA) is an appropriate technology for constructing such a scaffold, with both an interconnected macroporous architecture and a complex external shape. However, it is then necessary to undergo a high-temperature debinding and sintering thermal treatment to eliminate the organic phase and densify the material.
However, ß-TCP cannot be heated to a temperature higher than 1130°C to avoid the transition to the α phase. But at this temperature, HAp does not sinter.
In this work, Mg-doped β-TCP powder is used to manufacture biphasic parts by SLA. High temperature XRD has shown that Mg stabilises β -TCP at temperatures of up to 1400°C. As a result, biphasic bone substitutes prepared by SLA and sintered at 1230°C were produced.
Prospects for co-densification of Mg-β -TCP with yttrium stabilized zirconia are also presented.

PoA.20.24

The taiwanese cirripede exhibits promising bioceramic and bioactive properties in bone graft

Long Chang[1], Zhi-Hong Wen[1,2,3]

[1]Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan. [2]Institute of BioPharmaceutical Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan. [3]Institute of Medical Science and Technology, National Sun Yat-sen University, Kaohsiung, Taiwan

Abstract

In the human body, bones play a crucial role in providing structural support for the movement and to protect vital organs. However, they are susceptible to fractures or defects caused by external forces. Marine organism skeleton contains trace elements, unique architectures, and degradable compositions, giving them an excellent bioactive, strength, and ceramic properties. Thus, they have considerable success as bone graft materials. Currently, there is a limited research focusing on indigenous cirripede, which may hold the potential to develop a novel bone graft material. From biomedical analytic results, we discover that one Taiwanese cirripede-derived material exhibits osteoconductive and osteoinductive activities. Physical and chemical measurements have confirmed their bioceramic properties. This is the first time that a cirripede exoskeleton has been found to exhibit features of a bone graft. Future research into this topic is recommended.

PoA.21.03

Novel multi-azide polyoxazoline coating

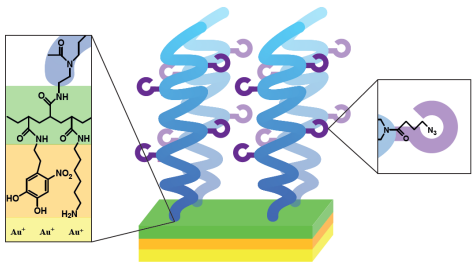
Tobias Komsthöft[1,2], Niccolò Bartalucci[1], Mark W Tibbitt[2], Samuele Tosatti[1], Stefan Zürcher[1]

[1]SuSoS AG, Dübendorf, Switzerland. [2]ETH Zurich, Zurich, Switzerland

Abstract

In the biomedical field a material's surface plays an essential role in determining the interactions with the surrounding biological environment. Thin-film polymeric coatings are often leveraged to prevent unwanted adsorption of proteins, macromolecules, and microorganisms [1,2]. Among various materials, polyoxazoline polymers are of great interest because of their resistance to oxidation and thus their durability in aqueous environments [3]. However, unlike other materials such as PEG, synthesizing polyoxazolines with additional functionalities, e.g. enabling bioorthogonal chemistry, is not trivial, and coating functionalizations have been primarily limited to their chain ends. Finding innovative strategies to improve their functionalization could help increase their utilization as biosensing and bioactive coatings. Azides have been widely employed in the biomedical field as versatile functional groups for bioorthogonal click-chemistry. Their reaction with alkynes provides beneficial condition for the coupling of a wide range of biomolecules [4,5]. Furthermore, using strain-promoted alkyne-azide cycloaddition (SPAAC) neither a toxic catalyst nor an additional reagent is required for the reaction to proceed, enabling simple, fast, and catalyst-free coupling. In this work we developed a coating that combines the non-fouling characteristics of polyoxazoline with the ability for subsequent functionalization via bioorthogonal SPAAC. A novel multi-azide polyoxazoline was synthesized and grafted onto a PAcrAm™ backbone resulting in a bottle brush polymer (Figure 1). The PAcrAm™ backbone was further modified with anchoring groups (i.e. amine and nitrodopamine) suitable for spontaneous assembly of a monolayer on gold surfaces that can withstand high ionic media. We investigated the influence of polyoxazoline molecular weight (M_w) and spacing on PAcrAm™ on the coating performance. The polyoxazoline was characterized with ^{1}H-NMR and GPC and its successful grafting onto PAcrAm™ was confirmed with ^{1}H-NMR. The azide's presence was confirmed by FTIR and XPS. The functionalization of the coating via SPAAC and protein resistance were investigated on gold surfaces with VASE. The resulting capacity for SPAAC was increased by almost factor of 4 compared to a coating with terminal azide groups while simultaneously being able to supress up to 90% of protein adhesion. These characteristics show great promise for potential applications as a highly sensitive biosensor.

Figure 1: PAcrAm™ backbone (green) with multi-azide (purple) polyoxazoline (blue) and anchoring groups (orange) on a gold surface (yellow).



[1] Maan, Adv Funct Mater. 2020; DOI: 10.1002/adfm.202000936
[2] Weydert, Langmuir. 2017; DOI: 10.1021/acs.langmuir.7b01437
[3] Zheng, React. Funct. Polym., 2017, DOI: 10.1016/j.reactfunctpolym.2017.07.006
[4] Breinbauer, Chembiochem. 2003; DOI: 10.1002/cbic.200300705
[5] Spicer, Chem Rev. 2018; DOI: 10.1021/acs.chemrev.8b00253

PoA.21.04

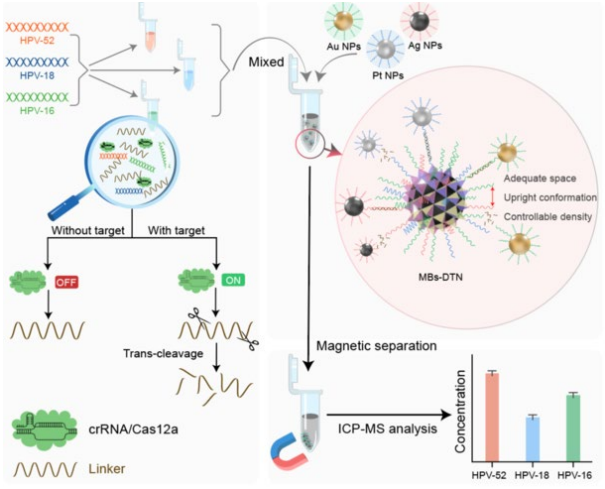
DNA tetrahedron and metal-nanoparticle tagging powered CRISPR/Cas12a-based biosensor for multiplex HPV-DNA genotype analysis

Xiaohui Zhan, Fang Lan, Yao Wu

Sichuan University, Chengdu, China

Abstract

Accurate quantification of multiple analytes is crucial for clinical diagnosis. The CRISPR-Cas platform offers a method for enhancing the speed, specificity, and sensitivity of nucleic acid-based diagnosis. However, their multiplex analysis capacity is challenging to achieve. In this study, we developed a novel DNA Tetrahedron (DTN)-supported biosensor for multiplex detection of HPV-DNA (HPV-16, HPV-18, and HPV-52) by utilizing the spatially separated CRISPR-Cas self-amplification strategy and multiple-metal-nanoparticle tagging coupled with inductively coupled plasma mass spectrometry (ICP-MS) detection. When the target DNA is present, Cas12a/crRNA duplex is activated, resulting in the robust trans-cleavage activity of the corresponding ssDNA linker. The short fragments of linker are unable to bond with metal-nanoparticle probes (197 Au, 107 Ag, 195 Pt) onto DTN-modified magnetic bead probes (MBs-DTN). This results in a significant ICP-MS signal change. Compared with ssDNA functionalized MBs, MBs-DTN amplifies the signal, resulting in a higher Signal-to-Noise Ratio in our system. Our approach achieved detection limits as low as 218 fM, which allowed for the multiplex assay of HPV-DNA with high accuracy and specificity. Furthermore, we demonstrated the feasibility of detecting HPV-DNA in cervical swab samples, which showed high consistency with DNA sequencing results. Our results show that this work provides a promising option for designing a CRISPR-based multiplex detection system with high sensitivity, specificity, and clinical molecular diagnostics.



PoA.21.05

Magnetic metal-organic framework composites for detection of circulating tumor DNA

Fang Lan, Peng An, Yao Wu

Sichuan University, Chengdu, China

Abstract

Circulating tumor DNA (ctDNA), derived from tumors of cancer patients, is an important biomarker for *in vitro* diagnosis of cancer. However, precise detection of target ctDNA remains a huge challenge due to the extremely trace ctDNA and strong backgrounds of interfering DNA. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system has attracted much attention as diagnostic tool due to the advantages of high accuracy, low cost, and easy operation. The CRISPR/Cas12a system exhibits ultrahigh trans collateral cleavage activity towards single-stranded DNA (ssDNA) indiscriminately. Metal-organic frameworks (MOFs) hold huge application potential in various fields due to the controllable structure, high specific surface area, high porosity, and thermal stability. Herein, we propose an ultrasensitive and convenient approach to detect ctDNA via integrating MOF-based enhanced fluorescent labels and the CRISPR/Cas12a system. First, UIO-66-NH2 with loading Cy5 fluorescein was prepared and blocked with hairpin DNA. Then, the MOF based fluorescent labels were linked onto magnetic microspheres via ssDNA. Through recognition and binding of crRNA toward to the target ctDNA, activated the CRISPR/Cas12a system cleave ssDNA linker. The released MOF-based fluorescent labels were collected using magnetic separation. Finally, the Open-ssDNA, which was complementary to the hairpin DNA, was added to open the hairpin DNA and release the fluorescent molecules. The intensity of released fluorescent molecules was measured to achieve quantitative detection of target ctDNA. The proposed strategy exhibited ultrahigh sensitivity for detecting target KRAS mutated ctDNA as low as 5.6 fM and could distinguish lung cancer patients from healthy person with high specificity and sensitivity, which had a broad application prospect in clinical diagnosis.

PoA.21.06

Highly sensitive detection of melanoma exosomal PD-L1 via a self-calibrated magnetic aptamer sensor with dual lanthanide-assisted time-resolved luminescence

Nanhang Zhu

Sichuan University, Chengdu, China

Abstract

Highly sensitive detection of PD-L1 in exosomes derived from melanoma cells is critical for the non-invasive diagnosis of melanoma. We propose a self-calibrated magnetic aptamer sensor (sCMAS) that uses dual lanthanide-assisted luminescence and ratiometric fluorescent probes to achieve sensitive detection of PD-L1 in melanoma exosomes. By overcoming limitations caused by systematic or environmental factors, our sensor provides a more stable and reliable detection output. The sCMAS typically uses Tb-based nanoparticles (TbNps) immobilized on magnetic Fe3O4 microparticles for a stable luminescent reference signal report. Additionally, the BHHCT-Eu^{3+} complex is used for the detection signal report of the specific exosomal PD-L1 recognition. The PD-L1-responsive aptamer and its complementary DNA hybridization chain bridge the dual time-resolved luminescent elements, in particular. Recognition of exosomes with the aptamer led to the expulsion of the detection signal (BHHCT-Eu^{3+}), resulting in an increase of the reference signal/detection signal ratio value. This increase was linear and dependent on the concentration of exosomes, in a broad concentration range of 1.05×10^5 to 1.05×10^{10} particles/mL, with an extremely low detection limit of 1.88×10^2 particles/mL. Furthermore, the sCMAS enables the rapid and efficient differentiation between healthy samples and melanoma samples, including early metastatic melanoma cases. Promisingly, the sCMAS developed in this work will provide a powerful tool for future evaluation of tumor markers, such as exosomes, cells, and proteins, and will offer a vast opportunity in liquid biopsies.

PoA.21.07

Organ origin identification of circulating tumor cells based on AND logic recognition-triggered hybridization chain reaction

Qiangying Yi

Sichuan University, Chengdu, China

Abstract

As a type of novel biomarkers for liquid biopsy, circulating tumor cells (CTCs) have become significant important in clinical activities, since the capture of circulating tumor cells (CTCs) may provide vital predictive information for early diagnosis and disease monitoring of cancer. As CTCs with complete cellular structure, there is still a lack of simple and effective methods to capture them and identify multiple surface biomarkers at the same time. In view of this, developing a new CTCs enrichment and identification of subpopulation strategy that can achieve high-efficiency capture and precisely identification of their organ origin is urgently needed. Accordingly, we proposed the nano-magnetic aptamer sensor platform incorporating AND logic recognition-triggered hybridization chain reaction to capture and identify lung and liver origin CTCs. The developed platform consisted of magnetic nanoparticles (MNPs), AND logic probes and HCR probes. Among them, MNPs and DNA probe with epithelial cell adhesion molecule (EpCAM) aptamer realize the capture function of broad spectrum of epithelial CTCs. Then, the logical recognition function is triggered on the surface of CTCs from different organ through the organ-derived aptamer probe (GPC3 for liver and TTF1 for lung), and the multi-channel hybridization chain reaction (HCR) is lunched with the HCR probe. Finally, corresponding amplified fluorescent signals are formed on the surface of different subtypes of CTCs, enabling capture of CTCs and identification of their organ origin. This strategy has been tested to identify the organ origin of CTCs in complex blood samples, including tumor-bearing mice and clinical patients, and even trace the origin of metastatic cancer in related organs.

PoA.21.09

Microfluidic chip-based optical sensing platform for ovarian cancer multimarkers

Neelam Vishwakarma[1,2], Suman Singh[1]

[1]CSIR-CSIO, Chandigarh, India. [2]RMIT, Melbourne, Australia

Abstract

Ovarian cancer is among the leading cause of death in women worldwide. With the continuous rise in lethal cases of ovarian cancer, the mortality rate of this cancer is projected to rise significantly in the next decade. Unfortunately, ovarian malignancies often go unnoticed due to a lack of symptoms, and their symptoms are often mistaken for gastrointestinal sickness or other common ailments. The 5-year survival rate of ovarian cancer is one the lowest amongst other cancer types in women and decreases rapidly with a delay in diagnosis of the disease. These statistics, however, can be improved if the patients are accurately diagnosed in stage I or II. With the current traditional methods of diagnosis available like ELISA, pelvic examination, CMIA, etc., early diagnosis is still a crucial bottleneck and the need for point-of-care sensing platforms for single and multiple biomarkers persists. In this study, multiple ovarian biomarkers were analyzed using microfluidic channels on paper-based substrates using optical signals produced by antigen-antibody interaction chemistry. The Cancer Antigen 125 (CA125) and Human Epididymis 4 (HE4) were analyzed on the proposed platform while covering a wide range of concentrations around the clinical limit of the antigens. A range of 6.25-400 unit/ml and 12.5-800 ng/ml concentrations were analyzed for CA 125 and HE4 respectively. The linear variation of optical signals obtained was observed to be proportional to the concentration change in analyte samples.

PoA.21.10

Oxidase-based enzymatic assessment of diabetes biomarkers

Andrea Rescalli[1], Luca Casanova[1], Elena Maria Varoni[2], Francesco Cellesi[1], Pietro Cerveri[1]

[1]Politecnico di Milano, Milan, Italy. [2]University of Milan, Milan, Italy

Abstract

Systematic glycemic control is pivotal to prevent diabetes long-term complications, such as cardiovascular disorders or kidney diseases [1]. To bridge the time gap between detecting abnormal variations in diabetes biomarkers and healthcare intervention, research focuses on point-of-care testing for rapid and convenient use outside the laboratory [2]. In particular, enzymatic biosensors have revolutionized the field of medical diagnostics, enabling rapid and accurate detection of various biomarkers. This work aims at developing a simple, but flexible biosensor using specific oxidases, such as fructosyl-amino acid oxidase (FAOx) or glucose oxidase (GOx), to detect different diabetes biomarkers (e.g., glycated hemoglobin, glycated albumin, glucose). A first version of the sensor has been developed using the GOx-glucose couple as a model to explore the feasibility and the performances of the setup. Interdigitated electrodes (IDEs) were selected as transducers for their capability to reduce capacitive signal effects and quickly achieve a steady-state working regime [3]. The electrodes were functionalized by depositing a solution of enzyme (GOx) and mediator (hexaammineruthenium(III) chloride) onto their active area. The enzyme facilitates substrate oxidation, while the mediator undergoes reduction. By applying a voltage potential, electron exchange between the mediator and the electrodes enables signal collection. Square wave voltammetry (SWV) was chosen as measurement technique due to its higher sensitivity compared to cyclic voltammetry or chronoamperometry [4]. The biosensors were evaluated by measuring their response to glucose at three distinct physiological concentrations, using SWV. Each test was conducted three times on separate sensors, and the outcomes are described in Figure 1.

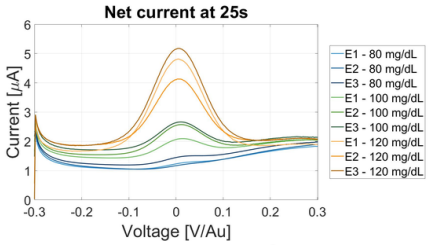


Figure 1: Net current response of GOx-based glucose biosensors. *Measurements performed 25s after sample deposition.*

Through a calibration, the concentration of the target was related to the intensity of the peak of the collected current, and the result is shown in Figure 2.

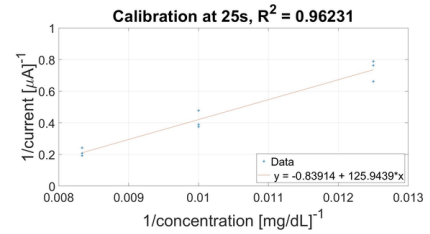


Figure 2: Calibration curve.

These findings showcase the potential of a simple system using oxidases for monitoring diabetes biomarkers. While GOx and glucose were used as models, the sensor's flexibility allows adaptation for different oxidases (such as FAOx) to detect other biomarkers (fructosyl-lysine obtained from glycated proteins) based on functional and structural similarities. Further investigations are required to enhance reproducibility and validate the technology through calibration in a wider range.

References

[1] World Health Organization (https://www.who.int/news-room/fact-sheets/detail/diabetes/)
[2] A. Rescalli et al., Biosensors, 2022
[3] K. Stulík et al., Pure and Applied Chemistry, 2000
[4] V. Mirceski et al., ChemTexts, 2018

PoA.21.11

Synthesis of a wearable strain sensor using AgNW and PANI

Halldór Kárason[1,2], Pierluigi Ritrovato[2], Francesco Tortorella[2], Nicola Maffulli[2], Aldo Boccaccini[1]

[1]University of Erlangen-Nuremberg, Erlangen, Germany. [2]University of Salerno, Salerno, Italy

Abstract

This study presents a wearable sensor design that incorporates polydimethysiloxane (PDMS) as an elastic component and a conductive composite of silver nanowires (AgNWs) and polyaniline (PANI). The primary goal of the study was to determine the potential advantages of a composite approach over the single-element application of AgNWs. The sensor was mechanically and electrically characterised under varying strain conditions and simulated human motion conditions. The composite sensor showed slight improvements in several key performance parameters, including sensitivity and stability. To test the durability of the composite sensor, it was subjected to multiple loading-unloading cycles. The results demonstrated increased resilience and longevity, indicating the beneficial role of PANI in this robust sensor system. The PANI and AgNW composite strain sensor wearable technology has the potential to provide more accurate, reliable, and persistent human motion detection. Because of these characteristics, it is an excellent candidate for use in rehabilitation devices, prosthetics, and human-machine interfaces, improving precision and user adaptability. Furthermore, the sensor's flexibility and robustness may make it easier to integrate into smart textiles, opening up new possibilities for wearable technology, sports science, and occupational health monitoring.

PoA.22.01

Atmospheric pressure plasma spraying of hydroxyapatite coatings w/o zinc alloying on 3D printed, temperature and distortion sensitive polymer and titanium implants for reconstruction of face, jaw and finger bone defects

Juergen M. Lackner[1], Dietmar F Kopp[1], Wolfgang Waldhauser[1], Reinhard Kaindl[1], Andreas Hinterer[2], Maximillian Stummer[2], Felix Spiess[3], Bernd Prettenthaler[3], Guenter Russmueller[4], Barbara Kapeller[4], Roman Major[5]

[1]JOANNEUM RESEARCH Forschungsges.m.b.H., Institute for Sensors, Photonics and Manufacturing Technologies, Niklasdorf, Austria. [2]Inocon Technologie GmbH, Attnang-Puchheim, Austria. [3]alphacam austria GmbH, Vienna, Austria. [4]Medical University of Vienna, Vienna, Austria. [5]Polish Academy of Sciences, Institute of Metallurgy and Materials Science, Krakow, Poland

Abstract

Facial implants are increasingly used surgically to correct trauma-related deformities (i.e. caused by accidents and tumours) and age-related degeneration of e.g. cheekbones, chin bones and nasal bones. Currently, mainly implants made of polymers are used, which are not accurately adapted to the patient's anatomy in advance by e.g. 3D printing, but only roughly and manually during the surgical intervention. Their often inadequate anchoring to the bone by means of e.g. screws bears the risks of migration and rotation of the implant, potentially resulting in a disfigured patient's face as well as bacterial infections in the barely perfused gap between implant and bone. The aim of the presented developments was the development of coatings and deposition technology, which allows the use of temperature-sensitive, even 3D-printable polymers as an optimal surface for bone growth as well as with antimicrobial properties. Industrially-scaled atmospheric pressure plasma spray (APS, InoCoat® 3, Inocon Technologie GmbH), based on established plasma spraying but with significantly reduced thermal load to the substrates, was chosen to deposit hydroxyapatite coatings (crystalline $Ca_{10}(PO_4)_6(OH)_2$, HAp) from biocompatible powder feedstock (Mediscan®). By integrating the digital twin of the plasma jet, it was possible to quickly find a coating regime that transfers the optimal biocompatible properties of the medically certified starting powder to layers up to 100 µm thickness on 3D printed polymers without chemical and structural changes. This was proven in extensive biocompatibility and microbial tests *in vitro* and verified *in vivo* in sheep models according to ISO 10993. Coatings without phase change from powder feedstock show fast covering with osteoblasts and wide, biologically optimal spreading of cells without any cytotoxic effects. While the pure HAp coatings are even anti-microbial to *S. aureus* and *E. coli* in ISO 22196 direct contact tests, the onset of the effect was boosted even in the surrounding medium by admixture of zinc particles to the powder feedstock, however, without negative influences to osteoblast adhesion and proliferation. The work shows additionally the extraordinary high potential of APS for biocompatible coatings on 3D patient-specific porous demonstrator implants with physiologically optimal topography, elaborated from patients' CT.

PoA.22.02

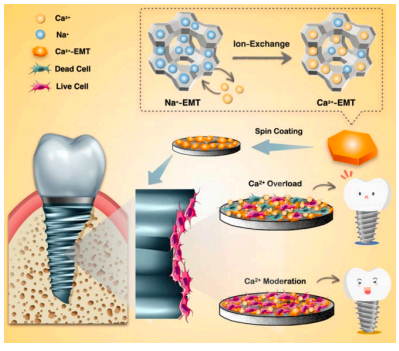
Positive regulation of osteogenesis on titanium surface by modification of nanosized Ca2+-exchanged EMT zeolites

Minghan Chi, Na Li, Neha Sharma, Florian Markus Thieringer

Department of Biomedical Engineering, University of Basel, Basel, Switzerland

Abstract

Cell–titanium interactions are of great importance for the clinical success of dental implants, while traditional titanium surfaces are affected by inertness and have poor osteogenicity. In this study, for the purpose of enhanced osteoconductive and osteoinductive properties, titanium (Ti) substrates modified with calcium ion-exchanged nanosized EMT zeolites (Ca2+-EMT) were developed and the effect on cell viability, proliferation, osteo-differentiation, and mineralization of MC3T3-E1 cells was systematically and quantitatively examined. For this purpose, firstly, we synthesized and characterized the nanosized EMT zeolites fabricated using a Na2O-Al2O3-SiO2-H2O precursor system without the organic template. Then calcium ion was exchanged at different levels within nanosized EMT zeolites (Ca2+-EMT) and spin-coated onto the Ti surfaces (EMT@Ti, EMT-3@Ti, EMT-6@Ti, EMT-12@Ti, and EMT-18@Ti). Comprehensive characterization was conducted to study the physicochemical properties of as-synthesized specimens. To determine the osteogenic effects of Ti substrates functionalized with Ca2+-EMT, we investigated the biological behavior of MC3T3-E1 cells cultured on Ca2+-EMT surfaces *in vitro* with pure Ti and EMT@Ti serving as negative control and experimental control groups, respectively. The results showed that the Ca2+ was successfully encapsulated in the sodalite cage of the EMT zeolite, while the overall microstructure was not compromised. Enhanced hydrophilicity and roughness were achieved by spin-coating the Ca2+-EMT to the surface of Ti specimens. *In vitro* biological experiments suggested that all Ca2+-EMT coated samples possess favorable osteogenicity, as evidenced by the *in vitro* osteogenic-related biological cell performance. Notably, a dose-dependent manner was detected, where the EMT-12@Ti group exhibited the greatest bone regenerative potential. Meanwhile, the mechanisms of promoting osteoblast differentiation by Ca2+ regulation were discussed in depth. The schematic illustration of Ca2+-EMT zeolite and its osteogenic properties as coating of titanium surfaces modified with Ca2+-EMT zeolite. Nanosized EMT zeolite with microporous and mesoporous structures serves as an ideal carrier for Ca2+ due to its high ion-exchanging properties. The released Ca2+ affects the biological behavior of osteoblasts to large extent, where a moderate concentration of Ca2+ would lead to favorable viability and osteogenic efficacy of osteoblasts. On the other hand, Ca2+ overload rather negatively affects the osteogenesis of osteoblasts. Finally, by regulating Ca2+ ion exchange time, optimized osteointegration properties of modified titanium surfaces are achieved.



PoA.22.03

Modelling heparin binding domain of fibronectin onto polymer surfaces by using molecular dynamics simulations.

Viswanath Vittaladevaram

University of Galway, Galway, Ireland

Abstract

Understanding the adsorption of proteins onto surfaces is central to the creation of biomaterials for use in medical devices and the fabrication of synthetic models of biological tissues, such as the blood-brain barrier. The adsorption process, however, depends on the microscopic details of the protein-surface interaction, which are challenging to investigate experimentally. Molecular dynamics simulations are ideally suited to provide this information, as they can directly investigate the molecular scale.

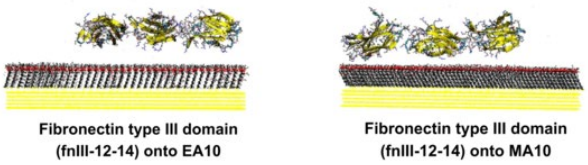


***Figure 1**: Fibronectin(fnIII-12-14)trimer adsorption onto SAM-EA10(Left) & SAM-MA10(Right)*

In this project molecular dynamics simulation were used to investigate the binding of the fibronectin fnIII(12-14) fragment onto synthetic surfaces. This fragment contains a heparin binding domain, which is key in promoting cell growth in the surface. The mechanism of protein adsorption onto surface was investigated to identify different conformational changes of protein and exposure of binding sites. Data analysis was performed to verify the residues that drives the process of adsorption. The results obtained facilitates towards understanding molecular mechanisms that control protein adsorption onto surfaces, which can be used to optimize formation of functional biomaterials.

PoA.22.04

Surface functionalization of cell culture plates for biomolecule immobilisation using plasma treatment

Clara Thao Hoang Tran[1], Xuege Feng[2], Stuart Fraser[3], Marcela Bilek[1]

[1]University of Sydney, NSW, Australia. [2]university of Sydney, nsw, Australia. [3]Culturon Pty Ltd, NSW, Australia

Abstract

In vitro cell culture is a critically important technology for a range of assay systems including high throughput drug screening, stem cell differentiation, cellular immunotherapy, and cellular agriculture. Commercially available cell culture plates are either non-treated plates and hydrophobic or “tissue culture-treated” which show increased hydrophilicity. These plates are typically made of polystyrene with high optical transparency. However, these plates lack the ability to covalently bind biomolecules to their surfaces. Surface plasma treatment profoundly changes surface chemistry and creates long-lived radicals, allowing for rapid covalent bonding of biomolecules to surfaces while maintaining functionality without the need for linker chemistry. We have created plasma-activated cell culture plates for cell culture. Plasma treatment of 96-well plates resulted in coatings across the bottom of the well with nanometer range thickness with minimal effect on transparency and fluorescence emission. We demonstrated the covalent attachment of functional biomolecules on the plasma treated wells in a one step process without the need for chemical linkers or any reagents. The covalency of the biomolecule bonding was tested with detergent washing at high temperature and compared with physical adsorption on untreated plates. We also evaluated cell adhesion on plasma treated plates in comparison with untreated commercial plates. One challenge of the technique is the accumulation of plasma dust inside the wells which may interfere with subsequent assays. When the well size increases (96 wells - 24 wells), more dust can be observed on the base of the well. We have designed an electrode and sample holder to prevent plasma dust precipitating onto the well surface and demonstrate here the efficacy of our new design.

PoA.22.06

Characterization of the functional layer formed on titanium bone wedge and drug stability in polymer after sterilization processes

Alicja Kazek-Kęsik[1,2], Weronika Maciak[1], Jakub Adamek[3], Monika Śmiga-Matuszowicz[4], Małgorzata Krok-Borkowicz[5], Anna Taratuta[6], Marcin Basiaga[6]

[1]Silesian University of Technology, Faculty of Chemistry, B. Krzywoustego Str. 6, Gliwice, Poland. [2]Silesian University of Technology, Biotechnology Centre, Krzywoustego Str. 8, Gliwice, Poland. [3]Silesian University of Technology, Faculty of Chemistry, B. Krzywoustego Str. 8, Gliwice, Poland. [4]Silesian University of Technology, Faculty of Chemistry, M. Strzody Str. 9, Gliwice, Poland. [5]AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Cracow, Poland. [6]Silesian University of Technology, Faculty of Biomedical Engineering, Zabrze, Poland

Abstract

Anodization process is used to form a porous oxide layer enriched with bioactive compounds on the surface of the titanium implants. Degradable polymers such as poly(sebacic anhydride)(PSBA) or poly(adipic anhydride)(PADA) are easily loaded with the drugs (amoxicillin, cefazolin) and deposited on the anodized implants using a dip coating. Final results of the surface treatment strongly depend on the implant requirements. For bone tissue, a bioactive surface, as well as bacteriostatic, is desirable for long-term implants. Formation of a hybrid layer provides a good opportunity to obtain a surface with favorable morphology for osteoblast adhesion, and fast drug release and protects the surface against bacteria adhesion and biofilm formation. In this work, we present the results of the hybrid layer formation on real shape bone wedges. The bone wedge was anodized in a solution of 0.1M $Ca(H_2PO_2)_2$ at 350V. Then, the implants were immersed in a solution of 1% PSBA or PADA in chloroform. Immersion was controlled using the dip coater, and the best result was obtained when immersion and withdraw speed was 100 mm/min. The polymer solutions were enriched with drugs and successfully deposited on a previously anodized surface. SEM analysis showed that the porous oxide layer is slightly covered by polymer with drug (Fig.1). The concentration of drug released from the coatings, and the stability of the drug in polymer storage at various conditions was analyzed using high performance liquid chromatography. It was found that the cefazolin show lower drug stability than the amoxicillin. However, the concentration of cefazoline released from the bone wedges after its 1h immersion in phosphate buffer solution was 4.91±0.87 µg/mL, whereas for the implant with amoxicillin 6.25±1.91 µg/mL. The exctract inhibited bacteria S.aureus ATCC25923, S.epidermidis ATCC12228 growth. On the other hand, the collected extract from the implants was cytocompatible with osteoblast-like MG-63 cells and mouse fibroblast L929.Additionally, the polymers loaded with drug were treated using ethylene oxide gas, irradiation process, UV, and the chemical composition of was analyzed using an FT-IR technique. The proposed surface treatment of titanium bone wedge may find application in medicine and in veterinary.

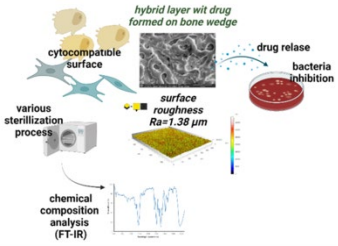


Fig.1. Surface morphology of hybrid coatings formed on Ti bone wedge, and provided analysis.

This work was supported by the National Centre for Research and Development, Poland, according to the LIDER XI programe. Project "Technology of hybrid coatings formation on titanium implants for animals" LIDER/12/0048/L-11/19/NCBR/2020.

PoA.22.07

Development of a photoresponsive drug delivery system targeting dopaminergic neurons

Hajar Alghamdi, Giuseppe Mantovani, Keith Spriggs, Pavel Gershkovich, Mischa Zelzer

University of Nottingham, Nottingham, United Kingdom

Abstract

INTRODUCTION

Parkinson's disease is a neurodegenerative disease marked by poor prognosis. Current treatment modalities administering dopamine (DA) in solution are affected by progressively deteriorating effectiveness due to overexposure to dopamine. Here, we aim to address this by developing an on-demand dopamine receptor stimulation system that reduces dopamine overexposure. By conjugating dopamine to nanoparticles via a light-responsive spiropyran (SP) linker [1],(Fig1) we are able to non-invasively control dopamine receptor activation on-demand in a cell model that we established for this project.[2,3] **MATERIALS AND METHODS** Molecular docking was used to explore the interaction of our SP-DA platform with a dopaminergic receptor. The SP-DA design was then realized by preparing silica nanoparticles via the Stöber method, followed by particle surface modification with SP and DA. To prepare an *in vitro* model system for PD, SH-SY5Y cells were treated with a neurotoxin (MPP+). cAMPGlo assays were performed on the cells to test the SP-DA modified particles. **RESULTS AND DISCUSSION** Light responsive material that display dopamine on demand upon activation by UV light were fabricated and characterised. Appling the open, active merocyanine (MC) form of this material to cells of the PD model with low level of cAMP results in a significant increase of the amount of cAMP compared to control samples (Fig2). Molecular docking of SP-DA to dopaminergic receptor shows high affinity of the active merocyanine compared to the inactive SP.

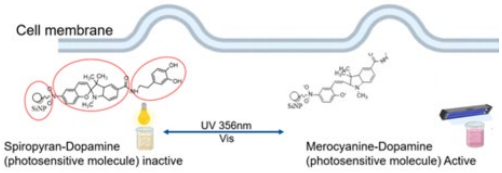


Figure 1. Conceptual Figure

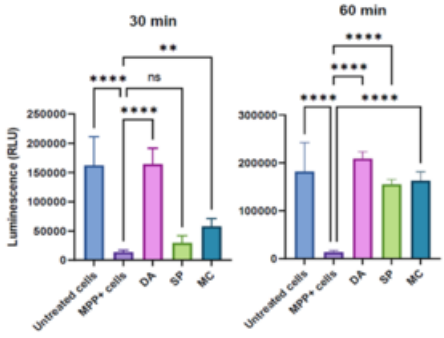


Figure 2. Effect of light induced conformational changes of dopamine-modified nanoparticles on dopamine receptor activation measured by cAMP levels. All Shsy5y cells except the control samples have been treated with 1 mM MPP+ (neurotoxin) which results in decrease cAMP levels in the cells. DA (Dopamine modified nanoparticles), SP (Spiropyran modified nanoparticles), MC (Merocyanine modified nanoparticles) applied to the cells for 30 min and 60 min results in elevated cAMP levels. For the MC samples (Merocyanine modified nanoparticles), after 60 min, cAMP levels were three times higher than after 30 min.

CONCLUSION

Light activation of our synthesized Dopamine-spiropyran-SiNP shows significant differences in cellular cAMP levels, which can be associated with different levels of receptor stimulation.

REFERENCES

1. Zhang X, Heng S, Abell AD.. Chem - A Eur J. Published online 2015.
2. Pettifer KM, Jiang S, Bau C, et al. 2007;3(4):399-409.
3. Jin Jung Y, Choi H, Oh E.. Neurosci Lett. 2021;765(September):136265.
4. Cardano F, Del Canto E, Giordani S. Dalt Trans. Published online 2019.

PoA.22.09

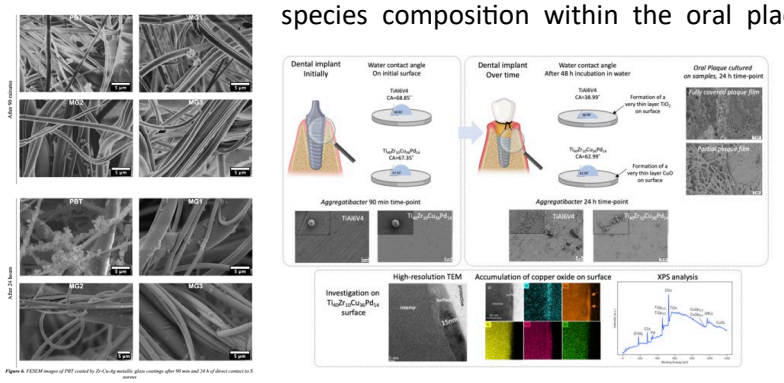
Metallic glass thin films, microfibers and bulk systems for biomedical applications

Elham Sharifikolouei[1], Baran Sarac[2], Sara Ferraris[1], Ziba Najmi[3], Andrea Cochis[3], Alessandro Calogero Scalia[3], Sergio Perrero[1], Maryam Aliabadi[4], Silvia Spriano[1], Lia Rimondini[3]

[1]POLITO, Turin, Italy. [2]Erich Schmid Institute of Materials Science, Leoben, Austria. [3]UPO, Novara, Italy. [4]German Institutes of Textile and Fiber Research, Denkendorf, Germany

Abstract

Metallic glasses are a revolutionary class of materials where crystallization upon solidification is suppressed, keeping them in a disordered state. Many phenomena such as wear release, or cytocompatibility depend on the surface properties and metallic glasses often have lower surface energies regulating their behavior and their interaction with cells and/or bacteria. We are studying and developing the potential use of metallic glasses as intrinsic antibacterial materials without a need for antibiotic or nanoparticle loading on their surface. In this work, we have discussed different metallic glass systems for their application as antibacterial coating, dental and orthopedic implants. First, we have presented a series of different compositions of Zr-Cu-Ag ternary metallic glass thin films, prepared by Physical Vapor Deposition magnetron by co-sputtering on Polybutylene terephthalate (PBT) substrate, showing a superhydrophobic nature and a ≈ 95% antibacterial activity. Human mesenchymal stem cells were used for direct cytocompatibility evaluation of coated samples and their metabolic activity was evaluated via relative fluorescence unit after 24 hours and 5 days. The results were further visualized by FESEM, Fluorescent staining by Live/Dead Viability/Cytotoxicity Kit and confirmed the cytocompatibity of all coated samples. In another system, we have introduced five new biocompatible Ti-based metallic glass (MG) compositions with different metalloid and soft metal content for a synergistic improvement in corrosion properties. Without any potentially harmful elements such as Cu, Ni or Be, these novel alloys can eliminate the risk of inflammatory reaction when utilized for permanent medical implants. Furthermore, bulk Ti40Zr10Cu36Pd14 metallic glasses as dental implants were investigated for its performance in the inhabitation of oral biofilm formation in comparison with the gold standard Ti-6Al-4V implant material. Metallic glasses cytocompatibility was first demonstrated towards human gingival fibroblasts, and then the antibacterial properties were verified towards the oral pathogen Aggregatibacter actinomycetemcomitans responsible for oral biofilm formation. After 24 h of direct infection, metallic glasses reported a >70% reduction of bacteria viability and the number of viable colonies was reduced by ~8 times, as shown by the colony-forming unit count. Oral biofilm obtained from healthy volunteers was cultivated onto specimens' surface, and proteomics was applied to study the surface property impact on species composition within the oral plaque. The underlying reason for this significantly improved antibacterial performance was investigated by high resolution transmission electron microscopy and XPS analysis. We have further used thermoplastic forming on Ti40Zr10Cu36Pd14 metallic glasses and studied its effect on the cytocompatibility.



PoA.22.10

Layer-by-layer deposition of silk fibroin aqueous solution: Mechanical and adhesion properties

Benedetta Isella[1,2], Alexander Kopp[2], Ted J Vaughan[1]

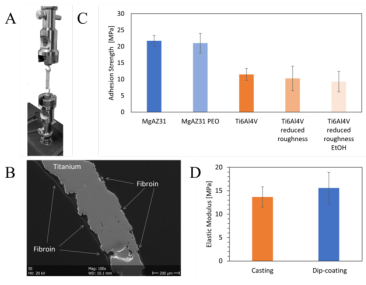
[1]Biomechanics Research Center (BioMEC), Biomedical Engineering, School of Engineering, College of Science and Engineering, University of Galway, Galway, Ireland. [2]Fibrothelium GmbH, Aachen, Germany

Abstract

Introduction Silk is a natural polymer that is composed of fibroin and sericin. Despite the biodegradability [1] and the useful functions that silk fibroin can provide when applied as a coating [2,3], its mechanical properties and the mechanics of its adhesion remain poorly understood. In this study, both the mechanical and adhesion properties on two separate metallic biomaterials of dip-coated silk fibroin were investigated. **Methods** Dip-coated coatings obtained from silk fibroin aqueous solution were first analysed in terms of roughness and distribution (SEM). Water contact angle and Fourier transform infrared spectroscopy were used to understand the structure of silk fibroin. Mg AZ31 and Ti6Al4V substrates were then dip-coated and studied to investigate the adhesion properties (Figure 1A) after coating and after incubation in a medium containing Protease XIV. The mechanical properties of the coatings were evaluated using tensile testing by using self-standing dip-coated structures and compared to casted membranes. **Results** Fibroin is able to significantly reduce the roughness of the substrate surface, a mechanism that suggests mechanical interlocking (Figure 1B). On both substrates, the dip-coating process showed high adhesion strength, being equal to 21.57 ± 1.38 MPa for Mg AZ31 and to 11.47 ± 1.82 MPa for Ti6Al4V (Figure 1C), which starts declining only after 8 weeks of incubation. The mechanical behaviour exhibited by fibroin in the tensile tests is elasto-plastic with an elastic modulus of 15.58 ± 3.38 MPa similar to the one obtained for casted membranes (Figure 1D). **Conclusion** The study characterised the mechanical performance of silk fibroin coatings and their surface adhesion properties to both Mg AZ31 and Ti6Al4V. The coatings showed high adhesion and reduced surface roughness substantially when applied to both metallic materials, which could be beneficial for cell-implant interactions. The mechanical interlocking mechanism explains the general adhesion behaviour of silk fibroin, but chemical interactions need also to be considered in the adhesion between Mg AZ31 and fibroin. The mechanical properties measured in the tensile tests showed the comparability between the structures obtained through dip-coating and casting, opening the possibility to obtain self-standing structures.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie grant agreement ID 956621.



References

[1] Kundu (et al.), 2013

[2] Popescu (et al.), 2020

[3] Xu (et al.), 2020

Figure 1 - (A) Adhesion strength setup (B) Silk fibroin distribution (C) Adhesion strength results (D) elastic modulus in tensile tests.

PoA.22.11

Erythrocyte-inspired lipid membranes for improved particle hemocompatibility

Francisca L. Gomes[1,2,3], Rick Edelbroek[2,3], Jasper van Weerd[3], Pascal Jonkheijm[2], Jeroen Leijten[1]

[1]Leijten Lab, Dept. of Developmental BioEngineering, Faculty of Science and Technology, Technical Medical Centre, University of Twente, Enschede, Netherlands. [2]Dept. of Molecules and Materials, Laboratory of Biointerface Chemistry, Faculty of Science and Technology, MESA+ Institute for Nanotechnology, University of Twente, Enschede, Netherlands. [3]LipoCoat BV, Enschede, Netherlands

Abstract

Introduction Intravenously injectable materials are the core components of nanotherapeutic systems, diagnostic mediators, or blood substitutes. To improve their hemocompatibility, surface coatings are often needed. Blood cell membranes are one of the most stealthy solutions, having shown promising results in cell viability and prolonged circulation time.[1] However, their potential immunogenicity and the well-known scarcity of blood lead to the need for similar blood-independent alternatives. In this work, we designed erythrocyte membrane-mimicking synthetic lipid coatings of different complexities, and investigated their influence on the hemocompatibility of different particles used in nanomedicine. **Methods** Green-fluorescent silica microparticles (SiO_2 MPs), silica nanoparticles (SiO_2 NPs) and polycaprolactone microparticles (PCL MPs) were coated through a solvent gradient-assisted method adapted from Ferhan et.al.[2] In short, lipid solutions of different compositions were mixed with particles in a solvent, followed by the induction of a buffer gradient. Coating formation was explored using confocal, epifluorescence, and cryo-transmission electron microscopies. Hemolysis assays were conducted by incubating SiO_2 NPs with human erythrocytes at 37°C (4 h) and quantifying hemoglobin release. Plasma coagulation time was determined through fibrin clot formation kinetics after SiO_2 NP incubation (0.5 h). **Results and Discussion** A solvent gradient-assisted lipid coating method (Fig.1a) was first optimized on SiO_2 MPs with a simple formulation. After optimizing the conditions, erythrocyte-mimicking lipid coatings (Fig.1b) were applied to SiO_2 MPs, SiO_2 NPs, and PCL MPs, confirming that all three substrates were successfully coated. Complex lipid formulations showed better suspension stability than simple coatings. Assays showed a ten-fold reduction in hemolysis in all conditions, while plasma coagulation kinetics resulted in delayed coagulation in all cases, particularly in complex coatings.

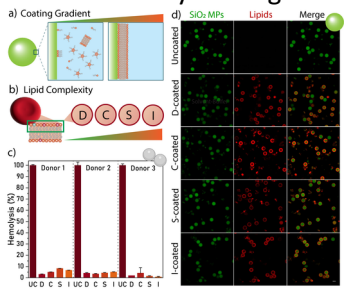


Figure 1. a) Solvent gradient coating principle. b) Erythrocyte-inspired formulations of increasing lipid complexity. C) Hemolysis of SiO_2 NPs. d) Confocal microscopy of coated SiO_2 MPs. Scale bar equals 5 µm.

Conclusions We report on an innovative method of lipid-coating particles of different size and surface chemistry with erythrocyte membrane-mimicking formulations. All coatings improved particle hemocompatibility, with complex coatings resulting in higher suspension stability and delayed coagulation. Ongoing studies focus on analyzing 25 blood biomarkers to gain a deeper understanding on the mechanisms of blood response to artificial erythrocyte membranes.

Acknowledgements F.L.G. acknowledges the TechMed Donor Service of the University of Twente and donors. Authors acknowledge financial support from Health~Holland (Project LSHM19074).

References

[1]Fang, R. H. *et al. Advanced Materials* 30, 1706759 (2018).

[2]Ferhan, A. R. *et al. Nature Protocols* 14, 2091-2118 (2019).

PoA.22.12

Plasma-activated coatings for rapid, covalent, linker-free biomolecule attachment to cell culture surfaces-from the research bench to the commercialisation space

Stuart T Fraser[1,2], Thao H Tran[1,3], Kanako Coffi Dit Gleize[4], Badwi B Boumelhem[1], Jameel Sardharwalla[1], Auriol Purdie[5], Aaron Gilmour[1], Xuege Feng[1], Shelley FJ Wickham[4,3,6], Marcela MM Bilek[1,3,6]

[1]University of Sydney, School of Biomedical Engineering, Camperdown, Australia. [2]CULTURON Pty Ltd, Sydney, Australia. [3]University of Sydney, School of Physics, Camperdown, Australia. [4]University of Sydney, School of Chemistry, Camperdown, Australia. [5]University of Sydney, School of Veterinary Science, Camperdown, Australia. [6]University of Sydney, Sydney Nano Institute, Camperdown, Australia

Abstract

One of the greatest barriers to progress in tissue engineering is the inability to easily generate bespoke cell culture surfaces to regulate and direct stem cell differentiation. Currently, critical microenvironmental elements such as extracellular matrix (ECM) components, growth factors, activators and inhibitors cannot be easily covalently bound to the surface of cell culture dishes or multiwell plates. Conventional approaches, such as coating cell culture plates with ECM, rely on time-consuming, costly protocols resulting in non-covalent physisorption. These products are prohibitively expensive with stability problems in culture. Other protocols require elaborate, often toxic, linker chemistry. To overcome this barrier to progress, plasma-activated coatings (PAC) have been developed for use in the non-flat geometry present in cell culture plates and dishes. These coatings, on the base of the well of cell culture plates, are nanometers-thick and result in rapid, covalent attachment of multiple cargo molecule types in a simple buffer. No further chemical reagents are required nor is linker chemistry is necessary. This facile, non-toxic coating is biomolecule-agonistic as the radicals embedded within the PAC can covalently bind proteins, peptides, simple and complex carbohydrates, nucleic acids, vitamins and drugs. Remarkably, biological function is maintained and indeed covalent protein attachment appears to increase protein stabilisation. PAC-treated plates are being used to develop bespoke complex microenvironments for stem cell differentiation. These same plates are being used to develop the next generation of ELISA tests with covalently attached antigens or antibodies. PAC-treated glass chamber slides are being developed to improve cell behavior on an optically superior interface typically challenging for cell culture. These coatings do not alter optical performance significantly. PAC-treated glass coverslips enhance attachment and maturation of stem cell cultures in response to covalently attached ECM, compared to physisorbed ECM. Here, we will present recent developments and show the pathway to commercialisation of this technology, leading to the creation of CULTURONTM, a new biotechnology company based in Sydney, Australia offering cell culture surfaces for optimal cell attachment, growth and differentiation.

PoA.22.15

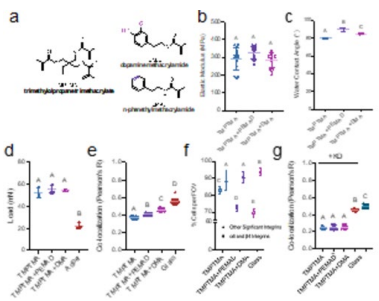
Bioinstructive light-curable coatings direct pericellular laminin to induce hemidesmosome formation on transepithelial implants

Nicholas G. Fischer[1], David A. De Jong[1], Conrado Aparicio[1,2,3]

[1]University of Minnesota, Minneapolis, USA. [2]UIC Barcelona, Barcelona, Spain. [3]IBEC-Institute for Bioengineering of Catalonia, Barcelona, Spain

Abstract

Introduction: Extracellular matrixes provide signals perceived by resident cells. The pericellular space is at the center of the dynamic and reciprocal relationship between cells and their environment. Elaborated pericellular matrix is continuously remodeled and reassembled by cells through bi-directional signaling. Implants disrupt this instructive matrix and may impair regenerative healing. Transepithelial implants(TrEpIm), those penetrating skin/mucosa have high failures rates; e.g., 1M/year dental implants fail worldwide. But teeth display marked longevity as resident keratinocytes secrete a matrix rich in laminin332(LN332) to ligate epsecialized integrin/hemidesmosomes(HDs) to link the gingival matrix and tooth. HDs impart soft tissue-tooth stability to prevent failure-causing infection. Biomaterials used to enhance TrEpIm healing suffer from dynamic interplay between cells and matrix; the nascent matrix enrobing cells masks and overrides material cues. An alternative strategy is engineering bioinstructive, synthetic materials to control pericellular matrix display thereby recapitulating processes that form reparative niches. We present a broadly-applicable meth/acrylate-based coating to leverage keratinocyte secreted matrix to instruct epithelial cell behavior toward extending the lifespans of TrEpIm. Methods: Trimethylolpropane trimethacrylate (TMPTMA), n-phenethylmethacrylamide (PEMAD) , dopamine methacrylamide (DMA), and other structurally-similar monomers were polymerized with a thiol-ene crosslinking system to systematically vary coating' surface chemistry, polarity, and charge. Formulations' thermal, chemical, mechanical, and surface properties were characterized. Keratinocyte HDs formation was quantified with immunofluorescence co-localization. The outside-in signaling mechanism responsible for out materials' bioinstruction was determined through systematic integrin blocking and transient LN-332 knockdown (KD). Results: All formulations (Fig-1a) displayed similar bulk mechanical properties (Fig-1b), varied water contact angles (Fig-1c) and, possessed a higher load at failure than a commercially-available adhesive (Fig-1d). Keratinocyte HD formation (Fig-1e) was highest on formulations with DMA (i.e., TMPTMA+DMA) compared to PEMAD (i.e., TMPTMA+PEMAD), which was higher than pure TMPTMA. Integrin blocking (Fig-1f) and LN-332 KD HD formation (Fig-1g) results demonstrated the interplay between the polymer and secreted LN332 in the pericellular matrix to direct HD formation. Pericellular LN332 structure was responsible for signaling events that determined feedback mechanisms between cells, matrix, and materials. *Figure 1: a) Base polymers b) Elastic modulus c) Water contact angle d) Scratch test load to failure e) HDs formation co-localization f) Integrin blocking g) HDs formation co-localization with LAMC2 transient KD. Statistics – ANOVA; triplicates*



Conclusions: Our materials provide insight into the nature of cell–materials interfaces, and mediation by pericellular matrix, offers a biologic-free, broadly-applicable strategy for extending lifespans of TrEpIm.

PoA.22.16

Differences of cell migration between ADSC and osteoblast differentiated from ADSC by micro-patterned surface and electrical stimulation

Mi Hee Lee, Jong-Chul Park

Yonsei University College of Medicine, Seoul, Korea, Republic of

Abstract

Cell migration is a phenomenon that can be observed naturally: in developmental, physiological, and disease-related processes. Electrical stimulation of cells induces a polarized state, influencing direction of cell motility. The extent varies across cell type. Adipose-derived stem cells (ADSCs) are an attractive subject because they are less intrusive to collect and have high proliferation, rendering them ideal for therapeutic applications. In this study, the difference in the direction and speed of motility in ADSCs, corresponding differentiated osteoblasts at 7 and 14 days were measured. To increase directedness, cells were seeded along a micro-patterned fibronectin stamp and compared with cells seeded randomly. Cells were stimulated in a custom agar-salt electotaxis chamber environment. Cells were stimulated with 1200 μA for 3 hours, with position being recorded at intervals of 10 minutes. The micro-patterning was shown to improve directedness significantly compared to cells seeded randomly, highlighting lower migration speed of osteoblasts compared to ADSCs. Therefore, not only does fibronectin micro-patterned stamping improve directedness of cell migration but notes lower speed ADSCs and osteoblasts differentiated for 7 and 14 days. This insight could potentially be used to easily separate differentiated cells from undifferentiated ADSCs.

PoA.23.02

Bilirubin-based nanomedicine for anticancer and anti-inflammation therapy

Sangyong Jon

Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of

Abstract

Despite the high potency of bilirubin (BR) as an endogenous anti-inflammatory compound, its clinical translation has been hampered because of its insolubility in water and potential toxicity on erythrocytes and immune cells. To overcome the critical issues, we attached polyethylene glycol (PEG) to BR, yielding PEGylated bilirubin (PEG-BR). The PEG-BR self-assembled into nanoscale particles with a size of approximately 110 nm, termed bilirubin nanoparticles (BRNPs). Unlike free BR, BRNPs are fairly water-dispersible and circulate much longer in blood, thus overcoming a critical issue associated with the clinical use of BR. Recently, we demonstrated that BRNPs had potent therapeutic efficacy in animal models of several inflammatory diseases, including inflammatory bowel disease, acute asthma and hepatic ischemic reperfusion injury. We also demonstrated that BRNPs can be used as a dual-stimulus (light and ROS)-responsive drug-delivery carrier, reflecting the fact that BR in NPs undergoes a switch in water solubility and degradation in response to these stimuli. Very recently, we recoginzed that BR, a bile pigment that exerts potent antioxidant and anti-inflammatory effects, is also a major constituent of black pigment gallstones found in bile ducts under certain pathological conditions. Inspired by the intrinsic metal-chelating power of BR found in gallstones, in this talk I will present various metals-chelated BR-based nanoparticle for use as a new theranostic nanomedicine for combined cancer imaging and photothermal therapy.

PoA.23.03

Improving hemocompatibility: Interactive coatings that can modulate hemostasis at the surface of blood-contacting medical

Lena Witzdam[1,2,3], Fabian Obstals[1,2], Manuela Garay-Sarmiento[1,4], Jonas Quandt[1,2], Nina Kostina[3], Oliver Grottke[5], Cesar Rodriguez-Emmenegger[1,3,6,7]

[1]DWI – Leibniz Institute for Interactive Materials e.V., Aachen, Germany. [2]Institute of Technical and Macromolecular Chemistry, RWTH Aachen University, Aachen, Germany. [3]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain. [4]Chair of Biotechnology, RWTH Aachen University, Aachen, Germany. [5]Department of Anesthesiology, University Hospital of the RWTH Aachen University, Aachen, Germany. [6]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain. [7]Biomedical Research Networking, Center in Bioengineering, Biomaterials and Nanomedicine, The Institute of Health Carlos III, Barcelona, Spain

Abstract

The contact of blood with the surface of medical devices inevitably causes the activation of coagulation leading to a number of serious effects. Immediately after the contact of blood with the surface, protein adsorption occurs. This leads to the reciprocal activation of factor XII and plasma prekallikrein generating large amounts of thrombin, which locally overwhelm the inhibitory effect of current anticoagulants. The formation of clots in blood-contacting medical devices can lead to life-threatening complications such as thrombosis and stroke. In nature, the lining of healthy endothelium is capable of sensing and maintaining a tightly regulated equilibrium, called hemostasis that prevents hemorrhages and excessive coagulation. Our goal is to develop coatings inspired by the endothelium that turn the surface of medical devices hemocompatible to prolong their use without negative outcomes. Towards this aim, we develop coatings that go beyond passivation but that interact with blood and orchestrate a cascade of reactions to enhance their hemocompatibility and performance. The coatings include three hierarchical levels: a passive, a modulatory, and an interactive one. The passive level consists of antifouling polymer brushes that create a physical barrier to protein adsorption and cell adhesion thereby prohibiting the surface-induced activation of coagulation. The modulatory level is achieved by decorating the brushes with small biomolecules capable of binding to key elements of the coagulation cascade and inactivating them directly at the surface of the device. In contrast to anticoagulants, this approach allows a local inhibitory effect at the surface and does not interfere with hemostasis. However, what happens if the clot is formed at a different site in the system? To address this we introduce an interactive level that can sense the presence of a thrombus and orchestrates its disintegration. We develop a fibrinolytic coating that is only active in the presence of a thrombus and orchestrate its destruction using components present in blood. After digestion, the coating return to its dormant state. In this talk, I will show the preparation of the coating, the demonstration of their functions, and the translation to oxygenators, catheters, and artificial hearts.

Acknowledgment: The authors acknowledge the financial support by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) via Schwerpunktprogramm “Towards an Implantable Lung”, 346972946 and by the German Federal Ministry of Education and Research (BMBF) with the project Heart2.0 within the “Bio4MatPro-Competence Center for Biological Transformation of Materials Science and Production Engineering” program (grant no. 031B1154B).

PoA.23.04

Development of convenient cell sorting column device using physiologically active copolymer.

Moe Kato[1], Tadashi H NAKAJI[1], Yuki Usui[2], Takahiro Kishioka[2], Taito Nishino[3]

[1]Univ. of Toyama, Toyama, Japan. [2]NISSAN Chem Co. Ltd, Toyama, Japan. [3]NISSAN Chem Co. Ltd, Saitama, Japan

Abstract

In order to implement and develop the cell therapy, we must overcome various challenges such as establishing a stable supply of cell sources, developing methods to strictly regulate the cell behavior, and ensuring the safety of cell sources. To overcome these challenges, it is necessary to develop technique for selectively separating and purifying the desired cell types. For instance, the transplantation of cells containing various cell types carries several large risks such as causing cancer and reducing the efficiency of differentiation into the desired cell types. There are several methods for cell separation and purification. However, existing methods have a problems as the technique of a cell isolation and purification for transplantation therapy, For example, fluorescence-activated cell sorting is excellent system for the isolation of specific cells, but it is concerned that fluorescent-labelled antibodies causing cytotoxicity reduce on the surface of separated cells and flow-induced shear stress causes the damage to the cells. In this study, we aim to develop a devise which can easily separate only a specific type of cells under minimizing shear stress to cells. Specifically, we developed silica beads modified with a physiologically active polymer mainly composed of zwitterionic monomer [1], which suppresses protein adsorption and non-specific cell adhesion. Additionally, this polymer has the cellular membrane receptor-binding peptide [2], which can selectively capture specific cells, as a side chain. The target cells are human bone marrow mesenchymal stem cells (hMSCs), which have the ability to differentiate into bone, cartilage, fat, and muscle. We added hMSCs and human embryonic kidney (HEK293) cells to the column filled with the silica beads modified with CD44-binding peptide-anchored zwitterionic polymers, and investigated the separation of hMSCs and HEK293 cells. As shown in Figure 1, HEK293 cells were recovered at 94% in fractions 1-30, whereas hMSCs were recovered at 67% after fractions 30. In addition, we evaluated the proliferation and differentiation potencies of the isolated hMSCs. It was indicated that cells separated by our column device showed no loss in proliferation ability or multipotency, indicating that the cells are safe for use.

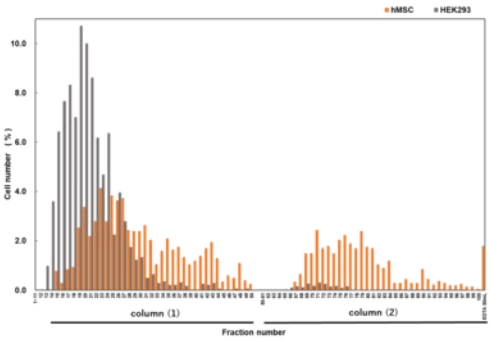


Figure 1. Separation profile of hMSCs and HEK293 cells. The gray bars represent HEK293 cells, and the orange bars represent hMSCs, indicating the percentage of each cell in each fraction. After fraction 50 of Column (1), a 5 mM EDTA solution was added to recover the remaining cells in the column. The collected cells was added to Column (2).

PoA.23.05

Endothelium-inspired synthetic coating for medical devices to reduce coagulation by catalytically releasing nitric oxide

Jenny Englert[1,2], Lena Witzdam[1,3,4], Cesar Rodriguez-Emmenegger[1,4,5,6]

[1]DWI – Leibniz Institute for Interactive Materials e.V., Aachen, Germany. [2]Chair of Biotechnology, Aachen, Germany. [3]Institute of Technical and Macromolecular Chemistry, Aachen, Germany. [4]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain. [5]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain. [6]Biomedical Research Networking, Center in Bioengineering, Biomaterials and Nanomedicine, The Institute of Health Carlos III, Barcelona, Spain

Abstract

We develop an endothelium-inspired synthetic coating to fight the detrimental effects of surface-induced coagulation on medical devices. When blood comes in contact with any surface different than the endothelium, coagulation begins, leading to the formation of deposits on blood that affects the performance of the medical device, can propagate to systemic coagulation and ultimately may pose risks to the lives of the patients. To date, the most successful coatings are based on the immobilization of heparin or its derivatives which hydrophilize the surface and partly neutralize in-situ generated thrombin. However, the activity of these coatings is seldom enough to prevent a specific adsorption of proteins, ultimately leading to the failure of the device. In this poster, we present a nanoscale coating that combines passive stealth properties with active modulation of hemostasis at the surface. Like the endothelium, our coating provides stealth properties of the medical surface to inhibit activation of coagulation. The passive properties are introduced by hydrophilic polymer brushes grafted from the surface. The strong hydration and entropic penalty prohibit factors and proteins of the contact and complement system from being activated, turning the surface "invisible" to blood. The brushes of *N*-hydroxypropyl methacrylamide and carboxybetaine methacrylamide reduce the fouling of blood plasma to less than 7 ng·cm^{-2} compared to 200 ng·cm^{-2} which correspond the a reduction of 97%. The active properties are based on the modulation of platelets by the release of nitric oxide, for which two routes are explored: the immobilization of inducible nitric oxide synthase (iNOS) and the immobilization of a synthetic enzyme. iNOS is an enzyme produced by the endothelium, which was bound to the polymer brushes (557 ng·cm^{-2}). The synthetic enzyme is formed by tri-tert-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate and copper chloride, which was immobilized on the polymer brushes. Ultimately, the coatings are translated to commercial venous catheters. In contrast to the systemic treatment with anticoagulants, this approach relies on the local release of nitric oxide and thus prevents the activation of coagulation on site. We envision that our coating will significantly contribute to the future of hemocompatible coatings for medical devices.

Acknowledgement: The authors acknowledge the financial support by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) via Schwerpunktprogramm "Towards an Implantable Lung", 346972946 and the German Federal Ministry of Education and Research (BMBF) with the project AntiBacCat within the "Bio4MatPro-Competence Center for Biological Transformation of Materials Science and Production Engineering" program (grant no. 031B1153A).

PoA.23.06

Evolution of a synthetic bone graft substitute over last decades – MBCP technology from osteoconductive to smart scaffold for bone repair

Cyril d'Arros, Pascal Borget

Advanced Medical Solutions Group plc - Biomatlante, Vigneux de Bretagne, France

Abstract

Biphasic Calcium Phosphate (BCP) material is currently one of the most common synthetic Bone Graft Substitute (BGS) used in clinical situation to replace the gold standard treatment (autograft). MBCP technology (Micro-macroporous Biphasic Calcium Phosphate) is recognized as osteoconductive, highly bioactive, and even possessing osteoinductive properties. Osteogenicity is still a property lacking for the synthetic bone graft substitutes. Thus, bone tissue engineering strategy combining cells and/or molecules allows to improve efficacy for bone repair. MBCP®+ granules are composed of 20% Hydroxyapatite and 80% beta-Tricalcium Phosphate and demonstrate a dynamic surface evolution when used in physiological environment (apatitic needles growing observed *in vitro* and *in vivo*). This behavior of MBCP®+ acts like a smart biomaterial and seems to be an ideal scaffold for tissue engineering strategies. These granules selected as scaffold for combination with B2A peptide (molecule which augments the osteodifferenciation via increasing BMP2 at local site of the implantation) were evaluated in two different clinical indications (Prefix study in lumbar spine fusion and with Amplex study in foot and ankle fusion) and have demonstrated safety and efficacy. The same BCP granules were also used associated with autologous mesenchymal stromal cells (MSCs) in clinical trials (European projects Reborne, Maxibone, and OrthoUnion) and have demonstrated promising results with bone regeneration in non-union long bone and in atrophied mandibular bone situations. A new generation of BGS called FDBS (Freeze Dried Bone Scaffold), based on the same smart biomaterial, was recently designed as a dried powder to be rehydrated with any solution chosen by the surgeon to get a highly moldable and cohesive putty. FDBS rehydrated with MSCs suspension demonstrated the new product as a viable moldable and cohesive platform with similar cell behavior compared to granule shape (proliferation and differentiation of the MSCs into the putty compared to MBCP®+ scaffold by seeding the MSCs onto the granules). Preliminary data on FDBS also demonstrated the capability to be used with blood, Bone Marrow Aspirate (BMA), or even PRP rehydration offering many opportunities for clinical unmet needs. A special focus seems important for medical device companies in the next years to design ATMP products easy to use as therapeutical solution for highly critical bone defects.

PoA.23.07

Development of antibacterial urinary catheters for the prevention of CAUTI using pH responsive hydrogel coatings

Jingyi Xu[1], Colin P McCoy[2], Matthew P Wylie[1]

[1]Queen's university of Belfast, Belfast, United Kingdom. [2]Queen's univeristy of Belfast, Belfast, United Kingdom

Abstract

Background Many patients who undergo long-term urinary catheterization become infected with bacteria which can lead to complications such as catheter-associated urinary tract infections (CAUTI). Catheters become blocked and encrusted due to bacterial species that produce urease. Urease can hydrolyze urea in urine to generate, raising the urinary pH and causing calcium phosphate and magnesium phosphate to deposit on the catheter surface. Hydrogels have been further developed over the last 2-3 decades to become 'smart' or responsive hydrogels in which polymers or crosslinkers with special chemical properties can allow the hydrogel to become responsive to specific environmental triggers such as light, temperature, pH, ionicity, and enzymes. In CAUTI, the alkaline shift in urinary pH can be exploited through development of catheter antibiotic-loaded hydrogel coatings which swell in response to alkaline pH, releasing antibiotics to eradicate adjacent bacterial cells. Therefore, pH responsive hydrogels as 'smart' materials have high potential research value in the treatment of CAUTI. Methods 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), 2,2'-azo-iso-butyronitrile (AIBN) and methacrylic acid (MAA) were used to form hydrogels. Release of rifampicin from pH-responsive hydrogels was determined at neutral (pH 7) and alkaline (pH 10) conditions to simulate healthy and infected urine, respectively. Bacterial adherence assay was performed for unloaded and loaded rifampicin in the pH responsive hydrogels against Escherichia coli (E. coli) ATCC NSM59 and Proteus mirabilis (P. mirabilis) ATCC 51286 to determine the bacteria viability. Results

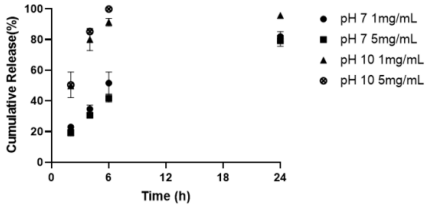


Figure 1. Cumulative release curves of 1 mg/mL and 5 mg/mL rifampicin from p(HEMA-co-10%MAA) at pH 7 and 10. Each time point represents an average ± SEM (n = 5).

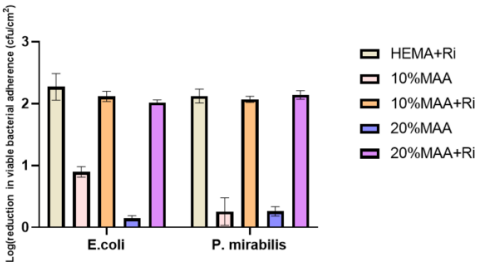


Figure 2. The microbial adherence of E. coli and P. mirabilis to polymers-based HEMA and polymers loaded rifampicin compared to p (HEMA) control, incubated at 37℃ for 24 h.

MAA possesses an ionizable carboxylic acid group which becomes anionic in alkaline pH leading to electrostatic repulsion between neighbouring MAA polymeric chains. This resulted in increased swelling of MAA-incorporated polymers in alkaline buffer compared to p(HEMA). The increased swelling of MAA-incorporated gels in alkaline pH resulted in a more rapid release of rifampicin compared to neutral pH media with >90% drug release within 6 h compared to 50% at pH 7 for 10% MAA gels. During initial bacterial studies the more rapid release of rifampicin from MAA-incorporated gels did not lead to a significant improvement in reducing bacterial adherence compared to non-stimuli responsive HEMA gels.

PoA.23.08

Fundamental study of $CaCO_3$ coating on Mg for improving corrosion resistance

Daichi Asai[1], Tomokazu Hattori[2,3], Nobunori Takahashi[4]

[1]Dept. of Materials Science and Engineering, Meijo University, Nagoya, Japan. [2]Dept. of Materials science and Engineering, Meijo University, Nagoya, Japan. [3]Dept. of Orthopedi, Aichi Medical Univercityc Surgery, Nagakute, Japan. [4]Dept. of Orthopedic Surgery, Aichi Medical University, Nagakute, Japan

Abstract

Introduction Mg and its alloys are very attractive as bioabsorbable metal for medical application. However, because of high corrosion rate, Mg and its alloys cannot be applied yet for fracture fixation. The limescale of the obstinate white spot is often observed around the wet area of mineral rich water, which is well-known as a sediment of calcium carbonate, where the calcium carbonate is also known as bioactive material. As inspired by the white spot, $CaCO_3$ coating is proposed to improve the Mg corrosion resistance. Materials and Methods Mg disk specimens with ϕ20 x 1 mm were prepared by cutting Mg ingot of 99.95%, and polished, then mirror finished by the buff polishing. Commercial mineral water of Contrex®, which contains Ca^{2+} of 468mg/l, Mg^{2+} of 74.5mg/l, was employed as a source mineral ionized water. And CO_2 gas was blown into the source water to add CO_3^{2+}. The disk specimen was placed on the hot plate heated at 200°C, and the source water was applied to the disk surface with a brush for 20 times. Consequently, a white coating layer of 8-15 µm was formed on the disk surface. In order to identify the chemical composition of the coating layer, XRD analysis was performed. And the peel-off strength of the coating layer was examined. Furthermore, the corrosion resistance of the $CaCO_3$ coated specimen was evaluated by Linear Sweep Voltanmetory, and the laser microscope observation of the corroded Mg surface after soaking in water for two months. Results and Discussion It is confirmed that the main component of the coating layer was $CaCO_3$, with small amounts of $Mg_3O(CO_3)_2$ and $Mg_3Ca(CO_3)_4$. The Peel-off strength was over 4.5MPa. In the initial electrochemical measurement, the $CaCO_3$ coated specimen showed higher corrosion potential and lower current as compared with the non-coated Mg specimen. In daily time course measurement, the corrosion potential gradually decreased, and the current was increased, both were toward the standard values of the non-coated Mg specimens. (Fig.1) As the corrosion due to the soaking in water for 2 months, after the $CaCO_3$ coating layer was removed by acid solution, the surface roughness of Mg substrate under the $CaCO_3$ coating was smaller with shallow dimples than that of the non-coated specimen. (Fig.2) It is demonstrated that the $CaCO_3$ coating can suppress the Mg corrosion. Furthermore, the coating layer can be improved with ionic components such as Zn^{2+}, PO_4^{-3}.

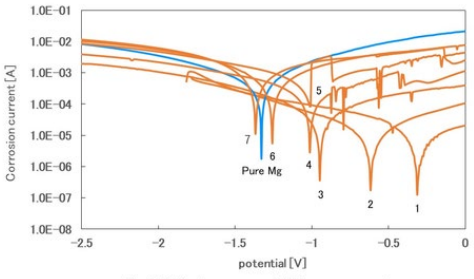
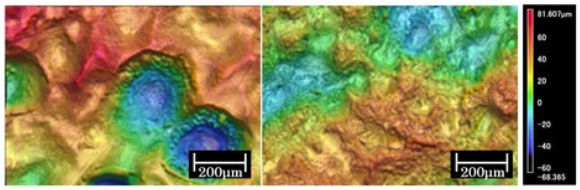


Fig.1 Daily time course LSV measurement



(a) The non-coated Mg specimen (b) The $CaCO_3$ coated specimen

Fig.2 Laser Microscopic Images of corroded Mg surface after soaking in water for 2 months

PoA.23.09

A silver lining: incorporation of hydrophobic quaternary ammonium-capped silver nanoparticles to augment the antimicrobial activity of photodynamic polymeric films.

Robyn N Irwin, Matthew P Wylie, Colin P McCoy

School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom

Abstract

Within nosocomial environments, antibiotic resistance is prevalent, emanating mainly from biofilm formation following medical device insertion. Antimicrobial biomaterial coatings represent a potential strategy for mitigation. Current coatings contribute to antibiotic resistance, governed by their inability to regulate release; a large initial burst subsequently followed by continuous elution at the subtherapeutic dose. Therefore, 'smart' materials; capable of eliciting their antimicrobial effect upon response to exogenous or endogenous stimuli while modulating their action via on/off control are proposed. Photodynamic antimicrobial chemotherapy (PACT) employing light activated photosensitisers has shown promise. Upon irradiation, reactive oxygen species: singlet oxygen and oxygen based radicals are produced, adopting a catalytic multi-mechanistic action; photo-oxidizing biomolecules, contributing to cell lysis and disrupting cell physiology, inhibiting bacteria growth. In this study, photosensitiser Toluidine Blue O (TBO) was immobilised within polycaprolactone, in conjunction with cetyltrimethylammonium bromide (CTAB) capped silver nanoparticles (Ag-NPs) via a solvent casting method. Physicochemical and microbiological characterisation were performed.

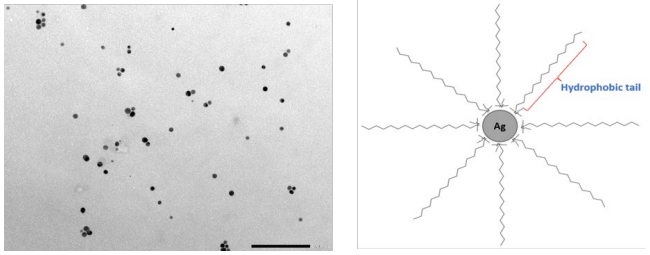


Figure 1. *Transmission electron micrograph of synthesised CTAB capped Ag-NPs with structural schematic representation.*

Previously, it was determined that leaching of the photosensitiser was the main antimicrobial mechanism of action, displaying a profile indictive of burst release. Thus, following immersion in physiological media under sink conditions resulted in a loss of antimicrobial activity. Consequently, to preserve the antimicrobial integrity of the films, CTAB capped Ag-NPs were employed. Due to its long apolar chain as portrayed in figure 1, CTAB invokes hydrophobicity. It was found that the dispersion of Ag-NPs throughout the polymeric matrix decreased the leaching of the photosensitiser due to the net hydrophobic effect, though slight, it could be improved by increasing the Ag-NP concentration.
However, following a bacterial adherence assay conducted under red light irradiation, the films comprising both photosensitiser and nanoparticles retained their antimicrobial activity against Staphylococcus aureus, displaying no growth following seven days of 'soaking' in phosphate buffered saline, highlighted by figure 2.

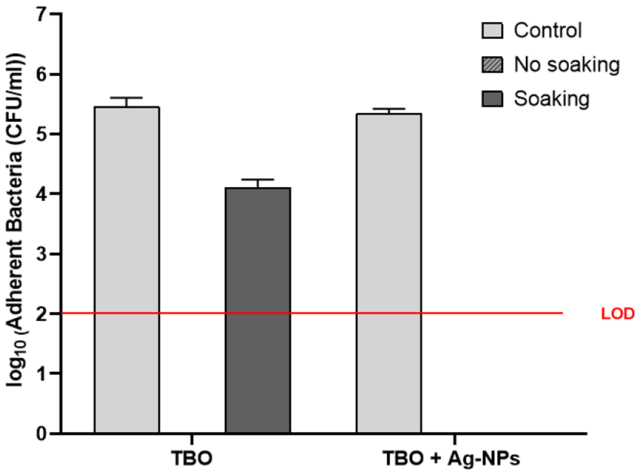


Figure 2. *Viable S.Aureus adhered to 0.5 wt% TBO films with/without Ag-NPs, challenged with 1 x106 CFU/ml. LOD indicates limit of detection.*

We propose the Ag-NPs prolong the antimicrobial activity of the films via three distinct mechanisms: Increased retention of the photosensitiser within the polymeric matrix. Synergistic dual action: enhanced photodynamic effect via increased quantum yield of singlet oxygen attributed to silver's surface plasmon resonance, in addition to oxidative dissolution of Ag+ via generated reactive oxygen species. Inherent antimicrobial activity following leaching of the photosensitiser.

PoA.23.10

3D printing of solid liquid composites for cell-like based artificial skin model

Kianna Cabral[1], Laureline Thiriot[1], Barbara Röhrnbauer[2], Mathias Bonmarin[1], Fabrizio Spano[1]

[1]Institute of Computational Physics, School of Engineering, Zurich University of Applied Sciences, Winterthur, Switzerland. [2]Institute of Mechanical Systems, School of Engineering, Zurich University of Applied Sciences, Winterthur, Switzerland

Abstract

Artificial skin models have become increasingly important in biomedical research. In recent years, 3D printing technology has emerged as a powerful tool for the fabrication of complex, biomimetic skin models. To address this challenge, researchers are exploring the use of solid-liquid composites in 3D printing to create more realistic and functional skin models. Therefore, the development of a skin model using a solid-liquid composite is an attractive approach to bridge the gap between the two types of models. This work presents a 3D printing-based approach to create a cell-like based artificial skin model using a solid-liquid composite material. The composite material is composed of a flexible polymeric matrix (polydimethylsiloxane) and a liquid substance (glycerol with blue dye). The flexible matrix provides structural support and stability, while the liquid substance mimics the presence of cells into the skin, providing an additional mechanical flexibility to the silicon-based material. Based on a previous development of the encapsulation of droplets into unpolymerized silicon-based material, here the 3D printing process allows for precise control over the architecture and composition of the skin model, enabling the creation of multilayered complex structures with cell-like arrangements (Figure 1). The printing process also allows for the incorporation of different substances, different sizes and densities of droplets and droplet arrangements. In a next step, even the incorporation of multiple cell types, such as keratinocytes and fibroblasts, to create a more realistic and functional skin model would be considered. We expect for the resulting skin model to exhibit characteristics like human skin, including mechanical flexibility. Overall, the 3D printing of solid-liquid composites for a cell-like based artificial skin model provides a promising platform for the development of a realistic and functional skin model that can be used for a range of applications, including tissue engineering and durotaxis. The approach described in this work provides a foundation for further optimization and development of skin models using solid-liquid composites. While there are still challenges associated with achieving optimal printing parameters, the potential benefits of these models make them an exciting area of research for the future. In parallel to the development of these 3D printed cell-like based skin models, additional experimental and computational works have been realized elsewhere to characterize the influence of the encapsulated droplets on the local mechanical properties of the obtained material.

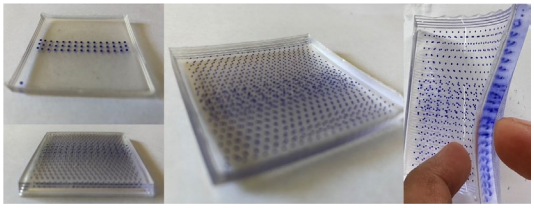


Figure 1: Illustrations of cell-like based artificial skin models obtained via 3D printing of solid liquid composites.

PoA.23.11

A combined experimental and numerical method for tailoring the multi-scale mechanical properties of soft solid liquid composites

Eléonore Cauquil[1], Luca Kiener[1], Jonas Hofmann[1], Fabrizio Spano[2], Barbara Röhrnbauer[1]

[1]Institute of Mechanical Systems, School of Engineering, Zurich University of Applied Sciences, Winterthur, Switzerland. [2]Institute of Computational Physics, School of Engineering, Zurich University of Applied Sciences, Winterthur, Switzerland

Abstract

Solid liquid composites are motivated by a variety of multi-physics applications including research in mechanobiology. From a mechanical perspective, liquid inclusions in a matrix affect both its global and local properties - the latter seen as local stiffness variations. It is known that stiffness variations in substrates are sensed by cells and incite cell migration so-called durotaxis. To investigate this complex interplay, detailed knowledge is needed on the local mechanical properties of the substrate. In this study, a combined experimental and numerical approach is proposed to characterize and tailor the local and global mechanical properties of a soft solid liquid composite. Polydimethylsiloxane (PDMS) membranes with a regular pattern of liquid inclusions of two different sizes (1.1mm, 0.5mm) were produced according to the procedure reported elsewhere. Planar tension tests were performed resulting in a biaxial state of stress, representative for loading conditions of biological membranes. After preconditioning during 9 cycles, samples were strained quasi-statically to 30% nominal strain. In addition to the global force and displacement data, local deformations were evaluated using a digital image correlation system. A numerical model based on a representative unit cell approach was built using a commercial finite element software. The unit cell was modeled as a 3D cuboid containing a spherical inclusion. For the PDMS, a Neo-Hookean material was chosen, which was fitted to test data of pure PDMS. The liquid inclusion was modeled using built-in element types. The model was validated applying both the global force response and the local deformation pattern. A numerical parameter study was performed, varying the size and density of the inclusions. The numerical model was shown to excellently reproduce both, the global force response, and the local deformation pattern (Figure). Apart from the parameter of the Neo-Hookean model, no fitting of parameters was needed resulting in a simple and robust modeling approach. The parameter study revealed the potential to tailor a wide variety of biaxial global stiffnesses (0.20-0.44 MPa) and to finetune local stiffness gradients. Current limitations are the reproducibility of the PDMS properties and the small experimental basis (n=3). However, the feasibility of the approach as well as the excellent predictive capabilities of the model have been shown. This experimental and numerical framework shall be used to investigate phenomena such as durotaxis incited by specifically tailored stiffness gradients and thus, contribute to quantitative research in mechanobiology.

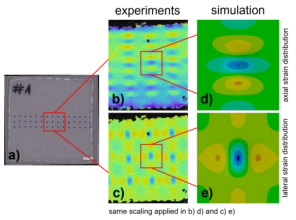


Figure: a) Soft solid liquid composite, b,c) experimental d),e) numerical results

PoA.23.12

Hybrid inorganic-organic smart biomaterials for biofabrication

Uxue Aizarna[1], Ane Urigoitia[1,2], Malou Henriksen Lacey[1,3], Dorleta Jimenez de Aberasturi[1,3,4]

[1]CICbiomaGUNE, San Sebastián, Spain. [2]POLYMAT, San Sebastián, Spain. [3]Centro de Investigación Biomédica en Red de Bioingeniería Biomateriales, y Nanomedicina (CIBER-BBN), San Sebastián, Spain. [4]Ikerbasque, Basque Foundation for Science, Bilbao, Spain

Abstract

Biomaterials presenting stimuli-responsive behaviours have gained importance as they offer the possibility to create models that realistically mimic the forces and stimuli that occur in biological systems. The combination of hybrid inorganic-organic materials that respond to externally applied stimuli could be a suitable method to achieve this. In this respect, we are interested in the synthesis of 3D printable hybrid bioinks that enable us to not only control the spatial resolution on a micro-scale and deposit multiple different inks in close proximity, but also incorporate specific responsiveness in 3D *in vitro* models of pathological interests in a controled manner. By combining cell-containing inks with such stimuli-responsive materials, stiffness, contractility or pulsatility effects can be achieved important for cellular mechanoadaptation processes. These printable materials have been applied to the fabrication of models designed to mimic the artery and alveoli wall, incorporating pulsatile and breathing forces, respectively. Additionally, the use of hybrid bioinks containing plasmonic nanosensors to fabricate multifunctional scaffolds for tumoral growth will be highlighted. [1,2] Within this talk I will discuss the varied roles of hybrid biomaterials and their importance in biofabrication, including recently published work on their use to study the evolution of cells in 4D, taking advantage of multimodal imaging techniques. [3,4]

[1] C. García-Astrain, et al., Adv. Funct. Mater. 2020, 30, 2005407.
[2] E. Lenzi, et al., ACS Appl. Mater. Interfaces 2022, 14, 20708.
[3] C. De la Encarnación, et al., J. Phys. Chem. C 2022, 126, 19519.
[4] D. Jimenez de Aberasturi, et al., Adv. Funct. Mater. 2020, 30, 1909655.

PoA.23.13

A bioelectronics approach for bone tissue engineering.

Donata IANDOLO[1], Darius Widera[2], Roisin Owens[3]

[1]INSERM, U1059, SAINBIOSE, Saint-Étienne, France. [2]University of Reading, Reading, United Kingdom. [3]University of Cambridge, Cambridge, United Kingdom

Abstract

Due to demographic and lifestyle changes, traumatic injuries have grown to become paramount medical and socio-economic challenges in affluent nations. Despite numerous advances in implant technology, grafts prepared using bone extracted from the patient are still the gold standard. However, the increasing life expectancy calls for innovative and effective approaches to compensate for bone loss. The knowledge of bone piezoelectricity has inspired the use of physical stimulation together with electroactive materials as smart alternatives for bone tissue engineering. The combination of smart substrates, stem cells, and physical stimuli to induce stem cell differentiation is therefore a new avenue in the field. Biomimetic scaffolds were prepared by combining the conducting polymer PEDOT:PSS with collagen type I, the most abundant protein in bone. Their pore size, mechanical and impedance properties were measured as a function of scaffold composition. Two populations of stem cells, namely human adipose-derived stem cells, and neural crest-derived stem cells were used to understand the impact of scaffold composition on cell behaviour. Osteogenic differentiation studies were run for 21 days and the different compositions were assessed for their impact on stem cell fate. SEM coupled with FIB was used as a powerful tool to look into the fine interaction between material and cells, highlighting an intimate contact of the cells lining the pore walls. Preliminary electrical stimulation experiments were run using human adipose-derived stem cells and the adopted capacitive coupling protocol proved to positively affect stem cell osteogenic differentiation with an increase in the mineralized matrix deposited by cells at d21 after 4 days of electrical stimulation.

PoA.23.14

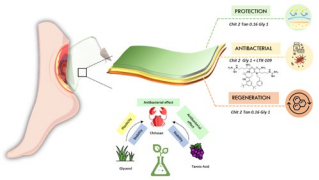
Multi-layered chitosan-based patch, loaded with antimicrobial peptide for the healing of chronic lesions

Sara Bernardoni[1], Lucia Ferrazzano[2], Chiara Palladino[2], Chiara Artusi[1], Francesca Bonvicini[3], Elisabetta Campodoni[1], Giovanna Angela Gentilomi[3], Alessandra Tolomelli[2], Monica Sandri[1]

[1]Institute of Science Technology and Sustainability for Ceramics, National Research Council (ISSMC-CNR), Faenza (RA), Italy. [2]Department of Chemistry "Giacomo Ciamician", Alma Mater Studiorum - University of Bologna, Bologna (BO), Italy. [3]Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Bologna (BO), Italy

Abstract

Chronic wounds represent a severe social and economic issue, impacting both the healthcare system and millions of people's quality of life, due to their increasing prevalence and cost. Existing treatments, including mechanical/surgical cleansing and antibiotic administration (both topical and systemic), are often painful, ineffective, or not advisable, especially for bedridden patients affected by comorbidities which worsen the clinical picture. Therefore, is crucial to find new strategies to overcome the existing hurdle associated with deep wound care. In that regard, biomaterials present themselves as promising candidates for this application since they can be carefully selected and blended to develop composite materials with chemical and physical properties finely tuned. Moreover, thanks to their composition and structure, biopolymeric matrices can host therapeutics which can be released in a controlled way directly on the infection site, thus containing the drawbacks associated with antibiotics administration, such as antibiotic resistance insurgence. In this work, we investigated an advanced solution to combine chitosan, a suitable biopolymer used in wound healing applications for its antimicrobial properties and the ability to create homogeneous layers, with the antimicrobial function of Lytixar (LTX-109), an antimicrobial peptide (AMP) displaying bactericidal lytic mechanism of action, by applying a biomimetic approach of the injured tissue. A multi-layer antimicrobial patch was designed with good biocompatibility and mimicry of the skin microenvironment, thus promoting wound management from homeostasis to skin remodelling. To this aim, together with chitosan, were used glycerol as a plasticizer to contrast the brittle nature of the films, and tannic acid as cross-linker to control their chemical stability when applied to wounds. Each layer was designed by choosing different compositions to obtain a tuneable device: the top layer is a protective layer with the function of shielding the wound and avoiding bacterial infection, followed by a medicated layer loaded with LTX-109, aimed at the release of the antimicrobial agent, and a bottom layer with the multiple functions of modulating the AMP release and provide regenerative stimuli. Extensive characterization demonstrated that the patch meets the essential requirements to assist wound healing such as exudates absorption, maintenance of good O_2 and water permeation, biocompatibility, and suitable degradation time. The incorporation of the LTX-109 in the middle layer guarantees a sustainable and slow release of the antimicrobial peptide over a period of time of 48h, with its potency fully preserved as demonstrated by MIC values obtained on the tested reference bacteria.



Multilayer patch's representation

PoA.23.15

Strategies to attain underwater actuation of solvent-responsive chitosan and its biomedical applications

Amit kumar[1], Nidhi Gupta[2], Pijush Ghosh[1]

[1]Indian Institute of Technology Madras, Chennai, India. [2]Dr. Shroff's Charity Eye Hospital,, Delhi, India

Abstract

Chitosan is a biocompatible smart polymer that responds to solvents. The chitosan thin film, when kept on a solvent surface, actuates due to the diffusion of solvent molecules, which creates a concentration gradient across the thickness. The developed concentration gradient results in a displacement field that leads to out-of-plane bending of the films. The actuation characteristics are governed by the film's stiffness, diffusion characteristics of the solvent, and geometry of the film. The majority of drug delivery and soft robotics applications, require under-solvent actuation control. When dipped inside the solvent, the single layer chitosan film does not actuate as the diffusion of solvent occurs from both sides, which does not form the concentration gradient required for actuation. In our lab, to achieve under-solvent actuation, we modified the surface to a) form a bilayer of chitosan with hydrophobic PMMA and b) layer-by-layer assembly of chitosan with different crosslinking densities thus, swelling properties. PMMA/chitosan bilayer has been formed with silane at the interface. Silane provides the necessary interaction to keep the bilayer stable inside the solvent. The PMMA/chitosan bilayer, when dipped inside the solvent, actuates as solvent diffusion occurs only through chitosan, creating a concentration gradient across the thickness. PMMA/chitosan bilayer has been used to study the effect of actuation-induced curvature in bacterial cell growth and its possible application as an antifouling surface. Another approach for under-solvent actuation is forming a bilayer with the same material but different crosslinking densities. Due to the same base polymer, the bilayer developed using this layer-by-layer assembly does not require additional interface treatment. In our lab, we have made a bilayer of chitosan with one layer uncrosslinked and another layer crosslinked with glutaraldehyde. When dipped inside the solvent, as the swelling ratio of the uncrosslinked layer is more than the crosslinked layer, the concentration gradient developed across the bilayer thickness leads to actuation. This layer-by-layer assembled bilayer system was used to understand the mechanism and mimic the actual scroll of DMEK grafts using a layer-by-layer assembled chitosan bilayer. Scrolling of DMEK graft had been a major problem while transplanting in a patient's eye. The scrolled grafts were tapped to straighten after being placed in the patient's eye, which reduces the overall endothelial cell count. The actuation of these bilayers can thus attain multiple cycles of folding/unfolding using ethanol, thus providing better control in soft robotics and drug delivery applications.

PoA.23.16

Development of thermoresponsive scaffolds for controlled and localized drug release

Adriana Gonçalves[1,2], Miguel Castilho[2,3], João Paulo Borges[1], Paula Isabel Soares[1]

[1]CENIMAT|i3N, Department of Materials Science, School of Science and Technology, NOVA University Lisbon and CEMOP/UNINOVA, Caparica, Portugal. [2]Department of Biomedical Engineering, Technical University of Eindhoven, Eindhoven, Netherlands. [3]Department of Orthopedics, University Medical Center Utrecht, Utrecht, Netherlands

Abstract

Conventional cancer treatments present a low degree of personalization to specific cancer types, leading to therapeutic inefficiency and severe side effects. The use of thermoresponsive scaffolds as drug carriers is a promising alternative since it allows a localized and controlled drug release, maintaining an effective concentration of the drug at the site, reducing side effects[1]. Poly(N-isopropyl acrylamide)(PNIPAAm) is a thermoresponsive polymer with a lower critical solution temperature (LCST) of 32ºC that can be easily tuned. Due to its ability to conformationally change with temperature variation, PNIPAAm has been widely studied in drug release applications[2]. The use of polymeric fibers as drug delivery vehicles has the potential to reduce the systemic absorption of the drug, offering a more localized treatment at lower concentrations[3]. The present work aims to explore the influence of fiber scaffold geometries on mechanical properties and drug release kinetics. We hypothesize that the use of various printing techniques can create fiber scaffolds with distinct geometries, enabling control over their mechanical properties and drug release rates[4]. Polymerization of PNIPAAm through free radical polymerization was confirmed through 1H NMR and FTIR. Copolymerization with N-(hydroxymethyl)acrylamide (NMA) showed an increase in LCST to 41ºC and allowed crosslinking of the polymer through self-condensation. Electrospinning and electrowriting were chosen as the techniques to produce thermoresponsive scaffolds (Figure 1 and 2). Random fibers with an average diameter of 306nm were obtained using electrospinning, while straight PNIPAAm fibers with diameters between 50 and 100um were obtained using electrowriting. Mechanical tests show that increasing the NMA ratio in the copolymer slightly increases the elastic modulus of the produced membranes and after crosslinking the scaffolds display brittle behavior. Meanwhile, controlled-shaped scaffolds with auxetic microstructures and pore sizes between 200 and 400um are currently being optimized. The present work shows the potential of different polymer processing techniques to develop scaffolds as drug vehicles and the possibility to tune the architecture of the fibers to allow for more personalized treatment with potential in the treatment of skin tumors.

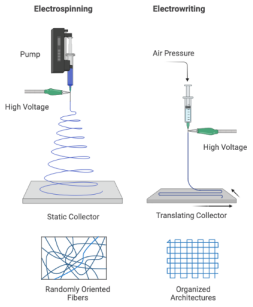


Figure 1-Schematic representation of electrospinning and electrowriting.

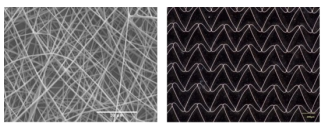


Figure 2-SEM image of PNIPAAm fibers obtained by electrospinning (A). Optical image of an auxetic microstructure of polycaprolactone fibers using electrowriting (B).

[1] R. De Souza et al., Drug Deliv 2010,17,365.
[2] M. A. Ward et al., Polymers (Basel) 2011,3,1215.
[3] A. Gonçalves et al., Gels 2021,7,28
[4] F. L. He et al., Polym Adv Technol 2019,30,425.

PoA.23.17

Laminarin-based snack for cells

Mehrzad Zargarzadeh[1], Maria Mendes[2], Catarina Custodio[1], João F. Mano[1]

[1]CICECO – Instituto de Materiais de Aveiro | CICECO – Aveiro Institute of Materials, Aveiro, Portugal. [2]University of Aveiro, Aveiro, Portugal

Abstract

In recent years, nano- and micromaterials have been widely used in tissue engineering and biomedicine. Polysaccharide-based particles are promising carriers for delivering physiologically and pharmacologically active compounds to target cells due to their versatility and tunable properties[1,2]. Here, we report a novel laminarin-based particles using microemulsion method and subsequent UV crosslinking. For this purpose, particles were formed based on the immiscibility between two aqueous phases of methacrylate laminarin and polyethylene glycol and crosslinked by UV radiation. Glucoamylase enzyme was entrapped inside the particles to gradually degrade laminarin to glucose. The efficiency of internalization and effect of glucose release of particles on cells was evaluated using Human Mesenchymal Stem Cells (hMSC). Initially particles were characterized in terms of size, morphology and surface charge. Series of particles with different sizes were produced by varying stirring rate and needle size (Figure 1A). SEM images demonstrated that the particles were spherical with a smooth surface (Figure 1B). Dynamic light scattering (DLS) showed that particles were negatively charged (-10mV). The dose-dependent toxicity of the particles was studied by measuring the hMSCs metabolic activity. As demonstrated in Figure 1C, only 300ug of particles caused toxicity and significant reduction in metabolic activity. The time course of particle internalization was studied from 4h to 3 days. As shown in Figure 1D, over 24h very few quantities of particles were internalized. After 3 days, there was a considerable increase in particles uptake for all the particle concentrations (Figure 1E). Furthermore, impact of glucose release from the internalized particles on cell's metabolic activity was assessed. Once hMSCs internalized enzyme-encapsulated particles, the media was switched to a glucose-free medium. As such, any difference in cell's response could then be attributed directly to the presence of glucose inside the cells. As it is shown in Figure 1F, most of cells which internalized particles without glucoamylase and cultured in glucose free medium were dead and detached. In contrary, cells that internalized enzyme encapsulated particles maintained their viability and showed elongated shape. This is the first report of particles being used to deliver glucose to cells through internalization. Such particles will make cells autonomous in providing their own source of energy. We believe this work will play a critical role for future advancements in tissue engineering by addressing the lack of glucose diffusion in 3D-structures and spheroids/organoids culture.

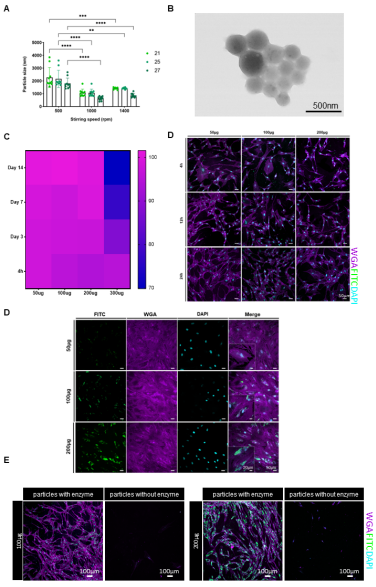


Figure 1. A)Effect of different needle size and stirring rate on particle size. B) SEM Images of fabricated particles using 27G needle and 1000rpm stirring rate. C) Metabolic activity of hMSCs after incubation with different concentration of particles. Confocal images of hMSCs particle's uptake over D) 24h E) 72h of incubation. E) Confocal images of uptake of particles with enzyme cultured in glucose free culture medium.

The Authors acknowledge project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, SFRH/BD/143883/2019 2020.01647.CEECIND.

PoA.23.18

CatchGel: A molecular to macroscale investigation of catch bond cross-linked polymer networks

Zarah Walsh-Korb[1,2], Michael A Nash[1,2]

[1]University of Basel, Basel, Switzerland. [2]ETH Zurich, Basel, Switzerland

Abstract

Nature's exquisite designs are a constant source of inspiration for materials scientists, yet our ability to replicate biological interactions in engineered systems is often limited. Biological systems exist in perfectly balanced environmental conditions that regulate their functionality. A major obstacle in exploiting biological interactions in macroscale materials is a lack of understanding of how environmental changes affect the intrinsic molecular functionality of the biological unit, ultimately impacting macroscale behaviour. From a material design standpoint bacterial adhesins, cell surface proteins allowing bacteria to bind to, and colonise, a host, are particularly interesting. Many of these adhesins exhibit so-called 'catch bond' behaviour. Essentially, the adhesive force of their receptor-ligand complexes increases at high shear, in contrast to traditional 'slip' bonds, which rupture under high shear. Transplanting catch bond-forming protein complexes onto polysaccharide chains opens up the possibility of creating bio-based hydrogel networks with unique tensile properties. Specifically, networks that display both shear-thinning and shear-thickening properties depending on the applied force. Taking the example of serine-aspartate repeat protein G (sdrG) from *Staphylococcus epidermidis* and its ligand fibrinogen beta strand (FgB), we explore how the receptor-ligand complex behaves as we move from the molecular to the macroscale. First, sdrG-FgB interactions were probed using single molecule force spectroscopy to understand fundamental molecualr scale behaviour. The mesoscale was emulated by expressing sdrG on yeast and probing interactions with FgB coated surfaces using spinning disk microscopy. Finally, the sdrG-FgB complexes were embedded into hydrogel networks and their macroscale behaviour examined by rheology, allowing us to build an understanding of the impact of protein orientation, chemical environment, cooperativity and stress dissipation in sdrG-FgB complexes across multiple lengthscales. Examining how protein behaviour and macroscale materials properties change as a function of protein composition and environmental complexity allows more accurate development and manipulation of adhesin-derived responsive biomimetic materials. Thus, leading to a greater understanding of the nano-macroscale relationships in complex multiphase systems and enhanced strategies for the translation of biological interactions into macroscale materials.

PoA.23.19

Cell-laden soft robot fabricated with extrinsic fields

Wei Gao[1,2], Riccardo Tognato[1], Tiziano Serra[1]

[1]AO Research Institute Davos, Davos, Switzerland. [2]School of Science, Lanzhou University of Technology, Lanzhou, China

Abstract

Magnetic responsive soft robots exhibit great potential for a multitude of applications such as biomedicine, automation, and industry. Under the influence of an external magnetic field, these robots can perform a variety of deformations such as elongation, contraction, bending, locomotion [1]. the common fabrication methods (e.g. molding, extrusion, 3D printing technologies) require long processing and harsh condition that are not compatible with the fabrication of cellularised construct [2]. To enrich the functionality of magnetic structures, here we proposed a new approach to fabricate magnetically sensitive morphologically-relevant soft tissues. The numerical simulation based on hydrodynamic theory and experimental testing of the formation process are carried out in detail, demonstrating that the magnetic responsive cues gather in the vibration process at the nodes of the surface wave. By placing the prepared magnetic soft robots in a time-changing magnetic field environment, we could program its deformation and movement properties. The diagram of preparation and magnetic field driving is shown in the figure 1. This technology, based on the standing waves formed on the free surface of a vertically vibrated liquid layer, can be utilized to simultaneously activate magnetic particles, cells, and other micron particles, and may offer an alternative contactless manufacturing of hybrid biorobot for stimuli-responsive cell carrier mission.

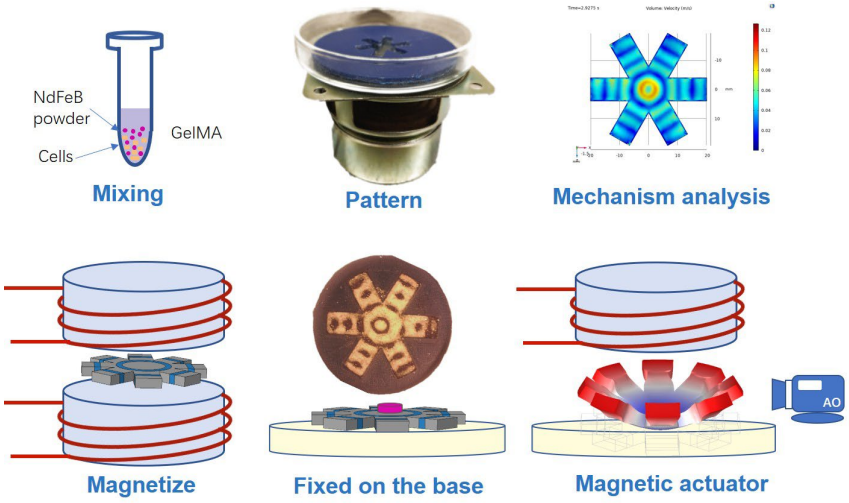


Figure 1 Schematic diagram of preparation and magnetic field actuation

Reference

[1] Kim J, Chung S E, Choi S E, et al. Nature materials, 2011, 10(10): 747-752.

[2] Wang H, Zhu Z, Jin H, et al. Journal of Alloys and Compounds, 2022: 166219.

PoA.23.20

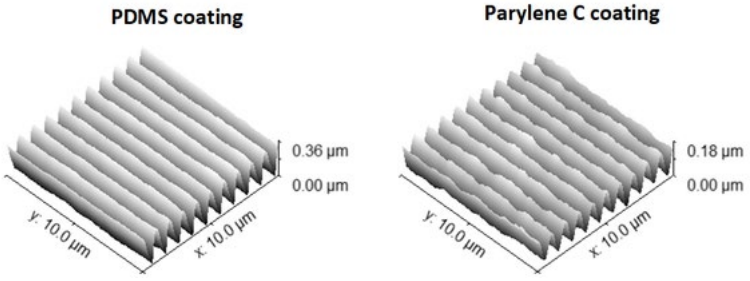
Polymer coating for improved biocompatibility and cell adhesion of light-responsive azobenzene-based thin films

Mari Isomäki[1], Lotta Kääriäinen[1], Chiara Fedele[1], Elina Mäntylä[2], Soile Nymark[2], Teemu O Ihalainen[2], Arri Priimagi[1]

[1]Faculty of Engineering and Natural Sciences, Tampere University, Tampere, Finland. [2]BioMediTech and Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

Abstract

Smart stimuli-responsive materials can react to external stimuli, which can cause material, for example, to change their shape. Light is an interesting energy source for smart biomaterials, as it can be localized over a substrate, it is non-invasive, and its properties can be varied.[1] Azobenzenes are one of the most commonly used molecules for photosensitive materials. The isomerization between the thermodynamically stable *trans* isomer and metastable *cis* isomer can create photoinduced motions. Thin amorphous azopolymer films can be surface patterned via light-induced mass migration to form sinusoidal surface relief gratings (SRGs). Azobenzene-based materials have been previously used for cell culture[2], however, these materials can begin to dissolve during long-term experiments in cell culture environment. Thus, polymer coating on top of azobenzene-based thin film would improve their biocompatibility. Here light-responsive azobenzene-based thin films were coated either with poly(dimethylsiloxane) (PDMS) or parylene C. The inscription and erasure of SRGs was studied with diffraction measurement and atomic force microscopy (AFM). To study biocompatibility and cell adhesion, Madin-Darby canine kidney type II epithelial cells (MDCK II) were seeded on top of non-coated and polymer coated films. The film quality and morphology of MDCK II cells was analyzed with light microscopy. The results show that SRGs were efficiently inscribed on thin films coated with PDMS and parylene C (Figure 1). Erasure of SRG topography and even multiple SRG inscription cycles were possible. Both polymer coatings protected the underneath azobenzene film only partially from dissolving into the surrounding cell medium. However, compared to non-coated and PDMS coated samples, cells stayed attached longer times on parylene C coated samples (Figure 2). Cells stayed viable up to 9 weeks of culturing on parylene C coated samples, thus this coating is suitable also for long term culture. The parylene C coating improved the biocompatibility of the azobenzene-based films without hindering the SRG formation or erasure. The coating also enables long-term cell culture experiments with light-responsive materials.



References
1. V. Y. Chang et al. Adv. Opt. Mater. 2019, 7, 1900091. DOI: 10.1002/adom.201900091
2. M. Isomäki et al. Small Sci. 2022, 2, 2100099. DOI: 10.1002/smsc.202100099

Figure 1. SRG topography on PDMS coated and parylene C coated azobenzene films.

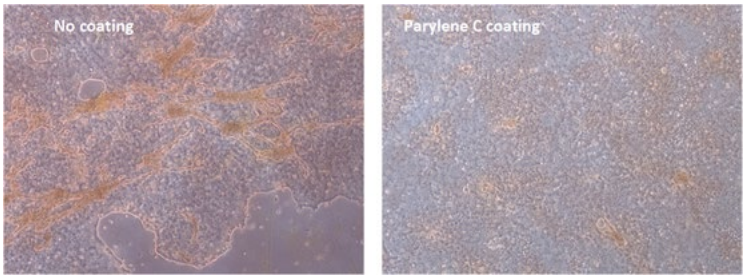


Figure 2. MDCK II cells detached from non-coated samples but stayed attached on parylene C coated samples after 9 weeks of culturing.

PoB.1.01

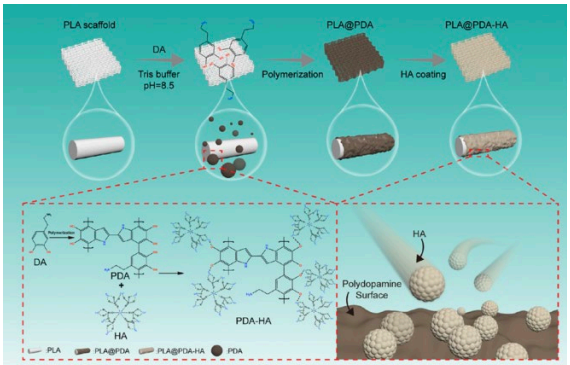
Biomimetic, mussel-inspired surface modification of 3D-printed biodegradable polylactic acid scaffolds with nano-hydroxyapatite for bone tissue engineering

Na Li, Minghan Chi, Neha Sharma, Florian Markus Thieringer

Department of Biomedical Engineering, University of Basel, Basel, Switzerland

Abstract

Polylactic acid (PLA) has been widely used as filaments for material extrusion additive manufacturing (AM) to develop patient-specific scaffolds in bone tissue engineering. Hydroxyapatite (HA), a major component of natural bone, has been extensively recognized as an osteoconductive biomolecule. Here, inspired by the mussel-adhesive phenomenon, in this study, polydopamine (PDA) coating was applied to the surface of 3D printed PLA scaffolds (PLA@PDA), acting as a versatile adhesive platform for immobilizing HA nanoparticles (nHA). Comprehensive analyses were performed to understand the physicochemical properties of the 3D-printed PLA scaffold functionalized with nHA and PDA for their potent clinical application as a bone regenerative substitute. Scanning electron microscopy (SEM) and element dispersive X-ray (EDX) confirmed a successful loading of nHA particles on the surface of PLA@PDA after 3 and 7 days of coating (PLA@PDA-HA3 and PLA@PDA-HA7), while the surface micromorphology and porosity remain unchanged after surface modification. The thermogravimetric analysis (TGA) showed that 7.7 % and 12.3% mass ratio of nHA were loaded on the PLA scaffold surface, respectively. The wettability test indicated that the hydrophilicity of nHA-coated scaffolds was greatly enhanced, while the mechanical properties remained uncompromised. The 3D laser scanning confocal microscope (3DLS) images revealed that the surface roughness was significantly increased, reaching Sa (arithmetic mean height) of 0.402 µm in PLA@PDA-HA7. Twenty-eight days of in-vitro degradation results showed that the introduction of nHA to the PLA surface enhances its degradation properties, as evidenced by the SEM images and weight loss test. Furthermore, a sustainable release of Ca2+ from PLA@PDAHA3 and PLA@PDA-HA7 was recorded, during the degradation process. In contrast, the released hydroxyl group of nHA tends to neutralize the local acidic environments, which was more conducive to osteoblastic differentiation and extracellular mineralization. Taken together, this facile surface modification provides 3D-printed PLA scaffolds with effective bone regenerative properties by depositing Ca2+ contents, improving surface hydrophilicity, and enhancing the in-vitro degradation rate.



Schematic illustration of surface modification process of three-dimensional (3D)-printed PLA scaffolds. The PLA scaffold was fabricated with a rectangular porous structure using material extrusion-based 3D printing technology. Afterward, polydopamine was homogeneously coated onto the surface by immersing PLA scaffolds into the dopamine solution (tris buffer, pH = 8.5) and simultaneously stirring for 24 h, during which PDA was developed by self-polymerization of DA particles. Furthermore, nHA particles were successfully immobilized onto the surface of as-synthesized PDA coatings via catechol functional groups. Finally, the nHA functionalized PLA scaffolds were obtained.

PoB.1.02

3D printed scaffolds with honeycomb patterning as prototypes for bioinspired implants and tissue engineering scaffolds

Yuliya Khrunyk[1], Philipp Zimmermann[2], Peter Schulze[2], Annette Gabriele Beck-Sickinger[1]

[1]Institute of Biochemistry, Faculty of Life Sciences, Leipzig University, Leipzig, Germany. [2]Leipzig University of Applied Sciences (HTWK), Engineering Faculty, Leipzig, Germany

Abstract

An increasing life span, which is one of the key risk factors for fragility fractures and delayed fracture healing, is expected due to demographic changes. Hence, the treatment of musculoskeletal injuries and diseases attracts a growing attention. In implantology, the treatment of larger bone fractures is still one of the main challenges as stiffness mismatch between the implanted material and a human bone often leads to the implant failure. Since recently, marine glass sponges (Hexactinellida) which underwent evolutionary selection over 500 MYA optimizing the hierarchical architecture of their scaffolds have been inspiring scientists and engineers to employ biomimetics strategies with the aim to produce novel structurally efficient matrices with outstanding mechanical properties. The goal of this research is to reveal the potential of a set of cloud sponge inspired 3D printed scaffolds having a unique honeycomb structure regarding the application in implantology and/or medical devices manufacturing. Using micro-computer tomography (Zeiss XradiaVersa 520, Carl Zeiss Microscopy) and software packages (In-Vesalius 3.0, SolidWorks) a series of CAD 3D scaffold models with honeycomb patterning (pores of 800-900 µm in diameter) that allows the bone ingrowth have been designed. 3D printing employing Clear Resin (FLGPCL04, Formlabs, Somerville, Massachusetts, USA) allowed us to successfully print five scaffolds with different mesoscale dimensions. The *in vitro* biocompatibility of the scaffolds printed from Clear Resin has been studied using bone cells (Saos-2, MG-63), endothelial cells (HMEC-1) and macrophage THP-1 cells. In comparison to the substrates from medical titanium the scaffolds composed from Clear Resin showed no increase in cytotoxicity, but lower levels of THP-1 adherence. In a set of comparative cell culture assays using bone cells it has been shown that three out of five scaffolds demonstrate significantly higher cell growth, which might be linked to the geometry of scaffolds´ units and a supply of nutrients. The present data suggest that Clear Resin has a potential to be applied in 3D printing of scaffolds for biomedical purposes and that three out of five tested scaffolds proved to be promising regarding further research aimed at their use in implantology.

PoB.1.03

State-of-the-art of commercial stereolithography 3D printers and their suitability for upscaled production of organ-on-chip platforms

Zoltán Guller[1,2], Rujing Zhang[2], Sandra Wilson[2], Niels Bent Larsen[1]

[1]Technical University of Denmark, Kongens Lyngby, Denmark. [2]Sophion Bioscience A/S, Ballerup, Denmark

Abstract

Stereolithographic (SLA) 3D printing has become widely commercially available over the past decade. This increased availability is not reflected in the biomedical industry, as the industrial 3D printing market mostly focuses on dental, jewellery, and audiology applications. Can we exploit SLA in medical technology research and anticipate the research results to be upscalable to meet manufacturing requirements? To address this question, a thorough system analysis has been carried out to assess the mass production suitability of SLA printing of organ-on-chip products. The evaluation is based on test prints encompassing overhanging parts, channels and other difficult-to-produce structures (Figure 1), received on request from 8 SLA printer manufacturers using Digital Light Processing (DLP). More recently introduced LCD-mask-based printers provide lower contrast than DLP printers and heterogeneous product quality over time, rendering them unsuitable for production.

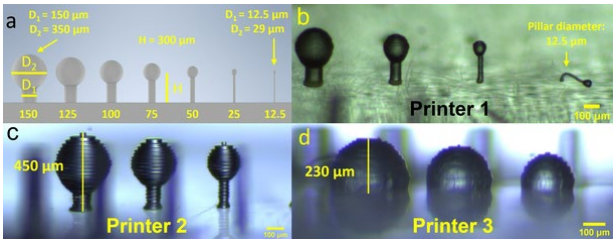


Figure 1: Pillars with hollow sphere on top. a) Design to be reproduced. b) Printed pillars, the smallest is 12.5 µm diameter. c) Printed as designed. d) Lost layers due to the squeeze-flow effect.

An apparent limitation for upscaled production is the inherent trade-off between lateral resolution and build area. The lateral resolution of a DLP printer depends on the pixel pitch size of the image-forming digital micromirror device (DMD) and on the optics projecting the DMD motif onto the light-sensitive resin. Texas Instruments is the only commercial provider of DMDs, and all systems are consequently bound by the same limits on pixel pitch. What separates 3D printer companies is the quality of the optical system, and their expertise to aid the fine-tuning to achieve the desired print features. As organ-on-chip products often consist of shallow, varying aspect ratio structures, the squeeze-flow effect needs to be compensated for to prevent losing layers. Moreover, upscaling of an organ-on-chip ideally adapts to standardized formats, e.g. microtiter plate, which introduces new challenges. The build platform should be replaced for each print, a demand that is seldom addressed by the 3D printer industry. These constraints formed the basis of a comprehensive criterion matrix for choosing a DLP 3D printer for upscaling organ-on-chip products. Although the 3D printing industry is not yet adapted to the needs of the biomedical market, this presentation will show that challenges of commercial printers can be overcome by a combination of print design considerations and a customisable printer configuration.

PoB.1.06

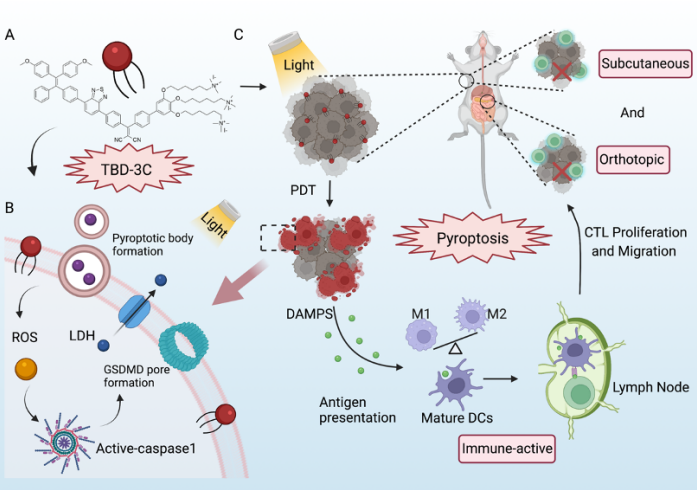
Pyroptosis remodeling tumor microenvironment to enhance pancreatic cancer immunotherapy driven by membrane anchoring photosensitizer

Meng Wang, Shiyi Shao, Junming Huang, Qida Hu, Tingbo Liang

Zhejiang University, First Affiliated Hospital, Hangzhou, China

Abstract

Introduction Immunotherapy, the most promising strategy of cancer treatment, has achieved promising outcomes, but its clinical efficacy in pancreatic cancer is limited mainly due to the complicated tumor immunosuppressive microenvironment. As a highly-inflammatory form of immunogenic cell death (ICD), pyroptosis provides a great opportunity to alleviate immunosuppression and promote systemic immune responses in solid tumors. Herein, we apply membrane-targeted photosensitizer (TBD-3C) with aggregation-induced emission (AIE) feature to trigger pyroptosis-aroused cancer immunotherapy via photodynamic therapy. **Experimental Methods** TBD-3C was synthesized and characterizated by 1H-NMR and UV/Vis. To investigate the feasibility of inducing pancreatic cancer pyroptosis by TBD-3C under light irradiation, we selected murine cell lines KPC. The tumor ablation ability and immune activation ability were demonstrated by *in vivo* studies up to now. **Results and Discussion** The UV/Vis absorption spectrum and the chemical structure were shown in Fig. 1A. TBD-3C was mainly localized on the cell membrane (Fig. 1B). Cell swelling and large bulging bubbles on the plasma membrane were observed upon light irradiation (Fig. 1C), and showed a higher level of N-GSDMD (Fig. 1D) and double-positive cells (pyroptotic cells) than the control sample (Fig. 1E). The TBD-3C-based strategy showed notable therapeutic effects (Fig. 1F,G) The TBD-3C PDT group showed increased pyroptosis-related protein, ICD-related protein expression and CD8+ CTL population (Fig. 1H,I). The heatmap (Fig. 1J) and two-dimensional t-SNE projections were constructed for visualization, and the TBD-3C PDT group showed notable decreased overall expression of PD-L1(Fig. 1K). **Conclusion** We report a strategy for immunotherapy through cell pyroptosis by a membrane-targeted AIE photosensitizer (TBD-3C), which demonstrated an excellent antitumor efficiency for pancreatic cancer.



Scheme. *Schematic illustration of anti-tumor immunotherapy induced by photodynamic pyroptosis.*

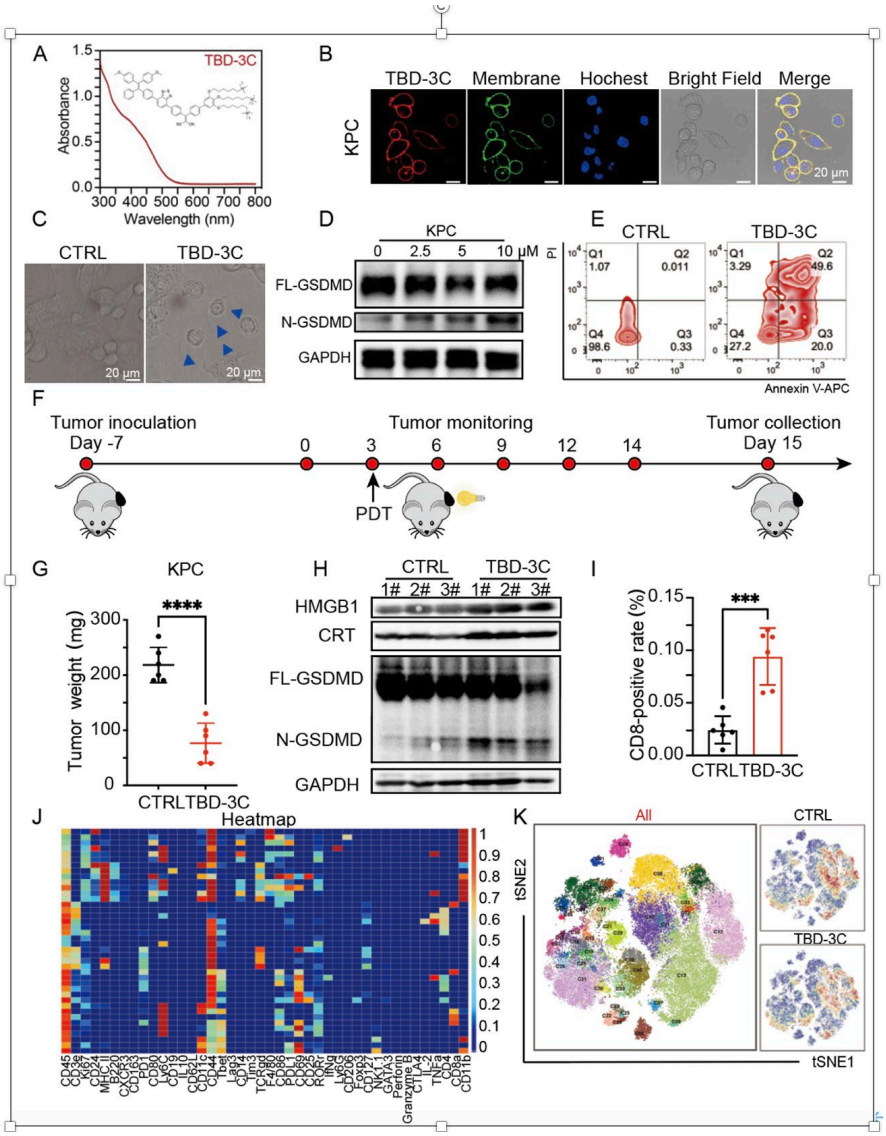


Figure 1. *A) The UV/Vis absorption and chemical structure of TBD-3C. B) CLSM images of KPC cells incubated with TBD-3C. C) Confocal images of KPC cells labeled with TBD-3C upon light irradiation. D) WB analysis of pyroptosis-related protein expression (GSDMD-FL, GSDMD-N) after treatment with TBD-3C (PDT). E) Flow cytometry of PI and Annexin V-stained KPC cells labeled with TBD-3C(PDT). F) Schematic illustration of TBD-3C-based PDT-arousing pyroptosis to inhibit tumor growth. G,H) The tumor weights, WB analysis of HMGB1, CRT, GSDMD in tumor tissue. I) CD8 positivity in the KPC model. J) A heat map showing the normalized expression of the markers. K) A tSNE map was colored by 40 clusters from all samples and the expression of marker PD-L1 in different groups.*

PoB.1.08

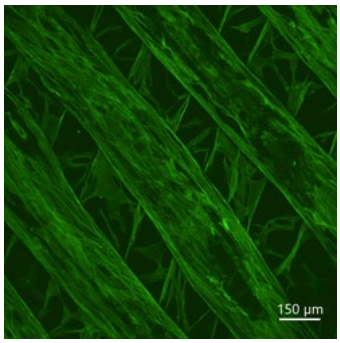
Stem cell-based 3D constructs for bone grafts

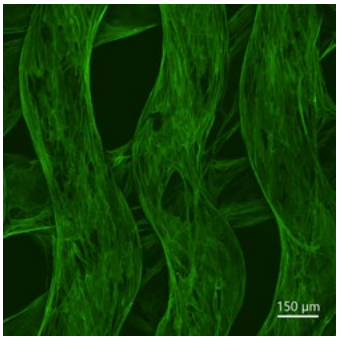
Jana Musilkova[1], Antonin Sedlar[1], Milos Beran[2], Zdenka Kolska[3], Petr Slepicka[4], Martin Molitor[5], Lucie Bacakova[1]

[1]Institute of Physiology CAS, Prague, Czech Republic. [2]Food Research Institute in Prague, Prague, Czech Republic. [3]3 J. E. Purkyne University, Usti nad Labem, Czech Republic. [4]University of Chemistry and Technology, Prague, Czech Republic. [5]Na Bulovce Hospital, Prague, Czech Republic

Abstract

There is an increasing need for appropriate vascularized bone tissue replacements. A promising approach to solve this task is to use cell-material constructs containing adult mesenchymal stem cells seeded in combination with endothelial cells on three-dimensional PLLA printed 3D scaffolds. It is known, that these cell types spontaneously assemble into tubular capilary-like structures, ensuring the pre-vascularization of the graft, and thus increasing the probability of its long-term suvival and good oseointegration. Moreover, the 3D printing enables to manufacture the replacements in any arbitrary shape. In our work, we tested 10 types of 3D-printed polymeric scaffold types with different inner architectures, based on two basic patterns – triangle (S1, S2, S6, S7) and „honeycomb“ (S3, S4, S5, S8, S9, S10), differing in exact orientation of single layers and width of spaces between the fibres. Scaffolds were prepared from a bioresorbable PLA/PCL copolymer (ratio 3:5) using SWETJA type AM001 3D printer (Swetja[BL1] , Prague, Czech Republic), were mineralized in simulated body fluid (SBF) solution or using carbodiimide chemistry. Scaffolds were characterized by SEM, FTIR and a pressure deformation test. As a cellular component, the adipose tissue-derived stem cells (ASCs) were used and cultured on scaffolds for 10 days. The cell number, adhesion, cell morphology, and cell penetration inside the scaffold were followed on days 2, 6 and 10 by fluorescence staining by phalloidin and DAPI using the Dragonfly 503 confocal microscope. Possible vascularization of the scaffolds was tested in experiments with co-cultivation of ASCs and human umbilical vein endothelial cells. We have observed improved cell adhesion and proliferation on the surface on the chemically mineralized scaffolds All the samples were very stiff and complied with demands for mechanical properties of bone implants; only sample S6 had slightly worse mechanical strength. Thus the basic criterion for the scaffold type selection was the density of the ASCs colonization of the scaffolds. We have found that the cells were spread along the fibers, proliferated well, and colonized the inside of the scaffold of all 10 types of prints. The images from the confocal microscope proved that the most efficient penetration occurred in types No. S3, S4 and S8, having the honeycomb pattern, and S7 of the triangle pattern. The honeycomb patterns thus appear generally advantageous.





S7 S8

Figure 1: Colonization of S7 and S8 scaffolds with ASCs on day 10. Dragonfly 503 (10x).

Supported by Ministry of Health of the Czech Republic (grant No. NU20-08-00208)

PoB.1.09

A New Generation Hernia Mesh

Seray Zora Tarhan, Sevil Dincer Isoglu, Ismail Alper Isoglu

Abdullah Gul University, Kayseri, Turkey

Abstract

A hernia is the protrusion of internal organs such as the intestines and urinary bladder from the cavity formed as a result of the weakening of the muscles surrounding the organs in the body. Hernia operations are one of the most frequently performed operations in general surgery in the world and polypropylene mesh implantation is the most widely used method to solve problems related to the hernia. However, hernia recurrence after the surgery due to the abdominal adhesions between the mesh and the visceral layer remains to be a concern for effective hernia treatment. In our study, a new approach has been employed to design a novel anti-adhesive visceral layer which was inspired by the nature of zwitterionic polymers for hernioplasty application. A zwitterionic polymer, namely, poly(sulfobetaine methacrylate) (PSBMA) was first synthesized via free radical polymerization and characterized by attenuated total reflection–Fourier transform infrared spectrometry (ATR-FTIR) and gel permeation chromatography (GPC). Then it was blended with polycaprolactone (PCL) polymer in different ratios, the printability of the material and the effects of the printing parameters on the resulting membrane were evaluated by changing the parameters (pressure, printing speed, material filling density, and material density) on the 3D bioprinter. The properties of the printed meshes were characterized by ATR-FTIR, scanning electron microscopy (SEM), and water contact angle measurements. Furthermore, static protein adsorption tests, cell viability, and mechanical tests were performed to determine the anti-adhesive properties, biocompatibility, and mechanical strength of visceral layers produced with a 3D bioprinter *in vitro* respectively. In these tests, printable PSBMA/PCL samples, a commercial polypropylene mesh, and PCL were used for comparison. The results show that the newly developed 3D printed-visceral layer for hernia mesh is a promising candidate to effectively prevent hernia recurrence derived from visceral adhesion after hernioplasty application.

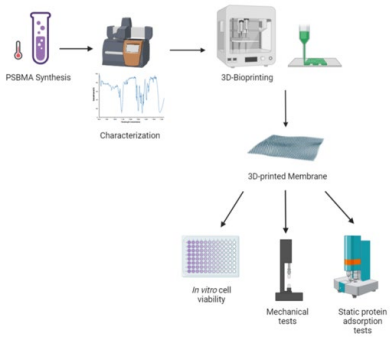


Figure.1 The workflow of the study (Created by Biorender)

PoB.1.10

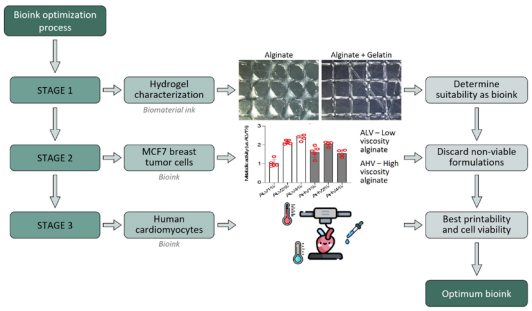
Optimizing the composition of alginate and gelatin bioinks to improve printability and cell viability for cardiac regenerative medicine

Ana Zubiarrain-Laserna[1], Izar Gorroñogoitia[1], Uzuri Urtaza[1], Olatz Guaresti[1], Olalla Iglesias-García[2], Manuel M. Mazo[2,3], Felipe Prosper[2,3], Ane Miren Zaldua[1]

[1]Leartiker S. Coop., Markina-Xemein, Spain. [2]Cardiac Tissue Engineering Lab, Division for Advanced Technologies, Cima Universidad de Navarra, Pamplona, Spain. [3]Hematology and Cell Therapy, Clínica Universidad de Navarra, Pamplona, Spain

Abstract

Myocardial infarction results in the formation of non-functional scar tissue, an irreversible process that can has fatal consequences. Cardiac tissue engineering seeks to create a biological substitute that can restore the lost functionality of the heart. Using extrusion based bioprinting, 3D cell-laden scaffolds can be fabricated with good resolution. The successful application of this technology requires a bioink that can offer good printability while ensuring maximum cell viability, two requisites that entail opposing material properties. Here, we present a three-stage approach to finding the optimum alginate/gelatin bioink composition for the bioprinting of cardiomyocytes. On the first stage, the composition of the natural hydrogels was systematically varied and its correlation with their rheological, mechanical, and printability properties was studied to determine their suitability as bioinks for cardiac regeneration. The effect of alginate concentration, molecular weight, and M/G ratio was addressed. The viscosity, compression modulus, and viscoelasticity could be tuned by changing both the concentration and the type of alginate, and their values fell within the range of the native heart. Two nozzle sizes and three flow speeds were used to build a printability map. The smallest nozzle gave the best resolution, while the largest nozzle offered the best shape fidelity. The printability was better with the higher viscosity alginate, but the addition of gelatin allowed for the printing of low viscosities that would otherwise be non-printable via extrusion based bioprinting. On the second stage, MCF7 breast tumor cells were mixed with the hydrogels and biocompatibility and bioprinting tests were conducted to discard non-viable formulations before attempting to bioprint human cardiomyocytes. The metabolic activity on day 7 increased with alginate concentration and there were differences between the two types of alginate. The differences in shear stress caused by the different nozzle sizes and flow speeds could be correlated with cell viability. On the third and final stage, the bioinks that best balanced printability and cell viability were used to bioprint cardiomyocytes. Overall, this paper presents a stagewise methodology that facilitates and systemizes the formulation of bioinks with good printability and cell viability. This approach can be crucial for scarce and sensitive cells such as cardiomyocytes. It also highlights the importance of alginate type and the advantages of adding gelatin.



Stagewise methodology to formulate an alginate/gelatin bioink with good printability and cell viability for cardiac tissue engineering.

PoB.1.11

Fabrication of bioresorbable cellularised scaffolds of PLA-PEG with optimised geometry for the reconstruction of auricular cartilage

Olatz Guaresti[1], Uzuri Urtaza[1], Emma Muinoz-Lopez[2], Tania Lopez-Martinez[2], Iñigo Arroyo[3], Manuel Mazo[4], Froilán Granero-Moltó[2], Ane Miren Zaldua[1], Bernardo Hontanilla[3]

[1]Leartiker S.Coop., Markina-Xemein, Spain. [2]Department of Orthopaedic Surgery and Traumatology & Regenerative Medicine Program, Clínica Universidad de Navarra, Pamplona, Spain. [3]Department of Plastic, Aesthetic and Reconstructive Surgery, Clínica Universidad de Navarra, Pamplona, Spain. [4]Regenerative Medicine Program, Cima Universidad de Navarra, Pamplona, Spain

Abstract

INTRODUCTION Three dimensional printed scaffolds have been reported to generate different kinds of connective tissues including cartilage, bone and skeletal muscle. Among scaffolds, hydrogels have been proposed for cartilage regeneration in many studies; however, it is still difficult to build large-scale tissues just from hydrogels due to their poor mechanical stability, printability, and inadequate structural integrity. Hence, combining hydrogels with polymeric scaffolds can meet the requirements of cartilage repair in terms of structure, scale, biological nature and mechanical properties. Consequently, the existing challenges regarding biomaterial-based scaffolds used in tissue engineering leads to further progress in new strategies that can attain broad clinical usage and commercialization. In the described context, the following study aims to present an innovative treatment for the reconstruction of the auricle to provide a solution for congenital anomalies of microtia and anotia, where novel 3D printed scaffolds were designed considering the complex biochemistry, morphology, mechanical behaviour and biodegradation characteristics of the target tissue. RESULTS Hybrid scaffolds based on alginate hydrogel and thermoplastic structure of poly(lactide-b-ethylene glycol) block copolymer (PLA-PEG) were identified as suitable material-geometry constructs for the creation of auricular cartilage tissue after an optimized printing fabrication process. Preliminary experiments [1] have shown PLA-PEG to be an attractive candidate for structural support; whereas alginate hydrogel provides the ideal environment for cells to adhere, grow and proliferate due to the similarity of its composition with the native extracellular matrix, promoting chondrogenesis. *In vivo* tests were performed in order to analyze the biocompatibility and potential chondrogenic induction in 3-,6-,12-weeks post-surgery in model animals. In addition, inserts were analyzed 12 weeks after surgery by performing mechanical tests as well as molecular and histological analysis. Indeed, the mechanical properties were studied through uniaxial compression tests, which enabled to identify new tissue formation (Figure 1). This research was the premise to define an accurate protocol for the design of personalized anatomical geometry scaffold, ear-shaped in this case, for a cellularised material suitable for *in vivo* implantation.

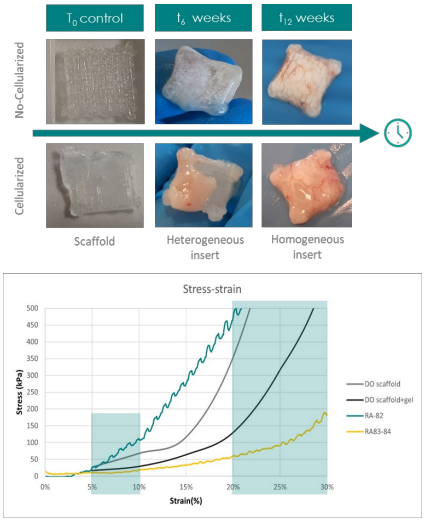


Figure 1. In vivo test images of hybrid scaffolds over time and uniaxial compression test results : PLA-PEG scaffold (DO scaffold), PLA-PEG + Alg scaffold (DO scaffold + gel), In vivo t12 no-cellularized PLA-PEG + Alg scaffold (RA-82), In vivo t12 cellularized PLA-PEG + Alg scaffold (RA83-84).

BIBLIOGRAPHY

[1] 3D printed bioresorbable scaffolds for articular cartilage tissue engineering: a comparative study between neat polycaprolactone (PCL) and poly(lactide-b-ethylene glycol) (PLA-PEG) block copolymer - IOPscience, https://iopscience.iop.org/article/10.1088/1748-605X/ac78b7/meta (accessed 15 March 2023).

PoB.1.12

3D Printing of an electroconductive scaffold for the promotion of axonal regrowth after spinal cord injury

Liam M. Leahy[1,2], Ian Woods[1,2], Jack Maughan[1,2,3], Javier Gutierrez-Gonzalez[1,2,4], Michael G. Monaghan[2,5], Adrian Dervan[1,2], Fergal J. O'Brien[1,2,5]

[1]Tissue Engineering Research Group, Dept. of Anatomy & Regenerative Medicine, RCSI University of Medicine and Health Sciences, Dublin, Ireland. [2]Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and Trinity College, Dublin, Dublin, Ireland. [3]School of Physics, TCD, Dublin, Ireland. [4]School of Chemistry, TCD, Dublin, Ireland. [5]Trinity Centre for Biomedical Engineering, TCD, Dublin, Ireland

Abstract

Introduction Spinal cord injury (SCI) induces paralysis by severing axons. Recovery is inhibited by poor axonal regrowth rates. Electrical stimulation (ES) when applied in conjunction with electroconductive biomaterial scaffolds, presents a promising method of promoting and directing axonal regrowth post-injury. This work aimed to develop novel neuro-compatible EC scaffolds to direct ES for SCI repair by coating 3D-printed polycaprolactone (PCL) with electroconductive polypyrrole (PPy). **Experimental methods** PPy was polymerised in situ, forming an electroconductive coating on PCL. Electroconductivity was measured via the 4-point method and production of PPy coating was verified by FTIR and SEM. Biocompatibility was tested by seeding SH-SY5Y neurons on PPy/PCL films (metabolic activity, total cellular DNA, immunofluorescence imaging). Scaffolds were 3D-printed with an Allevi 2 extrusion printer. A neurotrophic, extracellular matrix (ECM) was freeze-dried within scaffolds to improve biological functionality. Scaffolds of various channel sizes were produced to match native cord geometries. Neurons were electrically stimulated (IonOptix C-Pace EM) and imaged to assess development. **Results & Discussion** PCL was successfully coated with biocompatible, electroconductive PPy. FTIR spectroscopy and SEM imaging verified formation of PPy nanoparticles on PCL. Conductivity of the nanoparticle coating was measured to be 15±5S/m, 30 times higher than native tissue. SH-SY5Y neurons cultured on 2D PCL and PPy/PCL substrates exhibited no difference in cell metabolic rate, cellular DNA or neurite outgrowth, indicating that PPy/PCL is a suitable substrate. 3D-printed scaffolds were designed as collections of interlocking cylinders, mimicking human cord axonal tracts. Scaffolds of various channel sizes were produced without loss of cell activity or conductivity, demonstrating that scaffolds can be scaled to match human axonal tract dimensions, to effectively direct ES. Neurons were electrically stimulated on PPy/PCL substrates, and ES parameters were optimised. **Conclusion** This study produced 3D-printed, ECM-functionalised conductive PPy/PCL scaffolds with tuneable geometries. Scaffolds were successfully scaled to native axonal tract diameters while maintaining biological functionality and favourable material properties. PPy/PCL scaffolds supported neuronal outgrowth, demonstrating excellent biocompatibility and when combined with high conductivity, indicating that these scaffolds provide an excellent substrate for neuronal electrostimulation for SCI repair.

Funding Acknowledgements

Irish Rugby Football Union Charitable Trust and Science Foundation Ireland Advanced Materials and Bioengineering Research (AMBER) Centre (SFI/12/RC/2278_P2).

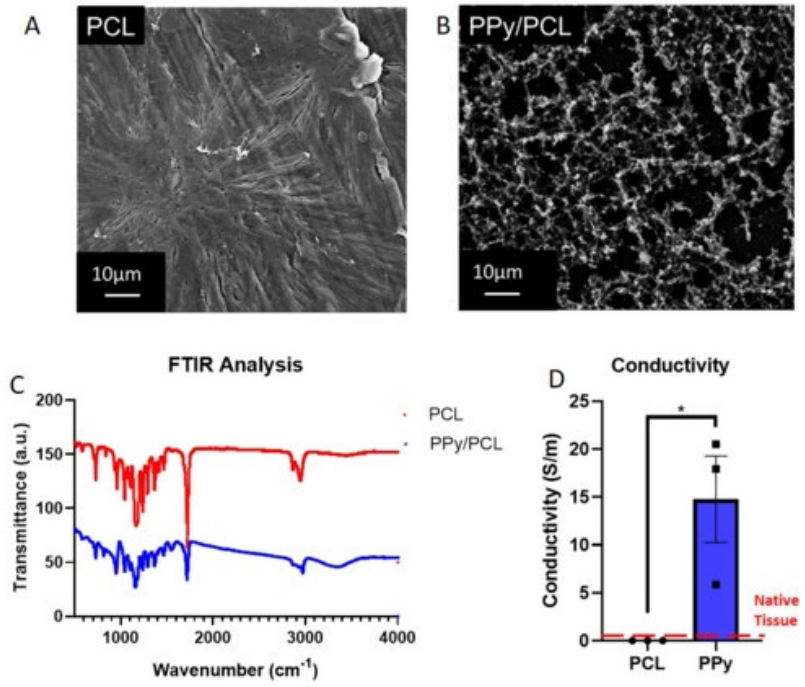


Figure 1 - A), B) SEM images C) FTIR spectroscopy D) Conductivity

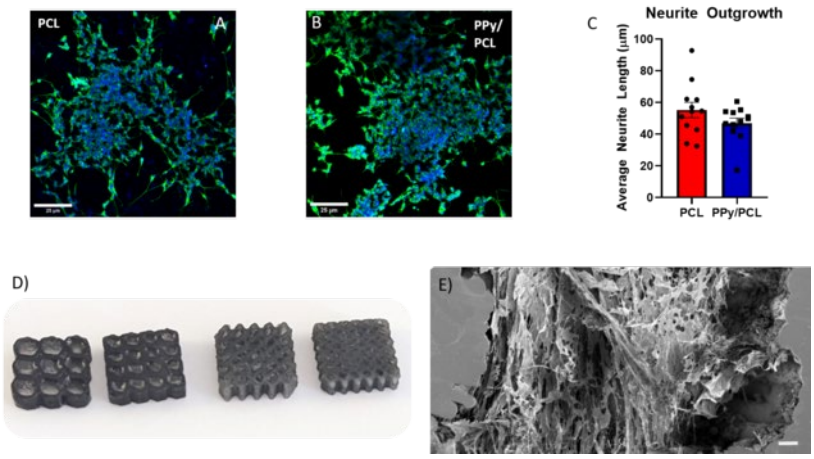


Figure 2 - A), B) Immunostained neurons C) Neurite outgrowth, D) ECM-Functionalised PPy/PCL Scaffolds, E) SEM image of freeze-dried ECM architecture within scaffold channels

PoB.1.13

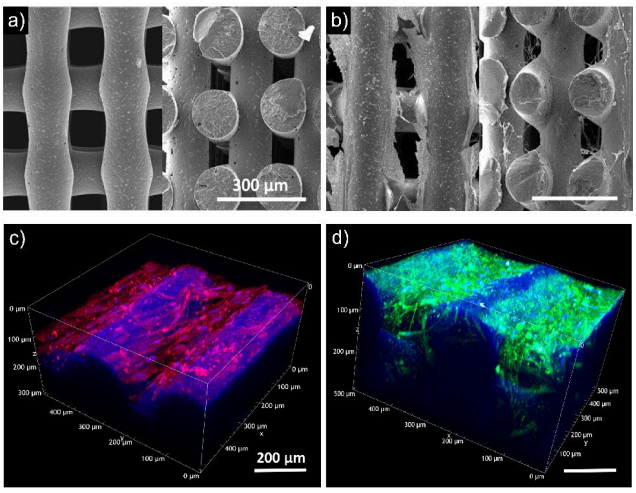
3D Melt-fabricated bio-hybrid scaffolds for the *in vitro* modelling of human cardiac fibrosis.

Mattia Spedicati[1], Irene Carmagnola[1], Mario Lavella[2], Francesca Tivano[1], Alice Zoso[1], Valeria Chiono[1]

[1]Politecnico di Torino, Turin, Italy. [2]Università di Bergamoo, Bergamo, Italy

Abstract

Myocardial infarction causes cardiomyocytes loss, and extracellular matrix (ECM) remodelling, resulting in a scar tissue mainly populated by cardiac fibroblasts, rich in collagens (Type I, II, IV) and stiffer [1]. Following the 3Rs principle, *in vitro* models of human cardiac post-infarct tissue may improve preclinical validation of new therapies for cardiac regeneration. This work focused on the design of bioartificial scaffolds providing support and mechanical stimuli for adult human cardiac fibroblasts (AHCFs) adhesion and activation into myofibroblasts to develop *in vitro* models of cardiac fibrosis. 3D polycaprolactone (Mw=43.000 Da, PCL) scaffolds with interconnected porosities were fabricated by melt extrusion additive manufacturing (MEAM) (Figure 1a) and, functionalized with type A Gelatin (G), exploiting mussel-inspired pre-coating. G grafting and its stability was confirmed by QCM-D analysis and by static contact angle measurement respectively. G coating improved long-term culture of AHCFs (up to 21 days) and, combined with PCL stiffness, stimulated fibrotic ECM deposition, as confirmed by SEM (Figure 1b) and two photon excitation fluorescence (TPEF) images (Figure 1c, 1d). The expression of myofibroblast markers (α-SMA) and the secretion of fibrotic ECM proteins (Fibronectin, Laminin, Tenascin and Collagen I, II and IV) by immunofluorescence analysis confirmed the engineering of a fibrotic tissue [2]. As a step forward to this work and previous literature [3], stretchable PCL wavy meshes were designed and fabricated by MEAM, and then embedded into AHCFs-cellularized GelMA hydrogels. Such structures provided mechanical resistance and biomimetic composition, sustaining long-term dynamic testing. PCL mesh geometry was designed by structural and FEM analyses. AHCFs morphology and distribution were analyzed by immunofluorescence analysis after 14 days culture time, confirming the successful development of human cardiac fibrotic tissue. Both works confirmed that mechanical stiffness affects AHCFs behaviour triggering their fibrotic activation. Models will be validated by testing drugs with well-know effect on human cardiac fibrotic tissue.



References:
[1] A. González et al., J of the American College of Cardiology 2018; 71.
[2] M. Spedicati et al., Front. Bioeng. Biotechnol., 2022.
[3] J. C. Deddens et al., Adv. Healthc. Mater., 6, 2017.

Acknowledgments:
BIORECAR project European Research Council (ERC) funded (European Union's Horizon 2020 research and innovation programme, grant agreement No 772168.

PoB.1.14

Cell type-dependent growth patterns on melt-electrowritten scaffolds

Magdalena Z. Gładysz[1], Marcus Koch[2], Marleen Kamperman[1], Małgorzata K. Włodarczyk-Biegun[1,3], Anika Nagelkerke[1]

[1]University of Groningen, Groningen, Netherlands. [2]Leibniz Institute for New Materials, Saarbrücken, Germany. [3]Silesian University of Technology, Gliwice, Poland

Abstract

Introduction Melt electrowriting (MEW) is a 3D printing technique that enables the fabrication of complex fibrous architectures with micrometre resolution. As such, MEW scaffolds are suitable for guiding cells towards efficient tissue formation. However, cell growth on scaffolds is cell type-dependent and should be studied to provide optimal conditions tuned to the particular application. This work focuses on guiding the growth of endothelial cells and astrocytes, constituents of the blood-brain barrier (BBB) - a semi-permeable interface separating capillaries from brain tissue. **Goal** The ultimate goal is to produce an optimized *in vitro* BBB model. To this end, here we aim at characterizing cell growth patterns of endothelial cells and astrocytes on MEW scaffolds. **Methods** Scaffolds were manufactured from medical grade poly(ε-caprolactone) using MEW (Spraybase). Two distinctive cell types were seeded on the scaffold: murine brain endothelial cells - bEnd.3 - and primary mouse astrocytes. bEnd.3 were cultured on scaffolds and on flat control surfaces for 7 days, followed by proteomic analysis. bEnd.3 and astrocytes' behaviour on scaffolds was monitored via live cell imaging over 19 days. Expression of astrocyte A1 and A2 type markers, such as C3, PTX3, S100a10, and EMP-1, along with YAP and TAZ mechanosensors in bEnd.3, were observed via immunofluorescence. Scanning electron microscopy was utilized to assess cells' morphology. **Results and Discussion** Astrocytes exhibited faster growth and higher surface coverage on scaffolds with 50 µm inter-fiber distance compared to bEnd.3 (Figure 1). Prolonged culturing time resulted in bEnd.3 detachment or cluster formation. The growth of bEnd.3 grown on top of the confluent astrocyte layer was inhibited, possibly due to harmful A1 polarization. In bEnd.3 cells YAP and TAZ translocation to the cytoplasm was activated by insufficient area available for cell attachment. Among the proteins identified in bEnd.3 cultured on scaffolds were VCAM1, PECAM1, TJP1, TJP2, ABCB7, ABCB8, ABCC3. The identified proteins are responsible for cell-cell recognition, cell adhesion, tight junction assembly, and substrate transport across plasma membrane and are needed for endothelial monolayer formation.

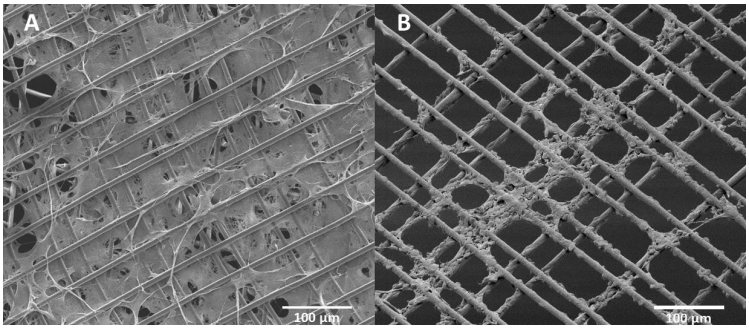


Figure 1. Cells cultured for 21 days on MEW scaffolds. A) astrocytes, B) bEnd.3.

Outlook Different cell types show different behaviour and morphologies on MEW scaffolds. In the following studies, the astrocyte layer could be used as a substrate for bEnd.3 monolayer growth after polarization into A2 type. Proteomics will benefit MEW-related research to understand differences in cellular response.

PoB.1.15

Fabrication of biodegradable high strength and shape-shifting polylactic acid membrane for reinforcement of a photo-curable composite bone fixation patch

Guillaume Patt-Lafitte[1], Daniel J. Hutchinson[2], Michael Malkoch[2], David Eglin[1]

[1]Mines Saint-Etienne, Univ Jean Monnet, INSERM, U 1059 Sainbiose, 42023, Saint-Etienne, France. [2]Division of coating technology, Royal Institute of Technology, Stockholm, Sweden

Abstract

A photo-curable thiol-ene composite fixation patch has been proposed as an alternative to Open Reduction Internal Fixation (ORIF) metal implants, for the fixation of hand fractures. The fixation patch is formulated from thiol-ene monomers, a photo-initiator, reinforcing hydroxyapatite particles and poly(ethylene terephthalate) (PET) fiber meshes. However, so far the formulation is not biodegradable, and removal surgery may still be required. Thus, the following work aimed to develop a biodegradable membrane to replace the PET meshes with matching mechanical properties, biodegradability and shape-shifting properties for improved surgical handling. Polylactic acid (PLA) membranes were produced by fused deposition modelling (FDM) 3D printing with different geometries and infills. Differential scanning calorimetry, mechanical testing, thermomechanical analysis, scanning electron microscopy and cytotoxicity assay were performed to characterize the PLA, the PLA membranes as well as the effect of selected PLA membranes on the properties of the photo-cured thiol-ene resin composite. Several infill patterns and densities have been explored as well as printing parameters, such as layer thickness, printing speed, nozzle, temperature, to optimize the FDM produced membrane's tensile strength and program the shape-memory polymer. Young's modulus of 0.6 GPa and tensile strength of 30 MPa has been achieved for 70% of pattern infill of the PLA membrane. Shape-shifting assessment was performed showing the deformation from a 2D flat to 3D bent conformation with amplitude dependent notably on the infill pattern orientation. The membrane combined with the thiol-ene/hydroxyapatite composite had a flexural modulus value of 6.2 GPa and flexural strength value of 63 MPa, equivalent to the resin composite fixation reinforced with PET meshes. This suggested that shape-shifting PLA membranes produced by FSDM 3D printing could advantageously replace PET meshes in the design of advanced customizable biodegradable bone fixation patches.

PoB.1.16

Development of a platform to test healing strategies for the healing of fetal membrane defects

Lukas Moser[1,2], Katharina Gegenschatz-Schmid[2], Nicole Ochsenbein[2,1,3], Martin Ehrbar[2,1]

[1]University of Zurich, Zurich, Switzerland. [2]University Hospital of Zurich, Zurich, Switzerland. [3]The Zurich Center for Fetal Diagnosis and Therapy, Zurich, Switzerland

Abstract

Introduction Preterm birth, which is before 37 weeks of gestation, occurs in 3 to 4% of all pregnancies. 50% of these cases are due to the preterm premature rupture of the fetal membranes (PPROM). PPROM can happen spontaneously (sPPROM) or after surgical intervention of the amniotic cavity (iPPROM). Despite affecting around 30% of all operations, there is still very little known about the cause for iPPROM. The defect mostly does not heal and increases in size until birth. This is in big contrast to the fact, that amniotic membranes are widely used for wound healing. Despite multiple efforts in the recent years no satisfying treatment exists currently. Therefore, we aim at developing a novel *in vitro* platform to screen for healing-inducing factors and to develop novel healing strategies. Method All FM samples were obtained with informed consent from all participants in accordance with the Ethical Committee of Zurich. We designed a three-dimensional printed device that allows the immobilization of small FM biopsies and the reproducible formation of a hole in the center of these biopsies. The hole will be filled with a enzymatically cross-linked poly(ethylene glycol) (TG-PEG) hydrogel. The cell ingrowth into the hydrogel will be observed for up to four weeks with bright field microscopy and the influence of various growth factor will be investigated. Further, the model will be investigated with life/dead and antibody staining for actin, nuclei and collagen. As a control, fetal membrane punches will be embedded directly into hydrogels. The survival in the 3D printed devices will be evaluated and the sealing capacity of intact FMs will be tested. Additionally, it will be investigated, if there is a difference in cell growth between fetal membrane pieces embedded into the hydrogel and the 3D devices. Results The 3D printed device allowed the immobilization of the FM biopsies, the reproducible creation of defects and their filling with TG-PEG hydrogels. Initial evaluations showed a good survival of the FM cell. Additionally the influence of PDGF-BB will be evaluated. Conclusion The 3D printed devices could offer a model that recapitulated the *in vivo* situation more accurate. Additionally, the design of the 3D printed devices would enable the possibility to investigate the influence of amniotic fluid on the amniotic side and pressure could be applied to add another parameter. In conclusion, this platform could be an interesting approach to investigate healing strategies and reduces animal trials.

PoB.1.17

3D printed medical-grade poly(lactic acid) scaffold loaded with chitosan-hydroxyapatite hydrogel: *in vitro* degradation and interaction with human mesenchymal stem cells

Leonard Bauer[1], Anamarija Rogina[1], Marko Zajec[2], Nenad Drvar[3], Katarina Caput Mihalić[4], Inga Urlić[4], Marica Ivanković[1], Hrvoje Ivanković[1]

[1]University of Zagreb, Faculty of Chemical Engineering and Technology, HR-10000 Zagreb, Croatia. [2]IZIT d.o.o., HR-10431 Sveta Nedelja, Croatia. [3]TOPOMATIKA d.o.o., HR-10431 Sveta Nedelja, Croatia. [4]University of Zagreb, Faculty of Science, HR-10000 Zagreb, Croatia

Abstract

Surgical procedures are performed daily to replace and restore damaged tissue in dental and orthopaedic medicine. Bone loss after tooth extraction, inflammation or trauma requires surgical bone augmentation. Current periodontal treatments use various inert structures such as titanium mesh, which is widely available in clinical applications due to the reduced immune response of patients and mechanical resistance [1]. The main disadvantage of titanium implants is the secondary surgical procedure for their removal. The use of synthetic biodegradable structures obtained by additive manufacturing enables the structure modulation of biodegradable implants, where their strength ranges in the order of magnitude of natural bone tissue [2]. This research presents the production and characterization of a three-component scaffold based on poly(lactic acid) (PLA), chitosan (CHT) and hydroxyapatite (HAp). Medical-grade PLA filament was 3D printed into a 1 mm pore-size scaffold. Medical-grade chitosan solution with 30 wt.% of HAp was vacuum infiltrated and lyophilized within the PLA structure. The incorporation of microporous bioactive CHT-HAp hydrogel within macroporous load-bearing mesh should enhance the osteogenic potential of PLA-based scaffold. The *in vitro* degradation behaviour of prepared composite scaffolds was investigated in phosphate-buffered saline solution with the addition of 1 mg mL^{-1} lysozyme and in Hank's balanced salt solution. The degradation was carried out according to ISO 13781:2017 (E) standard for 8 weeks. The compression tests revealed that the loading of CHT-HAp hydrogel reduces mechanical properties in comparison to the neat PLA 3D structure. The composite samples maintained mechanical properties during the *in vitro* degradation, while the neat PLA control showed a decrease in Young's elastic modulus and compressive strength. The degradation of mechanical properties is attributed to the hydrolytic degradation of PLA, which seems to be inhibited by the addition of CHT-HAp hydrogel. The osteogenic potential of PLA-CHT-HAp scaffolds was evaluated with the culture of human mesenchymal stem cells in an osteogenic medium for 21 days under perfusion conditions. The results showed the tissue growth during culture and the presence of osteogenic markers, which indicated a potential of PLA-CHT-HAp scaffold for further *in vivo* studies.

[1] Y.D. Rakhmatia, Y. Ayukawa, A. Furuhashi, K. Koyano, J. Prosthodont. Res., 2013, 57, 3-14.

[2] D.W. Hutmascher, J.T. Schantz, C.X.F. Lam, K.C. Tan, T.C. Lim, J. Tissue Eng. Rend. Med., 2007, 1, 245-260

PoB.1.18

Bone Microtissue construction using 4D Biofabrication

Ali Yousefinejad, Leonid Ionov

Faculty of Engineering Sciences, University of Bayreuth, Bayreuth, Germany

Abstract

The primary building blocks of cortical bone are osteons, which exhibit a hierarchical tubular microstructure consisting of collagen fibrils at the nano-scale level arranged in concentric circles. Among the methods of treatment of bone damage, tissue engineering has emerged as a promising approach for addressing bone defects. Construction of tubular structures like osteons as cortical bone microtissues is challenging due to the need for high resolution, perfusion of media, and cell guidance for the fabrication of such tissue. To overcome these problems, 4D biofabrication has emerged. This method involves the transformation of flat mono or bilayers containing various types of adhered cells into complex shapes that can replicate anisotropic and tubular structures found in human tissues. In this study, we report a novel approach for the fabrication of bone microtissues using electrospun bilayers and trilayers that are highly aligned and self-actuating. The study also studied the effect of the bioactive glass on bone formation capability and enhancement of osteoconductivity. The scaffolds are made of aligned polycaprolactone (PCL) fibers and non-aligned hyaluronic acid methacrylated (HAMA) fibers in the presence of 5% and 13.4-14.7% of bioactive glass for bilayer and trilayer scaffolds respectively. The scaffold has been changed into the tubular structure upon facing water/cell culture media with a diameter of 455 µm which is in the range of the diameter of the osteon. A higher diameter of the structure has been achieved by the increase of the ratio of the passive (PCL) to the active layer. The presence of hydroxyapatite has been confirmed after being immersed in the simulated body fluid for 4 weeks using SEM and XRD results. MC3T3-E1 cells which were seeded on the scaffolds had a viability of 84-94% after 7 days of being cultured in the media. Progressive cell proliferation has been achieved through days 1, 4, and 7, and the Alamar blue reduction has been significantly increased for bilayer scaffolds in presence of BGs (63.4-64.6%) in comparison to the scaffold without BG (38.6%). The alignment of the cells has been increased for all the scaffolds from day 4 to 7, and it could reach 81-100% for all the scaffolds. Furthermore, the presence of the bioactive glass in the scaffold in the bilayers and trilayer, not only increased the alkaline phosphatase (ALP) activity as an osteoblast differentiation marker but also enhanced the deposition of calcium phosphate which was measured by alizarin red assay.

PoB.1.19

High-resolution 3D-printing of biosynthetic vessels using Two-Photon-Stereolithography

Johanna Vetter[1], Anna Fritschen[1], Nils Lindner[2], Alina Filatova[3], Ulrike Nuber[4,3], Andreas Blaeser[1,4]

[1]Technical University of Darmstadt, Institute for BioMedical Printing Technology, Darmstadt, Germany. [2]Technical University of Darmstadt, Darmstadt, Germany. [3]Technical University of Darmstadt, Stem Cell and Developmental Biology, Darmstadt, Germany. [4]Technical University of Darmstadt, Centre for Synthetic Biology, Darmstadt, Germany

Abstract

Providing nutrient supply in macroscopic tissue precursors remains an unsolved challenge in tissue engineering. Despite promising biomimetic and self-assembly approaches, capillary vessels, which take weeks to mature, cannot be formed fast enough to close the supply gap. This work aims to improve nutrient supply in tissue precursors by incorporating biosynthetic vessel networks generated by Two-Photon-Stereolithography (TPS). TPS allows 3D-printing of complex geometries with a resolution down to 100 nm. Here, the technology was used to fabricate porous channels in the dimensions of human blood vessels. Firstly, the influence of printing parameters on shape accuracy and surface roughness of printed vessels was investigated for two resins (IP-S and IP-Visio). With both resins, transparent channels of diameters ranging from 10 µm to 2 mm could be readily fabricated. In addition, the walls of the conduits could be perforated with pores ranging from 1-50 µm. Furthermore, complex and branched vessel structures with varied diameters could be printed. In order to investigate the incorporation of vessel elements into matrix materials, the wettability of flat, curved and porous surfaces was tested with water, 0.5 % Agarose and 0,5 % hyaluronic acid. Partial wettability (contact angle of 70 ° ± 5) and no penetration of matrix materials into the pores could be observed. This demonstrates that matrix materials can be deposited around the biosynthetic network without clogging the porous channels. The cytocompatibility of the two resins was shown by culturing HUVECs on flat printed substrates. To further pursue the goal of a biosynthetic vascular network, HUVECs were introduced into printed channels in the absence and presence of flow. The experiments showed high viability, adhesion and spreading of cells on and in the printed structures. To study the permeability of printed vessels with pore diameters ranging from 5-50 µm, they were connected to a pump system and flushed with FITC labeled dextran molecules (70 kDa). The experiments revealed that the permeability depended on both the pore size and the surrounding matrix material. While penetration of FITC-dextran into an aqueous environment was already observed at a pore size of 15 µm, the pore size had to be increased to 50 µm to achieve particle transport into a surrounding hydrogel matrix. The results of this work demonstrate the general feasibility of using TPS for the fabrication of biosynthetic vessels with tailorable particle diffusion. The method offers great potential to close the nutrient supply gap in biofabricating macroscopic tissue precursors.

PoB.1.20

Combining 3D printing and cryostructuring to ensure spine fusion and prevent postoperative infection

Tiziana Fischetti[1,2], Gabriela Graziani[2], Daniele Ghezzi[3], Friederike Kaiser[4], Stefanie Hölscher-Doht[5], Martina Cappelletti[3], Giovanni Barbanti Brodano[6], Nicola Baldini[1,2], Uwe Gbureck[4], Tomasz Jüngst[4]

[1]University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy. [2]IRCCS Istituto Ortopedico Rizzoli, BST Biomedical Science and Technologies and Nanobiotechnology Lab, Bologna, Italy. [3]University of Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy. [4]University Hospital Würzburg, Department for Functional Materials in Medicine and Dentistry, Würzburg, Germany. [5]University Hospital Würzburg, Department of Trauma, Hand, Plastic and Reconstructive Surgery, Würzburg, Germany. [6]IRCCS Istituto Ortopedico Rizzoli, Department of Spine Surgery, Bologna, Italy

Abstract

Chronic back pain due to osteoarthritis is a main issue in spine surgery. The intervertebral disk may be removed and surgical fusion of adjacent vertebrae is often advocated in order to reduce pain and severe disability. Autologous or allogeneic bone or synthetic bone grafts with proper bone fixation is a successful solution that sometimes is complicated by the occurrence of postoperative infection. In this study, a novel dual-component device is proposed to ensure spine fusion and decrease the rate of infection. To ensure optimal fusion, 3D extrusion-based printing technology was used to manufacture magnesium-phosphate based scaffolds. Magnesium-phosphates are advantageous in terms of osseointegration and degradation properties compared to calcium phosphates. In fact, they have shown promising results in terms of bone tissue ingrowth *in vivo* in animal models. Here, for the first time, magnesium phosphate scaffolds were investigated to develop 3D printed custom-shaped cages for spinal fusion, and high printing accuracy (> 75%) was obtained. The 3D printed scaffolds were hardened by chemical reaction and the converted phases and Mg2+ and Ca2+ ions release profile were investigated by XRD and ICP-MS analysis, respectively. Additionally, stability and mechanical properties were studied over 28 days and found to be within the physiological ranges for bone tissue formation. To prevent infection, a custom-built device based on ice-templating technique (i.e., cryostructuring) is proposed to develop tailored porous alginate structures, to be loaded with vancomycin. This innovative cryostructuring technology is introduced for the first time for drug loading and antibacterial applications, in combination with 3D printing. The cryostructuring process was optimized to obtain optimal porous structures by studying the impact of different parameters (alginate concentration, temperature gradients) on pores size and orientation (SEM). Then, the cryostructuring process was successfully combined with the 3D printed scaffolds. Results show that magnesium-phosphates can be printed into complex geometries and that the 3D printed geometry affects pores orientation during cryostructuring. Antibacterial efficacy of vancomycin loaded within the alginate during and after cryostructuring process, as assessed for Escherichia coli and Staphylococcus aureus strains, showed promising results.

Funding acknowledgement: Starting Grant project " Novel multifunctional nano-coated 3D printed scaffolds for the treatment of bone defects" (SG-2021-12373011) by The Italian Ministry of Health.

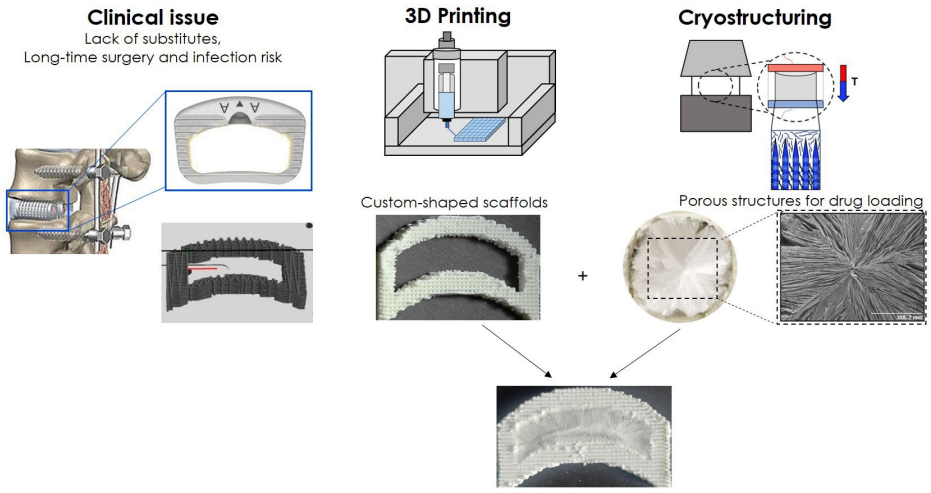


Figure. *Schematic representation of the clinical issue and the approach pursued in this study. In spinal fusion surgery, the common approach is the combination of bone autografts with synthetic cages. However, this approach is limited by the availability of synthetic cages and infection occurrence. Here, a dual-component device is proposed to achieve spine fusion and decrease infection rate. 3D printing and cryostructuring are investigated to obtain custom-shaped cages and porous materials for antibiotic drug loading, respectively.*

PoB.1.21

4D electroactive and microporous polyHIPE-PEDOT scaffolds as a dynamic *in vitro* cell culture platform

Franziska Hahn[1,2], Ana Ferrández Montero[1,2], Cédric Vancaeyzeele[1], Rémy Agniel[2], Cédric Plesse[1], Johanne Leroy-Dudal[2]

[1]Laboratoire de Physicochimie des Polymères et des Interfaces (LPPI) - CY Cergy Paris Université, Neuville sur Oise, France. [2]Equipe de Recherche sur les Relations Matrice Extracellulaire-Cellule (ERRMECe) - CY Cergy Paris Université, Neuville sur Oise, France

Abstract

In vivo, cells are surrounded by an extracellular matrix (ECM). Signals from the ECM such as biochemical or biophysical cues regulate cell behavior in physio-pathological processes. Therefore, mimicking this cell microenvironment is one of the most important challenges in the fields of physio-pathological research, tissue engineering or drug screening. Although 3D cell cultures offer biologically superior structures, there is still a lack in the transmission of dynamic mechanical signals that exist in the microenvironment of cells *in vivo*. The aim of this work is to develop a 4D microporous and electroactive scaffold as an innovative cell culture platform that enables the in situ electromechanical stimulation of cells and monitoring of the cell behavior. For this purpose, a 3D microporous scaffold is obtained from the polymerization of a high internal phase emulsion template. This so called polyHIPE is characterized by a high interconnectivity and a suitable porosity for a rapid cell colonization. In a further step the polyHIPE-scaffold was homogenously functionalized with a conducting polymer, the poly(3,4-ethylenedioxthiophene) (PEDOT), via vapor phase swelling and an oxidative polymerization process with iron(III) chloride. The functionalization leads to a 4D electroactive polyHIPE-PEDOT scaffold with stimuli-responsive properties such as changes in shape, morphology, pore size under time-dependent external stimulation. These properties are kept after sterilization via autoclavation, in cell culture medium and also in the presence of adherent cells and/or extracellular matrix proteins. Our results show that the stimulation process is reversible, stable and generates volume variations of 10% on the scaffolds. The polyHIPE-PEDOT scaffolds support cell adhesion, spreading and migration. Stimulations up to 5h were carried out on the polyHIPE-PEDOT scaffolds seed with human dermal fibroblasts. No difference in cell viability or cell density was found between stimulated and unstimulated scaffolds. We believe that our 4D scaffold is a promising dynamic cell culture platform which pave the way to a better understanding of cell responses to the dynamic of their microenvironment. Several applications could be possible as cell phenotype tuning, mechanotransduction analysis or drug testing under mechanical tension.

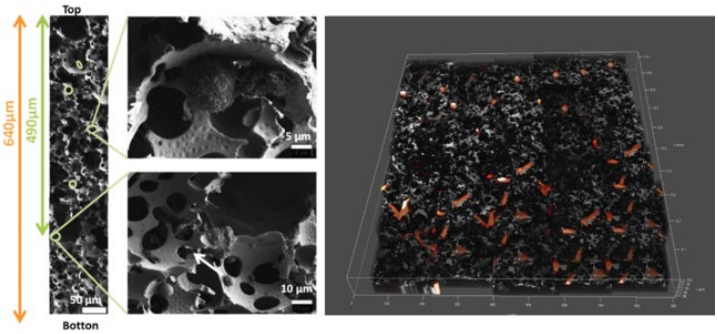


Figure 1: Human dermal fibroblast on top (right) and inside (left) a polyHIPE-PEDOT scaffold. Green circle represent location where cells could be found inside the microporous structure of the scaffold after 3h of incubation.

PoB.1.22

Advanced cerebral aneurysm clipping training– a hybrid approach including a realistic patient head phantom

Julia Maier[1], Thomas Thurner[2], Sebastian Lämmermann[1], Andreas Schrempf[2], Zoltan Major[1]

[1]Johannes Kepler University, Institute Polymer Product Engineering, Linz, Austria. [2]University of Applied Sciences Upper Austria, Research Group for Surgical Simulators, Linz, Austria

Abstract

Neurosurgical clipping of cerebral aneurysms to avoid subarachnoid haemorrhage is a very complex and challenging surgery even for experienced neurosurgeons. The individual skills and experience of the surgeon can decide a surgery's success. To increase both patient safety and confidence of surgeons, training of surgery procedures is an important discipline. In this project called MEDUSA, a hybrid simulator for cerebral aneurysm clipping has been developed. An interdisciplinary team consisting of neurosurgeons, material and software engineers and experts for regulatory aspects create a training platform for neurosurgeons. The simulator offers a hybrid approach combining the physical and the virtual world. The physical component of the simulator mimics a patient head designed based on the anatomy of an individual patient. All materials and production methods were chosen in order to replicate the properties of human tissues as accurately as possible. The head phantom consists of skin, skull, brain, blood vessels with an aneurysm and arachnoid. The single parts of the head phantom are reconstructed from CT, MR and DSA patient data and manufactured by using different 3D printing techniques. PLA as well as different silicone types are used to reproduce properties similar to material properties known in literature. Additionally, the neurosurgeons in the project give valuable feedback. A novel part of the head phantom is the arachnoid which is made of a custom-made hydrogel. Skills such as the preparation of the arachnoid and of the entry towards the aneurysm can be physically trained by the surgeons. The brain lobes are hollow bodies filled with water, allowing hydraulic control of the brain lobe movement and are equipped with electromagnetic tracking. This allows to e.g. measure the pressure applied on the brain during surgery or simulate a response of the brain to the opening of the skull. Moreover, by applying hydraulic control the level of difficulty can be adjusted by changing the brain pressure or opening angle of the Sylvian fissure during training. As soon as the neurosurgeon physically reaches the aneurysm the view through the microscope switches to the virtual part. Based on advanced non-linear and blood flow simulations, the success rate of clip placing is calculated in terms of percentage of the blood flow into the aneurysm which has been cut off successfully. Different strategies for an upcoming aneurysm clipping surgery can be trained in advance under realistic conditions to produce the best possible result for the patient.

PoB.1.23

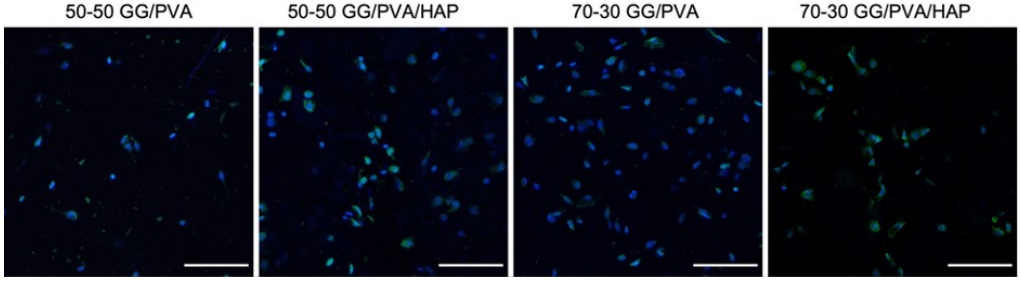
3D bioprinted constructs comprising gellan gum/PVA enriched with nanohydroxyapatite promote bone tissue regeneration

Konstantinos Loukelis[1], Georgia-Ioanna Kontogianni[1], Maria Chatzinikolaidou[1,2]

[1]Department of Materials Science and Technology, University of Crete, Heraklion, Greece. [2]Foundation for Research and Technology Hellas (FORTH)-Institute of Electronic Structure and Laser (IESL), Heraklion, Greece

Abstract

Bioprinting is one of the most versatile 3D printing techniques in the field of tissue engineering, combining cells and biomaterials into constructs with desired geometry. This in turn leads to an increasing demand for the development of new combinations of biomaterials and biological constituents in order to produce biomimicking devices, optimal for tissue regeneration. In this work, we prepared different compositions of bioinks comprising gellan gum (GG) and polyvinyl alcohol (PVA), two water-soluble biomaterials of natural and synthetic origin, respectively, with nanohydroxyapatite (HAP), one of the most thoroughly investigated bioceramic, known for its osteoinductive and osteoconductive capabilities. The bioinks were formulated after the mixing of the biomaterial solutions with MC3T3-E1 pre-osteoblastic cell suspension at a concentration of 5x106 cells/ml, and assessed in regard to their mechanical properties and their osteogenic potential. By mixing a 10% w/v PVA and a 4% w/v GG solution at different ratios, with and without the addition of a standard concentration of 2.5% w/v HAP, four scaffold compositions were prepared: (i) 50-50 GG/PVA, (ii) 70-30 GG/PVA, (iii) 50-50 GG/PVA/HAP and (iv) 70-30 GG/PVA. To physically crosslink the scaffolds, they were submerged in a 1% w/v CaCl2 solution for 10 min, followed by multiple rinsing cycles with ultrapure water. The biological evaluation of the bioinks includes Live/Dead staining protocol, DAPI/actin staining, collagen and alizarin red staining. Moreover, the bioinks have been characterized by examining their biodegradation rate and their rheological properties. At day 7, all bioinks had evident extracellular matrix formation, with the HAP containing bioinks depicting greater levels. Regarding biodegradation rate, the 50-50 GG/PVA bioink retained mass loss values between 14% and 28%, the 70-30 GG/PVA 7% and 23%, the 50-50 GG/PVA/HAP 9% and 17% and the 70-30 GG/PVA/HAP 5% and 16% after 7 and 21 days, respectively. Rheological analysis revealed that the presence of HAP only slightly affected the examined quantities. The bioinks retained a recovery rate of at least 89% of their viscosity after the application of 200% strain. Moreover, all scaffolds depicted great levels of biocompatibility with proliferating cells inside the bioink matrix and upregulation of bone related markers. With their excellent printability and biological response, these novel bioinks containing gellan gum, PVA, and hydroxyapatite present a favorable environment for bone regeneration.



Expression of alkaline phosphatase activity (green), cell nuclei (blue). Scale bar: 100 µm.

PoB.1.24

Bioprinting by light sheet lithography: engineering complex tissues with high resolution at high speed

Levin Hafa[1,2], Louise Breideband[1,2], Gustaf Mårtensson[3,4], Robert Eklund[3], Helmut Wurst[5], Angres Brigitte[5], Núria Torras[6], Elena Martinez[6], Ruby Shalom-Feuerstein[7], Francesco Pampaloni[1,2]

[1]Buchmann Institute for Molecular Life Sciences (BMLS), Frankfurt am Main, Germany. [2]Goethe University Frankfurt, Frankfurt am Main, Germany. [3]Mycronic AB, Taby, Sweden. [4]Div. nanobiotechnology, Royal Institute of Technology (KTH), Stockholm, Sweden. [5]Cellendes GmbH, Reutlingen, Germany. [6]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain. [7]Department of Genetics & Developmental Biology, the Ruth and Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel

Abstract

Three-dimensional bioprinting is constantly reaching new horizons in the field of tissue engineering, with various applications reaching the market every year. Nevertheless, systems are limited in resolution when required to print in a fast manner (e.g., extrusion-based) or limited in speed when required to print at high resolution (e.g., 2-photon-polymerization). Here, we present a bioprinting technique, which produces millimeter-sized objects in less than 3 minutes, using a digitally scanned light sheet. Hence, the resolution is dictated by the light sheet dimensions, as low as 50 µm for the z-axis and about 15 µm for the xy-axis, also depending on the used photo-crosslinkable hydrogel. In this study, a novel hydrogel was developed to fully exploit the light sheet resolution. Additionally, built-in light sheet microscopy enables live acquisition of the printing process in high resolution for real-time observation of the cell-laden hydrogel. Initial testing with human cell lines shows promising results in terms of cell outgrowth and interconnectivity. A human skin model was bioprinted and kept in culture for about 6 weeks, showing significant stratification allowing the pharmaceutical and cosmetics industry to choose an alternative to animal testing.

PoB.1.26

Radical-free photopolymerizable hyaluronan bioink for articular cartilage tissue engineering

Alisa Hangartner[1,2], Gregor Miklosic[1,2], Mauro Pavan[3], Devis Galesso[3], Riccardo Beninatto[3], Ezgi Bektas[1], Matteo D'Este[1]

[1]AO Research Institute Davos, Davos, Switzerland. [2]Institute for Biomechanics, ETH Zürich, Zurich, Switzerland. [3]Fidia Farmaceutici SpA, Abano Terme (PD), Italy

Abstract

Background: Photopolymerization strategies are commonly used to reinforce hydrogel-based constructs and maintain their shape fidelity after extrusion-based bioprinting [1]. Despite the precisely controllable and tuneable photopolymerization process, cytocompatibility is still a challenge due to the production of cytotoxic free radicals in the presence of a photoinitiator [2]. To address this, a range of photoinitiator-free biomaterial inks based on Diels–Alder reaction of coumarin derivatives of hyaluronic acid (HA) and gelatin (Gel) were introduced, which are photopolymerizable with UV (365 nm) without production of reactive species or radicals [3]. Methods: Triethylene glycol (TEG) or octyl (C8) spacers were used to link umbelliferone residues to Gel or HA respectively. Multiple formulations were studied rheologically to evaluate their viscoelastic properties, shear-thinning behavior and elastic recovery for extrusion. A monochromatic light source at 365 nm was used with a power output of 15 mW. Shape fidelity was evaluated based on printability assessments with optimized parameters. Cytocompatibility was tested with live-dead fluorescence using printed bovine chondrocytes. Results: All compositions demonstrated an immediate 20-90-fold increase in storage modulus and gel formation after light exposure (Fig. 1, A). Optimal shear-thinning behaviour and flow under high shear conditions above yield point was observed (Fig. 1, B), and elasticity maintained to a large extent after 3 high/low shear cycles (Fig. 1, C). The material was printable and showed uniform filament formation as well as bridging capabilities for some formulations (Fig. 1, D, E, F). Excellent cytocompatibility was shown with high cell viability and metabolic activity after 21 days of culture (Fig. 2).

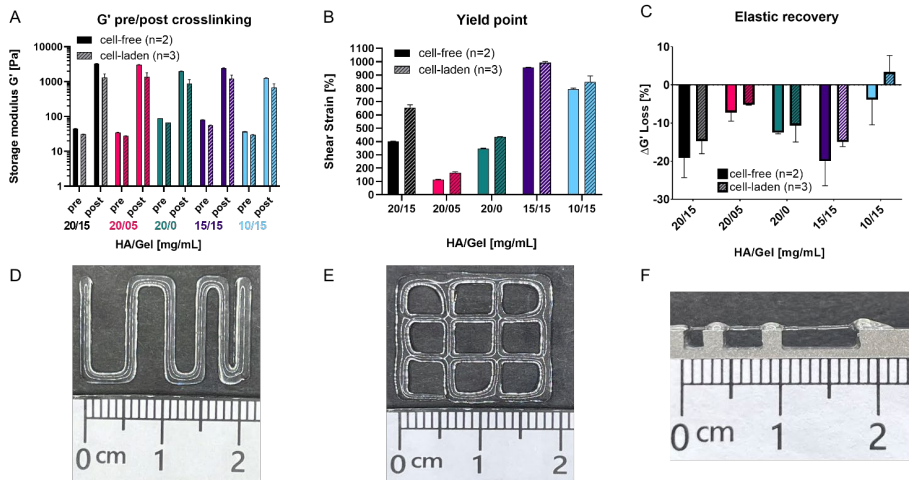


Figure 1: (A, B, C) Comparison rheological characterization of HA-Gel-inks. (D, E, F) 3D-bioprinted line spacing, grid structure and bridging of 20/05 precursor hydrogel.

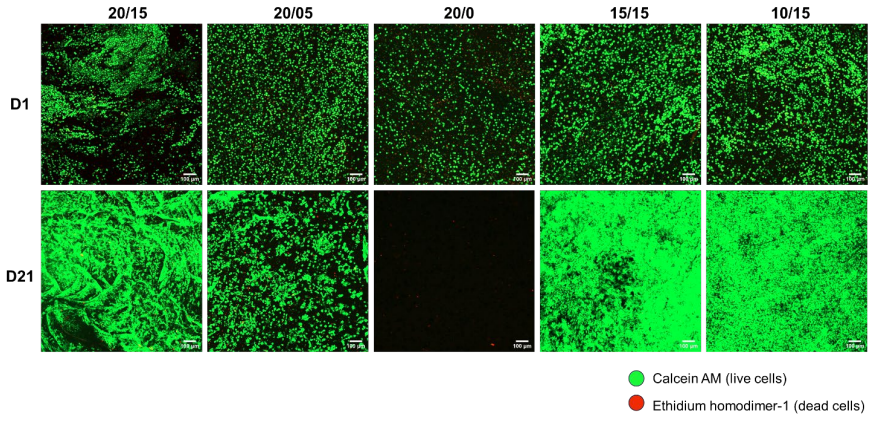


Figure 2: Representative live/ dead staining of bovine chondrocytes embedded in cast gel at days 1 and 21 [HA/Gel in mg/mL].

Conclusions and Outlook: This work demonstrates radical-free photopolymerization, good printability and excellent chondrocyte viability of the novel ink. As a next step, cell viability of cell-laden inks after printing as well as mechanical properties will be studied. This injectable bioink is cytocompatible, and supports cell growth in materials for cartilage tissue engineering, enabling safer clinical translation.

Acknowledgements: Funding and materials were kindly provided by Fidia Farmaceutici S.p.A.

References:

[1] Agarwal et al. Mat Sci Eng C, 2021, 123:112005.

[2] Beninatto et al. Mat Sci Eng C, 2019, 96, 625-634.

[3] Patent WO2021186335 (2020). Fidia Farmaceutici SpA.

PoB.1.28

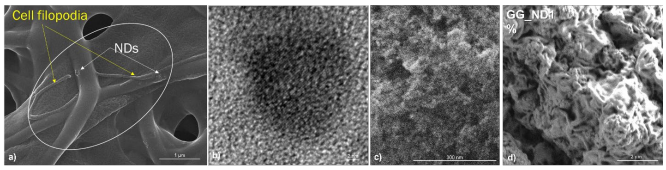
The potential of nanodiamond particles to orchestrate the cellular behavior and modulate the (nano)mechanical properties of 3D printed scaffolds

Carmen Valentina Nicolae[1], Elena Olăreț[1], Raluca Elena Ginghină[2], Sorina Dinescu[3,4], Andrada Serafim[1], Izabela Cristina Stancu[1]

[1]Advanced Polymer Materials Group, Faculty of Chemical Engineering and Biotechnologies, University Politehnica of Bucharest, Bucharest, Romania. [2]Research and Innovation Center for CBRN Defense and Ecology, Bucharest, Romania. [3]Department of Biochemistry and Molecular Biology, University of Bucharest, Bucharest, Romania. [4]Research Institute of the University of Bucharest (ICUB), Bucharest, Romania

Abstract

The potential of nanoparticles to guide cells interactions through numerous mechanisms, involving complex interplay between mechanical forces, biochemical signalling and cellular responses requires further understanding. Previous research or our group suggesting improved cell interactions (hASCs, fibroblasts, osteoblasts, neural precursors) with low concentrations of nanodiamond nanoparticles (NDs) (<1%) loaded in gelatin nanofibers, were inspiring for this study [1-4]. Scanning electron microscopy suggested cellular adhesion contacts intensified on locally exposed NDs in gelatin nanofibers (Fig.1a), while local increase in stiffness was detected by nanoindentation [2]. *Figure 1. a) NDs improved the innate cell-instructive character of electrospun gelatin scaffolds, cell filopodia oriented towards NDs aggregates; microstructural details: b) single ND particle (TEM), c) SEM image of NDs; d) GG_ND1% nanocomposite appearance (SEM).*



Here from a question on the mechanism inducing localized cell adhesion – composition-driven, nanomechanically-driven, or combined? The present research explores if such cell-instructive behavior is expressed in 3D printed scaffolds loaded with low amounts of NDs as well (Fig. 1d). Acellular inks were formulated for 3D printing, based on a gellan gum polysaccharide matrix incorporating 0.5%, 1%, 2% and 3% (wt/v) NDs (GG_ND0.5%, GG_ND1%, GG_ND2%, GG_ND3%). The control hydrogel was also prepared (GG_ND0%). The rheological behavior of the formulations was evaluated, along with their injectability and printability. Both 3D printed scaffolds and their casted counterparts were tested in terms of dimensional stability, swelling behavior, gel fraction, preliminary degradation in PBS. The morpho-structural characterization of the samples was performed through SEM and micro-CT. The reinforcing effect of the nanoparticle loading was investigated through compression tests, using a texture analyzer, while nanoindentation was used to explore local mechanical effects. Murine preosteoblasts MC3T3-E1 were used to assess scaffolds' biocompatibility, cytoskeleton investigation and ability to support osteogenic differentiation *in vitro*, revealing the effect of low concentration NDs to promote cell interactions.

[1]. Șelaru, A. et al. Int. J. Mol. Sci. 2022, 24, 555. doi: 10.3390/ijms24010555.
[2]. Olăreț, E. et al. Polymers 2021, 13, 407. doi: 10.3390/polym13030407.
[3]. Șelaru, A. et al. Mater. 2019, 12, 2933. doi: 10.3390/ma12182933.
[4]. Serafim, A. et al. RSC Adv. 2015, 5, 95467–95477. doi: 10.1039/c5ra14361f.

Acknowledgements: This work was supported from the project Integrating mechanically-tunable 3D printing with new bioactive multi(nano)materials for next functional personalized bone regenerative scaffolds, PN-III-P4-PCE-2021-1240, nr. PCE 88/2022. 3D printing and micro-CT analysis were possible through ERDF/COP 2014-2020, ID P_36_611, MySMIS 107066, INOVABIOMED.

PoB.1.31

Real-time system for in-flight droplet parameter monitoring in drop-on-demand bioprinting utilizing a dynamic vision sensor and semantic segmentation

Nils Lindner[1], Jonas Dietz[2], Andreas Blaeser[1,3]

[1]Technical University of Darmstadt, Institute for BioMedical Printing Technology, Darmstadt, Germany. [2]Technical University of Darmstadt, Institute of Printing Science and Technology, Darmstadt, Germany. [3]Technical University of Darmstadt, Centre for Synthetic Biology, Darmstadt, Germany

Abstract

Introduction: The use of drop-on-demand (DOD) processes is widespread in tissue engineering, enabling high throughput with high cell viability at low cost. Despite its popularity, the technology faces a number of challenges and technological hurdles, such as inconsistent droplet volumes or velocities, which negatively impact process stability and accuracy. Utilizing optical sensors and a real-time control system can help overcome these challenges. **Objective:** This work aims at establishing a system to provide real-time data acquisition of in-flight droplet parameters (velocity, eccentricity, volume) of different bio-inks and to instantaneously provide feedback to a higher-level control system. This is achieved by using a dynamic vision sensor, which has an extremely high temporal resolution and the advantage over conventional high-speed cameras of producing only small amounts of data, which is essential for the implementation of a real-time system. The reliable analysis of the collected data is enabled by the use of semantic segmentation based on a trained deep learning model. **Methods:** For the evaluation of the implemented system different hydrogels (e.g. agarose) with different material concentrations were utilized and printing parameters, including printing pressure (0.1 – 1 bar), printing temperature and valve opening time (300 µs – 1000 µs), were varied. The recorded data was then analyzed to validate the reliability of the sensor. In addition, the processing time was evaluated with respect to the real-time capability of the system. **Results:** The data recorded by the sensor and processed by the algorithm shows a high accuracy with respect to the detection of droplet velocity and eccentricity, is in itself consistent and comparable with expected values from the literature. However, the volume calculation shows a comparatively high average relative standard deviation of ~6% due to the two-dimensional approach. The system provided real-time feedback (< 40 ms) on droplet shape, size, and speed, allowing for the application in a process control system. **Conclusion and Outlook:** The presented work shows good suitability of the applied dynamic vision sensor for the monitoring of in-flight data for droplets printed in a microvalve based DOD process. The major advantage is the high accuracy and real-time capability of the system. In combination with further sensors for observation during droplet formation in the print head as well as the behavior when impacting a substrate or previously printed layers, the presented technology shows the potential to be part of a higher-level optimization control to improve 3D bioprinting.

PoB.1.33

Comparison of conventional sterilization methods for composite biomaterial inks for 3D printing for biomedical applications

Anna Woźniak[1,2], Monika Biernat[1], Magdalena Gizowska[3], Wojciech Święszkowski[2]

[1]Łukasiewicz Research Network - Institute of Ceramics and Building Materials, Cracow, Poland. [2]Warsaw University of Technology, Faculty of Materials Science, Department of Materials Design, Warsaw, Poland. [3]Łukasiewicz Research Network - Institute of Ceramics and Building Materials,, Cracow, Poland

Abstract

3D printing, or spatial printing, is a modern, low-cost alternative to earlier manufacturing technologies. It involves printing layer by layer an object designed in a suitable digital file. Various filaments, usually thermoplastic, which are melted in the printer head, are used as inks in 3D printers. However, the medical industry is increasingly using hydrogels as inks for 3D printers. Such inks, depending on the application, should exhibit appropriate properties such as viscosity, pH [1] and, in the case of composite inks, whole-mass stability. 3D printed hydrogel-models find applications in tissue engineering and regenerative medicine (e.g., as scaffolds to promote healing). Increasingly, not only biomaterial inks are used in this area, but also materials with cells based on hydrogels - bioinks [2]. It is crucial that the material intended for cell contact undergoes effective sterilization to minimize the risk of contamination of the sample. Indeed, prints using bioinks cannot be sterilized after the process of 3D printing. One of the problems associated with hydrogels is their sensitivity to the conditions experienced during conventional sterilization methods [3]. For this reason, each hydrogel material requires the individual selection of the most appropriate, effective sterilization method and the design of its process in such a way as to preserve the main desired properties of the material unchanged. The aim of this study was to select the best method for sterilizing composite biomaterial inks based on natural polymers and solid ceramic particles. The work included a comparison of two conventional sterilization methods – with steam heat or by electron beam irradiation. Results indicate that better method for considered materials is sterilization with steam heat regarding rheological characterization, pH tests, Zeta potential tests, particle size distribution, and FTIR analysis. Electron beam irradiation caused the significant change of material rheology, so it is not recommended for such hydrogel materials by us.

References:

[1] Malda J., et al. 25th anniversary article: Engineering hydrogels for biofabrication. Adv Mater 2013;25:5011–28

[2] Groll J., et al. Adefinition of bioinks and their distinction from biomaterial inks, Biofabrication, 2019, 11 013001

[3] R. Galante, T. J. A. Pinto, R. Colaco, A. P. Serro, Sterilization of hydrogels for biomedical applications: A review. J Biomed Mater Res Part B 2018: 106B: 2472– 2492

PoB.1.34

3D extrusion printing of marine collagen-chitosan-fucoidan inks: evaluation of shape fidelity and cytocompatibility

Duarte Nuno Carvalho[1,2,3], Sophie Dani[3], Carmen G. Sotelo[4], Ricardo I. Pérez-Martín[4], Rui L. Reis[1,2], Tiago H. Silva[1,2], Michael Gelinsky[3]

[1]3B´s Research Group, I3B´s – Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark 4805-017, Barco, Guimarães, Portugal, Guimarães, Portugal. [2]ICVS/3B´s – PT Government Associate Laboratory, Braga/Guimarães, Portugal, Braga, Portugal. [3]Centre for Translational Bone, Joint- and Soft Tissue Research, Technische Universität Dresden, Faculty of Medicine and University Hospital, 01307 Dresden, Germany, Dresden, Germany. [4]Group of Food Biochemistry, Instituto de Investigaciones Marinas (IIM-CSIC), C/Eduardo Cabello 6, Vigo, Pontevedra, Spain, Vigo, Spain

Abstract

The field of tissue engineering has had a significant progress in the last decade, remarkably thanks to advances in 3D printing using inks from natural and/or synthetic origin compounds due to their fast processing and reproducibility fidelity. However, achieving high structural shape fidelity is still a significant challenge when hydrogel-like materials are employed, as it directly impacts the construct's physical and eventual cell biological performance. On the other hand, some marine-origin polymers have gained attention as an alternative to land mammal-origin polymers due to the reduced risk of disease transmission and low ethical concerns, mimicking human extracellular matrix components while promising biocompatibility, biodegradability and low immunogenicity [1, 2]. The present study aimed the manufacturing of adequate biomaterial ink that could achieve high shape fidelity and reproducibility, using a blend of collagen from shark skin, chitosan from squid pens, and fucoidan from brown algae. The cohesion of the 3D printed scaffold was further improved by incubation in one of four non-synthetic crosslinker solutions: phosphate-buffered saline, cell culture medium RPMI, 6% CaCl2, and 5 mM genipin. Several tests were conducted to evaluate the ink's performance, including rheology, filament collapse and fusion behavior, as well as to characterize scaffold features, namely swelling behavior, compressive mechanical properties and cytocompatibility. The results demonstrated favorable shape fidelity, resulting in a stable structure without deformations and interesting shear recovery properties around 80%. Additionally, live/dead assays, conducted using an immortalized human mesenchymal stem cell line (MSC) seeded directly on the 3D-printed constructs, showed over 90% viable cells for all conditions, with apparent cell proliferation with time of culture (up to 7 days). In general, RPMI medium was found to be an adequate crosslinker for the biopolymer blend, taking advantage of its capacity as buffer to compensate the initial acidic behavior of the biomaterial ink, as could be easily monitored by the presence of the pH indicator phenol red in the cell medium. In conclusion, this research paves the way for developing biocompatible alternatives to traditional biomaterials for tissue engineering applications. The biopolymer blend produced from collagen, chitosan, and fucoidan offers printability with high shape fidelity and reproducibility, making it an excellent alternative to 3D printing inks based in land mammal-origin polymers.

Acknowledgments
Portuguese Foundation of Science and Technology for Ph.D. fellowship (D. N. Carvalho), ref. PD/BD/143044/2018, and The German Research Foundation (S. Dani).

References
[1] Doi: 10.1016/j.bioadv.2022.212843
[2] Doi: 10.1016/j.msec.2020.110963

PoB.1.35

Cancer 3D bio-printed models and their further studies in microfluidic devices

Claire Godier[1], Zakaria Baka[1], Daniele Pedroni[1], Lina Bezdetnaya[1], Caroline Gaucher[2], Eric Gaffet[1], Halima Alem[1]

[1]Université de Lorraine-CNRS, Nancy, France. [2]Université de Lorraine-CNRS, nancy, France

Abstract

When injected in the body, nanoparticulate-based therapeutic systems face a fast surface covering by various proteins (creating the so-called protein corona) 1. Nowadays, many reports claim that the adsorption of proteins has to be prevented while in contrast, other works claim that adsorption of proteins could help targeting the nanomedicine and hence posit it to be favorable. This big discrepancy is mainly due to the fact that there is no development of real systems that would enable reliable ex-vivo studies in a more realistic way2 . In the frame of cancer therapeutic development it is now crucial to develop *in vitro* cancer models to enable the efficient development of new anti-cancer drugs. To provide new insight we are developing a bio-mimetic Microfluidic based system intended to host a 3D bio-printed cancer model3 .In this presentation, we will also describe the development of a more realistic system based on the 3D bio-printing3. An ovarian and a pancreatic tumor models containing cancer cells and cancer-associated fibroblasts (CAFs) encapsulated in a gelatin-alginate hydrogel were fully characterized 3. By combining different technologies we could demonstrate that the 3D-bioprinted cancer models show high viability and maintained cell metabolic activity up to 15 days after bioprinting. The both models are intended to be incorporated in the microfluidic system to develop a so-called cancer-on-chip device. This microfluidic systems are expected to pave the way for the understanding of the interaction of the NPs surfaces in biomimetic conditions.

1. Ke, P. C. ACS Nano 11, 11773–11776 (2017).

2. Venditto, V. J. & Adv. Drug Deliv. Rev. 65, 80–88 (2013).

3. Baka, Z. et al. . Macromol. Biosci. 2023, 2200434.

PoB.1.36

How to improve the sodium alginate base hydrogels composition and preparation to get high cells' viability after 3D direct bioprinting process and effective proliferation in printouts?

Dorota Bociaga, Adrianna Wierzbicka, Mateusz Bartniak

Institute of Materials Science and Engineering, Lodz University of Technology, Lodz, Poland

Abstract

Currently developing on a large scale, the opportunities for 3D printing represent more and more perspective solutions in tissue engineering and personalized medicine. Hydrogels are popularly used materials to produce bioinks designated for 3D printing. Today, solutions based on sodium alginate are frequently used compositions for this purpose as it is a biocompatible material with very good printability. Unfortunately, due to its high hydrophilicity and lack of interactive domains, sodium alginate does not create an environment conducive to the adhesion and proliferation of eukaryotic cells [1]. The incorporation of other biomaterials of natural origin, such as collagen or gelatin, into the structure of alginate effectively improves its bioactivity through the action of adhesive peptides and cell ligands [2]. Also the solvent has a significant impact not only on the mechanical properties and crosslinking time of the hydrogel, which directly affects the parameters of the 3D bioprinting processes, but also on the cells proliferation ability. The use of a culture medium dedicated to a given type of cell line as a solvent can support cell growth [3]. The high viability of the cells incorporated into bioink is the key parameter determining the application opportunities of printed structures. The parameters of the process used for the preparation of hydrogel compositions may have a direct impact on the viability of the cells incorporated within the printed structure. This study aims to develop a protocol for the preparation of hydrogel materials based on alginate and gelatine, providing the highest viability of the model cell line incorporated directly into the bioink before the 3D bioprinting process. In the scope of this study, the analyzed process parameters of the preparation of the hydrogel bioinks are the method of combination of a polymer solution with biological material, the applied concentration, the cross-linking solution, the type of solvents used for hydrogel components preparation, and also the waiting time of the prepared hydrogel bioink for the 3D printing process. A key aspect of the study is the evaluation of the influence of 3D printing on changes in the survival rate of biological material directly after the manufacturing process and after individual incubation periods of the printouts in conditions reflecting the body's environment.

[1] Neves M. I. et al., Front. Bioeng. Biotechnol. 8 (2020) 665. [2] Sawyer S. W. et al., Biomed Mater. 18 (2022) 015016. [3] Rosinska K. at al., J Biomed Mater Res. 111:314–330, 2023.

PoB.1.37

Side-emitting optical fibers by multimaterial 3D printing

Zahra Kafrashian[1,2], Stefan Brück[1], Peter Rogin[1], Usama Farrukh[1,2], Samuel Pearson[1], Aranzazu del Campo[1,2]

[1]INM – Leibniz Institute for New Materials, Campus D2 2, 66123, Saarbrücken, Germany. [2]Saarland University, Chemistry Department, 66123, Saarbrücken, Germany

Abstract

Hydrogel optical fibers are receiving strong interest for phototherapies inside the body because of their high compatibility with soft tissue. In some applications light needs to be delivered to large areas or multiple discrete zones which is best achieved using side-emitting fibers,[1] but fabricating hydrogel fibers capable of spatially controlled side emission is challenging. Inspired by a recent multimaterial printing approach,[2] we report here the extrusion printing of segmented hydrogel fibers with tunable optical properties and demonstrate their use as optical waveguides with controllable side-emission for photoactivation in phantom tissue.The printing of segmented optical fibers required the selection of hydrogel inks offering: i. shear-thinning behavior with a shear yield stress to prevent backflow in the static channel, ii. high transparency at optical wavelengths (waveguiding ink), iii. a scattering component for side-emission (scattering ink), and iv. photocrosslinkable groups for stabilizing extruded fibers. PluDA was chosen as an ideal candidate due to its ability to form a physical gel with yield stress at room temperature, its high transparency at visible wavelengths, and its photocrosslinkable functions. Polystyrene nanoparticles (FluoSpheres) were selected as scatterers to provide both scattering and fluorescence (for imaging) without impacting ink rheology. Optimization of the curing conditions and printing pressures during multimaterial printing gave access to mechanically stable segmented fibers with segment lengths down to the sub-millimeter scale controlled by switching time (Fig. 1A). A customized side-emitting fiber intended to outcouple a pre-defined proportion of light evenly across 10 segments distributed over 3 cm was then designed. Centimeter-scale outcoupling of green light is appropriate for some photodynamic therapy scenarios.[3] The design was successfully printed with segment lengths and spacings closely matching target values (Fig. 1B). Green light could be coupled into the proximal end of the fiber and selectively outcoupled from the scattering domains with intensities approximating the intended design (Fig. 1C). Finally, the printed fibers embedded in a fluorescent phantom tissue could generate co-localized fluorescence adjacent to the side emission domains (Fig. 1D), demonstrating their potential for large-scale biomedical photoactivation.

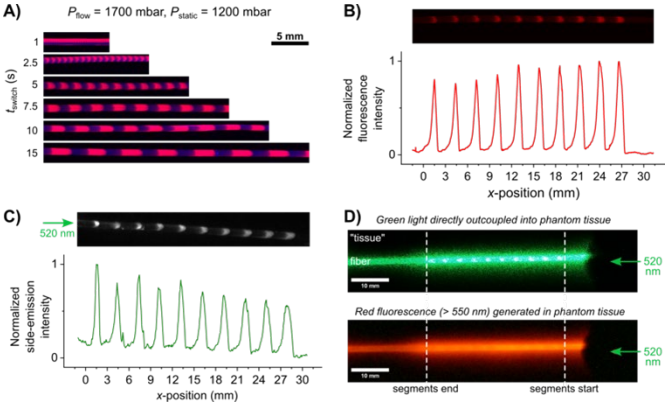


Figure 1. A) Fluorescence images of printed fibers with increasing switching times. B) Representative fluorescence image (n = 4) and segment distribution in 10-segment fiber. C) Representative image (n = 4) and intensity profile for side-emission of 520-nm light. D) Outcoupled green light (520 nm, top) and activated fluorescence (>550 nm, bottom) in a surrounding gel of fluorescent gelatin, imaged using a long-pass filter.

PoB.1.40

3D bioprinting of bone – combination of human bone marrow-derived stem cells, a novel hyaluronic acid-based bioink, and bioactive glass-derived ions

Roope Ohlsbom[1,2], Anni Mörö[1], Karoliina Hopia[1], Jonathan Massera[1], Susanna Miettinen[1,2]

[1]Tampere University, Tampere, Finland. [2]Tampere University Hospital, Tampere, Finland

Abstract

Bone defects are a global public health issue. The most challenging to treat are critical-sized bone defects. They are too large to be healed by bone's natural regeneration capacity. Critical-sized bone defects are treated with tissue substitutes, which aim to restore the function of the lost bone. Traditional tissue substitutes include bone grafts and implants. They have various drawbacks including limited bioactivity, risk of disease transmission and immune reaction, donor site morbidity, limited availability, and lack of degradability. 3D bioprinting provides a viable option for conventional tissue substitutes. In 3D bioprinting, a bioink comprising a hydrogel biomaterial and cells is deposited as a thin filament, layer-by-layer, in high spatial resolution; simultaneous bioprinting of different bioinks into a single structure is also possible. These features allow the formation of a custom-shaped tissue engineering (TE) construct, which contains different cell microenvironments in desired positions and orientations. Such TE construct is a promising starting point for recreating complex native tissue structures. In this study, we aim to bioprint a bone TE construct with the combination of human bone marrow-derived mesenchymal stem cells (hBMSCs) and a novel hyaluronic acid-based (HA) bioink, using Bioplotter Manufacturer series (EnvisionTEC) extrusion bioprinter. The bioink comprises hydrazone cross-linked and dopamine-modified HA and collagen I to achieve excellent printability, post-printing stability, and cytocompatibility. The bioink should also be a potential candidate for bone formation as HA and collagen I have been shown to support osteogenic differentiation. To further promote bone formation in the construct, we are using cell culture media containing bioactive glass (BAG)-derived ions. The addition of BAG-derived ions into the culture medium has been shown to promote osteogenic differentiation of mesenchymal stem cells. We have shown that hBMSCs printed in the HA bioink remain viable and form connections with each other during a 14-day osteogenic culture. In addition, we showed that adding BAG-derived ions to the medium increased the mineral formation in the printed constructs, indicating enhanced osteogenic differentiation of the hBMSCs. In summary, bioprinting hBMSCs in the novel HA bioink followed by maturation of the printed constructs in media containing BAG-derived ions might be a promising strategy for reconstructing bone tissue. However, further *in vitro* and *in vivo* analyses are still needed to confirm the osteogenic differentiation of the hBMSCs and the quality of the formed mineral tissue.

PoB.3.01

Ex vivo and *in vitro* wound healing models as development tool for medical device industry

Katharina Schuhladen[1,2], Anne Wibaux[2], Maike Kuhlmann[1]

[1]Medical and Clinical Affairs Department, Beiersdorf AG, Hamburg, Germany. [2]Research and Development Department, Beiersdorf AG, Hamburg, Germany

Abstract

Aim: Wound healing of acute wounds is a complex process of overlapping and coordinated phases, which can be disturbed among others by bacterial contamination. In the development of wound care products, it is crucial to understand and evaluate the factors that influence the wound healing process. In addition, due to MDR the need of clinical studies increased and therefore also the need to predict early the clinical outcome of new devices. To do so, advanced test systems such as ex-vivo and in-vitro models allowing the development of safe and efficient products are needed. Method: Therefore, the scope is to provide an overview of available *in vitro* and ex vivo wound healing models, which can be used for the development of new wound care devices. Suitable models were identified by a comprehensive literature search using the database Scopus and relevant keywords. Results / Discussion: The identified available *in vitro* and ex vivo wound healing models will be presented, and advantages and disadvantages of the different wound healing models will be compared. In addition, the presented models will be critically discussed in terms of their reliability and relevance for industrial companies/health authorities. Conclusion: This overview could serve as guideline for research institutes and companies developing new wound care products in order to find the most relevant wound healing model for their development processes. In addition, gaps of current existing wound models will be identified and possible directions for future improvement of wound healing models will be provided.

PoB.3.03

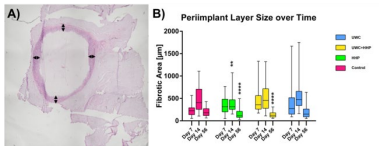
High Hydrostatic Pressure (HHP) decreases fibrotic area around xenogeneic cartilage implants in Lewis rats

Christopher Pohl[1], Nico Brandt[1], Charlotte Koppe[1], Jens van den Brandt[2], Sabine Berg[2], Daniel Behrend[1], Andreas Hoene[1]

[1]Department of General Surgery, Visceral, Thoracic and Vascular Surgery, University Medical Center Greifswald, Greifswald, Germany. [2]Central service and research facility for laboratory animals, Greifswald, Germany

Abstract

Tissue replacement by xenogeneic material is of increasing importance in clinical practice. To ensure optimal transplantation outcomes, it is of utmost importance to minimize adverse inflammatory effects in recipients by devitalization as well as decellularization. Fibrosis in the tissue-implant contact zone is an important parameter to evaluate immunogenicity. A thicker fibrosis layer leads to encapsulation of the implant and can result in decreased functionality. HHP is a gentle devitalization method for tissue. It utilizes high pressure levels to induce apoptosis in cells, while matrix integrity remains undiminished. This *in vivo* study aimed at investigating the immunogenic potential of HHP devitalized porcine cartilage implants regarding the formation of fibrosis in the peri-implant area. Following extraction, porcine cartilage samples were kept in PBS for a maximum of 24 hours and then exposed to either HHP, an ultrasound washing chamber (UWC), both HHP and UWC or were kept as an untreated control. 24 Lewis rats each received four implantations into the neck musculature via simultaneous implantation of the differently treated samples. On day 7, 14 and 56 implants were extracted. Morphometric analysis was performed on cryostat sections, stained with neufuchsin and hematoxylin (Figure 1 A). For morphometric analysis, fibrotic layer thickness was measured at four distinct points per sample and further processed with ImageJ and QuPath. Data was not normally distributed (Shapiro Wilk Test) and therefore analyzed by Kruskal-Wallis test, using GraphPad Prism. An initial significant increase of fibrotic area thickness on day 7 during the acute phase was observed in all treatments (Figure 1 B). This was seen in HHP as well as HHP+UWC samples (p=<0.0001) but was also true for the UWC group (p=0.0073). On day 14 HHP treated samples showed significantly decreased fibrosis thickness (p=0.0246) when compared to the control group. This effect could not be shown for other treatments. This decrease was most pronounced on day 56 during the chronic reaction phase. Here a highly significant decrease was observed both in the HHP group as well as in the HHP+UWC group (p≤0.0001). Overall HHP was able to diminish fibrotic area thickness, especially in chronical inflammation processes. However further analyses regarding locally involved inflammatory cells combined with serological parameters are needed to further evaluate this method immunologically.



Fibrosis of peri-implant area around xenogenic cartilage implants in rat neck musculature A) histological full image of cartilage. B) Fibrotic area thickness around differently treated cartilage implants over time.

PoB.3.04

How functionalization of implants with nisin improves their antibacterial properties?

Ziba Najmi[1], Mari Lallukka[2], Virginia Alessandra Gobbo[3], Francesca Gamna[2], Mirko Prato[4], Andrea Cochis[1], Lia Rimondini[1], Sara Ferraris[2], Silvia Spriano[2]

[1]Department of Health Sciences, Center for Translational Research on Autoimmune and Allergic Diseases—CAAD, Novara, Italy. [2]Department of Applied Science and Technology, Politecnico di Torino, Turin, Italy. [3]Faculty of Medicine and Health Technology, Laboratory of Biomaterials and Tissue Engineering, Tampere University, Tamper, Finland. [4]Materials Characterization Facility, Italian Institute of Technology, Genova, Italy

Abstract

Titanium and its alloys are suitable biomaterials for orthopedic applications due to their high biocompatibility, low toxicity and high fatigue strength. One of the main drawbacks of using biomaterials for orthopedic applications is to improve osteointegration and simultaneously prevent bacterial infection. On the other hand, the increasing number of antibiotic resistance has attracted many interests in antibiotic-free alternatives. Nisin is a promising and FDA-approved antimicrobial peptide that has been implemented as a food preservative. Recently, the potential of nisin in biomedical applications for its antimicrobial effects has gained interest. This work aimed to optimize nisin adsorption on titanium alloy surfaces with extra-low interstitials (Ti6Al4V-ELI) and investigate their antibacterial activity towards *Staphylococcus aureus*. Before functionalization of the surfaces, Ti6Al4V-ELI discs were mechanically polished (MP) and chemically treated (CT) for inducing bioactive properties. The samples were then UV-C irradiated to activate the surfaces and improve nisin adsorption from three different pH solutions (pH 5, 6 and 7). The effectiveness of different pH values on nisin adsorption were determined by means of EDS and XPS. Antibacterial properties of functionalized specimens were assessed towards Gram-positive *S. aureus* by following International Standard ISO 22196 protocol. After 24 hours incubation at 37 °C, bacterial metabolic activity and surface-attached colonies count were evaluated; then the results were visually confirmed by SEM. The EDS analysis of the bare and nisin-functionalized specimens revealed a noticeable increase in nitrogen and carbon percentages on the functionalized samples in comparison to bare ones at pH 6, indicating high effectiveness of nisin adsorption at this pH. The nisin-functionalized samples had a high surface wettability suitable for osteointegration and released slowly nisin till 7 days, suggesting a prolonged antibacterial activity (Fig.1). The results of metabolic activity and viable colonies count showed the beneficial impact of nisin on bacterial biofilm formation; these obtained results were confirmed visually with SEM and 3D reconstructed images of microcolonies on the samples' surfaces (Fig.2).

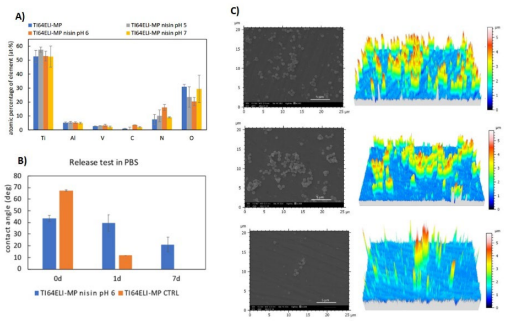


Figure 1: A)EDS analysis of MP samples; B)Measurement of contact angle; C)3D reconstructed images of SEM with the order from top: MP, MP nisin pH3 and pH6.

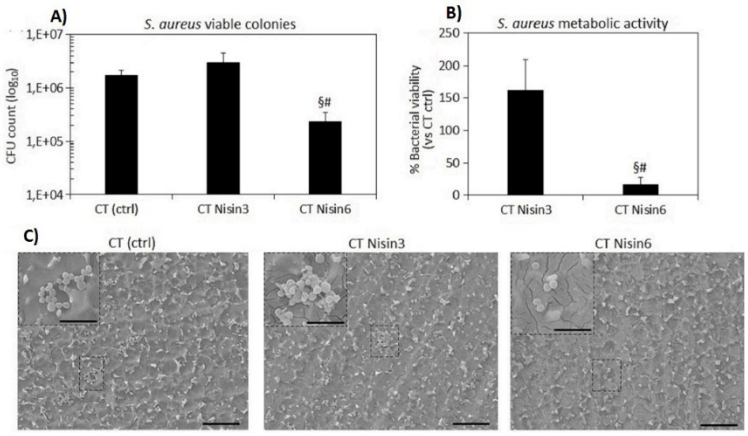


Figure 2: A)CFU count; B)Bacterial viability; C)SEM images (scale bars: 10,5µm).

As a conclusion, titanium functionalization with nisin at pH 6 revealed the exposure of hydrophobic and antibacterial functional groups toward the external environment that induced antibacterial propDerties to the samples.

PoB.3.05

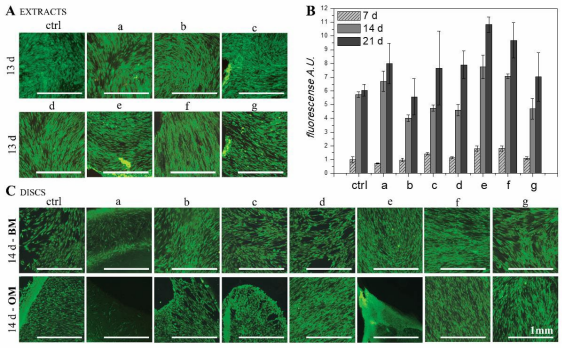
B12.5 borosilicate bioactive glasses; *in vitro* bioactivity and cell/material interactions

Jenna M. Tainio, Susanna Miettinen, Jonathan Massera

Tampere University, Tampere, Finland

Abstract

Bioactive glasses (BGs) are promising for regenerative medicine.[1] Borosilicate BGs are especially interesting, as they have been found suitable for both hard and soft tissue engineering applications.[2] Additions of therapeutic ions into the glass compositions can further enhance both glass properties and healthy tissue regeneration.[2,3] However, some boron containing glasses have been shown to inhibit cells proliferation.[4] The composition of the investigated borosilicate glasses is based on the well-known S53P4[5] (where 12.5% of the SiO_2 is replaced with B_2O_3), with molar composition $47.1SiO_2$-$6.7B_2O_3$-$22.7Na_2O$–(21.8-x-y)CaO-$1.7P_2O_5$–xMgO–ySrO, where x,y varied from 0 to 10 mol-%. The objective was to study glasses *in vitro* bioactivity and dissolution in simulated body fluid (SBF), and assess the materials/cells interactions using human adipose derived stem cells (hADSC). Both SBF study, and cell experiments were performed in static conditions. hADSCs were cultured *i*) in the presence of glass dissolution products (extracts), and *ii*) in direct contact. Viability, proliferation and differentiation of hADSCs were assessed. While Mg and/or Sr substitution resulted in a lower ion release in SBF and delayed formation of hydroxyapatite (HA), post dissolution structural analysis indicated that the typical BG dissolution mechanism was maintained, and reactive HA layer was formed on all B12.5 glasses.[6] B12.5-series and their dissolution products were found to be well tolerated by the hADSCs (Figure 1.) Especially the Mg/Sr substitutions for Ca promoted the cells osteogenic commitment. Along with an upregulation of the osteogenic markers, upregulation of angiogenic markers were also evidenced. B12.5 glasses were found to possess highly promising bioactive properties. ***Figure 1.*** *(**A**) Live-dead viability assay on dissolution products (extracts) hADSC cultures on culturing day 13. (**B**) Proliferation of extract cultures by CyQuant assay, up to 21 days of culturing. (**C**) Viability on top of discs; both basic culturing media (BM) and osteogenic (OM) on culturing day 14. Bioactive glass samples marked as (**a**) S53P4, (**b**) B12.5, (**c**) B12,5-Mg5, (**d**) B12.5-Mg10, (**e**) B12.5-Sr5, (**f**) B12.5-Sr10, (**g**) B12.5-Mg5-Sr10. Scale bar 1 mm.*



REFERENCES

1. Hench L.L., J. Mater. Sci. Mater. Med., 17, (2006), 967–978.
2. Ege, D. et al., ACS Appl.Bio Mater., 5(8), (2022), 3608−3622.
3. Hoppe, A. et al., Biomaterials, 32(11), (2011), 2757.
4. Ojansivu et al., PLoS ONE, 13(8), (2018), e0202740.
5. Jones J.R. et al., Int. J. Appl. Glass Sci., 7(4), (2016), 423–443.
6. Tainio, J.M. et al., J. Non-Cryst. Solids, 533, (2020), 119893.

PoB.3.06

Cytotoxicity and biocorrosion behavior of Mg-Zn-Ca-Mn-Y alloy containing long-period stacking ordered phase under physiological conditions

Diana C. Martinez[1], Alessia Paradiso[1], Anna Dobkowska[1], Emilia Choinska[1], Jiri Kubasek[2], Wojciech Święszkowski[1]

[1]Warsaw University of Technology, Faculty of Materials Science and Engineering, Warsaw, Poland. [2]University of Ljubljana, Institute of Metals and Technology, Ljubljana, Slovenia

Abstract

Magnesium (Mg) and its alloys are becoming alternatives for their use as temporary orthopedic implant materials. Mg–Zn–Y alloys containing the long-period stacking ordered (LPSO) phases (periodic arrangements of closed-packed atomic layers enriched with transition metals and rare-earth elements in the Mg lattice), play an important role in the outstanding corrosion resistance and mechanical properties of Mg alloys. The addition of Mn and Ca refines grain size and enhances corrosion resistance. Even though the microstructure of Mg-LPSO alloys has been studied, little is known about their biocompatibility and resistance to corrosion under physiological conditions, which are important for biomedical applications. Herein, the aim of the study was to investigate the cytotoxicity and biocorrosion behavior of the Mg-Zn-Ca-Mn-Y alloy *in vitro*. To determine the corrosion behavior of the alloy, a 72h immersion test was conducted with different electrolytes: phosphate-buffered saline (PBS), alpha minimum essential medium (α–MEM), and Dulbecco´s modified eagle medium (DMEM). The pH was monitored, the Mg and Y concentrations were measured using ICP-MS, and the corrosion rate was determined by the gravimetric method. The corroded surfaces were examined using electron microscopy, energy-dispersive X-ray spectroscopy (SEM/EDX) and Fourier transform infrared (FTIR). The biocompatibility of the Mg-Zn-Ca-Mn-Y alloy was assessed with murine fibroblast cells (L929) using an indirect cytotoxicity test in different diluted extracts for 1, 3, and 5 days, and a 24h direct cell attachment assay.

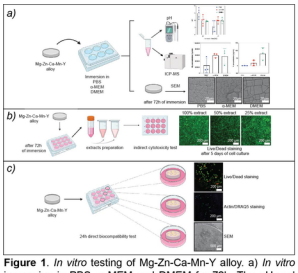


Figure 1. *In vitro* testing of Mg-Zn-Ca-Mn-Y alloy. a) *In vitro* immersion in PBS, α-MEM and DMEM for 72h. The pH and ICP-MS of Mg and Y concentration were measured and the SEM surface morphology observed after 72h of immersion in the three different solutions; b) Mg extracts preparation for indirect cytotoxicity test with L929 showing Live/Dead micrographs after 5 days of cell culture; c) 24 h direct contact test of L929 cells with the Mg-LPSO alloy with Live/Dead and actin/DRAQ5 staining images, and SEM morphology micrograph.

The results revealed the corrosion layers with a cracked dry mud-pattern formed on the Mg-Zn-Ca-Mn-Y alloy after 72 h of immersion. The pH was within the physiological range in both α–MEM and DMEM and lower in PBS. In the three immersion solutions, the corrosion rates varied between 0.92 and 1.32 mm/year. As determined by FTIR, hydroxyl, carbonate, and phosphate groups exhibited absorption bands, with phosphate peaks in α–MEM and DMEM being more intense. After 24h, the cytocompatibility of both the 100% and 50% extracts was slightly diminished compared to the 25% extract, whereas there were no differences after 5 days. The cell viability and adhesion were observed following direct cell seeding after 24h (Figure 1). Therefore, the Mg-LPSO alloy exhibited more than 90% cell viability and a corrosion rate with broad variability. Further immersion periods and mechanical testing are required to better understand its degradation in physiological environments.

This research has been funded from the National Centre for Research and Development in Poland under the project (V4-JAPAN/2/15MagMAX).

PoB.3.07

3D printing of medical grade polycaprolactone scaffolds with elevated porosity for bone tissue engineering

Theo Luciani, Mehmet Serhat Aydin, Samih Mohamed-Ahmed, Kamal Mustafa, Ahmad Rashad

University of Bergen, Bergen, Norway

Abstract

Polycaprolactone (PCL), a biocompatible thermoplastic, offers wide possibilities for the biofabrication of load-bearing organs such as bones. In addition to its high mechanical properties, its low melting point is appropriate to engineer defect/patient-specific bone scaffolds using 3D printing technologies. However, 3D printed scaffolds composed of this synthetic polymer alone do not trigger bone healing, and require to be blended with inorganic compounds, and functionalized with bioactive materials. For non-osteoconductive materials such as PCL, modifications of scaffolds porosity and its effect on their surface properties on cell behaviour have started to drag attention recently. An effective method to introduce porosity within a material is by blending the initial material with salts which are leached out afterwards, leaving a porosity in the material defined by the initial salt particle size. In this study, we mixed different concentrations (0% / 47% / 93% w/w) of sodium chloride particles (40-90 µm) in a solution of medical grade PCL in acetone. After complete evaporation of acetone, the material was 3D printed at 150°C with a nozzle diameter of 400 µm, using the 3D Bioplotter from EnvisionTEC. 3D printed scaffolds were washed in water to remove the salt and create the porosity, which was characterized by stereo microscopy, scanning electron microscopy (SEM) and micro computed tomography (micro-CT). Finally, the effect of the porosity on human bone marrow-derived mesenchymal stromal cells (hBMSCs) were investigated in terms of cell viability, attachment and proliferation up to 14 days. Regardless of the NaCl concentration, stereo-microscopy images showed that all scaffolds possessed good printability factor and shape fidelity. In addition, SEM images confirmed that leached NaCl particles altered filaments' surface morphology, and led to evenly distributed porosity within the printed filaments. Micro-CT quantitative analysis of the porosity within scaffolds' filaments showed that leached NaCl particles created a porosity ranging from 10 µm to 100 µm, which increased with the initial NaCl ratio. Finally, hBMSCs seeded on scaffolds demonstrated good cell adherence, spreading and viability, with proliferation up to day 14. In conclusion, the conventional salt leaching method was successfully combined with 3D printing technology to create medical grade PCL scaffolds that support hBMSCs viability, spreading and proliferation. Engineering such desired porosity within medical grade PCL scaffolds hold promises and open possibilities to improve cell-scaffold interactions for bone regeneration applications.

PoB.3.08

Effect of poloxamer 407 on cytocompatibility of calcium phosphate cement for bone regeneration

Yeeun Kim, Kenichi Hamada, Kazumitsu Sekine

Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan

Abstract

Calcium phosphate cement (CPC) is generally used for bone repair and augmentation. Poloxamers are tri-block copolymers used as surfactants typically applied in drug and antibiotic delivery. However, their biological effects on bone regeneration systems remain unelucidated. Here, we aimed to understand how supplementing the prototype CPC with poloxamer would impact cytocompatibility and function as a bone-grafting material. A novel CPC, modified beta-tricalcium phosphate (mβ-TCP) powder, was developed through a planetary ball-milling process using a beta-tricalcium phosphate (β-TCP). The mβ-TCP dissolved rapidly and accelerated hydroxyapatite precipitation, successfully shortening the cement setting time and enhancing the material strength. Furthermore, adding poloxamer 407 to mβ-TCP could reduce the risk of leakage from bone defects, improving fracture toughness while maintaining mechanical properties. In this study, the poloxamer addition effects (0.05 and 0.1 g/ml) on cytocompatibility of MC3T3-E1 cells cultured *in vitro* were investigated. The cell viability of mβ-TCP containing poloxamer 407 was similar to that of mβ-TCP. This material showed great cell attachment and healthy polygonal extension of the cytoplasm firmly attached to hydroxyapatite (HA) crystals. The addition of poloxamer to mβ-TCP resulted in an increased capacity to support cell attachment, proliferation, and differentiation compared to mβ-TCP. These data demonstrated that the addition of poloxamer 407 to mβ-TCP might be considered a potential therapeutic application for the repair and regeneration of bone defects.

PoB.3.09

Cytocompatibility and osteogenic differentiation of mesenchymal stem cells within phototunable polyester-based PEGylated dendritic hydrogels

Francesco Torelli[1], Samih Mohamed-Ahmed[1], Ahmad Rashad[1], Noemi M Cabeza[2], Daniel J Hutchinson[2], Michael Malkoch[2], Kamal Mustafa[1]

[1]University of Bergen, Bergen, Norway. [2]KTH Royal Institute of Technology, Stockholm, Sweden

Abstract

The synergic contribution of signalling events, in proper combination with discrete cell populations and their microenvironment during tissue development, is responsible for patterning and various morphogenetic phenomena. As a matter of consense, regenerative medicine and tissue engineering efforts should thus aim to replicate these events in standardized conditions and in a predictable fashion1. Thus, according to current paradigms, bone regeneration, when defined as re-establishment of anatomical and physiological integrity, finds its potential in the combination of those models, tools and technologies that might help targeting and reproducing developmental biology1. Hence, the potential for mimicking extracellular matrices of native tissues is gaining significant attention and novel biomaterials, including hydrogels, are deemed necessary for investigation either in terms of biocompatibility and biofunctionalization and tissue biomimicry, cell adhesion, proliferation and differentation2. Here we present findings and results from a series of *in vitro* biological characterization assays targeting the cytocompatibility and overall biological viability of a library of phototunable polyester-based PEG-ylated hydrogels. These hydrogels are designed to be translated as cell-delivery systems for bone regenerative applications via high-energy-visible light induced thiol-ene coupling (HEV-TEC) crosslinking3. 3D microenvironments have been reproduced *in vitro* to mimic native progenitor cell niches via human Bone Marrow Stem Cells (hBMSCs) encapsulated in cylindrical models4,5. These have been molded starting from various combinations of dendritic-linear-dendritic polymers decorated with peripheral alkene functionalities with LAP (Lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate) as a photoinitiator3. Gelatin methacryloyl (GelMA) was used as a control4,5. Cell viability imaging via confocal and inverted microscopy, metabolic activity evaluation, proliferation and osteogenic differentiation assays via photometric microplate readings have been performed to identify the best suitable combination of prepolymer solution, crosslinker and photoinitiator, providing new insights that might encourage further translational investigations4,5. The so composed polymeric cell-delivery systems have therefore been ascertained as potentially promising biomimetic solutions for bone tissue engineering, *in vitro* modeling and translational applications.

PoB.3.10

Ascorbic acid 2-phosphate embedded poly(L-lactide-co-ε-caprolactone) membranes for treating pelvic organ prolapse: an *in vitro* study

Alma Kurki, Reetta Sartoneva, Kaarlo Paakinaho, Susanna Miettinen

Tampere University, Tampere, Finland

Abstract

Even up to 40% of women worldwide suffer from pelvic organ prolapse (POP), in which pelvic organs uterus, bladder or rectum bulge to or through vagina. POP highly impacts patient's quality of life and even 13% of women end up having a surgery due to POP. Surgical repair of POP is primarily done using patients own tissue, yet recurrent prolapse often requires the use of non-absorbable mesh implants. However, those operations are highly prone for complications as infection, chronic pain and mesh erosion and therefore developing new techniques is crucial. In this study, we studied absorbable ascorbic acid 2-phosphate embedded poly(L-lactide-co-ε-caprolactone) membranes ($PLCL_{A2P}$) as an alternative for treating POP. Ascorbic acid and its derivatives, such as A2P, enhance collagen production and proliferation of stromal cells. Our aim was to assess the effect of A2P embedded $PLCL_{A2P}$ on human vaginal fibroblast (hVF) and human adipose-derived stromal cell (hASC) viability, proliferation, and collagen production. Monocultures of hVF and hASC were cultured on $PLCL_{A2P}$ and PLCL membranes and holed membranes. Effect of A2P on cell growth was evaluated by assessing cell viability and proliferation with Live/dead and CyQuant assays. Collagen production was studied with immunofluorescent staining and Sircol total collagen assay. In addition, expression of elastin, αSMA, collagen type I and III was evaluated with qRT-PCR. Both cell types remained viable on $PLCL_{A2P}$ and holed $PLCL_{A2P}$ membranes. Proliferation of hASCs was significantly increased on $PLCL_{A2P}$ membranes, whereas hVFs proliferated similarly on $PLCL_{A2P}$ and holed PLCL membranes. Collagen production of both hVFs and hASCs was increased on $PLCL_{A2P}$ membranes. Moreover, hVFs showed possible signs of enhanced collagen maturation since collagen was secreted to the extracellular space on A2P containing materials (figure 1). Further, expression of all tested stromal markers was increased in hASCs on $PLCL_{A2P}$ membranes. Our results demonstrate that A2P embedded $PLCL_{A2P}$ and holed $PLCL_{A2P}$ membranes support hVF and hASC growth and increase the collagen production of both cell types. The $PLCL_{A2P}$ membranes show promising results for enhancing the support of the pelvic floor stromal tissue. In future research, the significance of our findings needs to be further verified in *in vivo* research.

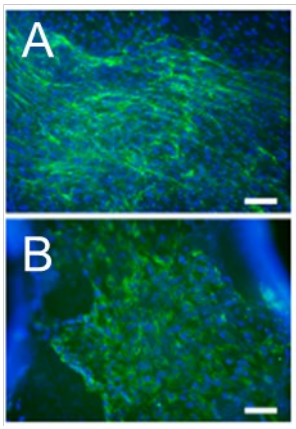


Figure 1 In the culture of vaginal fibroblasts, fibers of extracellular collagen type I were visible on A2P embedded $PLCL_{A2P}$ membrane (A), whereas on plain PLCL membrane collagen I appears to be intracellular (B).

PoB.3.11

Evaluating the *in vitro* degradation of medical devices with a physiologically relevant enzyme mixture

Cristina Oldani[1,2], Nina Bono[1], Alice Caldiroli[2], Stefania Adele Riboldi[2], Gabriele Candiani[1]

[1]Politecnico di Milano, Milan, Italy. [2]Dialybrid S.r.l., Cantù, Italy

Abstract

Introduction: When dealing with the characterization and validation of a novel biodegradable medical device, the assessment of its degradative profile represents a crucial step for its clinical translation [1]. *In vitro*, enzymatic degradation assays are used for this purpose, yet they generally do not mimic physiological conditions with high fidelity in terms of enzymes' source and concentrations [2]. In this context, we evaluated the enzymatic degradation kinetics of an enzyme mixture designed *ad hoc* to degrade a medical device in human-physiological conditions. **Methods**: Two fluorogenic substrates (MMP and Lysozyme assay kit, Abcam, UK) were used to monitor the activity of an enzymatic cocktail (composition reported in **Fig.1A**) prepared in sterile phosphate-buffered saline. Metalloproteinases (MMPs) and chymotrypsin concentrations were varied from the physiological value to give an equal contribution to the overall activity of the enzyme pool, while lysozyme activity was evaluated on its own. To determine the effect of storage temperature and incubation time on enzymes' activity, the enzyme cocktail was i) incubated for 3 days at 4 °C, 25 °C and 37 °C and ii) incubated at 37 °C under shaking (300 rpm) and assayed after 0, 4, 24, 48, 96, and 168 hrs. The activity of the untreated cocktail was considered the positive control of activity (100 %), while the denatured enzymatic mixture was used as the negative control (0 %). Fluorescence measurements were performed with both substrates. **Results**: Storage temperature affected MMPs and chymotrypsin activities but was less significant for lysozyme (**Fig.1B**). The enzyme pool activity decreased over time but was detectable up to 1 week even in the worst-case scenario in terms of enzymes auto- and cross-lysis considered (**Figs.1C-D**). This result suggested that replacing the enzyme pool once per week would ensure the continuous degradation of the tested device. **Conclusion**: We developed a versatile methodology to easily monitor the general activity decay over time of a mixture of enzymes designed for the *in vitro* physiological degradation of Silkothane® Arteriovenous Graft fabricated by Dialybrid [3]. Of note, although the enzyme cocktail used in this study was designed to meet specific requirements related to the device of interest, the method herein described can be conveniently adapted to another set of enzymes and used to validate the constant degradation of devices *in vitro*.

References: [1] ISO Standard 10993-13, 2010. [2] C. Li *et al.*, *Nat. Rev. Mater.*, 2020. [3] S. van Uden *et al.*, *Biomed. Mater. Bristol Engl.*, 2019.

A

Enzyme	Substrate	Physiological concentration	Concentration in same level activity mixture
MMP-1	MMPs	7 ng/mL	1 µg/mL
MMP-3	MMPs	200 ng/mL	2 µg/mL
MMP-9	MMPs	400 ng/mL	400 ng/mL
Chymotrypsin	MMPs	3 ng/mL	50 ng/mL
Lysozyme	Lysozyme	20 µg/mL	20 µg/mL

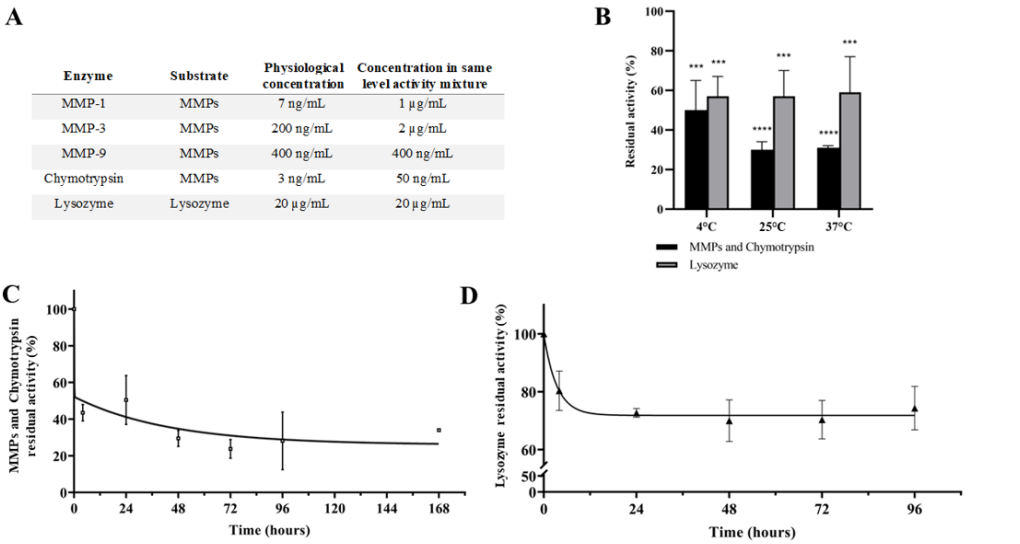


Figure 1. ***A*** *Table representing enzymes with fluorogenic substrate used and respective physiological and working concentrations.* ***B*** *Residual activity of MMPs, Chymotrypsin, and Lysozyme after 72 hrs after being stored at 4, 25, and 37 °C. ***p< 0.001, ****p < 0.0001 between every condition and positive control (100 % activity) (two-way ANOVA).* ***C*** *Residual activity over time of the MMPs and Chymotrypsin in the enzymatic mixture (a two-phase decay curve was fitted with GraphPad Prism to the data obtained). After an abrupt decrease during the first hours of incubation, the enzyme's activity slowly decreases until 30 % of residual activity.* ***D*** *Lysozyme's residual activity over time in the enzymatic mixture. After a sudden decrease during the first hours of incubation, the residual activity followed a one-phase decay and plateaued at 70 %.*

PoB.3.13

Suitability of additive manufacturing for biodegradable urinary devices: an *in vitro* study

Margarida Pacheco[1,2], Sviatlana Lamaka[3], Mikhail Zheludkevich[3], Jan Bohlen[4], Maria Nienaber[4], Dietmar Letzig[4], Carl Johan Hassila[5], Francesco D'Elia[5], Estêvão Lima[6], Cecilia Persson[5], Alexandre Barros[1,2], Rui Reis[1,2]

[1]3B's Research Group—Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal. [2]ICVS/3B's-PT Government Associate Laboratory, Braga, Portugal. [3]Institute of Surface Science, Helmholtz-Zentrum hereon GmbH, Geesthacht, Germany. [4]Institute of Material and Process Design, Helmholtz-Zentrum hereon GmbH, Geesthacht, Germany. [5]Division of Biomedical Engineering, Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden. [6]School of Health Sciences, Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal

Abstract

A biodegradable metallic ureteral stent (BMUS) would be a new and innovative concept in urology since it is expected to have a slower degradation than the current biodegradable polymeric ones. Therefore, a BMUS will offer a new alternative for longer-time treatments, widening the utilization of biodegradable ureteral stents. This work explores the suitability of additive-manufactured Mg1Y for BMUS development. The corrosion layer and corrosion of Mg1Y constructs are evaluated in urinary tract conditions, and their influence on cell viability was accessed. The process parameters optimization for thin structures using modulated laser was done using Mg1Y powder through additive manufacturing (AM) using laser powder bed fusion. The best process parameters were 50W laser power, 0.01 MHz laser frequency, 75 µs laser modulation width, and 50 mm/s mark speed. Several tubular-shaped printing methods were tried, and the most successful ones for ureteral stents applications were selected for further studies (Figure 1). These structures were studied under simulated urinary tract environment, *in vitro*, in dynamic conditions with a constant flow of fresh artificial urine solution (AUS) - ASTM F 1828-97 (2006). The corrosion layer formed and corrosion of the constructs were accessed and the biocompatibility of the developed structures with L929 and GG cell lines was evaluated. The results showed that the meshed tube could be a promising approach for ureteral stents design since it liberates very small fragments that should not be problematic. In fact, obtaining homogeneous degradation is a primary challenge when developing biodegradable urological devices, and herein promising results in this aspect were obtained. The corrosion layer formed on the structures seems thin and homogeneous for the first week of incubation, compared with the previous results with extruded metals, in the same conditions. Further studies are being done. This is also expected to be an exciting finding since a low accumulation of corrosion products on the metal surface can indicate a low propensity for encrustation, one severe problem associated with ureteral stents. Furthermore, AM-developed Mg1Y did not show toxicity to the cells with a maximum concentration of 13.75 mm2/mL. Further tests, such as corrosion layer composition, corrosion rate, and AUS composition along the experiment, are currently being carried out. The results indicate that Mg1Y could be a possible base material for BMUS, and AM seems to be a new and promising approach for the exploration of suitable BMUS designs.

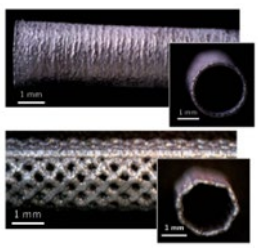


Figure 1 *Tubes produced by additive manufacturing using Mg1Y powder*

PoB.3.14

Comparing the effects of amorphous calcium phosphate crystallization on human osteoclast resorptive activity *in vitro*

Jana Vecstaudza[1,2], Christoph Martin Sprecher[3], Matteo D'Este[3], Janis Locs[1,2], Elena Della Bella[3]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia. [3]AO Research Institute Davos, Davos, Switzerland

Abstract

Amorphous calcium phosphate (ACP) is an under-investigated biomineral that is metastable and undergoes crystallization in wet conditions. The crystallization media may influence the outcome of ACP's crystallization and subsequently cell responses. Here we hypothesized that ACP crystallized in cell culture media (ACP-CCM) or water (ACP- H_2O) might have a different effect on osteoclast behavior *in vitro*. ACP was synthesized through wet precipitation. The obtained ACP was divided into two parts and freeze-dried right after the synthesis or after partial crystallization in water (Fig.1). For the *in vitro* experiments the freeze-dried powders were compacted into discs and conditioned in cell culture media. Mononuclear cells were isolated from the bone marrow of two human donors with full informed consent and ethical approval. The mononuclear cells were pre-incubated in M-CSF supplemented media for 7d. The obtained osteoclast precursors were seeded on discs made from ACP-H_2O or ACP-CCM (Fig.1). RNA was isolated to analyse the expression of osteoclast markers using quantitative polymerase chain reaction (qPCR). The cellular discs were observed with epifluorescence microscope (EFM) and scanning electron microscope (SEM). Acellular discs, incubated alongside cell-seeded ones, were analysed with Fourier transform infrared spectrometry (FT-IR), x-ray diffraction (XRD) and SEM to count the resorption pits.

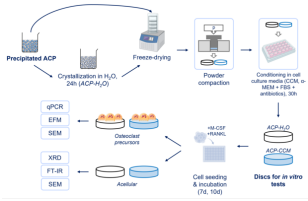


Fig.1. Experimental overview of the study.

XRD and FT-IR confirmed the crystallization of ACP into partially crystalline calcium phosphates both for ACP-CCM and ACP-H_2O. Based on SEM images taken at every timepoint, the number of resorption pits was higher for ACP-CCM than for ACP-H_2O (Fig.2). The expression of different osteoclast markers was analysed. At 10d *ACP5*, *MMP9* and *CA2* were upregulated for both ACP groups, with no difference between ACP-CCM and ACP-H_2O. *CTSK* remained at the level of positive control for both ACP-CCM and ACP-H_2O.

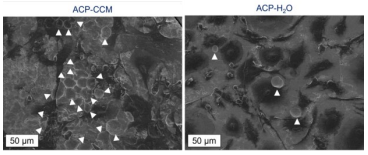


Fig.2. Surfaces of calcium phosphate discs resorbed by osteoclasts, where arrows indicate resorption pits.

Overall, both cell culture media- or water-crystallized ACP discs allowed osteoclast differentiation. Surfaces of differently crystallized ACP discs differed in the number of resorption pits, however, there was no significant difference in the expression of osteoclast markers. As ACP is considered the first mineral phase preceding bone mineralization, which later crystallizes into biological apatite, *in vitro* studies on bone cells and ACP and its crystallized products are important for future biomaterials development.

PoB.3.15

Development of a novel multicellular *in vitro* model of intestinal inflammation

Maria Helena Macedo[1], Adriana Pereira[1,2], Lorenzo Pastrana[1], Catarina Gonçalves[1]

[1]INL - International Iberian Nanotechnology Laboratory, Braga, Portugal. [2]UM - Universidade do Minho, Braga, Portugal

Abstract

Inflammatory bowel disease (IBD) is a chronic condition that affects approximately 7 million people, causing life-impairing symptoms. Poor drug delivery can lead to reduced efficacy and side effects from repeated administration. Biomaterial-based drug delivery platforms, such as nanoparticles, microparticles, and hydrogels, hold promise for improving drug efficacy and reducing adverse effects. *In vitro* models of intestinal inflammation are promising tools for studying IBD pathogenesis and treatment, and for screening drugs at early developmental stages. In this work, we developed a new *in vitro* model of human intestinal inflammation to evaluate the anti-inflammatory properties of bioactives in food/pharmaceutical applications. The model comprises four different cell lines representing enterocytes, goblet cells, M cells, and macrophages, and to our knowledge it is the first model of its kind to include M cells in the epithelium and immune cells on the basolateral side. M cells have a key role in intestinal inflammation due to their antigen-presenting capacity, inhibiting or triggering immune responses. Preliminary assays in a simple Caco-2 model showed a reduction in transepithelial electrical resistance (TEER) after stimulating the cells with 50 ng/mL of TNF-α for 48 hours on the basolateral side. Surprisingly, the co-culture with HT29-MTX cells further increased inflammation, resulting in a sharper TEER decrease, an increase in Lucifer Yellow (marker of paracellular transport) permeability, and an increase in the release of the pro-inflammatory cytokine IL-8. Successful differentiation of Caco-2 cells into M cells was confirmed by a decrease in TEER, since M cells present less tight junctions, and the absence of microvilli, a characteristic of M cells. Interestingly, in terms of inflammation, M cells appeared to have a protective role, with no effect in TEER or permeability and no IL-8 production after the inflammatory stimulus. Conversely, the addition of THP-1 cells resulted in a 100-fold increase in IL-8 production, a more pronounced decrease in TEER, and increased permeability. A Multiplex Immunoassay was employed to identify, among 20 common cytokines involved in inflammation, the ones that are most produced during healthy and inflamed states. Finally, the commercial anti-inflammatory compound Budesonide was used to validate the model's ability to produce an anti-inflammatory response. Overall, this model comprising different cells with key roles in inflammation is a valuable tool for testing biomaterials with anti-inflammatory properties, ultimately contributing to bridge the *in vitro - in vivo* gap.

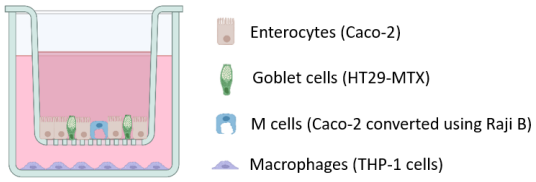


Figure 1 – Schematic representation of the in vitro model comprising enterocytes, goblet cells, M cells and macrophages.

PoB.3.16

Evaluating the immune response and cytotoxicity of degradation products from crosslinked silk fibroin scaffolds.

Jiranuwat Sapudom[1], Jeremy Teo[1], Peerapat Thongnuek[2]

[1]New York University Abu Dhabi, Abu Dhabi, UAE. [2]Chulalongkorn University, Bangkok, Thailand

Abstract

Silk fibroin (SF) scaffolds are widely used in tissue engineering and implantation. However, the long-term impact of their degradation products on the host response remains poorly understood. This study investigated the effect of two different crosslinkers, EDC and GA, on the topology, mechanical stability, and enzymatic degradation of SF scaffolds. Results showed that GA-treated SF scaffolds had a thicker sheet and higher elastic modulus than EDC-treated ones at a similar degree of crosslinking. The degradation of SF scaffolds was found to be dependent on the crosslinking mechanism. SF scaffolds treated with an intrafibrillar crosslinker (EDC) had higher resistance to enzymatic degradation than those treated with an interfibrillar crosslinker (GA). Enzyme-specific degradation was also observed, with high degradation observed in proteinase K but not in collagen type IV or trypsin. Predicted cleavage sites using computational tools were well-correlated with the experimental results. The cytotoxicity and genotoxicity of the degradation products of both uncrosslinked and crosslinked SF scaffolds were evaluated using primary human cells, including fibroblasts, mesenchymal stem cells, and adipocyte-derived stem cells. The degradation products were found to have minimal cellular toxicity and genotoxicity, indicating biocompatibility. However, they appeared to be bioactive in modulating the immune response. Our results showed that the degradation products of GA-SF scaffolds promoted pro-inflammatory phenotypes and activated pro-inflammatory macrophages, while those from EDC-SF scaffolds enhanced polarization towards anti-inflammatory macrophages. In contrast, the degradation products of uncrosslinked SF did not actively regulate macrophage activation. In sum, our study highlights the importance of understanding the degradation products of crosslinked SF scaffolds in modulating the immune response. This information could be utilized to develop strategies for controlling the long-term immune response during implantation, ultimately improving the success of SF scaffold transplantation.

PoB.3.17

Response of non-cytotoxic concentrations of various metal ions on adipose-derived mesenchymal stem/stromal cells

Olga Hahn[1], Katharina Ekat[1], Okikiola Tawakalitu Waheed[1], Brigitte Müller-Hilke[2], Anika Jonitz-Heincke[3], Rainer Bader[3], Tomas Fiedler[4], Thomas Eickner[5], Kirsten Peters[1]

[1]Department of Cell Biology, Rostock University Medical Center, Rostock, Germany. [2]Institue for Immunology, Rostock University Medical Center, Rostock, Germany. [3]Department of Orthopaedics, Biomechanics and Implant Technology Research Laboratory, Rostock University Medical Center, Rostock, Germany. [4]Institue for Medical Microbiology, Virology and Hygiene, Rostock University Medical Center, Rostock, Germany. [5]Institue for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany

Abstract

Wear and corrosion of metallic endoprosthetic implants are the main risk factors for aseptic loosening and implant failure. At the implant surface, electrochemical reactions can take place continuously, resulting in an exchange of electrons and metal ions in various oxidation states [1, 2]. The released metal ions, such as nickel, cobalt, and chromium, can damage surrounding tissues by inducing cell death or cell stress through various mechanisms [2, 3, 4]. The aim of this *in vitro* study was to investigate the low-threshold, "adapted" cellular responses to non-cytotoxic concentrations of the bivalent metal ions Ni2+ (tested as NiCl2) and Co2+ (tested as CoCl2) and the trivalent Cr3+ (tested as CrCl3) in mesenchymal stem/stromal cells (MSC) *in vitro*. Analyses focused on the effects on proliferation, inflammation, cell stress, energy metabolism and differentiation. Treatment with the selected metal ion concentrations induced no changes in cell morphology and cell number for all conditions. Furthermore, the exposure of MSC to the bivalent metal ions Ni2+ and Co2+ resulted in a slight decrease in the release of the pro-inflammatory molecules interleukin-6 (IL-6) and IL-8. At the same time, an NF-kB translocation did not occur, indicating that the Ni2+ and Co2+ concentrations used did not induce pro-inflammatory activation in MSC or even probably reduce the pro-inflammatory response. Cr3+ had no effect at all on the inflammatory state of MSC *in vitro*. Bivalent metal ions (mainly Co2+) are known to influence gene expression of a wide variety of signaling molecules related to tissue regeneration by stabilizing the transcription factor hypoxia-inducible factor (HIF)-1α. However, HIF-1α stabilization was not demonstrated at the selected ion concentrations, whereas vascular endothelial growth factor (VEGF), a typical HIF-1α-dependent factor, was shown to be increased after exposure to both bivalent ions (Ni2+ and Co2+). Also, these two ions led to a clear shift of the energy metabolism toward a glycolytic metabolism. Both aspects induced by the bivalent ions support the induction of oxygen deficiency signaling. In all cases, trivalent Cr3+ did not show any effects. This study clearly demonstrates that even non-cytotoxic concentrations of metal ions can have significant effects on cells. Thus, the complex mechanisms of biological responses induced by metal ions remain to be fully elucidated in order to prevent or treat aseptic loosening of endoprosthetic implants made of cobalt-chromium alloys.

PoB.4.02

Self-assembled oxidized dextran nanogels for cisplatin delivery

Lukáš Münster, Vítek Hrbáček, Jan Vícha

Centre of Polymer Systems, Tomas Bata University in Zlín, Zlín, Czech Republic

Abstract

INTRODUCTION Cisplatin (CP, cis-[Pt(NH_3)$_2$$Cl_2$]) is a first-line chemotherapeutic used for patients diagnosed with various types of cancer. However, its use is accompanied by neuro-, nephro- and ototoxicity as a result of its non-specific action. CP conjugation to polysaccharides advantageously reduces off-toxicity, protects the conjugated CP, and improves accumulation in tumors (enhanced permeability and retention effect). Dextrans are α-(1→6) glucans with α-(1→3) branching and are used as e.g. plasma volume expanders. Dicarboxylated dextran (DXA) prepared via periodate-chlorite oxidation (Fig. 1) was conjugated with CP and showed the highest overall cellular uptake and high toxicity towards malignant cell lines among the other dicaboxylated polysaccharide-CP conjugates.[1] The migrastatic and anticancer efficacy was found to be dependent on the formation of CP-DXA nano-assemblies.[2] Thus, the DXA macromolecules containing high –COOH group density were crosslinked via CP bidentate binding to form CP-DXA nanogels, and their physico-chemical (Fig. 2) and biological properties were evaluated.

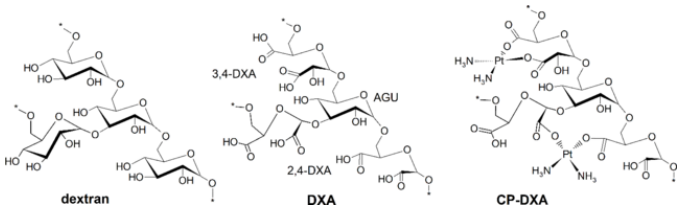


Fig. 1 Structure of dextran, DXA, and CP-DXA.

EXPERIMENTAL METHODS Dextran was oxidized by periodate (primary oxidation), and formed –CHO groups were converted to –COOH by chlorite (secondary oxidation). The prepared derivative was loaded using various CP:DXA ratios, increased temperature (*T*) and volume (*V*), and aqua-CP complex and characterized by DLS, TEM, cumulative release, and migrastatic potential.

RESULTS AND DISCUSSION i) CP:DXA ratio influences the nanogel hydrodynamic radius (R_h), higher ratio = smaller size. ii) High stability of all prepared nanogels (ζ < -50mV) confirmed. iii) Both increased temperature (*T*), volume (*V*), and their combination enable the preparation of smaller nanogels. iv) Use of aqua-CP complex significantly slows the release rate (direct reaction of aqua-CP with DXA's –COOH groups). v) Migrastatic potential increased with the decrease in nanogel size.

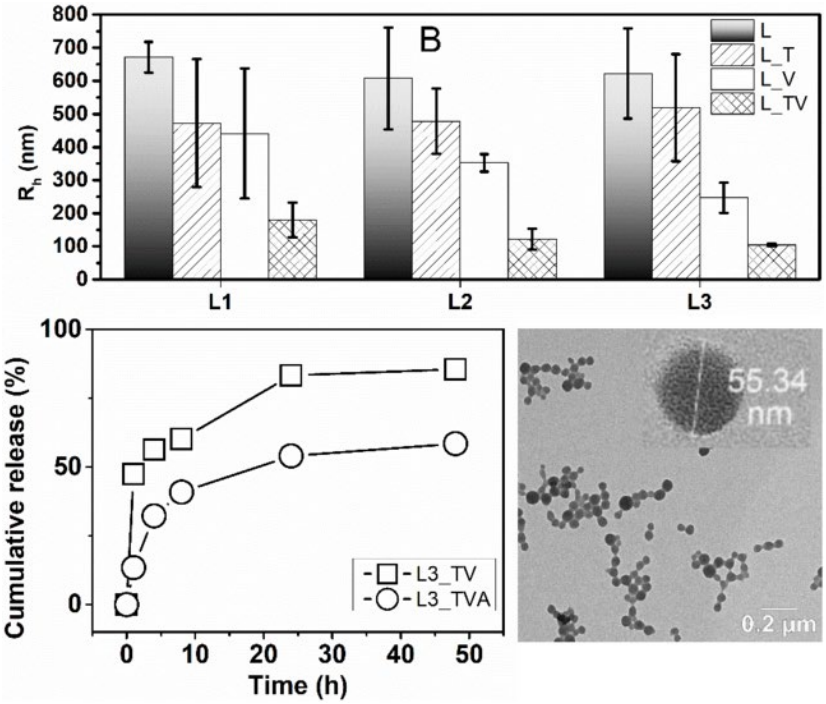


Fig. 2 Top – different conditions used in the preparation of CP-DXA nanogels affecting their R_h. Bottom left – CP cumulative release from CP-DXA (L3_TV) and aqua-CP-DXA (L3_TVA).Bottom right – TEM analysis of CP-DXA.

CONCLUSION

The CP-DXA was found to form nanogels under certain CP loading conditions. The size of nanogels can be modulated down to R_h = 100 nm which showed significant migrastatic potential towards A2780 A2780/CP, PC3, and A549 cancer cell lines.

REFERENCES

[1] Münster et al., Carbohydr Polym 2021, 257, 117562.
[2] Münster et al., Carbohydr Polym 2021, 272, 118461.

ACKNOWLEDGMENT

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic project DKRVO RP/CPS/2022/007.

PoB.4.03

A direction for reliable osteosarcoma research: a 3D tumour model

Jasmina Stojkovska[1], Ivana Banicevic[1], Jelena Petrovic[2], Luka Bojic[3], Milena Milivojevic[3], Milena Stevanovic[3,4,5], Radmila Jankovic[6], Bojana Obradovic[1]

[1]University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia. [2]Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia. [3]University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia. [4]University of Belgrade, Faculty of Biology, Belgrade, Serbia. [5]Serbian Academy of Sciences and Arts, Belgrade, Serbia. [6]University of Belgrade, School of Medicine, Belgrade, Serbia

Abstract

Over the last decade, 3D *in vitro* cancer models have been recognized as relevant and reliable models for better understanding etiology, development and growth of cancer, as well as for anti-cancer drug screening. The aim of this work was to create and validate a novel, simple, relevant, and robust 3D osteosarcoma model based on highly macroporous composite alginate scaffolds with embedded hydroxyapatite (HAP) particles as an artificial bone extracellular matrix (ECM) for osteosarcoma cell immobilization, and biomimetic perfusion bioreactor that imitates the environment of highly vascularized tissues. The composite scaffolds were produced by simple, controlled gelation of a suspension of HAP particles in Na-alginate solution (2 wt.% alginate and 2 wt.% powder), followed by freeze-drying and rehydration in the culture medium. Then, a suspension of murine K7M2-wt osteosarcoma cells was seeded manually onto the scaffolds (~9.5 mm in diameter, ~4.5 mm thick, $15x10^6$ cells cm^{-3} scaffold volume) and left for 24 h in the culture medium under static conditions. Next, the cell-seeded scaffolds were cultivated in perfusion bioreactors at continuous medium flowrate of 0.27 cm^3 min^{-1} (superficial velocity: 40 µm s^{-1}) up to 7 days, while cultures under static conditions served as a control. The scaffolds were assessed regarding the cell metabolic activity (viability) by MTT and Live/dead assay, morphology and distribution within the scaffolds by histological (H&E stain), immunohistological (α-tubulin) and field emission scanning electron microscopy analyses. Histological Masson-trichrome and reticulin staining were used for ECM analysis within the cancer cell clusters. Expression of markers related to osteosarcoma was assessed by quantitative real-time PCR (qRT-PCR). The seeded cells adhered to the scaffolds on the surfaces and in the pores as individual cells and aggregates retaining their viability and metabolic activity. After short-term cultures, the cells stayed viable, metabolically active, and expressed osteosarcoma markers, while spheroids were spontaneously formed within scaffold pores under both culture conditions. However, cells in the perfusion culture exhibited higher metabolic activity and better-oriented α-tubulin, while the spheroids were more compact with higher amounts of reticular fibers as compared to the static control. The obtained results could be explained by positive effects of continuous medium flow on cells, due to providing efficient mass transport and adequate shear stresses. The overall results of the present study demonstrated benefits of the novel 3D model based on macroporous alginate scaffolds containing mineral particulates as cell carriers in conjunction with a perfusion bioreactor for relevant and reliable osteosarcoma research.

PoB.4.04

Temperature-controlled photothermal therapy to induce immunogenic cell death in breast cancer cells

Emilia Happonen, Yiannis Constantinou, Konstantin Tamarov, Vesa-Pekka Lehto, Wujun Xu

University of Eastern Finland, Kuopio, Finland

Abstract

Introduction Black porous silicon (BPSi) nanoparticles were recently developed to efficiently generate heat under near-infrared irradiation to realize photothermal therapy (PTT) against cancer [1]. In the present study, the temperature used in PTT was controlled to induce immunogenic cell death (ICD) in the MDA-MB-231 breast cancer cells with ICD reporters expressing green and red fluorescence for HMGB1 and CRT, respectively. The changes of HMGB1 and CRT after PTT were monitored, as the released HMGB1 and ATP and the surface-exposed CRT are vital DAMPs that can activate an antitumor immune response in cancer cells [2]. Methods 15000 MDA-MB-231 cells per well were cultured overnight in a 96-well plate. The cells were incubated with the BPSi nanoparticles dispersed in cell medium (0.1 mg/ml) for 4 hours. Photothermal treatment with an 808 nm laser was conducted inside an incubator using a system controlling the temperature of the well precisely. Irradiation time was 10 min. After 24 h, the cell nuclei were stained with Hoechst 33342 (10 µg/ml) for 15 min in 37 °C. Fluorescence microscopy images were taken with a confocal laser scanning microscope (Zeiss LSM800 Airyscan). ATP content was analyzed with the CellTiter-Glo assay (Promega). Results Different temperatures were tested with the PTT system to find the optimal temperature for ICD. The temperature of 48 °C appeared to be the most effective for obtaining ICD. At this temperature, the ATP content decreased to 30% of the control demonstrating the reduction of viability after PTT. Microscopy images show that HMGB1 was released from the nuclei to the cytosol (Figure 1A) and intensity of CRT was increased on the surface of the cells (Figure 1B). The changes of HMGB1 and CRT, and the decreased viability indicated the incidence of ICD in breast cancer cells after PTT.

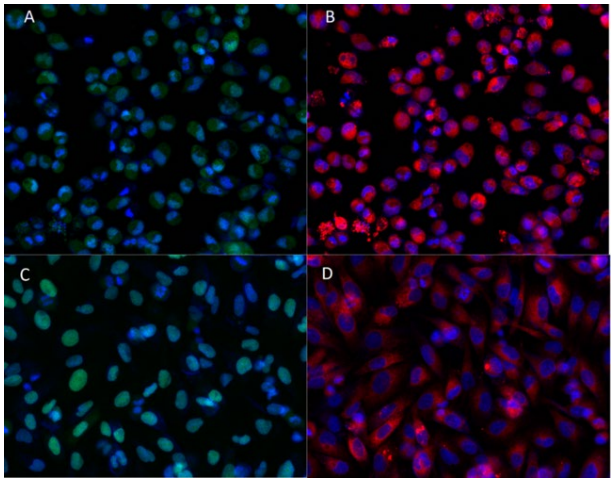


Figure 1. Confocal microscopy images of the MDA-MB-231 cells with ICD reporters and Hoechst 33342 (blue) nuclear staining. After PTT at 48 °C, A) HMGB1 (green) is released from the nuclei to the cytosol, and B) intensity of CRT (red) is increased on the surface of the cells. C) and D) show the nontreated cells as a control.

References

1. Xu W, et al., ACS Appl. Mater. Interfaces (2018) 10:23529-23538.
2. Fucikova J, et al., Cell Death and Disease (2020) 11:1013.

Acknowledgements

E.H. acknowledges Finnish Cultural Foundation for financial support. Fluorescence microscopy was carried out with the support of UEF Cell and Tissue Imaging Unit.

PoB.4.08

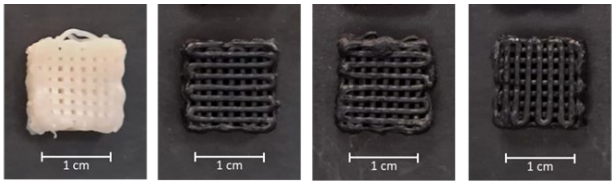
Development and *in vitro* characterization of 3D-printed composite scaffolds with superparamagnetic iron oxide nanoparticles for bone regeneration and magnetic hyperthermia

Francisco J. T. M. Tavares[1], Paula I. P. Soares[1], Jorge C. Silva[2], João P. Borges[1]

[1]i3N/CENIMAT, Department of Materials Science, NOVA School of Science and Technology, Campus de Caparica, Caparica, Portugal. [2]i3N/CENIMAT, Department of Physics, NOVA School of Science and Technology, Campus de Caparica, Caparica, Portugal

Abstract

Conventional bone cancer treatment, such as surgery, chemotherapy, and radiotherapy, often result in unwanted side effects, critical-sized bone defects, and inefficient cancer-cell targeting. Innovative approaches are necessary to better address bone cancer treatment and patient's recovery. One solution may reside in the combination of bone regeneration scaffolds with magnetic hyperthermia (MHT), which can be attained through the incorporation of superparamagnetic iron oxide nanoparticles (SPIONs) into additively manufactured scaffolds. The present study aimed at developing 3D-printed composite magnetic scaffolds with potential for bone cancer therapy and regeneration. These structures (Figure 1) were obtained by 3D printing a polymeric blend composed of chitosan (CS) and poly(vinyl) alcohol (PVA) with integrated hydroxyapatite (HA) particles and SPIONs, at three different concentrations (1.92, 3.77, and 5.54 wt.%). *Figure 1 – 3D-printed scaffolds: (a) CS/PVA/HA control; CS/PVA/HA/SPIONs scaffolds with SPIONs concentrations of (b) 1.92 wt.%, (c) 3.77 wt.%, and (d) 5.54 wt.%.*



These scaffolds with excellent stability under a saline medium, exhibited a mechanical behaviour like that of the organic phase of bones. FTIR data confirmed the existence of electrostatic interactions between the SPIONs and CS, which affected the swelling capacity of the 3D printed scaffolds. Furthermore, due to that same interaction, the incorporation of the SPIONs into the 3D printed CS/PVA/HA scaffolds acted as a mechanical reinforcement. The magnetic hyperthermia studies showed that the SPIONs-containing scaffolds displayed a temperature increase under the application of an alternating magnetic field, dependent on NPs concentration, thus demonstrating potential for hyperthermia treatment of cancer - scaffolds containing 3.77 and 5.54 wt.% of SPIONs, attained temperature increases of 6.6 and 7.5 ºC, respectively. *In vitro* studies using human osteosarcoma Saos-2 cells indicated that all tested scaffolds displayed no cytotoxicity for concentrations up to 50 mg/mL, having the incorporation of SPIONs significantly stimulated cell adhesion and proliferation when compared to CS/PVA/HA controls. Moreover, the expression of alkaline phosphatase by the cells grown on the SPIONs-containing scaffolds indicates that bone cells grown on these structures may be capable of forming an apatite layer that is essential for bone regeneration. Overall, these results support that CS/PVA/HA/SPIONs scaffolds with SPIONs concentrations above or equal to 3.77 wt.% have the potential to be used for MHT and bone regeneration, through a synergy between its magnetic properties and osteogenic activity enhancement capabilities.

PoB.4.10

Nitric oxide-scavenging hydrogels as promising therapeutics for triple-negative breast cancer

Amir M.A Alsharabasy[1], Sharon Glynn[2,1], Pau Farràs[3,1], Abhay Pandit[1]

[1]CÚRAM, SFI Research Centre for Medical Devices, University of Galway, Galway, Ireland. [2]Discipline of Pathology, Lambe Institute for Translational Research, School of Medicine, University of Galway, Galway, Ireland. [3]School of Biological and Chemical Sciences, Ryan Institute, University of Galway, Galway, Ireland

Abstract

In triple-negative breast cancer (TNBC), nitric oxide (●NO) orchestrates the angiogenesis, depending on its levels and the duration of exposure. These are essential for enhancing the cancer cell spreading. ●NO-scavenging is a potential strategy to restore these cells' homeostasis. In breast cancer, specific low MW-hyaluronic acid (HA) fragments have pro-inflammatory functions, while HA with M.W above 200 kDa suppresses the expression of many inflammatory cytokines, with potential interactions with ●NO, as we reported before. For this purpose, the development of high MW-based HA-hydrogel systems can be therapeutic as a carrier system for different ●NO-scavengers' payloads. The specific objectives of this study were: (i) to construct HA-hydrogels loaded with different hemin conjugates and examine their physicochemical properties; (ii) to test their ●NO-scavenging potential electrochemically and using luminescence reactions; (iii) to evaluate the effects of hydrogels on ●NO-induced migration of TNBC cells and tumour progression in a TNBC mouse model. Experimental methods: (i) Synthesis of a number of hemin conjugates and choosing two of them for further loading to hydrogels. (ii) Fabrication of ●NO-scavenger-loaded Hydrogels: these were chemically crosslinked, and their ●NO-scavenging efficiency and physicochemical properties were measured towards choosing of candidate formulations. (iii) Evaluating of the effects of candidate hydrogels on the ●NO-induced migration of TNBC cells. (iv) Investigation of the influence of candidate ●NO-scavenging hydrogels on the growth rate and size of breast tumours in Immunocompetent mice. Results and discussions: A number of hydrogel formulations were fabricated with different properties and interactions with ●NO dependent on the crosslinking degree. This also governed the probability of distribution of the ●NO-scavenging molecules on the surface and within hydrogel matrix, with less surface distribution detected upon increasing of crosslinking degree. The hydrogel formulations characterized as injectable, slowly degrading with the highest ●NO-binding efficiency, and slowly release the loaded compounds were selected for further testing. The experiments revealed certain hydrogel formulations could protect the loaded ●NO-scavenging compounds without influencing their functions and maintain their mechanical properties. This controlled hydrogel degradation was further confirmed in *in vivo* experiments by iron quantification in tumour tissue. Meanwhile, modulation of tumour growth was observed, following the hydrogel injection, compared to the unloaded compounds. Conclusions: Injectable hydrogels loaded with different hemin conjugates were fabricated. The hydrogel formulation can be tuned to maintain the ●NO-scavenging efficiency by loading the lowest possible ●NO-scavenger concentrations, mechanical properties and degradability. Hence, these hydrogels can be used as potential therapeutics for treating breast cancer.

PoB.4.13

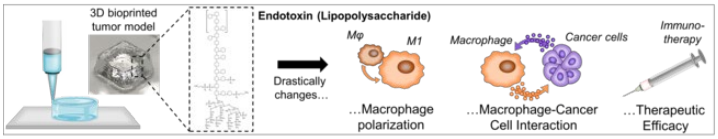
Endotoxin contamination alters macrophage-cancer cell interaction and therapeutic efficacy in pre-clinical 3D *in-vitro* models

Catarina Silva[1], Praneeth Reddy Kuninty[2], Jos Olijve[1]

[1]Rousselot, Son, Netherlands. [2]Rousselot BV, Gent, Belgium

Abstract

The rapid developments in biofabrication systems such as 3D bioprinting, organoids and organ-on-chip for drug testing have increased the need for novel biomaterials. The main focus in the development of biomaterials is on replicating the target tissue's composition, mechanical properties and providing cells with the optimal environment to proliferate, differentiate and communicate. However, a potential endotoxin contamination of these biomaterials and the consequent cellular impact is often overlooked. Endotoxins or lipopolysaccharides (LPS) are found in the outer membrane of gram-negative bacteria and have profound *in vivo* responses. They can trigger strong immune responses and therefore are unwanted contaminants in (bio)materials. Their activity must be as low as possible. Hence, FDA defined 2.15-20 EU/medical device or 0.06-0.5 EU/ml as limits depending on the type of application. In the last years, it has become evident that tumor-associated macrophages in the tumor microenvironment along with other immune cells such as T cells, play a crucial role in the progression, invasion and development of drug resistance in several cancer types. In this study we demonstrate the effects of endotoxins in commercially-available gelatins on the macrophage (RAW264.7) - cancer (4T1) cell crosstalk in a 3D bioprinted co-culture model by measuring relevant macrophage and cancer cell biomarkers. We observed that, while having the same mechanical and structural hydrogel properties, high levels of endotoxin can have significant influence on the metabolic activity of macrophages and cancer cells. Furthermore, this study shows that high endotoxin contamination causes a strong inflammatory reaction in macrophages and significantly inhibits the effects of a paracrine macrophage-cancer cell co-culture. This impacts the physiological relevance of the 3D in-vitro system. Furthermore it was also demonstrated that the differences in endotoxin levels can drastically alter the efficacy of novel macrophage modulating immunotherapeutic drug, 3-methyladenine. Which might lead to misinterpretation of the potency and safety of novel immune therapeutic compounds.



PoB.4.15

Photo-processed decellularized matrix hydrogels for engineering organotypic human tumor-stroma 3D biomodels

Vítor M. Gaspar, Maria V. Monteiro, Luís P. Ferreira, João F. Mano

University of Aveiro, Aveiro, Portugal

Abstract

High-throughput compatible models seeking to recapitulate major components of the tumor microenvironment interplay between cancer-stromal cells and their surrounding ECM remain challenging to develop. Within this context, decellularized scaffolds provide a new pathway to integrate ECM-specific cues and bioactive components in bioengineered 3D *in vitro* tumor models as a mode to increase their physiomimetic potential. Seeking to overcome these challenges, herein photo-processable decellularized matrix hydrogels are engineered as cell supporting platforms for *in vitro* cancer modelling. The precise combination of tissue sepecific dECM hydrogels with cancer and stromal cells and their rapid processed into spherically structured 3D tumor models leveraging on biomimetic superhydrophobic surfaces is presented. Biomolecular characterization, omics analysis and drug screening using standar of care and precision medicine therapeutics evidences the importance of introducing native ECM components in bioengineered 3D *in vitro* models of human neoplasias.

PoB.4.17

Development of a new composite injectable system for cancer treatment

Cezar Tipa[1], Paula Soares I. P. Soares[1], João P. Borges[1], Luis C. Costa[2], Jorge C. Silva[1], Adriana Gonçalves[1]

[1]CENIMAT|i3N, Department of Materials Science School of Science and Technology NOVA University Lisbon, Caparica, Portugal. [2]I3N and Physics Department University of Aveiro,, Aveiro, Portugal

Abstract

Cancer is an alarming and increasing worldwide cause of death. Its most common treatment approach, systemic chemotherapy, has several limitations including toxicity to healthy tissues, severe side effects to patients and low therapeutic concentrations at tumor sites. This study focuses on the development of a multifunctional magnetic system for cancer treatment. With the thermo-responsive nature of the selected hydrogel and the multifunctional properties of SPIONs as well as clay particles, we aim to produce an injectable magnetic system able to control and target-release a chemotherapeutic drug along with other synergistic therapeutic effects. Clay minerals (in particular montmorillonite) are biocompatible and present unique properties of great interest for drug delivery systems. Magnetic nanoparticles (mNPs) demonstrate huge potential for cancer treatment including peroxidase-like activity that can act against the tumor. The synthetized mNPs were successfully characterized and efficiently intercalated into nanoclays´ interlaminar space by cation exchange reactions, without compromising its properties and therapeutic hyperthermia feature. The developed and already characterized magnetic clays (mClay) were integrated into an optimized F127/F68 Pluronic formulation to produce a suitable thermo-responsive and injectable composite hydrogel. The rheological behaviors of the composite systems were extensively studied to confirm suitable injectable flow resistance, appropriate gelation temperature and sufficient hydrogel structural integrity to support the produced magnetic clay. A model drug has been used to assess the long-term drug release profiles from clays, magnetic clays, and final composite system. In the future, this system is expected to be able kill tumor cells by a synergistic combination of controlled delivery kinetics of suitable chemotherapeutic drugs, with ferroptosis and starvation of tumoral cells. We will evaluate the intrinsic peroxidase-like activity of clay-intercalated SPIONs and functionalize magnetic clays with glucose oxidase to deprive tumor cells of crucial nutrients.

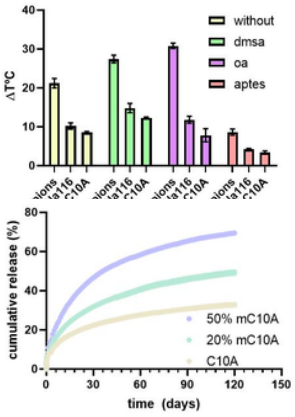


Fig. 1 - Comparison between the ΔT (ºC) values from the magnetic Hyperthermia assays of : 5mg/ml SPIONs mNPs), 10mg/ml of 50wt.% mNa116 and 10mg/ml of 50wt.% mC10A with the respective mNPs ´ surface coatings

Fig. 2 - Cumulative release of methylene blue-loaded mC10A (1:2 ratio) with diferent wt.% of mNPs without coating intercalated.

PoB.5.01

The combination of passive and active mechanical loadings improves contractility and maturation of three-dimensional engineered cardiac tissues

Antonio Sileo[1,2], Alessia Pisanu[1,2], Deborah Fusco[1,2], Stefano Gabetti[3], Diana Massai[3], Anna Marsano[1,2]

[1]University of Basel, Basel, Switzerland. [2]University Hospital of Basel, Basel, Switzerland. [3]Department of Mechanical and Aerospace Engineering and PolitoBioMed Lab, Torino, Italy

Abstract

INTRODUCTION: The establishment of functional three-dimensional engineered cardiac tissue (ECT) models is crucial for effective drug screening and for deepening the understanding of cardiac regeneration/maturation mechanisms. The application for several weeks of physiological cues mimicking those typical of native myocardium promotes cardiac maturation and functionality *in vitro*[1,2]. Current commercially available bioreactors deliver only one/two physical stimulation types at a time, often with a limited loading range[3]. To overcome these limitations, a millimetric-scale microscope-integrated bioreactor was developed to deliver passive and active mechanical loading (in physiological/pathological ranges) and electrical stimulation to ECTs[4]. Pisanu *et al* showed that ECT exposed to passive stimulation possessed a superior functionality, a superior sarcomere organization, and increased cell elongation compared to static controls in 8-day-culture[4]. However, cell elongation and maturation were predominantly observed at one edge of the ECT. In this study, we hypothesized that the application of passive mechanical loading followed by an additional active cyclic mechanical loading further enhances ECT functionality and cardiac maturation in 8 days. **METHODS:** ECTs were generated by seeding rat cardiomyocytes (70%) and fibroblasts (30%) in a 100µL fibrin gel solution (25mg/mL fibrinogen, 5U/mL thrombin, cell-density: $20x10^6$cells/mL). Passive loading (PL) for 8 days alone or combined to active cyclic loading (AL) for the last 4 days of culture (10% deformation at 1Hz) was applied. Excitation Threshold (ET, the minimum electrical field to induce ECT synchronous beating), Maximum Capture Rate (MCR, the maximum pacing frequency), and force of contraction were assessed for ECT functionality. Cardiac maturation was evaluated by immunofluorescence-staining for specific cardiac markers. **RESULTS:** Compared to PL alone, the combined application of passive and active loadings (PL+AL) further enhanced significantly ECT functionality (ET: PL=8.11±5.87V/cm, PL+AL=3.31±1.83V/cm, p=0.044; MCR: PL=3.00±1.07Hz, PL+AL=4.00±0.76Hz, p=0.049) and the developed force (PL=0.04±0.03mN, PL+AL=0.22±0.25mN, p=0.008), which resulted to be in the range of other ECTs generated under long-term PL[1,2]. Cell elongation and cardiac maturation (based on sarcomere organization) appeared also improved and more uniform throughout the construct compared to ECT exposed to only PL, where sarcomeric structures were present only at one edge[4] (Figure1). **CONCLUSIONS:** *In vitro* application of PL+AL appears to greatly improve the contractility, functionality and spatial uniformity of ECT cardiac maturation, leading to *in vitro* cardiac models of superior quality in only 8 days of culture.

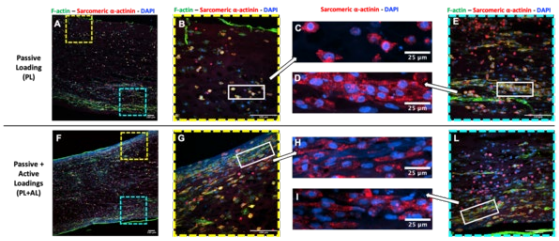


Figure1. Representative immunofluorescence images of sarcomere organization for passive stimulated ECT (A) and the combination of passive and active stimulated ECT (F). Top (B,C) and bottom (D,E) areas in PL and top (G,H) and bottom (I,L) areas in PL+AL, stained for sarcomeric α-actinin cardiac marker (red) and F-actin (green). Nuclei were stained in blue (DAPI). Scale bar (A,B,E,F,G,L) 100 µm, (C,D,H,I) 25 µm.

ACKNOWLEDGEMENTS: Swiss National Science Foundation (310030_172989 to A.M.). **REFERENCES:** [1]Goldfracht,I.(2019), [2]Zhao,Y.(2019),[3]Mittal,R.(2019), [4]Pisanu,A.(2022).

PoB.5.02

Development of a completely biological heart valve repair strategy for fallot's tetralogy correction

Manisha Singh[1], François Roubertie[2,3], Paul Borchiellini[4], Adeline Rames[4], Caglar Ozturc[1], Jean Bonnemain[1], Sophie Wang[1], Jérôme Naulin[2], Dounia El Hmrani[2], Nicolas L'Heureux[4], Ellen T Roche[5], Fabien Kawecki[4]

[1]Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, USA. [2]IHU Liryc, Electrophysiology and Heart Modeling Institute, Pessac, France. [3]Congenital Heart Diseases Department, CHU de Bordeaux, Pessac, France. [4]Univ. Bordeaux, INSERM, BIOTIS, UMR1026, Bordeaux, France. [5]Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, France

Abstract

Tetralogy of Fallot (ToF) is the most common cyanotic heart malformation in newborns. It can be corrected by surgical management, which involves enlarging the right ventricular outflow tract (RVOT). This approach generates pulmonary valve leakage that is currently repaired by creating a monocusp valve made of synthetic material or chemically-treated animal tissue. However, these materials are associated with severe postoperative complications. This project aims to evaluate the effectiveness of a Cell-Assembled extracellular Matrix (CAM) sheet for ToF repair. We first demonstrated that a CAM sheet cultured for 16 weeks is compatible with surgical manipulations and sutures required to create a monocusp valve and a transannular patch in an explanted animal heart ex vivo. Using an organosynthetic dynamic heart model, combined with computational models, we evaluated different designs of CAM-based monocusp valves to prevent pulmonary regurgitation and restore baseline hemodynamic pressures. The optimal CAM-based monocusp valve design was implanted in clinically relevant ovine models (n = 3). The effectiveness of the valve was evaluated using epicardial echocardiography, magnetic resonance imaging, and hemodynamic pressure measurements. The CAM-based monocusp valve was hemodynamically effective (transvalvular pressure gradient ≤ 6 mmHg) and provided a competent pulmonary valve (regurgitation < 5%) after seven days of implantation. Finally, the CAM biomaterial represents a promising therapeutic solution for the pediatric population suffering from ToF.

PoB.5.03

Assembly of multilayered tissue engineered vascular equivalent with a biomimetic approach

Gozde E. Kole[1,2], Yilmaz O Koyluoglu[3], Halime Kenar[2,4,5], Vasif Hasirci[2,4,5], Deniz Yucel[1,2,3,5,6]

[1]Acıbadem Mehmet Ali Aydınlar University (ACU), Graduate School of Health Sciences, Department of Medical Biotechnology, Istanbul, Turkey. [2]ACU, Biomaterials Center, Istanbul, Turkey. [3]ACU, School of Medicine, Istanbul, Turkey. [4]ACU, Faculty of Engineering and Natural Sciences, Department of Biomedical Engineering, Istanbul, Turkey. [5]ACU, Graduate Department of Biomaterials, Istanbul, Turkey. [6]ACU, School of Medicine, Department of Histology and Embryology, Istanbul, Turkey

Abstract

Cardiovascular diseases are the number one cause death worldwide. Large artery replacements are successfully performed in the clinic; of however, smaller caliber (<6 mm) tubular equivalents are still a challenge. To address this, we aimed to construct a small caliber vascular equivalent mimicking layers of native coronary artery. Multilayered tubular equivalent was composed of 4 layers and had a 3 mm lumen diameter (Fig1a). The innermost layer was a porous tubular film of PCL-PLGA obtained by dip-coating of a tubular mold. Randomly oriented elastin-PCL-PLGA fibers covered with, and then circumferentially aligned fibers of PCL-PLGA-P(L/D-L)LA were collected over by electrospinning. Afterwards, collagen-based hydrogel was integrated as an outermost layer over the electrospun mat. Human umbilical cord vein endothelial cells (HUVECs) were seeded on the lumen side of the tubular film, human Wharton`s Jelly mesenchymal stem cells (WJ MSCs) differentiated into smooth muscle cells (SMCs) and fibroblasts were seeded into the middle and outer layer of tubular equivalent, respectively. HUVECs, WJ MSC derived SMCs and fibroblast were then co-cultured on tubular equivalent for 4 days. HUVEC and WJ MSC adherence and proliferation on the tubular scaffold were evaluated with phalloidin-DAPI staining and MTS. It was observed that both cell types adhered and spread on the corresponding layers of the scaffold. After co-culture, immunocytochemistry results showed that the cell specific expressions of CD31 for HUVEC, α-SMA and calponin for SMC, and collagen type III and vimentin for fibroblast were conserved (Fig2b). In conclusion, a multilayer, 3D, small-medium diameter vascular equivalent carrying endothelial cells, smooth muscle cells, and fibroblasts were successfully developed under *in vitro* conditions. Considering the ultimate translational medicine goal, this vascular equivalent has the potential to be further evaluated under *in vivo* conditions. Another potential use of the vascular equivalent developed would be as *in vitro* models for the treatment of vascular diseases.

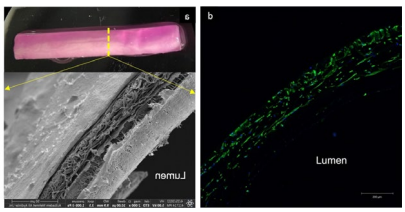


Figure 1. Multilayer, 3D small caliber vascular equivalent. (a) SEM and stereomicroscope images of multilayered tubular equivalent. (b) Confocal images of WJ MSC derived fibroblast within the outermost layer of the vascular equivalent after immunostaining for vimentin (green) and counterstained with DAPI for nucleus (blue).

ACKNOWLEDGMENTS

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) SBAG 118S587 and TUBITAK 2211-A/BIDEB. The authors acknowledge ACU Biomaterials Center for the use of equipments and facilities

PoB.5.04

An anatomically inspired and spatially heterogeneous mitral valve scaffold via melt electrowriting

Kilian Maria Arthur Mueller, Matthias Pixner, Paula Castejón Echevarne, Petra Mela

Chair of Medical Materials and Implants, Department of Mechanical Engineering and Munich Institute of Biomedical Engineering, Technical University of Munich, Garching, Germany

Abstract

Introduction The mitral valve (MV) is a highly complex apparatus that provides annulo-ventricular (AV) continuity in the left heart via a multitude of chordae extending from the two asymmetric leaflets towards the papillary muscles. Although imperative to native valve functionality, most clinically available MV implants neglect AV continuity. Here, we propose to exploit melt electrowriting (MEW) to fabricate a microfiber-based, anatomically relevant scaffold for MV tissue engineering, recapitulating the anterior and posterior leaflets and the chordae. Methods An intuitive MATLAB user interface was developed that outputs patient-specific fiber deposition commands and respects the MV anatomy by i) assigning an anisotropic serpentine architecture to two independently designed asymmetric leaflets and ii) a straight fiber pattern to the chordae section. The fabricated scaffolds were extensively evaluated for tensile properties and bending stiffness. Fiber diameters and pore morphologies were studied via SEM. Optimized scaffolds were embedded in isotropic hydrogel matrixes via a D-shaped mold to evaluate their hemodynamic performance in a mock circulation system. Results The as-coded fiber patterns were precisely translated into seamless tubular scaffolds via MEW as verified by SEM. The serpentine fiber design resulted in a tunable J-shaped stress-strain response, individual for each leaflet, and distinctly different from the linear response obtained from the straight multifiber pattern of the chordae zone. Fiber diameter and layer number affected tensile strength and bending stiffness. Embedding the scaffolds in hydrogels resulted in near net shaped MVs of anatomical relevance. The composite valves met the requirements of ISO 5840 for hemodynamic functionality. Conclusion This work leverages the accurate fiber placement of MEW to fabricate an anatomically inspired MV scaffold that provides uninterrupted load transmission along the entire valve via fiber continuity between two differently designed scaffold zones.

PoB.5.05

Platelet-inspired nanoparticles for targeted drug delivery to the atherosclerotic plaque

Yangshuo (Jessie) Hu, Adam Celiz

Department of Bioengineering, Imperial College, London, United Kingdom

Abstract

INTRODUCTION Atherosclerosis is a chronic inflammatory disease that underpins most coronary heart diseases. An occlusive lipid plaque develops in the arteries increasing the risk of fatal ischaemic events.[1] Systemic drug treatments are associated with off-target side effects, calling for more specific drug delivery to the atherosclerotic site.[2] Inspiration is drawn from platelets' natural ability to marginate and adhere to injured vasculature, via adhesion to collagen and von Willebrand Factor under pathologically elevated shear stress.[3] This study aims to develop a nanoparticle deformable under shear stress to enhance adhesion to von Willebrand factor, and a microfluidic based platform to assess adhesion of the particles in an atherosclerosis mimicking environment. EXPERIMENTAL METHODS Microfluidic chips were produced based on a previously published design,[4] (Figure 1). Channels with one-sided stenosis of 80% lumen reduction were patterned on a silicon wafer with photolithography.

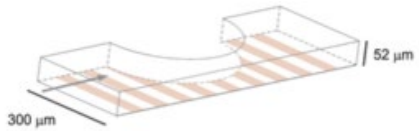


Figure 1. Design and dimension of the microfluidic chip.[4]

For vWF morphology under elevated shear rate, microfluidic channels were pre-coated with collagen I, Alexa 488 tagged human plasma vWF was perfused through at a peak shear rate of 70,000 s^{-1} and then washed. Images were obtained on a confocal microscope. RESULTS AND DISCUSSION A microfluidic chip mimicking atherosclerosis was successfully made. Live imaging of the chip showing particle-channel interaction was demonstrated with a model antigen-antibody system. Immobilization of vWF within the channel was confirmed through imaging. Under elevated shear stress in the apex region (70,000 s^{-1}), vWF adhered to the collagen coated channel underwent elongation (Figure 2), as reported to be a key feature of the atherosclerotic microenvironment.[4]

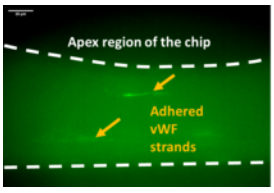


Figure 2. Adhesion of vWF on collagen-coated stenosis channel. Arrows indicate elongated vWF multimers. The dashed white line indicates the outline of the channel. Scale bar = 20 µm.

CONCLUSION A microfluidic chip mimicking the geometry and microenvironment of atherosclerotic stenosis was successfully made with evidence of immobilized vWF. Further studies will focus on the development of the shear stress-deformable nanoparticle system, and assessment of its adhesion performance with the developed chip.

REFERENCES 1.Badimon, L et al., Eur. Heart J. Acute Cardiovasc. Care, 1:60-74, 2012 2.Groner, J. et al., J. Controlled Release, 333:536-559, 2021 3.Wellings, P. J et al., Eng. Technol., 3 (2): 161–170, 2012 4.Westein, E et al., Proc. Natl. Acad. Sci., 110 (4): 1357–1362, 2013

ACKNOWLEDGMENTS The authors would like to thank the Imperial College President's PhD Scholarship for providing financial support to this project.

PoB.5.06

An improved radiolabeling strategy of extracellular vesicles (EVs) for prolonged *in vivo* monitoring in the heart using PET imaging

Arnab Banerjee[1], Ivanna Hrynchak[2], Karina Simon[1], Francisco Tejo[3], Quélia Ribeiro[1], Antero Abrunhosa Abrunhosa[2], Lino Ferreira[1,4]

[1]CNC-Center for Neurosciences and Cell Biology, University of Coimbra,, Coimbra, Portugal. [2]ICNAS – Institute for Nuclear Sciences Applied to Health; University of Coimbra, Coimbra, Portugal. [3]Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal. [4]Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Abstract

Worldwide, 26 million people suffer from heart failure (HF), and annually in Europe alone, about 3.6 million people are diagnosed with HF. EVs are biological nanoparticles, with sizes between 30 to 200 nm, secreted by most cells1. EV-based therapies for HF have grown over the years, necessitating the use of PET imaging to monitor EVs for extended periods of time. 89Zr4+has a higher half-life of 78.4 h with a relatively low positron energy of 395.5 keV , cheaper to produce makes it a favourable choice for PET imaging2. The most common metal chelator for 89Zr4+ is deferoxamine (DFO) which forms the 89Zr4+-complex very fast at room temperature3. However several preclinical animal models show the leaching of 89Zr4+ from the DFO-89Zr4+ after one hour of administration4. Alternatively, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) forms a stable 89Zr4+-complex at high temperature (90oC). Our objective was to develop a chelator for 89Zr4+ that could form a stable complex at room temperature and be used in PET imaging. The chelator was able to complex 89Zr4+ in one hour. The highest complexation was observed in Tris-buffer. The EVs were radiolabeled in two steps, the first of which included conjugating the chelator with the EVs and the second of which involved radiolabelling with 89Zr4+. EV-chelator-Zr had a radio-chemical yield of ~60% and ~ 80% stability in plasma for 7 days. Our modification did not affect the morphology, surface protein and internal RNA content of EVs. Finally, we successfully imaged EVs in healthy and myocardial infract mice after injecting EV-chelator-89Zr intravenously and measured the bio-distribution of it in different organs including the different parts of the heart for a prolonged time. Funding: Project ‘LABEL’ (POCI-01-0247-FEDER-049268) under the Portugal 2020 program, and FCT project reference 2022.02803.PTDC. Authors have no conflicts of interest to declare.

1. M. Tkach and C. Thery, Cell, 2016, 164, 1226-1232.
2. J. P. Holland, V. Divilov, N. H. Bander, P. M. Smith-Jones, S. M. Larson and J. S. Lewis, Journal of Nuclear Medicine, 2010, 51, 1293-1300.
3. D. N. Pandya, N. Bhatt, H. Yuan, C. S. Day, B. M. Ehrmann, M. Wright, U. Bierbach and T. J. Wadas, Chemical science, 2017, 8, 2309-2314.
4. J. P. Holland, Y. Sheh and J. S. Lewis, Nuclear medicine and biology, 2009, 36, 729-739.

PoB.5.07

Novel injectable chitosan-based cryogel loaded with cytokines and growth factors restores heart function after myocardial infarction

Kamila Raziyeva, Zharylkasyn Zharkinbekov, Yevgeniy Kim, Arman Saparov

Nazarbayev University School of Medicine, Astana, Kazakhstan

Abstract

Composite chitosan-based biomaterials have been successfully utilized for multiple biomedical applications including tissue regeneration and sustained drug delivery. We have recently designed a novel chitosan cryogel containing heparin and poly-vinyl alcohol with high porosity and interconnective pore architecture. Our previous research has demonstrated that the aforementioned cryogel could act as an efficient drug delivery platform and induce tissue regeneration. Thus, it has supported the controlled and sustained delivery of specific growth factors and cytokines to cutaneous wounds resulting in significant regeneration of the damaged area. In this research, we have deployed this chitosan-based drug delivery system to improve the regeneration and repair of the cardiac tissue after myocardial infarction (MI) in a mouse model. Specifically, the cryogel was loaded with two sets of cytokines and growth factors, namely, one with interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) (Cryo/IL-10/TGF-β), and another with vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) (Cryo/VEGF/FGF-2). The Cryo/IL-10/TGF-β therapeutic system was intramyocardially injected immediately after MI (day 0) induced by a left anterior descending artery ligation, followed by the injection of Cryo/VEGF/FGF-2 on day 4 after MI. The initial treatment time was chosen for injection due to the massive inflammation in the first hours of MI, which as we hypothesized could be dampened by the sustained release of anti-inflammatory cytokines IL-10 and TGF-β. On the other hand, the time of the second treatment was selected for therapeutics administration because tissue regeneration and neoangiogenesis that occur around that time and could be supported by controlled delivery of pro-angiogenic and regenerative factors VEGF and FGF-2. According to the preliminary results, cryogel with factors has significantly enhanced regeneration and repair of infarcted myocardium four weeks after MI compared to no treatment or cryogel only treatment. In particular, a considerable improvement in the contractile properties of the heart was observed as judged by the increase in cardiac output, ejection fraction, fractional shortening, and stroke volume obtained by echocardiographic measurements. Moreover, reduced fibrotic areas were observed on the Masson Trichrome staining of the left ventricles. Overall, we present a novel drug delivery platform based on chitosan and loaded with anti-inflammatory and proangiogenic factors, which is capable of maintaining a controlled release of therapeutics and significantly improving tissue repair and regeneration after MI.

PoB.5.08

Vascular access for hemodialysis: the response of endothelial cells to turbulent flow shear stress

Chiara Emma Campiglio[1], Matteo Tironi[2], Andrea Remuzzi[1]

[1]University of Bergamo, Bergamo, Italy. [2]Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy

Abstract

Arteriovenous fistula (AVF) is recommended as first choice to create vascular access for hemodialysis. However, 40% of AVFs fail within one year due to stenosis caused by neointimal hyperplasia (NH) [1]. Despite intensive research, there is still a limited understanding of the underlying pathophysiology and molecular mechanism. Recently, it has been demonstrated that AVF surgery causes near turbulent blood flow conditions and it has been shown that endothelial cells (ECs) are regulators of hemodynamically-induced vessel remodeling [2]. The aim of the study is to evaluate the biological response of ECs to shear forces similar to those that develop in the AVF, using a purpose-designed cone-and-plate device. ECs were cultured on 150 cm^2 culture dishes and exposed to hemodynamic shear stress using a cone-and-plate device [3]. After 48 hours cell morphology was investigated, and the expression of different proteins evaluated by immunofluorescence staining (YAP, FAK, Caspase-3). The effect of laminar and turbulent flow on ECs migration was analyzed using *in vitro* wound-healing test. To investigate the role of ECs in NH, the proliferation of smooth muscle cells (SMCs) cultivated with conditioned media (i.e., medium collected during experiments of flow exposure on ECs) was studied. After 48 hours exposure to laminar flow, ECs aligned in the flow direction, while they maintained a random orientation when subjected to turbulent flow. The two flow regimens develop cytoskeleton reorganization.

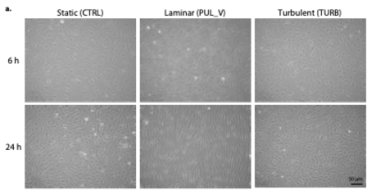


Figure 1. (a) Optical and (b) confocal images of ECs after exposure to shear stress.

The cytoplasmatic expression of YAP-1 protein was significantly higher when ECs were exposed to laminar flow, while YAP-1 nuclear translocation was observed after turbulent flow exposure.

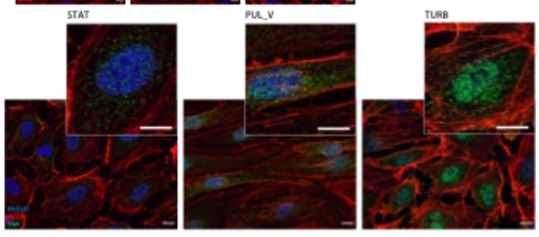


Figure 2. Immunofluorescence staining of YAP in stimulated ECs.

This condition may induce the production of pro-inflammatory molecules and cells migration, effects that may be involved in NH. *In vitro* wound-healing test showed a faster ECs migration and proliferation under turbulent flow, suggesting a quiescent state when physiological conditions are replicated. Culture medium conditioned by ECs exposed to turbulent flow selectively increased SMCs proliferation compared with unidirectional flow.

Our findings indicate that an important modulation of mechanoresponse to shear stress is induced by turbulent flow in ECs, suggesting a mechanism by which this condition may induce the development of NH in AVF and vascular access failure.

[1] Roy-Chaudhury, P. (2009) Nephrology Dialysis Transplantation, 24(9), 2786-2791.
[2] Bozzetto, M. (2016) Annals of biomedical engineering, 44, 2388-2401.
[3] Franzoni, M. (2016) Cytotechnology, 68, 1885-1896.

PoB.5.09

ADVANCED BIOACTIVE COATING TO IMPROVE THE BIO-INTEGRATION OF SYNTHETIC VASCULAR GRAFTS

Lydia Marinou[1], Vineetha Jayawarma[1], Niall Patterson[2], Robbie Brodie[2], Matthew Dalby[1], Manuel Salmeron-Sancez[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Terumo Aortic, Glasgow, United Kingdom

Abstract

Introduction Synthetic vascular grafts have transformed the management of cardiovascular disease. However, graft failure is a persistent issue, that may be caused by anastomotic intimal hyperplasia, thrombogenesis and infection[1]. It has been reported that in-vivo endothelialisation results in higher patency rates and reduced intimal hyperplasia[2]. Here, we propose a bioactive coating – a material-based strategy for the local presentation of relevant growth factors in synergy with integrins, to promote healthy endothelialisation *in vivo*. Briefly, it comprises an elastomer coating, poly (ethyl acrylate) (PEA), functionalised sequentially with extracellular matrix protein, fibronectin, and selected stimuli, currently vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2), acting as the driving force for improved cell adhesion and motility. In parallel, the coating is implemented on the inner surface of woven PET synthetic grafts to ensure its clinical relevance. Initial studies focus on the effect of VEGF and FGF-2 and their potential combined effects on human umbilical vein endothelial cells (HUVECs). Methods The polymer coating was deposited via plasma polymerisation in a custom-built inductively coupled plasma chamber. The physical and chemical properties of the bioactive coating were fully characterised prior to any cellular investigations. Cell metabolic activity, proliferation, and cell-to-cell communication (CD31) were investigated *in vitro*. Additionally, a range of growth factor ratios were investigated. Cell motility was examined with in-house 3D-printed migration barrier inserts, which were appropriately validated. Results The efficiency of the coating processes was investigated by XPS for the PEA layer and colorimetric assays for the fibronectin and growth factor functionalisation shown in Figure 1a, b and c respectively. Cell metabolic activity was improved in the presence of growth factor functionalisation, which was also correlated with higher CD31 expression (Figure 1d). Additionally, when both growth factors are present in various ratios, this appears to significantly increase CD31 expression (Figure 1e). Finally, cell motility is increased when cells are presented with functionalised surfaces.

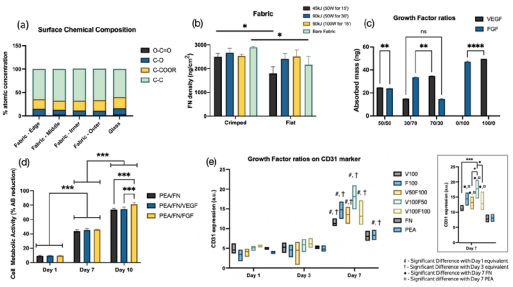


Figure 1: Characterisation of the Bioactive Coating (a)Surface Chemical Composition, (b) Fibronectin density and (c) Growth Factor Binding; Investigation of Cellular Interaction with the Bioactive Coating (d) Cell Metabolic Activity and (e) CD31 Expression

Conclusion The functionalised coating is successfully incorporated with the complex topography of the synthetic vascular grafts. Overall, the engineered coating provides suitable conditions for increased cell adhesion and motility.

References
1) Yuan et al., J. Biomed. Mater. Res, 108, 426-455, 2020
2) Pernagallo et al., Adv. Healthc. Mat., 1, 646-656, 2012

PoB.5.10

Design, fabrication and *in vivo* testing of thick human cardiac engineered tissues

María Flandes-Iparraguirre[1], Olalla Iglesias García[1], Pilar Montero-Calle[1], Andrea Sánchez[1], Ricardo M Rosales[2], Nicolás Laita[2], Elena M de-Juan-Pardo[3], Estefanía Peña[2], Esther Pueyo[2], Manuel Doblaré[2], Felipe Prósper[1,4], Manuel M Mazo[1,4]

[1]Regenerative Medicine Program, Cima Universidad de Navarra, Foundation for Applied Medical Research, Pamplona, Spain. [2]Aragón Institute for Engineering Research (I3A) University of Zaragoza, Zaragoza, Spain. [3]T3mPLATE, Harry Perkins Institute of Medical Research, QEII Medical Centre and UWA Centre for Medical Research, The University of Western Australia, Perth, Australia. [4]Hematology and Cell Therapy Area, Clínica Universidad de Navarra, Pamplona, Spain

Abstract

Introduction: Cardiovascular diseases remain the leading cause of death worldwide. Across its thickness, native myocardium is built of several layers where cardiomyocytes (CMs) are aligned to maximize contraction. Herein we aim at (1) mimicking this laminar architecture, by producing tissue engineered constructs with a preferential direction of contraction, and (2) using sequencing technology (total RNA-seq-backed spatial RNA-seq) to analyze in-depth the influence of the micro- and macro-mechanical environment, at unprecedented resolution. Methodology: Fibrous scaffolds with a diamond pattern were manufactured by melt electrospinning writing (MEW) of medical grade polycaprolactone, mechanically characterized and subsequently seeded with a mixture of 90% CMs and 10% cardiac fibroblasts, both obtained from human induced pluripotent stem cells (hiPSCs). Samples were kept in culture and fully characterized, and their beating compared to that of other pore geometries, as well as histology, staining, structure, plus electrophysiological and genomics analysis (RNAseq in bulk and spatial). *In vivo* testing was performed through transplant in athymic rats. Results: A design with diamond shaped pores was predicted to produce an in-plane contraction and accurately printed with MEW, showing adequate levels of compliance when mechanically tested. Engineered cardiac minitissues exhibited relevant cardiac-like biological features, including beating rate, sarcomere length and gene expression, as well as good viability and metabolic activity. When compared to other pore geometries, diamond-patterned scaffolds not only contracted along a preferential direction we had anticipated (that of lower mechanical resistance), but also displayed greater magnitude and velocity of contraction than squared or rectangular scaffolds. Furthermore, optical mapping of the constructs showed better electrophysiological properties for the diamond-patterned samples, with values closer to native human cardiac tissue. Genomics analysis with bulk RNAseq indicates an increased expression of genes related to cardiac maturity, lipid and calcium metabolism in the 3D tissues compared to cells cultured in 2D. Interestingly, we detected an increased expression of extracellular matrix related genes in 2D cultures compared to 3D tissues. Finally, we analyzed the fiber-reinforced samples using spatial RNAseq, of high relevance due to the different mechanical regions in the composite tissues providing novel information. *In vivo* transplantation of the tissues was also performed. Conclusion: In this work we developed a diamond patterned, MEW scaffold, showing a superior performance *in vitro* and were also tested *in vivo*, and great potential for cardiac tissue engineering and regenerative medicine applications. This information will help us devise advanced tissues with better mimicry, increasing their therapeutic capacity.

PoB.5.12

Characterisation of tobramycin-loaded tubular grafts: a potential infection-proof alternative to autologous vessels in vascular surgery

Mariella Rosalia[1], Pietro Grisoli[2], Rossella Dorati[1], Giovanna Bruni[3], Ida Genta[1], Bice Conti[1]

[1]University of Pavia, Department of Drug Sciences, Pharmaceutical Section, Pavia, Italy. [2]University of Pavia, Department of Drug Sciences, Pharmacological Section, Pavia, Italy. [3]University of Pavia, Consorzio per lo Sviluppo dei Sistemi a Grande Interfase (C.S.G.I.) & Department of Chemistry, Physical Chemistry Section, Pavia, Italy

Abstract

INTRODUCTION In this work, the fabrication and characterisation of two different types of antibiotic-loaded vascular grafts for the prevention of surgical site infections is presented. The grafts are intended as synthetic tissue engineering-suitable alternative to autologous vessel implants for reperfusion by-pass surgery in severe peripheral vascular occlusive disease. **MATERIALS AND METHODS** PR1 and PR2 graft prototypes were produced by electrospinning 12.5%w/v and a 10%w/v poly lactide-co-glycolide solutions in dichloromethane/dimethylformamide, loaded with 0.2%w/w tobramycin. A 2.5mm diameter rotating mandrel was used as collector. Uniaxial mechanical testing was performed in both longitudinal and circumferential directions to establish the prototypes' Young moduli (YM). *In vitro* drug release studies were carried out for 35 days in PBS in sink conditions, quantifying tobramycin by a spectrofluorimetric technique. Drug release kinetic modelling was performed by non-linear curve-fitting trials with DDSolver Excel add-in. Antibacterial efficiency was tested over 5 days on Staphylococcus aureus ATCC 6538 and Escherichia coli ATCC 10536 by means of a time-kill assay. **RESULTS AND DISCUSSION** Vascular grafts with an internal dimeter of 2.62±0.07mm and a wall thickness of 266±35mm and 144±12mm were achieved respectively for PR1 and PR2. Both grafts were composed by fibres in the nanometer range. YM values of PR1 were 130.7(long)/30.6(circ)MPa, whereas PR2 had YM values of 31.1 (long)/6.9 (circ)MPa. Tobramycin was successfully encapsulated in the electrospun fibres, ensuring an extended drug release that reached 86.6±15.3% for PR1 and 44.0±3.3% for PR2, within 35 days of testing. Release kinetic modelling showed that PR1 drug release profile could be described by Peppas-Sahlin model, whereas PR2 was described by Probit model, suggesting different drug release driving forces. Moreover, both release profiles showed to be effective in preventing bacterial growth, achieving a 8log reduction of microbial population after 5 days, for both bacterial strains. **CONCLUSIONS** The fabricated grafts showed to be effective in the prevention of bacterial growth, thanks to the efficient and extended local release of tobramycin. Further tuning of the YM of the grafts will be carried out to obtain optimal compliance and investigation on the hemocompatibility will be performed evaluating whole blood clotting and platelet activation.

PoB.5.13

Native fibroin-like protein as a base for the fabrication of an aortic heart valve replacement

Amanda Schmidt[1,2], Alex Greenhalgh[2], Stefan Jockenhoevel[1], Martin Frydrych[2], Alicia Fernández-Colino[1]

[1]Department of Biohybrid & Medical Textiles (BioTex), AME-Institute of Applied Medical Engineering, Helmholtz Institute RWTH Aachen University, Aachen, Germany. [2]Spintex Engineering Ltd, Oxford, United Kingdom

Abstract

Latest research on tissue-engineered valves focuses in overcoming limitations of current heart valve replacements. Promising results were obtained in the development of prosthesis reinforced by synthetic polymeric fibres, and a bioactive scaffold (Moreira, 2015). However, synthetic fibres were shown to increase stiffness of valve leaflets. Studies previously recognised fibroin as a promising biomaterial for tissue-engineering (Brif, 2020). This study focuses on the development of a native fibroin-like protein (NFLP) tubular scaffold, biomimetically reinforced, for in-situ tissue regeneration. A custom-made device was designed for the fabrication of the scaffolds. Photosensitivity was introduced to NFLP solution, provided by Spintex Engineering Ltd, through the incorporation of Riboflavin, as previously described (Brif, 2020). Scaffolds were produced from NFLP by depositing the protein solution onto a rotating and axially reciprocating mandrel, and NFLP fibres were embedded by winding onto the structure's surface. Samples were stabilised through UV crosslinking and 70% Ethanol treatment. Fibre reinforced scaffolds were characterized through tensile tests (Zwick Roell, Zwicki, 20N load cell). Samples were tested in the radial and circumferential directions (n = 5 for each condition). Fibre reinforced scaffolds were produced by depositing NFLP solution and fibres onto a rotating and axially reciprocating mandrel. The fabrication approach enabled to finely controlled the fibre angle and the fibre density. Tensile testing of the resulting scaffold allow us to correlate the disposition of the fibres and the mechanical properties. Specifically, we frame the conditions required for mimicking native-like anisotropy of the aortic native heart valve. Cytocompatibility of primary human smooth muscle cells was assessed through an indirect test, in accordance with ISO-10993-12:2019 guidelines (International Organisation for Standardisation, 2019). The smooth muscle cells presented excellent viability after 24 hours incubation with extracted media, showing a well-spread morphology and high metabolic activity, consistent with previous studies of protein-based scaffolds (Sanchez-Ferrero et al., 2015). The methodologies presented showed ease of manufacturing, ability to fine tune and native-like mechanical performance. Next steps include further hydrodynamic and biological analyses for proof-of-concept of the in situ tissue engineering capabilities of the designed scaffold.

REFERENCES
Moreira R., (et. al.) Tissue Engineering - Part C: Methods. 21(6):530-540, 2015.
Brif, A., (et. al.) ACS. 6(1), 705–714, 2020.
Balguid, A. et al. Tissue Engineering. 13(7), 2007.
Sanchez-Ferrero. et al., Biomaterials. 68, 42-53, 2015.

ACKNOWLEDGEMENTS
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Skolodowska-Curie grant agreement ID: 956621.

PoB.5.14

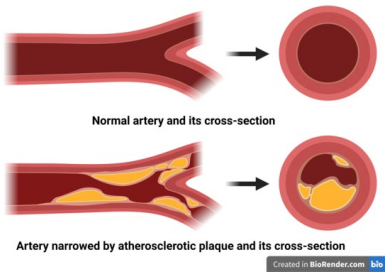
Everolimus loaded pluronic P123 self-assembled micelles as a potential drug delivery system for drug-eluting balloons

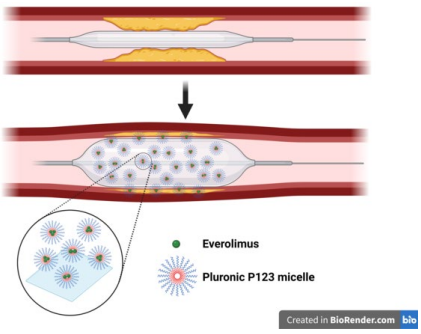
Mohammad AKRAMI-HASAN-KOHAL, Tahmer SHARKAWI

ICGM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

Abstract

INTRODUCTION Atherosclerosis is a common cause of cardiovascular disease that affects many individuals yearly [1] (Fig. 1). Drug-eluting balloons (DEBs) have been developed to deliver drugs to promote healing and inhibit the proliferation of smooth muscle cells in the vessel wall. Unfortunately, current DEBs have limitations like drug washout and suboptimal delivery [2, 3]. To address these issues, we are studying self-assembled micelles made from Pluronic P123 (P123) loaded with Everolimus (EV) as a controlled drug delivery system for DEBs. Our goal is to improve drug delivery to the treatment site and enhance the effectiveness of DEBs in treating atherosclerosis. *Fig. 1. A schematic representation of a normal artery and an artery narrowed by atherosclerotic plaque, including cross-sectional views. Created with BioRender.com.* **EXPERIMENTAL METHODS** Balloon material specimens were coated with -loaded P123 with various P123 to EV ratios. Initially, P123 and EV were dissolved in EtOH at a concentration of 10% (W/V). Subsequently, the prepared formulations were used to coat the balloon material samples through micropipetting, with P123 to EV ratios of 90:10, 75:25, and 50:50. The coated films were then analyzed by SEM and DSC to examine surface morphology and the miscibility of the EV-loaded P123 and control P123 coatings, respectively. Moreover, self-assembled micelles were characterized through TEM and DLS to determine the size and dispersity of the micelles. The drug-elution studies were conducted using HPLC UV-Vis to evaluate drug release behavior. **RESULTS AND DISCUSSION** The present study has revealed that the use of EV-loaded P123 coatings can be used as a micelle drug delivery system for drug-eluting balloons (Fig. 2). *Fig. 2. The concept of Everolimus loaded Pluronic P123 self-assembled micelles as a potential drug delivery system for Drug Eluting Balloon (). Created with BioRender.com.* **CONCLUSION** This study suggests using P123 as a coating for drug-eluting balloons (DEBs) to deliver drugs to the arterial wall. This approach may mitigate the issue of drug loss during balloon tracking and improve drug distribution during balloon inflation and treatment, leading to more precise and effective drug delivery for vascular diseases.





REFERENCES

1. Woolford, Steven E., et al. International Journal of Pharmaceutics 554 (2019): 312-321.
2. Huang, Li, et al. Bioactive Materials 23 (2023): 526-538.
3. Anderson, Jordan A., et al. Acta biomaterialia 29 (2016): 333-351.

PoB.5.16

Enhancing the endothelialization of bare metal stents with endothelial colony forming cells

Isra Marei[1,2], Matthias Gabriel[3], Blerina Ahmetaj-Shala[1], Omar Chidiac[2], Binitha M Thomas[2], Charbel Abi Khalil[2], Ramzi Khamis[4], Jane Mitchell[1], Chris Triggle[2]

[1]Imperial College London, London, United Kingdom. [2]Weill Cornell Medicine Qatar, Doha, Qatar. [3]Universitätsmedizin Berlin, Berlin, Germany. [4]Imperial College London, London, Qatar

Abstract

Introduction: In-stent thrombosis can lead to stent failure following percutaneous coronary intervention, and the incidence is doubled in diabetes. Late stent thrombosis could be caused by the delayed endothelialization of the stent lumen. Biofunctionalization using specific molecules to induce endothelialization can prevent thrombosis. The endothelium has a key role in maintaining homeostasis. Restoring a functional endothelium is important to prevent stent thrombosis. Blood endothelial progenitor cells (EPCs) represent a potential source for in situ-endothelialization of vascular stents. In this study, we biofunctionalized bare metal stents (BMS) using the EPCs specific peptide (TPSLEQRTVYAK)(1). Methods: Cobalt chromium stents were dip coated with polycaprolactone (5%). Coated stents were aminated using 20% ethylendiamine. NHS/maleimide crosslinker was added followed by TPSLEQRTVYAKGGGSC(2). FITC-tagged peptide was used to confirm peptide immobilization. Endothelilization of stents was tested using endothelial colony forming cells (ECFCs) isolated from type 2 diabetes patients. Results: Immobilization of FITC-tagged peptide on stents produced homogenous fluorescent signal indicating a successful modification. Proliferation of ECFCs was enhanced on modified stents when compared to untreated and NHS/Maleimide controls following 4 and 7 days of culture as estimated by AlamarBlue ($p=0.0002$ and $p=0.02$ at 4 and 7days respectively, ANOVA, n=4; OD 570-620 for UT, NHS/Maleimide and TPS stents at day 4 were: 0.18±0.03, 0.11±0.02, and 0.29±0.01; ODs at day 7 were: 0.13±0.03, 0.09±0.03 and 0.25±0.02 respectively). Staining with phenotypic/structural markers including CD31, VEGFR2 and phalloidin revealed an enhanced coverage of the modified stents with ECFCs when compared to untreated stents. Conclusion: Biofunctionalization of PCL coated BMS with the EPC specific peptide TPSLEQRTVYAKGGGSC through covalent crosslinking resulted in induced proliferation of diabetic ECFCs and full coverage of the stents by day 14 of culture. Studies are ongoing to validate the functionality of the stents and their interaction with platelets. Acknowledgments: This study was approved by WCMQ IRB board (IRB:22-00005, approved 31 March 2022). This work was made possible by an Early Career Researcher Award ECRA02007-3-006 from the Qatar National Research Fund (a member of The Qatar Foundation).

PoB.5.17

On the way to develop a degradable small diameter vascular graft: *in vitro* characterization, *in vivo* degradation and graft production

Duarte Moura[1,2,3], Helena P. Ferreira[1,2,4], Sabrina Rohringer[5,6], Andreia T. Pereira[1,2], Cristina C. Barrias[1,2,4], Fernão D. Magalhães[7,8], Petra Mela[9], Helga Bergmeister[5,6], Inês C. Gonçalves[1,2]

[1]INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen, 208, 4200-180, Porto, Portugal. [2]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208, 4200-180, Porto, Portugal. [3]FEUP – Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e de Materiais, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal. [4]ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4050-313, Porto, Portugal. [5]Center for Biomedical Research and Translational Surgery, Medical University of Vienna, Vienna, Austria. [6]Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria. [7]LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal. [8]ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal. [9]Medical Materials and Implants, Department of Mechanical Engineering. Munich Institute of Biomedical Engineering, TUM School of Engineering and Design, Technical University of Munich, Garching, Munich, Germany

Abstract

Small diameter vascular substitutes are often required to reestablish blood flow in blocked arteries. However, there are no available synthetic small diameter vascular grafts (SDVG)(I.D.<6 mm) with satisfying patency rates[1]. Poly(2-hydroxyethyl methacrylate) (pHEMA), a bio/hemocompatible and biostable hydrogel, has been explored together with graphene-based materials to improve the mechanical properties of SDVGs[2]. Herein, we propose to turn pHEMA in combination with graphene oxide into a degradable hydrogel to fabricate a degradable electrospun SDVG. Degradable pHEMA (dpHEMA) films were produced by adding pentaerythritol tetrakis(3-mercaptopropionate) (0.25% v/v) to a previously described pHEMA formulation[3], with 1wt.% few-layer graphene oxide with 5µm lateral size (dpHEMA/M5ox). Tensile tests were performed using a A.XTplus texture analyser and hydrophilicity by optical contact angle measurements. Degradation studies were performed in PBS over 6 months (6M) (37ºC;100rpm). Cytotoxicity of degradation products (24h and 6M) was evaluated with endothelial cells (HUVECs), assessing metabolic activity and morphology. Hydrogels' non-fouling character was evaluated by direct seeding of HUVECs ($1x10^6$cells/mL), human platelets ($3x10^8$platelets/mL) and S. aureus ($1.3x10^6$bacteria/mL). For *in vivo* assays, scaffolds were implanted subcutaneously in Sprague Dawley rats. At 1M, 3M, 6M, animals were sacrificed and retrieved hydrogels evaluated by H&E, immunohistochemistry and RT-qPCR. Conduits production was tested by electrospinning using 4-27.5%(w/v) hydrogel dispersions in different solvents at tip-collector distance of 9-15cm and flow rates of 1-9mL/h. *In vitro* results showed that inert pHEMA hydrogel was turned into a degradable material by the incorporation of tetrakis, and presence of 1wt.% M5ox increased the tensile strength up to 0.2MPa, while maintaining water uptake, wettability, cytocompatibility (short and long term), and non-fouling behavior of neat pHEMA. Regarding the *in vivo* implantation, H&E of 6M implants showed no inflammation in the implant area. Immunohistochemistry revealed dpHEMA and dpHEMA/M5ox are highly anti-adhesive, with no cells present in the hydrogels. CD80 and CCR7 (pro-inflammatory macrophages) as well as CD206 (anti-inflammatory macrophages) were down-regulated after 6M for dpHEMA and dpHEMA/M5ox. Cytokine gene expression demonstrated a similar response. Maneuverable conduits (length=4cm; wall

thickness=100µm; I.D.=5mm) were achieved by electrospinning using a 17.5%(w/v) hydrogel dispersion in ethanol, 5mL/h and a tip-collector distance of 15cm. Altogether, these results proved dpHEMA/M5ox as a promising biomaterial for fabrication of electrospun SDVGs.

[1] M.A. Hiob et al. ACS Biomater Sci Eng (2017) 712-723.
[2] A.T. Pereira et al. Biomaterials Science (2021) 3362-3377.
[3] I.C. Goncalves et al. Biomaterials (2009) 5541-51.

PoB.6.01

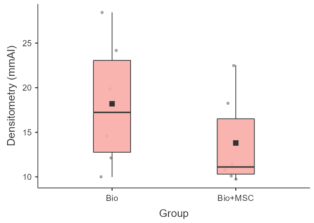
Radiographic analysis of chitosan, hydroxyapatite, and carbon nanotube composite with or without mesenchymal stem cells in critical size defects in sheep tibia after four months

Thamires SPM Koga[1], Anderson F Souza[1], Danielli CB Silva[1], Grazieli CM Silva[1], Marília N Cardoso[1], Geissiane M Marcondes[1], Nicole F Paretsis[1], Joice Fülber[2], Beatriz T Maia[1], Silvia RG Cortopassi[1], Ana MG Plepis[3], Virginia CA Martins[3], André LV De Zoppa[1]

[1]Department of Surgery, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, SP, Brazil. [2]Department of Clinical Medicine, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, SP, Brazil. [3]Department of Chemistry and Molecular Physics, Institute of Chemistry of São Carlos - University of São Paulo, São Paulo, SP, Brazil

Abstract

The use of composite biomaterials to aid bone regeneration is an efficient strategy in cases of large defects in long bones, where the association with stem cells can reduce repair time. Radiographic evaluation associated with optical densitometry is a non-invasive method that makes it possible to investigate the characteristics of the repair of these defects. The objective of this study was to radiographically analyze the repair of critical size defects in sheep tibia treated with a chitosan, hydroxyapatite, and carbon nanotube composite associated or not with mesenchymal stem cells (MSC) after four months. Twelve adults Suffolk ewes were undergoing a 3 cm osteotomy in the right tibial shaft, fixed by a 4.5 mm narrow locking compression plate. Two groups were formed (n = 6): Group Bio, implanted of chitosan, hydroxyapatite, and carbon nanotube composite, with the same dimensions of the removed bone segment, and group Bio+MSC, implanted the same composite, associated with the application of MSC of bone marrow, four weeks after the procedure, guided by ultrasound. At 120 days, radiographs (68 kVp, 1.5 mAs, 30 mA) were taken in the mediolateral and caudocranial projections. Based on the images, a description of the radiographic appearance and radiographic optical densitometry (ROD) were performed. This was made by converting the pixel intensity of the bone defect area (8-bit image) into aluminum millimeters (mmAl), using a standardized scale attached to the detector plate during the radiographic examination. The ROD data were compared between groups using Student's T test and the effect size was estimated using Cohen's D test. P-values < 5% were considered significant. The mean ROD for the group Bio was 17.23±7.21 mmAl (95% CI [10.62 - 25.76]) and for the group Bio+MSC it was 11.11±5.31 mmAl (95% CI [8.23 – 19.37]), being similar to each other (p = 0.257). The effect size was moderate (0.69). The radiographic aspect varied among the animals, with no pattern being identified within each group, with some showing the defect area almost completely filled with radiopaque tissue close to the bone, others only with focal points with this characteristic. Also, animals with little radiopacity in the region of the defect and rounded edges of the bone stumps were observed. It is concluded that the composite used produced, in most animals, radiographic characteristics compatible with the bone repair process and this seems not to have been different when MSCs were associated.



PoB.6.02

Customized PCL-based biomaterial effectiveness in the regard of osteoblastic cells

Benedetta Ghezzi[1,2], Matteo Meglioli[1], Biagio Matera[1], Donatella Duraccio[3], Maria Giulia Faga[3], Francesca Rossi[2], Andrea Zappettini[2], Guido Maria Macaluso[1,2], Simone Lumetti[1,2]

[1]Centro Universitario di Odontoiatria, Dipartimento di Medicina e Chirurgia, Università di Parma, Parma, Italy. [2]IMEM-CNR, Consiglio Nazionale delle Ricerche, Parma, Italy. [3]Istituto di Scienze e Tecnologie per l'Energia e la Mobilità Sostenibili, Consiglio Nazionale delle Ricerche, Torino, Italy

Abstract

INTRODUCTION: Bone regeneration requires peculiar biomaterials showing features as biodegradability, interconnectivity, surface wettability and resistance, which together play a pivotal role in the clinical performance of the material itself. β-tricalcium phosphate (β-TCP) and Poly-Ɛ-caprolactone (PCL) are biocompatible and degradable materials suitable for the development of three-dimensional scaffolds able to be used in tissue regeneration. The objective of this study is to optimize printable PCL/β-TCP composite biomaterials with a high percentage of β-TCP and presenting balanced mechanical characteristics to resemble human cancellous bone, maintaining optimal strength and eliciting positive cellular responses. METHODS: PCL/β-TCP scaffolds have been obtained starting from filaments suitable for 3D fused deposition modelling (FDM) previously obtained by medical grade PCL and β-TCP powders. Relative ratios of 100%/0% (control) and 30%/70% PCL/β-TCP have been used for FDM printing to obtain cylindrical scaffolds with 5mm in diameter and 2mm in height. Samples mechanical characteristics and surface topography have been evaluated as well as surface wettability. Moreover, cytocompatibility assays have been performed with osteoblast-like murine cells (MC3T3-E1) as well as cell adhesion and cell differentiation investigations. RESULTS: The settled parameters were optimal for the creation of 100% PCL and 30%/70% PCL/β-TCP filament and scaffolds. Composite surface shows a higher hydrophilicity when compared to the control ($p=0.0172$) and surface roughness appear sharply increased due to the presence of β-TCP powder. Mechanical tests underline how the Young's Modulus of the composite was higher than that of pristine PCL, shifting from 577 MPa to 2320 MPa, more than four times higher than that of unfilled PCL. In terms of biological behaviour, the biomaterials act as suitable surfaces for osteoblastic proliferation and differentiation underlining a higher cellular growth and a stronger osteoblastic differentiation on the composite material if compared to the control. Microscopy images allow to observe the specific surface topography of the biomaterial, that seems to be adequate for osteoblastic adhesion, which occurs preferentially over the embedded salt granules peaks. DISCUSSION & CONCLUSIONS: 30%/70% PCL/β-TCP composite material possess optimal printing properties and elicited an enhanced cellular response in the regards of both osteoblastic proliferation and differentiation. Moreover, the presence of β-TCP dramatically enhanced the intrinsic strength of the scaffolds and their hardness, leading the structure to be as similar as possible to the one of trabecular bone. Taken together our data show that 30%/70% PCL/β-TCP composite biomaterial might be an adequate candidate for bone regenerative applications.

PoB.6.03

Bioresorbable and bioactive polycaprolactone/silicate, zinc Co-substituted hydroxyapatite nanocomposites with enhanced mechanical properties

Tim Kreuz[1], Marcus Gaiser-Porter[1], David Zermeño Pérez[2], Ruth E Cameron[1], Serena M Best[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]Ashland Specialties Ireland Limited, Mullingar, Ireland

Abstract

Introduction Composites comprising a biodegradable polymer matrix reinforced with bioactive ceramic filler particles are promising materials for bone tissue engineering. The ability to disperse the filler particles, avoiding agglomeration, is critical for the composite properties and processability. The objective of this work is to explore the incorporation of silicon and zinc substituted hydroxyapatite (Si-Zn-HAp) into a bioresorbable polycaprolactone (PCL) matrix using a facile antisolvent precipitation method to achieve homogenous particle dispersion.[1][2] **Experimental Methods** Si-Zn-HAp was synthesised using a wet precipitation method. PCL/Si-Zn-HAp nanocomposites with 10 and 20 wt% filler content were prepared by precipitating dissolved polymer directly onto suspended HAp nanoparticles through a stepwise reduction of solvent power by the addition of a solvent-miscible antisolvent (Figure 1). Micro-injection moulding and 3D printing were used to prepare samples for further characterisation. The filler loading after processing was determined using thermogravimetric analysis (TGA). The particle dispersion and tendency for agglomeration were evaluated using scanning electron microscopy and micro computed tomography (µCT). Thermal properties and crystallinity of the composites were assessed using differential scanning calorimetry (DSC) and X-ray diffraction (XRD). Mechanical properties were determined using tensile testing. **Results and Discussion** PCL/Si-Zn-HAp nanocomposites produced using the precipitation method and subsequent injection moulding showed a well dispersed particulate phase (Figure 2) and a filler weight fraction that matched well with nominal values (maximum deviation 0.9 %). Particle agglomeration was prevented through the rapid nucleation of PCL on the suspended nanoparticles that occurred after supersaturation of the solvent-antisolvent system was achieved. Characteristic reflections of semicrystalline PCL and hexagonal HAp could be detected in the XRD patterns of the composites (Figure 2). The addition of Si-Zn-HAp led to significant mechanical reinforcement, with an increase in the elastic modulus from 129.4 ± 5.11 MPa for PCL to 216.3 ± 11.73 MPa for the 20 wt% composite.

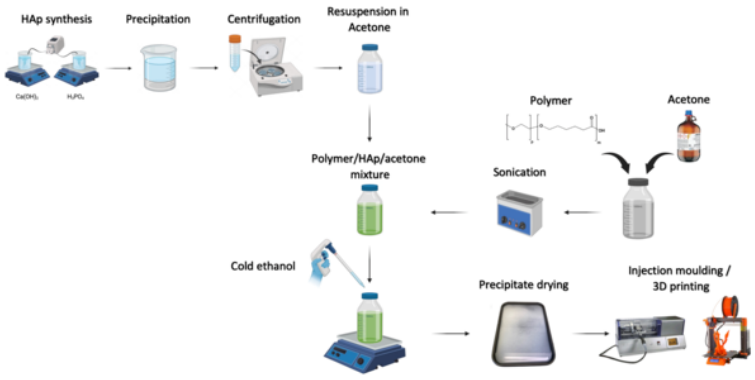


Figure 1: Schematic of composite antisolvent precipitation. HAp is synthesised using a wet precipitation method, centrifuged, and resuspended in acetone. PCL is dissolved in acetone. HAp/acetone slurry is added to PCL solution to establish a defined PCL/HAp ratio. Antisolvent is added to precipitate PCL directly onto suspended HAp particles. Composites are further processed using µ-injection moulding and 3D printing.

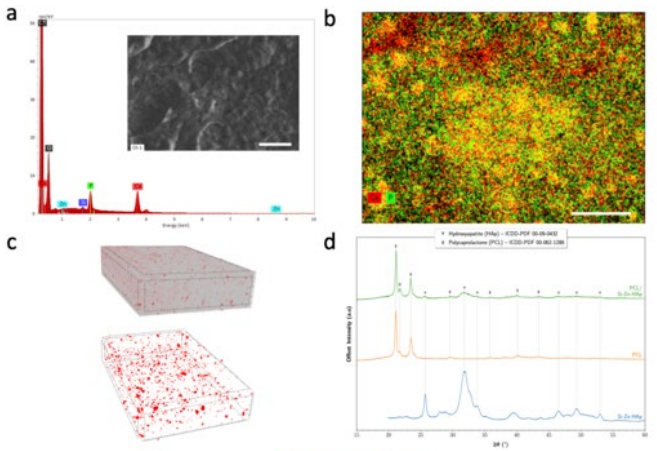


Figure 2: EDS spectrum (a) and calcium/phosphorous map (b) of cryo-fractured surface for PCL/Si-Zn-HAp (80/20) showing a homogenous dispersion of filler particles (Scale bar: 20 µm). X-ray µCT-based volume renders of a 0.8 mm x 1.2 mm x 0.2 mm slice of PCL/Si-Zn-HAp (80:20) (c). Offset diffraction patterns of nanocrystalline Si-Zn-HAp (blue), PCL (Orange), and PCL/Si-Zn-HAp (Green) are shown in (d).

Conclusion Nanosized Si-Zn-HAp was prepared using a wet precipitation method and incorporated successfully into a PCL matrix using an antisolvent precipitation method. Rapid precipitation of the PCL onto ceramic particles ensured homogenous dispersion of the particulate phase leading to significant mechanical reinforcement and potential to create uniform 3D printed structures for bone tissue engineering applications.

References

[1] R. J. Friederichs et al., *J. R. Soc. Interface, Vol. 12, No. 108* (**2015**) 20150190

[2] R. N. Oosterbeek et al., *J. Mech. Behav. Biomed. Mater., Vol. 123* (**2021**) 104767

PoB.6.06

Characterisation of Poly(3-hydroxybutyrate)/Graphene oxide (P(3HB)/GO) composite films and scaffolds for bone tissue engineering

Soponvit Larpnimitchai, Andrea Mele, Ipsita Roy

University of Sheffield, Sheffield, United Kingdom

Abstract

Introduction: Burkholderia sp. was found to produce polyhydroxyalkanoates (PHAs) using different of carbon sources such as fatty acids, sucrose and glucose. Recently, PHAs have been used as biological materials because of their characteristics such as biodegradability, biocompatibility and sustainable materials. Moreover, GO has been used to improve the properties of the material such as the mechanical properties and cell viability. This study aims to develop the scaffolds using PHAs and its composites to achieve suitable mechanical, physical and chemical properties required for bone tissue engineering. Methods: Neat P(3HB) and P(3HB)/GO films were prepared using solvent casting method. P(3HB)/GO composites were prepared in proportions of 1, 2, 5 and 10% w/w and aged for 1-month prior to further characterisation. Wettability of the films were determined using water contact angle (WCA). Morphology of the films were also determined using scanning electron microscope (SEM). Mechanical tensile testing was used to verify the properties of neat P(3HB) and P(3HB)/GO composite films. Moreover, neat P(3HB) and P(3HB)/GO scaffolds were prepared using 3D printing method. P(3HB)/GO composite scaffolds were prepared and optimized in proportions of 1, 2, 5 and 10% w/w and further characterised using mechanical tensile testing. Results: Wettability analysis confirmed that the hydrophobicity of the samples was decreased when adding the GO. The morphology of film samples were no significant different when adding GO in the P(3HB). The wt% of GO in P(3HB) also affected the mechanical properties of the composite films, leading to tailorable mechanical properties. The mechanical properties of P(3HB)/1%GO aged 1-month film exhibited the highest Young's modulus which was around 1300 MPa, suitable for cortical bone repair. Furthermore, mechanical properties of P(3HB)/GO composite scaffolds presented higher than the P(3HB)/GO composite films. Discussion & Conclusions: In this study we studied an in-depth characterisation of neat P(3HB) and P(3HB)/GO composite films and scaffolds to be used in the bone tissue engineering applications. The properties were found to be suitable for various processing techniques and could lead to bespoke mechanical properties depending on the filler concentration. In future these composites will be tested for their biocompatibility and antimicrobial properties.

PoB.6.07

MXene-based magnetic composites for highly effective enrichment of phosphopeptides

Lingzhu Yu, Yicheng Liu, Fang Lan, Yao Wu

National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, China

Abstract

In vitro diagnosis is a non-invasive detection method with enormous application prospects in prevention and early diagnosis of diseases. Reversible protein phosphorylation, as a ubiquitous post-translational modification, plays vital role in regulating multiple biological processes and related to various diseases. However, direct detection of phosphoproteins/peptides by Mass Spectrum (MS) faces great challenges. Therefore, effective separation and enrichment of phosphopeptides prior to MS analysis is the prerequisite to research phosphoproteomics-related biological and pathological processes. MXenes, a new family of two-dimensional materials, have been applied to construct a novel phosphopeptides enrichment platform (denoted as $Ti_3C_2T_x$@PAMAM@Fe_3O_4) approaching to selective enrichment of phosphopeptides with excellent enrichment specificity and efficiency. EXPERIMENTAL METHODS 2D and 3D $Ti_3C_2T_x$ were separately obtained through selectively etching Ti_3AlC_2 and further delaminating. Subsequently, PAMAM dendrimer was modified on the surface of $Ti_3C_2T_x$ MXene for further immobilizing Fe_3O_4 nanoparticles through a layer-by-layer surface modification method. Then, the prepared $Ti_3C_2T_x$@PAMAM@Fe_3O_4 were added to the tryptic digested standard proteins and complex biological samples. After incubation, the composites were magnetic separation, following washing and eluting. The elution was collected and analyzed by MS. RESULTS AND DISCUSSION SEM and TEM characterizations indicated the prepared $Ti_3C_2T_x$@PAMAM@Fe_3O_4 composites presented well morphology and dispersibility. Zeta potential, FT-IR, XPS and TGA measurements proved the successful preparation of each step. VSM measurement showed the $Ti_3C_2T_x$@PAMAM@Fe_3O_4 composites had well saturation magnetization, contributing to rapid magnetic separation.

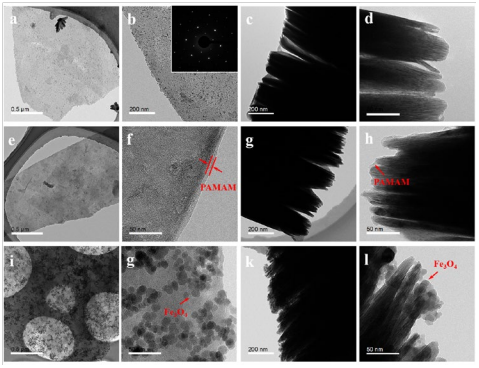


Figure 1. TEM images of 2D and 3D $Ti_3C_2T_x$ (a-d), $Ti_3C_2T_x$@PAMAM (e-h) and $Ti_3C_2T_x$@PAMAM@Fe_3O_4 composites (i-l).

The enrichment results showed that the $Ti_3C_2T_x$@PAMAM@Fe_3O_4 composites possessed high sensitivity (1 fmol/μL), excellent selectivity (β-casein: BSA=1:800), large enrichment capacity (200 mg/g) and great practicability in identifying low-abundance phosphopeptides from nonfat milk, human serum, human saliva and rat brain lysate. More importantly, the 2D and 3D $Ti_3C_2T_x$@PAMAM@Fe_3O_4 composites exhibited dimension-dependent enrichment performance.

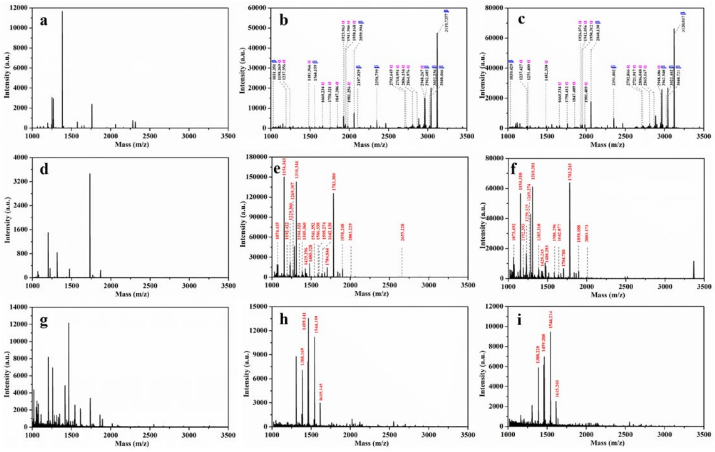


Figure 2. Mass spectra of nonfat milk, human saliva and serum before enrichment (a, d, g) and after enrichment by 2D (b, e, h) and 3D (c, f, i) $Ti_3C_2T_x$@PAMAM@Fe_3O_4 composites.

CONCLUSIONS The $Ti_3C_2T_x$@PAMAM@Fe_3O_4 composites were ingeniously designed and prepared through a facile layer-by-layer modification method with decreased time and chemicals consumption for possible large-scale production and application. The as-prepared $Ti_3C_2T_x$@PAMAM@Fe_3O_4 composites possessed the excellent properties of large specific surface areas, magnetic response and dispersibility, exhibiting high enrichment efficiency and great practicability in both trypsin digested standard proteins and complex biological samples. The work held great promise for future practical applications in comprehensive phosphopropteomics analysis.

PoB.6.09

Bacterial cellulose/Polyhydroxyalkanoate composite-based patches for bone tissue engineering

Zeming Cheng[1], Jonathan David Hinchliffe[1], Soponvit Larpnimitchai[1], Mahendra Raut[2], Ipsita Roy[2]

[1]University of Sheffield, Sheffield, United Kingdom. [2]SHEFFIELD, SHEFFIELD, United Kingdom

Abstract

Bacterial cellulose (BC) is a natural polymer that has low biotoxicity, strong hydrophilicity, good cell adherence, and a relatively simple production methodology in comparison to some other natural materials. Hence, BC has great potential as a tissue engineering material. Also, BC is one of the stiffest materials, hence it is highly suitable for bone tissue engineering. Poly(3-hydroxy octanoate-*co*-3-hydroxy decanoate), P(3HO-*co*-3HD) is a bacterial-origin polymer from the family of middle-chain-length polyhydroxyalkanoates (mcl-PHAs), which has non-immunogenicity, high biocompatibility, and biodegradability. This work detected BC hydrogel's capacity of P(3HO-*co*-3HD) and evaluated whether the composite patch could be applied to bone tissue engineering. The composite patches were produced from acetone-swollen BC membranes and different concentrations of P(3HO-*co*-3HD) solutions. The composites were characterized by Thermogravimetric Analysis, Scanning Electron Microscope, Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Water Contact Angle test, and mechanical tests including Atomic Force Microscopy and Tensile testing. The composites were seeded with MG63 cells for biocompatibility testing. Finally, BC hydrogels were proved to have huge P(3HO-*co*-3HD) capability, and the composite's mechanical properties were improved in comparison to pure BC and P(3HO-*co*-3HD). The patches were observed of high biocompatibility and turned out to be good candidates for bone tissue engineering.

PoB.6.10

MRI-visible and multifunctional melt-electrowritten scaffolds for tissue engineering applications

Salma Mansi[1], Kilian M.A. Mueller[1], Geoffrey J Topping[2], Zahid Hussain[3], Sarah Dummert[3], Carolin Rickert[4], Sebastian Schwaminger[5], Sonja Berensmeier[5], Roland Fischer[3], Oliver Lieleg[4], Franz Schilling[2], Petra Mela[1]

[1]Chair of Medical Materials and Implants, Department of Mechanical Engineering, Munich Institute of Biomedical Engineering and TUM School of Engineering and Design, Technical University of Munich, Garching, Germany. [2]Department of Nuclear Medicine, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany. [3]Chair of Inorganic and Organometallic Chemistry, TUM Department of Chemistry, Technical University of Munich, Garching, Germany. [4]Biomechanics, Department of Materials Engineering, TUM School of Engineering & Design, Technical University of Munich, Garching, Germany. [5]Chair of Bioseparation Engineering, TUM School of Engineering and Design, Technical University of Munich, Garching, Germany

Abstract

Introduction Melt electrowriting (MEW) is a powerful additive manufacturing technique for the fabrication of complex 3D scaffolds. MEW has proven its potential in numerous *in vivo* studies. Successful clinical translation of such constructs will, inter alia, require the compatibility with a clinically accepted imaging option, such as magnetic resonance imaging (MRI). However, current scaffolds from the MEW gold standard material polycaprolactone (PCL) are not suitable as they suffer from low MRI contrast. Therefore, we present strategies to obtain PCL-based composites with improved imaging capabilities and, additionally, antibacterial properties, by exploiting ultrasmall superparamagnetic nanoparticles (UPSIOs) and metal-organic frameworks (MOFs). Methods PCL composites with two types of functional additives were produced via a solvent route: Firstly, USPIO nanoparticles, and, secondly, an iron-based, silver-loaded MOF were embedded in PCL at different weight percentages. MEW parameters were optimized to result in accurately printed scaffolds that were evaluated via scanning electron microscopy. Pure PCL and composite scaffolds were embedded in agar for MRI and visualized with a 7 T magnet and 10 mm RF solenoid coil. T1-T2*w images, and R2, R2*, and R1 maps were acquired. Cytocompatibility was assessed via indirect eluate testing with smooth muscle cells following ISO 10993. PCL/MOF composites were additionally evaluated for antibacterial efficacy against the strains S. epidermidis, S. aureus, and E. coli by plating serial dilutions of bacterial suspensions on incubated samples and counting colony forming units (CFU). Results PCL composites with up to 0.3% USPIOs and with up to 20% MOFs were successfully processed via MEW. Both scaffold groups showed consistent fiber diameters and accurate layer stacking. PCL/USPIO composites were cytocompatible when tested according to ISO10993. In case of the MOF composite, 5% loading was found to be an optimal concentration, as the resulting scaffolds exhibited antibacterial efficacy against S. aureus, E. coli and S. epidermidis with an average reduction of 99.5%, while maintaining the PCL cytocompatibility. In MRI visualization, both strategies lead to up to ten-fold increased R2* relaxation rates compared to pure PCL scaffolds. Therefore, composite scaffolds presented strong hypointense contrast. Conclusions This work contributes to expanding the biomaterial library for MEW by demonstrating the potential of PCL composites to enable efficient visualization of complex scaffold architectures via the clinically established and non-invasive imaging method MRI. Although in this work exploited for the added antibacterial efficacy, MOFs provide an exciting material platform for the design of multifunctional scaffolds with a wide range of applications.

PoB.6.13

All-cellulose composites made from wood-based textiles using NaOH-urea solvent system

Eija-Katriina Uusi-Tarkka[1], Mikael Skrifvars[2], Antti Haapala[1], Pooria Khalili[2], Henrik Heräjärvi[3], Nawar Kadi[2]

[1]University of Eastern Finland, Joensuu, Finland. [2]University of Borås, Borås, Sweden. [3]Natural Resources Institute Finland, Joensuu, Finland

Abstract

All-cellulose composites (ACCs) are manufactured using only cellulose as a raw material. Biodegradable composites are alternatives to petroleum-based composites that can be used in many life-science applications. Textile-based ACCs can be used as break, strains, and bone protection or as shin pads or groin guards. In this study, an aquatic NaOH-urea solvent system was used to produce sustainable ACCs from wood-based woven textiles. Wood-fiber-based fabrics containing Lyocell yarn in the warp and Spinnova–Lyocell (60%/40%) yarn in the weft, are used to form unidirectional all-cellulose composites (ACC) through partial dilution in a NaOH–urea solution. The aim is to investigate effects of dissolution time, temperature during hot press, and the role of the yarn orientation in composites and how these affects the mechanical and thermal properties of the composites. As a reference, thermoplastic biocomposites were prepared from the same fabrics, with biobased polypropylene (PP) as the matrix. We also compared the mechanical and thermal properties of the ACC and PP biocomposites. The results showed a significant change in the tensile properties of the layered textile composite at dissolution times of 30 s and 1 min, while ACC elongation was the highest after 2 and 5 min. Changes in hot press temperature from 70 °C to 150 °C had a significant effect: with an increase in hot press temperature, the tensile strength increased and the elongation at break decreased. The study found no significant difference in tensile strength regarding the Spinnova–Lyocell orientation between ACC and PP biocomposites, while the composite tensile strength was clearly higher in the warp (Lyocell) direction for both composite variants. Elongation at break doubled in ACC in the Lyocell direction compared with the other samples. Thermal analysis showed that mass reduction started at a lower temperature for ACC, but the thermal stability was higher compared with the PP biocomposites. Maximum thermal degradation temperature was measured as being 352 ◦C for ACC and 466 ◦C for neat PP, and the PP biocomposites had two peaks in the same temperature range (340–474 ◦C) as ACC and neat PP combined. ACCs absorbed 93% of their own dry weight in water in just one hour, whereas the PP biocomposites BC2 and BC4 absorbed only 10% and 6%, respectively. The study highlights the different properties of ACC and PP reference biocomposites that could lead to further development and research of commercial applications for ACC.

PoB.6.14

Effect of dual-ring composite chitosan/brushite scaffolds enriched with Ce-oxide nanoparticles on human periodontal ligament cells viability

Maria Bousnaki[1], William Oates[2], Ioannis Tsamesidis[1], Anastasia Beketova[1], Antonios Anastasiou[2], Eleana Kontonasaki[1]

[1]Aristotle University of Thessaloniki, Thessaloniki, Greece. [2]The University of Manchester, Manchester, United Kingdom

Abstract

The development of advanced biomimetic composite scaffolds that combine the biodegradability of polymers with the antibacterial, angiogenic, and osteogenic capacities of certain metal oxide nanoparticles paves the way for new tissue regeneration approaches. In the present study, dual-ring cylindrical composite scaffolds were fabricated for periodontal and bone tissue regeneration, and their biocompatibility was evaluated with human periodontal ligament cells (hPDLCs). For the fabrication of the chitosan scaffolds, brushite powder was dispersed in a 2% v/v aqueous acetic acid solution and sonicated for 10 min for homogenous dispersion of the particles. Medium molecular chitosan powder was added to the solution (3%wt/v) and left to dissolve under stirring at 50 °C. The chitosan/mineral mixture was injected into appropriate molds (to form the inner ring) and placed in a freezer overnight (−20 °C) before being freeze-dried for 24 h. A similar procedure was followed for the outer ring but instead of brushite, Ce-oxide nanoparticles were used. Biocompatibility of the fabricated scaffolds was assessed via the MTT assay with the use of hPDLCs, while quantitative polymerase chain reaction (qPCR) for specific osteogenesis-related genes (such as BMP2, RUNX, BGLAP, and ALPL) was performed. Prior to cell culture experiments, scaffolds were treated with 1M NaOH for 1 min, followed by washes with PBS. Sterilization was performed in 70% ethanol solution for 15 min, followed by UV irradiation for 30 min. Scaffolds were then placed in 24-well plates and incubated at 37 °C and 5% CO_2 overnight in complete culture medium (CCM= high glucose DMEM with 10% fetal bovine serum and 1% penicillin, streptomycin) to allow pH adjustment and serum protein absorption onto the scaffolds. 90% confluent cell cultures of hPDLCs were trypsinized and 50.000 cells were loaded onto the different scaffolds (3%Chitosan-CS, 3%CS/1.25%Brushite-Br, 3%CS/7%Br, 3%CS/1%CeO, 3%CS/10CeO). The MTT assay revealed decreased cell viability on cell-loaded scaffolds compared to cells cultured in TCP (Fig. 1). Increasing the concentration of Br and CeO NPs leads to decreased cell viability. PCR analysis showed decreased expression of RUNX2 after 7 and 14 days in culture, but significant increase in BMP2 expression from hPDLCs cultured onto the 3%CS scaffolds. Additionally, there was a slight increase in BGLAP expression in scaffolds with the increased concentration of NPs. Further experiments with osteoinductive media and long-term culture are necessary to fully unravel the regenerative potential of those novel scaffolds.

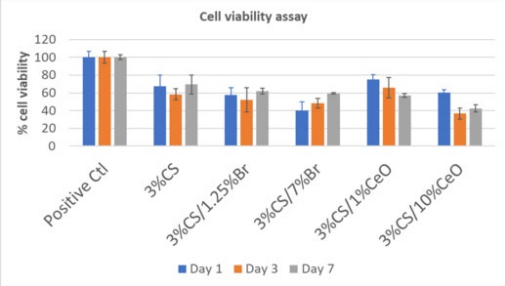


Fig. 1. MTT assay of hPDLCs cultured onto the different scaffolds.

PoB.7.01

Precise nanostructuring of polymer brush-based nanoparticles for gene delivery

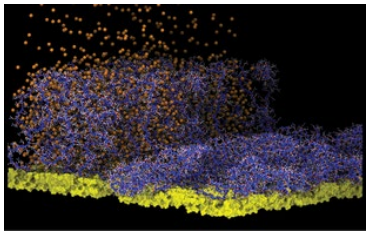
Carlos E Neri Cruz, Lan Chang, Julien E Gautrot

Queen Mary University of London, London, United Kingdom

Abstract

Although polycationic vectors display excellent performance *in vitro* with many cellular systems, their clinical use remains very restricted. To some level, this is due to the poor compatibility and stability of such systems with biological fluids and tissues. Specifically, the impact of competitive binding and interactions with a broad range of macromolecules present extracellularly and within the cytosol was recently found to regulate dissociation from polymeric gene delivery systems (1). Hence, the interactome associated with gene delivery nanomaterials may control not only their biodistribution and internalisation, but also mediate disassembly and timely release of the genetic materials. Evidence suggests it may also underpin some of the off-target effects of such materials. Polymer brushes offer unique features for the design of gene delivery systems (2). In particular, the high density of polymer chains that can be reached using controlled polymerisation techniques enables the control of their architecture in the direction normal to the surface of a nanoparticle with unprecedented precision. Such z-structuring, combined to precise chemical design, not only enables very stable high density loading of oligonucleotides, but also the control of competitive binding and further cytosolic interactome assembly. In this work, we demonstrate the z-structuring of block copolymer brushes for the effective loading of oligonucleotides and their controlled cytosolic release. We combine ellipsometry, surface plasmon resonance and neutron reflectometry to study the structural and chemical parameters that regulate oligonucleotide complexation and the morphology of the resulting assemblies (1, 3, 4). We use proteomics and gel electrophoresis to characterise the interactome forming upon cytosolic entry and characterise the impact of polymer brush z-structuring on competitive binding, using a recently developed biochemical fluorescence microscopy assay allowing the characterisation of exchange kinetics. Finally, we investigate how such processes and their control through z-structuring enables the sustained release of oligonucleotides for siRNA-mediated knock down and the delivery of therapeutic miRNA. For example, we find high levels of knock down are maintained over prolonged times, following a single transfection, even with highly cycling primary cells (including endothelial cells), with cell toxicities reduced to basal levels.

REFERENCES
1. Raynold et al. Nat. Commun. 12 (2021) 6445.
2. Li et al. Adv. Healthcare Mater. 2021, 10 (5), 2000953.
3. Qu et al. Biomacromolecules 20(6) (2019) 2218-2229.
4. Gautrot et al. Advanced Materials Interfaces 2022, 9 (33).



Atomistic model of a polymer brush protonated and collapsing in response to pH change

PoB.7.02

Microneedle-guided lymphatic delivery of a natural immune modulator toxin for enhanced cancer immunotherapy

Suyeon LIM, Dahwun KIM, Siyan LYU, Jihoon JEONG

SUNGKYUNKWAN UNIV., SUWON, Korea, Republic of

Abstract

Cancer immunotherapy has demonstrated significant potential as a cancer treatment by enhancing the immune system's ability to recognize and eliminate cancer cells. However, its efficacy can be limited by factors such as tumor heterogeneity, immunosuppressive tumor microenvironments, and systemic toxicities. Recent advances in drug delivery systems have facilitated the development of more targeted and personalized cancer therapies. Microneedles have emerged as a promising platform for non-invasive and highly localized drug delivery, directly administering drugs, vaccines, and other therapeutic agents to the skin. In this study, we designed dissolving microneedles (dMN) based on a biocompatible amphiphilic tri-block copolymer, which enables the self-assembly of nano-micelles containing hydrophobic drugs when applied to the skin. We used the dMN technology to formulate SKKU-06, a hydrophobic natural immune modulator toxin derived from fungi, which exhibits anti-cancer and immunomodulatory properties in melanoma (SSKU-06@dMN). After intratumoral application of SSKU-06@dMN to skin tumors, the drug-loaded nano-micelles can migrate to tumor-draining lymph nodes (TDLN). The dMN-guided delivery of SKKU-06 to skin tumors and TDLN induced immunogenic cell death and stimulated the activation and maturation of antigen-presenting cells (APCs), promoting the development of humoral and cellular anti-tumor immunity. Furthermore, the immunomodulatory effects of SSKU-06@dMN were enhanced when combined with anti-PD-1 treatment, impacting the tumor microenvironment through increased intratumoral CD8+ T cell infiltration and reduced Treg populations. This resulted in efficient growth inhibition of established skin cancer and metastatic cancer, as well as prolonged survival. The dMN-guided lymphatic delivery of SKKU-06 demonstrates the potential for treating metastatic solid tumors and improving cancer immunotherapy efficacy by modulating the tumor microenvironment.

PoB.7.03

Development of a nanoparticle for early diagnosis of Alzheimer's disease

Beatriz T Simões[1], Catarina Chaparro[1,2], Marco C. Cavaco[1], Miguel A. R. B. Castanho[1], Pedro Ramos Cabrer[3], Paula I.P. Soares[2], Vera Neves[1]

[1]Instituto de Medicina Molecular João Lobo Antunes, Lisbon, Portugal. [2]NOVA School of Science and Technology, Almada, Portugal. [3]CICbiomaGUNE, San Sebastian, Spain

Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder that affects the brain, causing progressive memory loss and cognitive decline. It is the most common cause of dementia, with no successful cure so far. Available treatments are only able to manage symptoms, so early diagnosis is essential to slow down AD progression and improve patients' quality of life. The hallmark characteristic of AD is the accumulation of abnormal protein deposits in the brain, such as, amyloid beta plaques (Aβ). It has been suggested that Aβ oligomers (AβO) can be used as a biomarker for early diagnosis of AD, before the appearance of clinical symptoms. In this work we aim to develop a multifunctional nanoparticle as a tool for early diagnostic of AD. This diagnostic nanoparticle (dNP) will allow translocation across the blood-brain barrier (BBB), further binding to AβO for detection of AD, with an additional feature of MRI contrast agent. The dNP is composed of an superparamagnetic iron oxide core synthesized by thermal decomposition, for image contrast, and coated with PEGylated lipid chains by thin-film hydration, that add stability to the system and allow further functionalization with moieties to cross the BBB and detect AβO. For spectroscopy detection purposes, part of the lipidic composition (≈ 0.8%) contain a fluorescent label. The final dNP was characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM), having hydrodynamic sizes of 130 nm with a polydispersive index of 0.2, the final dNP formulation size is 160 nm, after surface functionalization. The dNP are stable in human serum. In addition, protein corona formation around the surface of the dNP was limited, with an initial 20 nm increase in size that was maintained after during the 24h time-point incubation in human serum, 37°C. Cell based assays were carried out in cellular models of the BBB using human brain endothelial cells (HBEC-5i cell line). The dNP showed no toxicity in a concentration range of 12.5 to 25 ug/ml.

PoB.7.04

Local drug delivery systems for dealing with Peri-Implantitis: Evaluation in novel microfluidic tools.

William Oates, Antonios Anastasiou

University of Manchester, Manchester, United Kingdom

Abstract

Introduction Bacterial biofilm formation around an implant can compromise the healing process of surrounding soft and hard tissue, and severe cases can lead to implant failure and eventually revision surgery. A promising solution is development of Local drug delivery (LDD) systems, where a biomaterial releases active compounds directly to the site of action preventing infection. The development of effective LDD system requires understanding of both the drug carrier associated properties and its responsiveness to expected *in vivo* microenvironments. The study objective is the design and evaluation of a microfluidic tool in which we can replicate *in vivo* oral microenvironment (pH, temperature and flow) and study pharmacokinetics during the development of LDD systems. After design and fabrication of the proposed device, chitosan-based biomaterials have been tested as potential LDD systems to deal with periimplantitis. **Methods** Commercial CFD software was used for simulating flow in the device and initial design. Biocompatible resin and high-resolution 3D printing were used for fabrication. For preparation of LDD samples, chitosan powder was dissolved in aqueous 2% v/v acetic acid solution with appropriate quantities of doxycycline. Solutions were injected into a cup sample holder that fits the microfluidic device and after freezing to -25 C placed overnight into a freeze drier. For real time monitoring of drug concentration, a mini-UV-Vis spectrometer and a flow cell connected to the outlet of the microfluidic device were utilised. **Results and Discussion** To verify the accuracy of our measurements, average drug release was compared with HPLC data and was found for normal flow conditions deviation between the two was less than 5%, making our system highly reliable. Pharmacokinetic profiles were obtained under different scenarios to evaluate the LDD systems. It was found that ignoring continuous flow of liquid around the medical device after implantation can highly underestimate the drug release (even by 100%) as the driving force for mass transfer (gradient of drug concentration) is lower (Figure 1). A dynamic pH assay, mimicking repeated infection onset, was tested (Figure 2). The biomaterial responds to pH alterations by increasing the release of drug (increased dissolution rate) and returns back to steady state when the pH is restored.

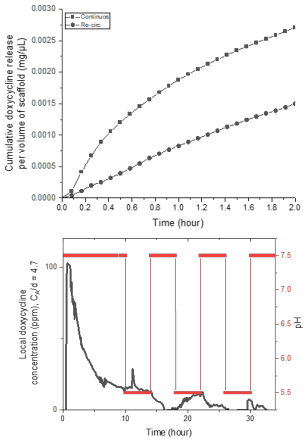


Figure 1: *release profile for loaded scaffolds under stimulated continuous flow and re-circulatory flow.*

Figure 2: *release profile for loaded scaffold exposed to pH pulses.*

Conclusion Using a new microfluidic tool, we were able to evaluate different chitosan based LDD systems for peri-implantitis treatment.

PoB.7.05

Inhalable microparticles embedding calcium phosphate nanoparticles for cardiac drug delivery

Lorenzo Degli Esposti[1], Federica Mancini[1], Eride Quarta[2], Jessica Modica[3,4], Alessio Adamiano[1], Anna Tampieri[1], Claudio De Luca[5], Paolo Colombo[2,6], Alessio Alogna[7], Daniele Catalucci[3,4], Michele Iafisco[1]

[1]Institute of Science, Technology and Sustainability for Ceramics (ISSMC), National Research Council (CNR), Faenza, Italy. [2]University of Parma, Food and Drug Department, Parma, Italy. [3]Institute of Genetic and Biomedical Research (IRGB)-UOS Milan, National Research Council (CNR), Milano, Italy. [4]IRCCS Humanitas Research Hospital, Rozzano, Italy. [5]NanoPhoria Srl, Milano, Italy. [6]PlumeStars Srl, Parma, Italy. [7]Medizinische Klinik m. S. Kardiologie Charité, Berlin, Germany

Abstract

This presentation reports on the generation of novel highly respirable dry powders embedding calcium phosphates nanoparticles for the delivery of therapeutic biomolecules into cardiomyocytes aiming to treat cardiovascular diseases. Rusconi et al. (Rusconi et al., Circulation 2016, 134, 534–546) have demonstrated that a cell-penetrating mimetic peptide (R7W-MP) targeting the Cavβ2 cytosolic subunit of the L-type calcium channel (LTCC) restored cardiac contractility in the pathological conditions of LTCC-based cardiomyopathy. However, the active Mimetic Peptide (MP) without the R7W cell-penetrating sequence loses the capability to enter the cardiac cells. In a follow-up paper, we achieved *in vivo* MP administration by nebulization of MP-loaded calcium phosphates nanoparticles (MP-CaPs) enabling their internalization into cardiomyocytes and restoring cardiac function in a mouse model of cardiomyopathy (Miragoli et al., Sci. Transl. Med. 2018, 10, eaan6205). This unconventional heart targeted treatment took advantage of the pulmonary administration route for cardiac accumulation of MP-CaPs. However, nebulization and inhalation of MP-CaPs present complexity due to difficulty to control their nano-size before and after delivery. Our technological solution to achieve lung deposition of MP-CaPs was their transformation into a microparticulate dry powder to be used in dry powder inhaler devices (Quarta et al., Pharmaceutics 2021, 13, 1825). Herein I will present data on the preparation and characterization of the nanostructured inhalable powders of CaP loaded with therapeutic peptides and small microRNAs for cardiovascular diseases, as well as the preclinical data on the effect of MP-CaP in large animal model.

PoB.7.07

Cartilage-penetrating zwitterionic polymer-drug conjugates for ultra-low dose glucocorticoid delivery

Patrick Weber, František Surman, Maryam Asadikorayem, Marcy Zenobi-Wong

ETH Zurich, Zurich, Switzerland

Abstract

INTRODUCTION Intraarticularly injected glucocorticoids (GC) are potent anti-inflammatory drugs that are frequently used to treat sports injuries and osteoarthritis (OA). Due to their rapid clearance from the joint cavity, they are clinically administered at a very high dose which can lead to long-term detrimental effects on cartilage health[1]. Here, we report on a cartilage-penetrating polymer-drug conjugate with sustained release kinetics with which the required GC dose could be decreased by several orders of magnitude below today's clinical standard. **METHODS** The polymeric backbone of the system consists of zwitterionic poly-carboxybetaine acrylamide (pCBAA), a material known for its excellent biocompatibility and non-fouling properties[2]. A new methacrylate-coupled dexamethasone (DEX) molecule was synthesized that allows for incorporation of the GC into the pCBAA backbone. Via hydrolysis of the linking ester bond, the DEX was continuously released from the polymer with a $t_{1/2}$ of 12-15 days at synovial pH of 7.4-7.6. To test our constructs, we used a model of inflammatory OA in which bovine cartilage explants were treated with IL-1β for two weeks leading to depletion of proteoglycans. **RESULTS AND DISCUSSION** The pCBAA polymers were found to diffuse into full-thickness cartilage explants within 1 hour thereby outperforming conventional polymers such as PEGMA or Dextran (Fig. 1A). Moreover, more than 50% of the polymer was retained over several weeks of extensive washing in PBS independently of the cartilage state (untreated vs. inflamed). When treating cartilage explants with a single, ultralow dose of pCBAA-co-DEX conjugate prior to IL-1β stimulation, DEX was continuously released and remained in the therapeutic window throughout the experiment (Fig. 1B). Free DEX however was completely washed out after 5 days. On the histological level, the sustained release kinetics prevented depletion of surface proteoglycans for the pCBAA-co-DEX group compared to free DEX (Fig. 1C). Continuous treatment with free DEX at a higher concentration led to even greater depletion of proteoglycans. We are convinced that with its optimized pharmacokinetics, our polymer-drug conjugate represents a powerful alternative to free GC formulations to improve the long-term outcomes in joint disease treatment.

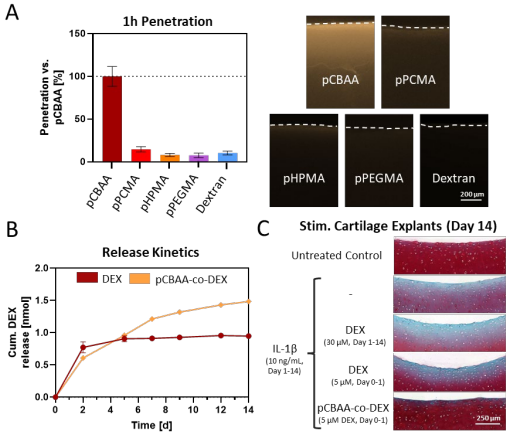


Figure 1: A) Penetration of pCBAA into cartilage explants compared to conventional polymers. B) Improved release kinetics of DEX from polymer-treated explants. C) Safranin O staining of treated explants in inflammatory OA model.

REFERENCES

1. T. McAlindon et al., Jama (2017)
2. L. Zhang et al., Nature biotechnology (2013)

ACKNOWLEDGEMENT

This work was supported by the Swiss National Science Foundation (Nr. 192656 to MZW).

PoB.7.08

Exploiting *in vitro* mechanical stimulation of cells to refine the transfection efficiency of non-viral gene delivery vectors

Beatrice Ruzzante, Stefano Parazzini, Emiliano Votta, Gabriele Candiani, Nina Bono

Politecnico di Milano, Milano, Italy

Abstract

Introduction: Non-viral gene delivery employs positively charged lipidic or polymeric vectors to drive exogenous genetic material into cells (a process called transfection) to alter specific cell functions [1]. Much effort has been made since their first introduction, though the presence of biological barriers that complexes must overcome still reduces their ultimate transfection efficiency (TE) [2]. In this scenario, we propose a new technology to boost the TE of gold standard non-viral polymeric vectors branched polyethyleneimine (*b*PEI) by modulating the cell behavior with cyclic mechanical strain. **Methods:** The stimulation device consists of an electro-mechanical actuator controlled by an Arduino microcontroller able to exert equibiaxial cyclic deformation to silicone cell culture chambers through the vertical displacement of a puncher (nominal deformation (NE) range:0-20 %; frequency (f) range: 0-2 Hz, step: 0.1 Hz) (**Fig. 1A**).The variation of the NE and the resulting strain profile obtained varying the puncher z-axis displacement were assessed through a Finite Element Analysis (FEA) (Abaqus®) and validated experimentally. To evaluate the cell response to mechanical stimulation and its effects on the uptake of non-viral gene delivery complexes, TE of gold standard *b*PEI/pGL3 complexes on stimulated C2C12 cells (stimulation time (t): 30 min, NE: 5-10 %, f: 0.5-1.5 Hz) was compared to that of statically-transfected cells. **Results:** The equibiaxial deformation of culture substrates was confirmed by the overlapping of the experimental NE measurements in 2 orthogonal directions (i.e., NE_{11} and NE_{22}) at different levels of puncher displacement (**Fig. 1B**). The overlap of experimental and computational NE data assessed the consistency of FEA (**Fig.1C**). FEA revealed the uniformity of NE distribution in the middle region of culture substrates (corresponding to 1/3 of the total culture chamber) (**Fig. 1D**). This area was hereinafter considered as the cell-seeding area for further *in vitro* transfection experiments using *b*PEI/pGL3 complexes in stimulated conditions. Of note, we found a 4-fold increase of the TE in cyclically stretched cells as compared to unstimulated cells (**Figs. 1E-F**), and this increase was strain- and frequency-dependent. **Conclusion:** We developed a versatile stimulation device exerting a homogeneous equibiaxial cyclic strain on 2D cell cultures. Overall, coupling well-defined mechanical cell stimulation cues with chemical vectors was effective in driving gene transfer.

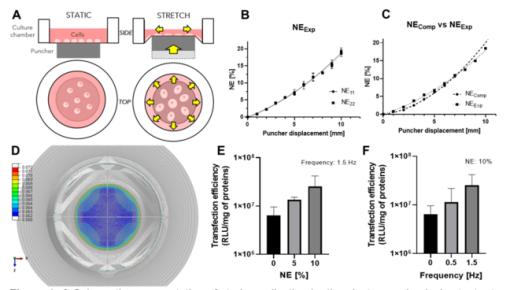


Figure 1. A *Schematic representation of strain application by the electro-mechanical actuator to a cell culture chamber. The equibiaxial deformation of the silicone-made culture chamber is induced by the vertical slide of the puncher.* ***B*** *Experimental NE measurements in 2 orthogonal directions (i.e., NE_{11} and NE_{22}) varying the puncher displacement.* ***C*** *Experimental (NE_{Exp}) and Computational (NE_{Comp}) strain data at different puncher displacements. NE_{Exp} is the mean of NE_{11} and NE_{22}.* ***D*** *FEA in the region of the membrane's top surface is characterized by almost homogeneous NE values. Computational data are exemplified for a puncher displacement equal to 5 mm and expressed in real value (0.072 NE is 7.2 %).* ***E*** *Effect of variation of NE on Transfection Efficiency (TE) (t: 30 min, f: 1.5 Hz, NE: 5-10 %).* ***F*** *Effect of variation of strain frequency on TE. TE is expressed in terms of luciferase activity of stretched cells with respect to unstimulated controls (0 %) (t: 30 min, f: 0.5-1.5 Hz, NE: 10 %). Data are expressed as mean ± SD.*

References: [1] Bono N *et al*. Pharmaceutics. 2020:183. [2] Aied A *et al.* Drug Discov. Today 2013:1090-1098

PoB.7.09

Development of magnetic-polymeric nanoparticles as a theranostics strategy for alzheimer's disease

Catarina Chaparro[1,2], Marco Cavaco[2], Miguel Castanho[2], João Paulo Borges[1], Vera Neves[2], Paula Pereira Soares[1]

[1]CENIMAT/I3N, Materials Science Department, Nova University of Lisbon, Almada, Portugal. [2]Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Abstract

Alzheimer's disease (AD) is the second most prevalent neurological disorder and the major cause of dementia [1]. The most acceptable theory for AD neuropathology is the amyloid beta (Aβ) protein-based hypothesis. Nanoparticles (NPs) appear as an essential tool when developing new systems for the efficient delivery of diagnostic molecules and potential therapeutic drugs to the brain. Poly(lactic-co-glycolic) acid (PLGA) nanoparticles enhance drug pharmacodynamics and bioavailability, and when loaded with magnetic nanoparticles (MNPs) they can act as contrast agents for magnetic resonance imaging (MRI) [2]. These characteristics make them attractive for brain imaging and therapy. However, the application of nanoparticles (NPs) for brain drug delivery is hindered by the presence of the blood-brain barrier (BBB). [3]. BBB peptide shuttles (BBBpS), are small peptides that engage adsorptive mediated transport (AMT) across the BBB and allow brain uptake. In this work, we propose a theranostics system that comprises the combination of MNPs-loaded PLGA NPs able to recognize Aβ oligomers using a therapeutic antibody against Aβ (VL). In addition, BBBpS will be used to functionalize nanoparticles' surface, providing the nanosystem the ability to translocate across the BBB and improving Aβ detection through MRI. We produced nanoparticles with a size range of 125-200 nm. Both BBBpS and VL moieties were conjugated to NPs' surface (BBBpS-VL-NPs), resulting in size alteration. To test the activity of NPs we first investigated the interaction of BBBpS-VL-NPs with human brain endothelial cells (BECs) that make up the BBB. NPs internalization in BECs was evaluated through flow cytometry and fluorescence microscopy. The results revealed that BBBpS promotes internalization, with an increase in BBBpS modified NP, in comparison with both bare and VL-conjugated NPs. Using an *in vitro* model of the BBB, we confirmed that the BBBpS-VL-NPs were able to translocate the barrier. We are now studying the neuroprotection effect of the nanosystem in neuronal cells in a co-culture model of the BBB. We will further evaluate the *in vivo* biodistribution of BBBpS-VL-NPs through MRI.

[1] R. Brookmeyer, E. Johnson, K. Ziegler-Graham, H. M. Arrighi, Alzheimer's Dement. 3, 186 (2007).
[2] C. I. P. Chaparro, L. R Loureiro, M. A. Valente, P. A. Videira, J. P. Borges, P. I. P. Soares, 2019 IEEE 6th Portuguese Meeting on Bioengineering (ENBENG) (2019).
[3] V. Neves, F. Aires-da-Silva, M. Morais, L. Gano, E. Ribeiro, A. Pinto, S. Aguiar, D. Gaspar, C. Fernandes, J.D.G. Correia, M. Castanho, ACS Chemical Biology 12, (2017).

PoB.7.10

Vescalagin and castalagin present anti-amyloidogenic activity towards pre-formed fibrils of α-synuclein

Ana Rita Araujo[1,2], Rui Luís Reis[1,2], Ricardo Alexandre Pires[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal. [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Abstract

Parkinson's (PD) and Alzheimer's disease (AD) are two of the most common neurodegenerative disorders. They are both characterized by extensive neuron loss caused by the pathological deposition of proteins/peptides aggregates in the intra/extra-cellular environment. Importantly, the protein/peptide is disease specific, i.e. alpha-synuclein (aS) in the case of PD and amyloid β (Aβ42) in the case of AD.(1) To date, the pharmacological strategies for both diseases target the relief of symptoms, and there is no clinical strategy that is able to halt or reverse the neurodegenerative process. In the case of PD, aS, a protein composed by 140 amino acid residues and a hydrophilic tail, has been shown to generate amyloid-like cytotoxic fibrils in the form of intracellular Lewis bodies (LBs) and neurites. These aggregated species are associated with the onset and progression of neurodegeneration in specific brain areas (i.e. basal ganglia and the substantia nigra), and are at the basis of the impairment of the neuronal activity. *In vitro* studies copycat the aS cytotoxicity using preformed fibrils (PFFs). Natural polyphenols are widely reported to present a wide range of bioactivities, e.g. anti-microbial, anti-oxidant, among others. More recently, they have been shown to modulate the assembly of cytotoxic amyloid-like fibrils into non-cytotoxic forms, as well as to disrupt the preformed amyloid aggregates.(2) Herein, we extracted/isolated two cork-based polyphenols (i.e. vescalagin and castalagin) and evaluated their ability to interact with the PFFs of aS, altering their aggregation state into non-cytotoxic forms. CD data show that both vescalagin/castalagin can modulate the supramolecular organization of PFFs. Fluorescence spectroscopy data (i.e. using Thioflavin-T) and STEM corroborate these findings, indicating that both polyphenols disrupt the PFFs and generate smaller aS species. Additionally, we assess the cytotoxicity of PFFs in SH-5YSY culture. By immunocytochemistry we confirmed that cells pre-treated with vescalagin/castalagin present a lower amount of PFFs in the cellular environment. Likewise, the polyphenol-modulated PFF species are non-cytotoxic to SH-5YSY cells during 5 days of culture. Our data confirms that both vescalagin and castalagin are able to modulate the aggregation pathway of aS and induce the formation of non-cytotoxic forms, demonstrating a relevant potential for the treatment of PD.

ACKNOWLEDGEMENTS: The authors acknowledge financial support from ReStart project (M-ERA-NET3/0007/2021). RAP acknowledges the Portuguese FCT for financial support (CEECINST/00077/2018).

References:

1. Xu L, et al. Parkinsons Dis. 2016;2016
2. El Gharras H. IInt. J. Food Sci. 2009;44(12)

PoB.7.12

The potential of fully biodegradable dendritic vectors to deliver nucleic acids to cartilage tissue

Sara Zarzo-Arias[1,2], Victoria Leiro[1], Ana Paula Pêgo[1,3]

[1]i3s/INEB, Porto, Portugal. [2]FEUP, Porto, Portugal. [3]ICBAS, Porto, Portugal

Abstract

INTRODUCTION: Cartilage tissue degeneration represents a major source of high impact diseases (mainly, osteoarthritis and discogenic low back pain) within our society. However, the treatments of these diseases are focused only on minimizing the symptoms, arising the need to find regenerative approaches to treat them. Gene therapy represents a promising approach for tissue regeneration, alleviate the symptoms and cure these conditions. One of the research areas that gene therapy arises is focused on developing nucleic acid delivery systems. Herein we propose to explore new proprietary fully biodegradable PEG-GATGE (Poly(Ethylene Glycol) Gallic Acid-Triethylene Glycol Ester) dendrimers, to develop a non-toxic fully biodegradable vector for RNA delivery to relevant cells of the cartilage tissue, such as chondrocytes, nucleus pulposus cells and mesenchymal stem cells (MSCs). METHODS: Fully biodegradable G3 PEG-GATGE dendrimers were synthesized, functionalized with benzylamine groups, and characterized by NMR. Afterwards, dendriplexes complexing siRNA were prepared at different N/P ratios (dendrimer amines/siRNA phosphate groups). Their RNA complexation efficiency was studied using the SYBRTMGold exclusion assay, their size was measured by dynamic light scattering and zeta-potential by laser doppler electrophoresis. Finally, dendrimer and dendriplexes cytotoxicity, dendriplex internalization and transfection studies, were tested in a chondrocyte cell line expressing the luciferase protein (C28/I2, provided by M.B. Goldring, Weill Medical College of Cornell University, New York, NY, USA). RESULTS: PEG-GATGE-based dendriplexes showed an siRNA excellent complexation (>70%) at different N/P ratios tested (5 to 80), showing also a suitable size (40-65nm), and an overall positive charge. Subsequently, dendriplexes at N/P 5, 10 and 20 were tested and proven non-cytotoxic for chondrocytes, showing higher siRNA internalization levels even than the positive control (lipofectamine RNAiMAX) (>90%, analysed by confocal microscopy and FACS). Transfection efficiency studies are currently ongoing, also using lipofectamine RNAiMAX as control.

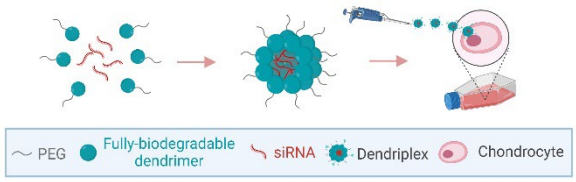


Fig. 1: Schematic representation of dendriplex formation and their application in vitro.

DISCUSSION & CONCLUSIONS: G3 PEG-GATGE dendrimers were successfully synthesized in very good yields, showing excellent siRNA complexation efficiencies. Furthermore, they show a very good biocompatibility and excellent internalization in chondrocytes, making them promising siRNA delivery vectors to the cartilage tissue. Further transfection studies with siRNA in chondrocyte cell line expressing luciferase and relevant biological *in vitro* studies in primary chondrocytes, MSCs and nucleus pulposus cells are currently ongoing.

PoB.7.13

Testing novel oligosaccharide-based drugs on polyoxazoline carriers for pharmacological inhibition of galectin-3 in an *in vitro* and *in vivo* rat model of pulmonary hypertension

Antonin Sedlar[1], David Vrbata[2], Katerina Pokorna[1], Frantisek Kolar[1], Pavla Bojarova[2], Lucie Bacakova[1]

[1]Institute of Physiology CAS, Prague, Czech Republic. [2]Institute of Microbiology CAS, Prague, Czech Republic

Abstract

Pulmonary hypertension is a serious cardiovascular disease characterized by elevated blood pressure in the pulmonary arteries. Symptoms of pulmonary hypertension include fatigue, weakness, chest pain, and eventually heart failure. To date, there is no reliable cure for pulmonary hypertension. Treatment focuses on lowering blood pressure and preventing heart failure. Galectin-3, a β-galactoside-binding protein, has been found to be involved in the vascular and myocardial remodeling associated with pulmonary hypertension and in the development and progression of cardiovascular disease, specifically heart failure, atherosclerosis, and systemic and pulmonary hypertension. Pharmacological targeting of galectin-3 with oligosaccharide-based drugs is promising in the treatment of pulmonary hypertension [1]. Oligosaccharide-based drugs usually exhibit relatively poor pharmacokinetic properties in organisms. Drugs can be attached to biocompatible synthetic polymers to increase their half-life and solubility in the bloodstream. Specifically, polyoxazoline polymers show low toxicity *in vitro* and *in vivo* as well as tunable pharmacokinetic properties [2]. We have tested several novel oligosaccharide ligands of galectin-3 covalently bound to polyoxazoline carriers. Their cytocompatibility was evaluated *in vitro* on vascular smooth muscle cells isolated from rats suffering from hypoxic pulmonary hypertension. The effect on the expression of markers associated with pulmonary remodeling (i. e. galectin-3, alpha-smooth muscle actin, collagen I) was also evaluated. Selected inhibitors were fluorescently labeled to determine their pharmacokinetic and biodistribution properties in rats. The results show that inhibitors conjugated to polyoxazoline carriers are generally non-toxic for vascular smooth muscle cells in concentrations up to 100 µM. Biodistribution studies in rats showed significant accumulation of the polymer in the liver and kidney, regardless of the presence of the galectin-3 ligand on the polymer (Fig. 1). The newly prepared ligands appear promising for pharmacological inhibition of galectin-3, but further experiments are needed to fine-tune their pharmacokinetic properties.

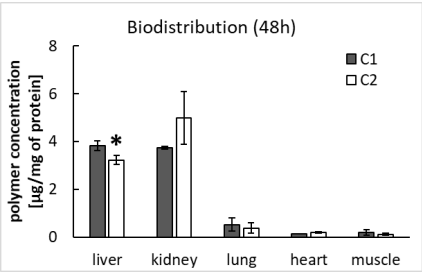


*Fig. 1: Biodistribution of fluorescently labeled polyoxazoline polymers in rat organs. The fluorescence signal was determined in tissue homogenates from rat organs 48 hours after intraperitoneal administration of fluorescently labeled polyoxazoline polymer conjugated with Gal-3 ligand (C2). Polyoxazoline without Gal-3 ligand (C1) was used as a control. Mean ± SD, n = 3, One way ANOVA, Student-Newman-Keuls test. * - Statistically significant difference between C1 and C2 ($p \leq 0.05$).*

This work is supported by the Czech Science Foundation (grant No. 22-00317S).

References:

[1] Barman, Scott A et al. American journal of physiology. Lung cellular and molecular physiology vol. 316,5 (2019): L784-L797. doi:10.1152/ajplung.00186.2018

[2] Humphries, James et al. Biomacromolecules vol. 21,8 (2020): 3318-3331. doi:10.1021/acs.biomac.0c00765

PoB.7.14

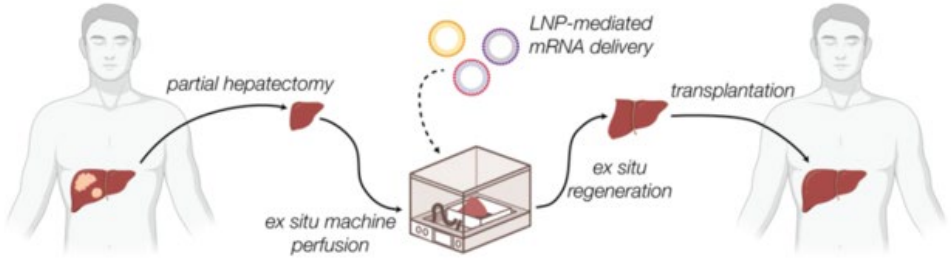
LNP-mediated mRNA delivery toward ex vivo liver regeneration

Leslie Cunningham[1], Florian Huwyler[2], Jonas Binz[2], Mark W. Tibbitt[1]

[1]ETH, Zurich, Switzerland. [2]ETH, Zürich, Switzerland

Abstract

Many organ grafts are not suitable for transplantation due to excessive ischemic injury. In an effort to save these discarded grafts, ex vivo perfusion systems have been developed to extend the time window for organ repair. The liver, in particular, has a remarkable regenerative capacity and its ex vivo perfusion provides a unique opportunity to trigger regeneration pathways. Thus far, advanced perfusion technologies have enabled the preservation of the human liver outside of the body for up to two weeks with use of a normothermic perfusion machine developed by Liver4Life [1]. Until now, this liver perfusion machine has only been employed to treat bacterial infections, determine tumour malignancy and assess liver function, yet the ability to stimulate growth and repair of liver grafts ex vivo remains unexplored [2]. In order to effectively develop potential regeneration strategies for perfused organs, *in vitro* liver models are needed since human liver experiments are low-throughput, confounded by patient-to-patient variability and costly. Thus, we aim to develop an *in vitro* screening platform for the identification of pro-regenerative biomolecules and optimal combinations thereof. Precision cut liver slices (PCLS) present a simple and cost-effective screening tool since they preserve the intact liver microarchitecture and basic physiological functions. In parallel, we are exploring lipid nanoparticle (LNP) mRNA delivery systems as they allow more targeted and sustained therapeutic effects during ex vivo perfusion. By combining PCLS culture and LNP-mediated mRNA delivery systems, we aim to identify combinations of proteins or other molecules that could be used to stimulate repair or regeneration in the PCLS. Identified combinations of therapeutics will be targeted for the treatment of discarded liver grafts with the aim of counteracting the worldwide transplant shortage.



[1] D. Eshmuminov et al., “An integrated perfusion machine preserves injured human livers for 1 week,” Nat Biotechnol, vol. 38, no. 2, pp. 189–198, Feb. 2020, doi: 10.1038/s41587-019-0374-x.

[2] P.-A. Clavien et al., “Transplantation of a human liver following 3 days of ex situ normothermic preservation,” Nat Biotechnol, May 2022, doi: 10.1038/s41587-022-01354-7.

PoB.7.15

Exploring the therapeutic potential of a new design of tetrahedral DNA nanostructures with a framework-integrated anti-microRNA antisense sequence

Sara D. Reis[1], Ana S.G. Martins[1,2], Ruxandra Baboi[1], João Cortinhas[1], Filomena Carvalho[3], Nuno C. Santos[3], Ana P. Pêgo[1,4], Pedro M.D. Moreno[1]

[1]i3S/INEB - Instituto de Investigação e Inovação em Saúde / Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [2]FEUP - Faculty of Engineering of the University of Porto, Porto, Portugal. [3]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal. [4]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Abstract

In the last years, DNA and RNA nanostructures have shown a great potential as delivery vehicles of drugs, in particular oligonucleotide therapeutics (OT). Their biocompatibility, easy self-assembly with controllable size and shape, and the possibility of integration of multiple cargos or targeting ligands with spatial and stoichiometric control, are some of the most advantageous properties of these nanostructures. Tetrahedral DNA nanostructures (TDN) are one of the most explored types of DNA nanostructures, with high structural stability in physiological conditions. Generally, OT have been integrated in TDN within the sequence of protruding single-strand loops (hairpins) or of dangling single-strand extensions with a free extremity, or even functionalized by hybridization to single-strand extensions. Here, we developed a novel design of TDN integrating an antisense oligonucleotide (ASO), with a gapmer DNA/RNA design, within the structural framework of TDN. The aim of this ASO is the suppression of a microRNA (anti-microRNA), and we have selected microRNA21 (miR21), a well-studied micro-RNA reported to be upregulated in many cancers, as a proof of concept. We have designed the ASO with a gapmer design to promote the recruitment of the RNAse H enzyme to induce nanostructure rearrangement and ultimately microRNA suppression. The TDN integrated with anti-miR21 (TDN-amiR21) has been successfully characterized by gel electrophoresis, dynamic light scattering (DLS) and atomic force microscopy (AFM). The efficiency of RNAse H recruitment and miR21 degradation of TDN-amiR21 was assessed by PAGE, revealing an efficient catalytic miR-21 cleavage. TDN-amiR21 demonstrated stability in 10% serum until 24-48h, in opposition to a single-strand amiR21 from TDN. In U87 human glioblastoma cell line, TDN-amiR21 were shown to be successfully intracellularly delivered, without lipid-aided transfection, by demonstrating an efficient downregulation of miR21 expression in comparison to free amiR21. Thus, TDN-anti-miR-21 reveals potential to be used as a therapeutic to downregulate miR-21. Furthermore, its design can be adapted to different ASO sequences, opening possibilities for application to different therapeutic targets. Additionally, we demonstrate an evolution of TDN design with the integration of an anti-miRNA ASO, with a gapmer design, enabling a more efficient mechanism of actuation of the ASO, without affecting the original geometry of TDN, and still enabling future potential multifunctionalization with targeting ligands or other drugs.

PoB.7.17

Near-infrared light-activated formulation for the spatial controlled release of CRISPR-Cas9 ribonucleoprotein for brain gene editing

Susana Simões[1,2], Miguel Lino[1,3], Angela Barrera[1,3], Catarina Rebelo[1,4], Francesca Tomatis[1,5], Andreia Vilaça[1,2], Andrea Neuner[6], Ricardo González[1,2], Alexandra Carvalho[1,2], Stefan Stricker[6], Lino Ferreira[1,3]

[1]CNC-Centre for Neuroscience and Cell Biology of University of Coimbra, Coimbra, Portugal. [2]Institute of Interdisciplinary Research of University of Coimbra, Coimbra, Portugal. [3]Faculty of Medicine, University of Coimbra, Coimbra, Portugal. [4]Institute of Interdisciplinary Research of University of Coimbra, Coimbra,, Coimbra, Portugal. [5]PhD Programme in Experimental Biology and Biomedicine of the Institute for Interdisciplinary Research (IIIUC) of the University of Coimbra, Coimbra, Portugal. [6]Epigenetic Engineering, Institute of Stem Cell Research, Helmholtz Zentrum, Munich, Germany

Abstract

The CRISPR/Cas9 genome editing technology opened the door to provide a versatile approach for treating multiple brain diseases. Many of the advances made in brain delivery systems used viral vectors. However, these vectors may cause genomic undesirable side effects because of the prolonged expression of the CRISPR system. Non-viral CRISPR/Cas delivery systems have emerged as alternative to viral delivery approaches. The non-viral vectors, administered by the intracerebral route, have successfully gene-edited different cerebral cell types, however one of the limitations of the current non-viral vectors is their limited spatial resolution. The control in spatial gene editing requires light-activatable NPs and in particular, near-infrared (NIR)light-activatable NPs to increase tissue-penetration. In addition, brain application of NIR-activatable-CRISPR formulations raise significant challenges taking in account that: (i)the laser should penetrate the skull and activate brain cells transfected with NPs, (ii)the triggering system should not activate gliosis processes and, (iii)they should allow spatial resolution. Here, we report a NIR light-triggerable NP formulation for brain gene editing with spatial control. For proof of concept, the gene editing was evaluated by the eGFP knockout in d2eGFP-HeLa cells. Then, we evaluated the *in vitro* gene editing in different brain cell populations (subventricular zone (SVZ)cells, astrocytes and neuros). The different brain cell populations were isolated from transgenic mice Ai9 with a cassette inserted into the Gt(ROSA)26Sor locus with a LoxP-Stop-LoxP-tdTomato-sequence. To demonstrate *in vivo* gene editing, AuNRs were stereotaxically injected into the SVZ of both hemispheres of adult Ai9 brain mice. The gene editing expression was evaluated two weeks after. D2eGFP-HeLa cells treated with the light-activable formulations showed more than 70% of eGFP knockout activity. The AuNR formulation enabled gene editing with single-cell resolution when confocal microscopy was used for NIR activation. In the brain cell population, the gene editing was determined by the quantification of tdTomato-positive cells. The formulation was able to edit SVZ cells, astrocytes and neurons. In *in vivo* the expression of tdTomato fluorescence was higher (~ 4-fold) in irradiated versus non-irradiated hemisphere. In conclusion, we have developed a gene-editing formulation for on-demand release into deep tissues with spatial resolution upon exposure to transcranial NIR light.

Acknowledgements.L.F. acknowledges the funding by COMPETE2020 and FCT programs[PTDC/NAN-MAT/28060/2017(acronym: BrainEdition), POCI-01-0145-FEDER-016682], Portugal2020-COMPETE funding throughCENTRO2020(CENTRO-01-0145-FEDER-000014) and NeuroAtlantic, ref:EAPA_791/2018), and EC projects ERAatUC (Ref:669088) and RESETageing (Ref:952266). S.S.acknowledge the FCT fellowships (SFRH/BPD/105327/2014) and the funding byFCT program (EXPL/BIA-CEL/0989/2021(acronym:Editing_Vision).

PoB.7.18

To reset the senescence program in senescent stem/progenitor cells by extracellular vesicles

Cláudia M. Deus[1], Susana Soares[1], Magda Ferreira-Rodrigues[1], Akhilesh Rai[1], Miguel Lino[1], Lino Ferreira[1,2]

[1]CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, UC, Coimbra, Portugal. [2]Faculty of Medicine, University Coimbra, Coimbra, Portugal

Abstract

Introduction: People worldwide are living longer. Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death. This deterioration is the primary risk factor for several human pathologies. With aging comes a progressive accumulation of a wide variety of molecular and cellular damage. Senescence is a well-studied ageing hallmark. Senescence is a cell fate that involves loss of proliferative potential of normally replication-competent cells, with associated resistance to apoptotic cell death, and generally increased metabolic activity. Thus, it is imperative to discover new therapeutic approach to target the cellular processes underlying aging compressing the period of functional decline in old age. EVs are nano-size vesicles secreted by cells, acting as intercellular communicators. Small EVs (sEVs) (50–200 nm), are the most studied EVs. Their physiological nature and content related to the condition of the cell of origin hints at an interesting potential use as biomarkers of disease progression and therapeutic activity. The aim of our work was to demonstrate that sEVs collected from neonatal tissues may decrease cellular senescence. Material and Methods: sEVs were isolated from WJ-MSC by differential ultracentrifugation and characterized using nanoparticle tracking analysis, Zeta potential and dynamic light scattering analysis. EVs internalization was labeled with DioRed staining. Senescence was induced in WJ-MSC using 5 Gy irradiation or 25 nM Doxorubicin and characterized at day 3. Two administrations of EVs were performed for 48 h each. Markers of senescence, including SA-β-Gal, proliferation and cell cycle arrest markers, including Ki67 and p21 were evaluated by microscopy. Moreover, transcripts levels of SASP, cell growth and proliferation were also evaluated by qPCR. Results: Our results showed that irradiation-and DOX-induced senescence in WJ-MSC 3 days after the induction increased SA-β-Gal and decreased the expression of anti-inflammatory genes. Also, a decrease in proliferation and cell cycle arrest markers, including Ki67 and p21 fluorescence were showed in senescent WJ-MSC. The opposite effect was demonstrated by the administration of EVs in senescent WJ-MSC. WJ-MSC-derived EVs decreased senescent cells measured by SA-β-Gal and increased the expression of anti-inflammatory genes in the same cells. Our results point out that WJ-MSC-derived EVs are highly effective senotherapeutics slowing the progression of aging and diseases driven by cellular senescence.

Funding: By project POCI-01-0247-FEDER-069790-“RESET_BONE_AGEING” and “BIO-MED”-CENTRO-01-0145-FEDER-181228, both funded by European Regional Development Fund through PT2020, the european project-“RESETageing”-(ref.952266) and Programa de Recuperação e Resiliência project-“HfPT- Health from Portugal”.

PoB.7.19

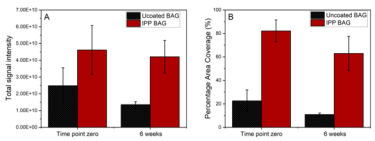
Multifunctional Baghdadite ceramic for local chemotherapy and bone regeneration

Sally Kortam[1], Zufu Lu[1], Pooria Lesani[1], Seyed-Iman Roohani-Esfahani[1], Ghazal Shineh[1], Leila Mamizadeh Janghour[1], Behnam Akhavan[1,2,3], Hala Zreiqat[1]

[1]School of Biomedical Engineering, The University of Sydney, Sydney, Australia. [2]School of Engineering, University of Newcastle, Callaghan, Australia. [3]Hunter Medical Research Institute (HMRI), New Lambton Heights, Australia

Abstract

Osteosarcoma is a primary malignant bone tumor leading to large bone defect that are challenging to treat, particularly when under load. Standard treatment for osteosarcoma involves surgery, and post-surgical chemotherapy, essential to prevent recurrence and metastasis. However, systemic chemotherapy has major drawbacks. Recent research has focused on local drug delivery systems utilizing bone scaffolds that aim to refill bone defects and provide localized chemotherapy. However, several challenges and limitations exist within this approach, originating from both the scaffolds and the loaded drugs, including burst release, drug instability, and rapid degradation. Furthermore, currently available scaffolds lack the necessary mechanical rigidity and strength required for effective bone repair. In this study, we aim to overcome these challenges by integrating highly efficient liposomal nanoparticles loaded with doxorubicin (DOX) into mechanically robust ceramics. This approach seeks to achieve a localized and sustained release of DOX, leading to the eradication of residual cancer cells, reduced side effects, and enhanced bone regeneration. **Methods** We had developed a bioactive ceramic (Baghdadite, Ca3ZrSi2O9) (BAG) and demonstrated its outstanding bioactivity and mechanical properties. Here, we functionalized BAG by incorporating DOX-loaded liposomes. To facilitate enhanced binding of liposomes and prolonged DOX release, we employed an innovative ion-assisted plasma polymer (IPP) film to coat the BAG surface and examined the drug release profiles of the DOX-liposome from IPP-coated BAG. Furthermore, we assessed the cytotoxic effects of this system on MG-63 osteosarcoma cells using MTS assay after 7 days. **Results** Analysis of confocal large scan z-stack images of Cy5-labeled liposomes on the BAG surface reveals that IPP-coated BAG exhibits approximately double the fluorescence intensity compared to uncoated BAG. Additionally, IPP-coated BAG displays significantly higher liposome coverage than uncoated BAG (**Fig.1**). Both uncoated and coated BAG exhibit an initial burst release of liposomes/ DOX within the first few days, followed by sustained continuous release over a period of one month. IPP-coated BAG shows a higher release of liposomes/ DOX due to the increased liposome binding. Cell viability assays show that DOX released from liposomes attached to the IPP-coated BAG, exerts a potent cytotoxic effect on MG-63 cells (**Fig.2**).



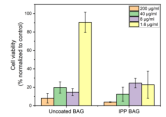


Figure 1. *IPP-coated BAG demonstrated enhanced attachment of liposomes than the uncoated BAG.*

Figure 2. *IPP-coated BAG exhibits higher toxicity towards MG63 cells compared to the uncoated BAG.*

Conclusion

This study demonstrates the significant potential of incorporating DOX-loaded liposomes with IPP-coated BAG system in addressing the treatment of tumor-induced bone defects.

PoB.9.02

Engineering hydroxyapatite-loaded scaffolds with melt electrowriting

Joanna Babilotte[1], Marta Ferreira[1,2,3], Monize Caiado Decarli[1], Rita Sobreiro-Almeida[2], Paul Wieringa[1], João Mano[2], Susana Olhero[3], Lorenzo Moroni[1]

[1]Complex Tissue Regeneration department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands. [2]Department of Chemistry, CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal. [3]Department of Materials and Ceramics Engineering, CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal

Abstract

Melt-electrowriting (MEW) is an emerging technology that uses an electric field to melt and extrude a polymer filament to create intricate and precise 3D structures, featuring fiber sizes smaller than 100 µm. This technology has the potential to fabricate scaffolds that mimic the complex architecture of natural joint tissues like cartilage, bone, and ligaments. In this sense, MEW holds promise as a tissue engineering strategy for the temporomandibular joint (TMJ), one of the most complex joints in the human body. However, MEW is still in its early stages, and there is a lack of compatible materials for this technology. Previous research mainly focused on the use of thermoplastic polymers, which are known to demonstrate good mechanical properties, but low bioactivity. To improve the bioactivity of scaffolds, calcium phosphates such as hydroxyapatite (HA) are good candidates for joint tissue engineering, namely for the bone part connection with the soft tissues. In this direction, we developed a composite material, compatible with MEW and relevant for TMJ applications. Blends of polycaprolactone (PCL) and Polylactic acid/Polycaprolactone copolymer (PCL/PLA) with 3.5% wt micro-sized HA were produced using a solvent mixing approach. Thermogravimetric analysis showed that the hydroxyapatite loading previously optimized was correctly accomplished and reproducible. Additionally, the presence of HA increased the young modulus compared to the plain polymer. Fibrous scaffolds were successfully fabricated using MEW, featuring regular and well-defined structures and fiber diameters ranging from 20 to 50 µm. Furthermore, *in vitro* cell colonization was evaluated over 21 days with human mesenchymal stem cells (hMSCs). We compared cell morphology and colonization on scaffolds produced with the plain polymer or the HA polymer blends. The *in vitro* results demonstrated that all types of scaffolds supported the attachment and proliferation of hMSCs. Currently, the cell differentiation potential is evaluated on the different scaffolds. Our study demonstrates new possibilities for producing fibrous scaffolds by MEW with HA-loaded polymer. Preliminary *in vitro* results showed promising outcomes for TMJ engineering. In future studies, the fibrous scaffolds will be included in a relevant hydrogel for TMJ joint regeneration. Overall, this study shows the potential of using MEW with HA-loaded polymer for TMJ tissue engineering, which can lead to the development of improved treatment strategies for joint disorders.

Acknowledgments

The authors acknowledge the support of the European Union's Horizon 2020 research and innovation program under grant agreement No 953169 (Interlynk).

PoB.9.03

Development of biofunctionalized membranes for the reconstruction of critical size segmental bone loss

Hugo Bossut[1], Cédric Zobrist[1], Feng Chai[2], Marc Saab[2], Bernard Martel[1], Nicolas Blanchemain[2]

[1]University of Lille, UMET, Lille, France. [2]University of Lille, U1008, Lille, France

Abstract

Critical bone loss can be managed in orthopaedic and trauma surgery by the induced membrane technique developed by Masquelet. At first a PMMA spacer is casted into the bone defect. This spacerwill be fully covered by an induced membrane (IM) throught a foreign-body reaction This IM serve during the second step as a vascularized receptacle for a bone autograft. Despite the high success rate (up to 90%) of this technique, the IM is formed over several weeks before bone grafting, and it requires two surgical interventions followed by prolonged hospitalizations [1]. On this basis, this project focuses on the development of an electrospun membrane (EM) that would accelerate the IM formation . Finally, this EM could be stuffed with bone autograft, allowing a one-step Masquelet procedure. First, resorbable Polycaprolactone (PCL) based polyurethanes (PU) were synthesized and analyzed by 1H NMR (300MHz Avance III HD, Bruker®) and FTIR (Spectrum Two, Perkin Elmer®). Then, they were dissolved in DMSO/DMF and electrospun through an homemade device. The resulting membranes were observed by scanning electron microscopy (FLEXSEM 1000, Hitachi®) and Fiber diameters were quantified using ImageJ (v1.42q, NIH).Resorption kinetic was studied in PBS, and the mechanical properties of the pristine and aged membrane were studied. The *in vitro* cytotoxicity of fibers was assessed with a pre-osteoblast MC3T3-E1 cell line, according to ISO 10993-5 standard, with extraction method by AlamarBlue® Assay. NMR and FTIR showed a clear structure of our synthetized PU, with various range of resorbable segement. The SEM analysis helped us to determine the optimal electrospinning parameters for obtaining membranes with well-defined fibers with a diameter of 0.5 to 1 µm and no beads. Some of these membranes have shown very good mechanical properties, equivalent to that of the periosteum which is similar to the IM [2]. Ageing tests excluded some membranes due to a lack of sustainability. However, some of them have kept a good morphology and the mechanical properties have been very little impacted. The cytocompatibility of fibers was proved by cell viability (>70% with regard to control) of pre-osteoblast cells.Resorbable polyurethane electrospun membranes have been developed with good mechanical properties close to those of the periosteum with a good behaviour in time in PBS. The next step will be to investigate the capacity of the electrospun membrane to provoke the induced membrane formation *in vivo* on rats.

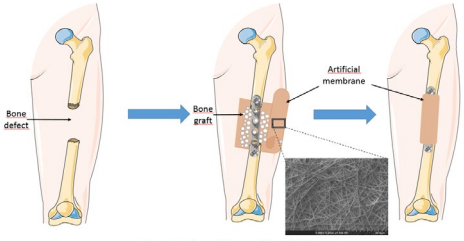


Figure 1 : Scheme of the use of the artificial membrane

Table 1 : References

Numbers	References
[1]	A. I. Alford, D. Nicolaou, M. Hake, et S. McBride-Gagyi, « Masquelet's induced membrane technique: Review of current concepts and future directions », *J. Orthop. Res.*, vol. 39, n° 4, p. 707‑718, 2021, doi: 10.1002/jor.24978.
[2]	S. H. McBride, S. F. Evans, et M. L. Knothe Tate, « Anisotropic mechanical properties of ovine femoral periosteum and the effects of cryopreservation », *J. Biomech.*, vol. 44, n° 10, p. 1954‑1959, juill. 2011, doi: 10.1016/j.jbiomech.2011.04.036.

PoB.9.08

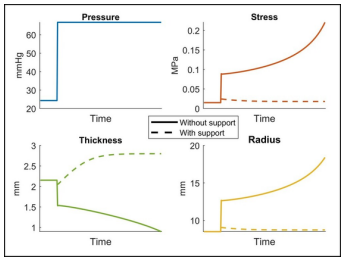
Electrospinning a mechanocompatible external support for the ross procedure

Thibault Vervenne, Lauranne Maes, Filip Rega, Nele Famaey, Arn Mignon

KU Leuven, Leuven, Belgium

Abstract

Introduction In the Ross procedure, the patient's own pulmonary valve is used to replace the aortic valve. However, dilatation is a common adverse effect leading to failure, as the autograft is unable to adapt to its new high-pressure environment. Textile mesh wrapping can stabilize this dilatation, but can also cause stress-shielding on the long term, leading to a loss of wall thickness and mechanical compliance. Here, we develop and produce a biodegradable external support as a trade-off between prevention of dilatation and maintenance of arterial wall integrity. Materials and methods omputational modelling can predict the outcomes of an artery exposed to different pressure levels, helping to derive the optimal mechanical and degradation properties of a textile reinforcement. For simulation purposes, we assume the artery to be an isotropic thick-walled cylinder, and tissue mass deposition or removal to be driven by a deviation from homeostatic stress [1]. An electrospinning setup is used to produce a polyester porous mesh, mimicking the extracellular matrix and promoting cell ingrowth during support. In a first try-out, biodegradable polycaprolactone (PCL) was dissolved in chloroform (CF), where the electrical field evaporates the solvent and PCL fibers are deposited from the needle onto the collector [2]. Results, discussion, and future work Figure 1 depicts two model outcomes, one without and one with a modelled external support around the artery. The acute pressure increase in the Ross procedure puts the artery under supraphysiological stress, which can cause maladaptation over time due to elastin damage. An external support can reduce the overstress and bring the artery back to its healthy stress regime, promoting benign remodelling. Indeed, smooth muscle cells can then recruit new collagen fibers, which will thicken and strengthen the arterial wall. Future work will include mechanical testing of the electrospun fibrous structure shown in Figure 2, in order to optimize and iterate on the experimental and simulated mesh mechanical properties. In a later stage, tuning the polymer biodegradability will prevent detrimental stress-shielding effects over time, and as such, ensure arterial pulsatility is restored. To this end, scaffold degradation time will be estimated through simulations and again validated experimentally. Changing the considered electrospun polymer structure will be key to realise the latter.



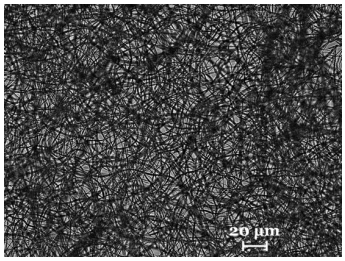


Figure 1: Computational predictions of arterial growth and remodelling

Figure 2: Zeiss Axioscope image of electrospun scaffold

References
[1] L. Maes et al., Biomech. Model. Mechanobiol., 2023.
[2] M. J. Mochane et al., Appl. Sci., 2019.

PoB.9.09

Comparing the performance of electrospun methacrylated alginate hydrogels with and without additional crosslinkers for wound healing application.

Carolina Gutierrez Cisneros, Elise Venken, Arn Mignon, Veerle Bloemen

KU Leuven, Leuven, Belgium

Abstract

Introduction Hydrogels are widely used wound dressings because they maintain moisture, allow oxygen exchange, and aid in tissue debridement. Alginate is especially used to make them given its bioactivity which stimulates repair of damaged tissue by moisture vapor-transmission and exudate absorption.[1] However, by itself, alginate presents an insufficient mechanical performance. So, the introduction of covalently crosslinkable moieties enhances this while reducing degradation.[2] Additionally, processing the polymer by electrospinning creates a dressing with high surface-to-volume ratio and porosity which mimic the extracellular matrix.[3] Objective Optimization study of hydrogels for skin healing applications by crosslinking methacrylated alginate (AlgMA) though UV-light exposure with different crosslinkers. Further performance comparison of electrospun AlgMA with and without crosslinker. Materials and Methods AlgMA was produced as previously reported.[4] Combinations of AlgMA with one out of four crosslinkers were analyzed through swelling studies, compression and tensile tests (dynamic mechanic analyzer), statistically evaluated (T-test, $p<0.05$, $n\geq3$). Electrospinning was optimized on an in-house device by the addition of poly(ethylene glycol) 1 Mo. Da, Triton X-100 and ethanol. Fiber formation was microscopically confirmed. The chemical modifications were confirmed by Fourier transform infrared and proton nuclear magnetic resonance. Results and Discussion After successful AlgMA synthesis, hydrogels were formed by film casting or electrospinning, combining AlgMA with one out of four acrylate crosslinkers (fig 1).

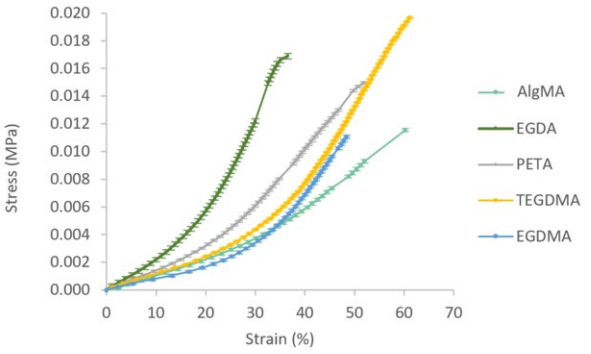


Fig 1. Stress-strain curve of film-casted AlgMA hydrogels upon crosslinker (ethylene glycol diacrylate, EGDA; ethylene glycol dimethacrylate, EGDMA; triethylene glycol dimethacrylate, TEGDMA and pentaerythritol triacrylate, PETA) addition in 1:1 acrylic moieties ratio.

The most promising crosslinker was found to be ethylene glycol diacrylate (EGDA), given its statistically significant increase in tensile (fig 1) and compressive strength, despite a decrease in swelling capacity. AlgMA+EGDA were successfully processed through electrospinning (see fig 2) and crosslinking was confirmed. In the present research, alginate wound dressings with improved mechanical capacity were obtained by combining AlgMA with crosslinkers. This was successfully electrospun with and without crosslinker and UV crosslinked.

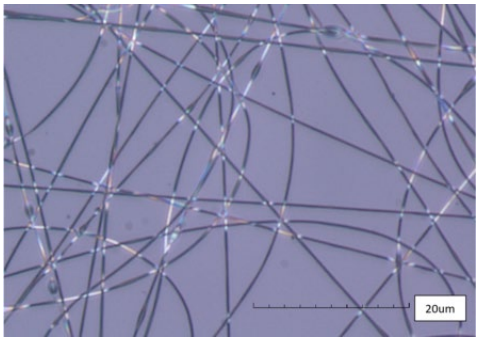


Fig 2. Electrospun AlgMA with addition of polyethylene glycol (PEO, 1 Mo.), Triton X-100 and ethanol.

References

1. K. Varaprasad, T. Jayaramudu, V. Kanikireddy, C. Toro, and E. R. Sadiku, Carbohydr Polym, 236, 116025 (2020).
2. R. Galli et al., Sci Rep, 8, 10841 (2018).
3. X. Zhang, C. Huang, Y. Zhao, and X. Jin, RSC Adv, 7, 39349–39358 (2017).
4. A. Mignon et al., Carbohydr Polym, 168, 44–51 (2017).

PoB.9.10

Electrospinning methods for the production of poly(ε-caprolactone) nanofibers for biomedical applications

Andjela N. Radisavljevic[1], Vukasin Ugrinovic[1], Dusica Stojanovic[2], Vesna Radojevic[2], Petar Uskokovic[2], Mirjana Rajilic-Stojanovic[2]

[1]University of Belgrade, Innovation Centre, Faculty of Technology and Metallurgy, Belgrade, Serbia. [2]University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia

Abstract

There is a great potential to use polymeric nanofibers as carriers of biologically active substances intended for biomedical applications, due to their possibility to deliver drugs at a controlled rate to the desired location. In this work novel poly(ε-caprolactone) (PCL) nanofibers as drug carriers intended for the prevention of infections were developed. Cefazolin-loaded PCL nanofibers were produced by using three electrospinning methods allowing for different dynamics of drug release. After a period of five days ~40 %, ~30 %, and ~2 % of the drug was released from the nanofiber mats obtained by blend electrospinning, co-axial, and emulsion electrospinning, respectively. As an alternative to synthetic drugs with one type of defined activity, medicinal plants have a broad spectrum of biologically relevant activities, such as antibacterial, antifungal, antiviral, and antioxidant. PCL nanofiber mats containing dry yarrow extract were processed by using blend electrospinning. In scientific literature, the antioxidant, antibacterial, and anti-inflammatory activity of yarrow has been demonstrated. In addition, yarrow extract is traditionally used for wound healing (this is also recognized by the European medicines agency - EMA). The positive effects of yarrow are attributed to the presence of bioactive molecules, such as polyphenols. Up to 98 % of the total polyphenols were released from the electrospun PCL nanofibers after a period of five days. In all processed samples, cefazolin and dry yarrow extract retained their biological activity and have not reacted with the PCL, nor degraded during the electrospinning process. Furthermore, silicone and rubber catheters were coated with PCL nanofiber mats with cefazolin or dry yarrow extract by using adhesive n-butyl-2-cyanoacrylate. The coated catheters showed good mechanical stability and could inhibit the growth of relevant human pathogens *Staphylococcus aureus* and *Escherichia coli* (Fig. 1). Newly developed PCL nanofiber mats with cefazolin could be used as a coating for urinary catheters with pronounced antibacterial activity, while PCL nanofiber mats with yarrow extract powder could be used for the same purpose with additional antioxidant activity and wound healing properties.

Figure 1. Inhibition zone diameter of nanofiber mat coatings onto rubber and silicone urinary catheters against S. aureus *and E. coli*

Based on the mechanical properties the produced materials are suitable for the production of antibacterial gauzes, patches, wound dressings, as well as coating materials.

PoB.9.11

Three dimensional melt electrospinning of poly-ε-caprolactone for future tissue engineering applications in meniscal repair

Anna-Maria Gröll, Carsten Linti, Michael Doser

Deutsche Institute für Textil- und Faserforschung (DITF), Denkendorf, Germany

Abstract

Melt electrospinning (MES) enables the generation of porous structures with tuneable fiber diameters and is a promising technique for the production of scaffolds for tissue engineering applications. In this work, MES of poly-ε-caprolactone was conducted creating three dimensional porous structures by stacking the fibers in a layer by layer approach (Fig. 1). Exemplary, a scaffold in the shape of the meniscus was created. In the future, such scaffolds could be used for the regeneration of meniscus tissue after meniscectomy. Using a conventional 3D printer (x350 pro, German RepRap GmbH) connected to a high voltage generator (KNH34, Eltex-Elektrostatik GmbH), MES of a Medical Grade poly-ε-caprolactone filament (ITV Denkendorf Produktservice GmbH) was carried out with a grounded nozzle and a negative voltage of 15kV applied to the printing bed. Adjusting the path of the extrusion head and the parameters of the process, scaffolds in different shapes and heights could be generated. The fabricated scaffolds were sputter coated and analysed using Scanning Electron Microscopy (SEM). Fiber diameters were determined with an image processing software (MAVIfiber2d, Fraunhofer ITWM). Capillary flow porometry was used to examine pore sizes and pore size distributions. Using the described 3D printing system, it was possible to produce scaffolds up to a height of 10 mm in a reproducible manner. The size of the fibers was highly dependant on the material flow rate through the nozzle, resulting in average fiber diameters in the range of 5 to 15 µm. Average pore sizes of approximately 22 µm were obtained at average fiber diameters of 10 µm. With an increase in fiber diameter an increase in pore sizes could be observed. Meniscus shaped, end contour near structures for potential application in meniscus tissue engineering could be generated by moving the extruder head along a circular path. Building on these results, further research is needed to create scaffolds with prescribed dimensions. Residual charges on the fibers lead to repelling forces affecting the fiber deposition in higher layers and remain a challenge that needs to be overcome.

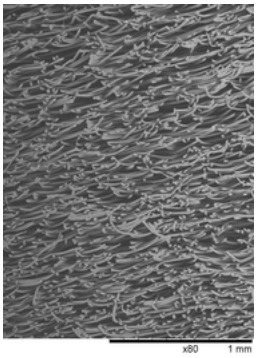


Figure 1: Cross-section SEM image of the stacked fiber layers

PoB.9.12

Effect of electrospun PVA- salmon gelatin- chitosan nanofibers using different types of collectors on muscle cell alignment

Dragica Bezjak[1], Benjamín I Schleyer[1], Cristian Acevedo[1,2], Tomás P. Corrales[1]

[1]Universidad Técnica Federico Santa María, Valparíso, Chile. [2]Centro de Biotecnología "Dr. Daniel Alkalay Lowitt", Valparíso, Chile

Abstract

Electrospinning is a manufacturing technique that consists of the use of a polymer solution that passes through a needle that is positively charged and a grounded collector, which creates an electric field that causes the solution to be ejected from the tip of the needle to the collector, resulting in nanofibers that are being deposited on the collector. This technique can be used for tissue engineering, since it allows the use of biomaterials that are biodegradable and biocompatible. In this work, films manufactured with the electrospinning technique were made to be use in the growth of muscle cells. Muscle cells have the characteristic of being elongated and multinucleated fibers. To achieve this morphology, cells must go through a fusion process, and the first step for this to happen is the alignment of cells, so in this work we fabricated electrospinning films with different topographies that were expected to guide muscle cells in the alignment process. Here we present the use of a new mixture of biomaterials that have proven to be suitable for use with the electrospinning technique, such as Polyvinyl alcohol (PVA), Salmon Gelatin and Chitosan. In addition, in this work a comparison of two nanofiber alignment methods using different types of collectors was made: microstructured static collector and rotating collector. Through SEM images nanofibers could be observed, and their orientation and distribution were evaluated using ImageJ software. The use of both types of collectors resulted in nanofiber alignment. Muscle cells of C2C12 cell line were seeded onto the films, proliferate for 3 days, and the growth of the muscle cells was observed through epifluorescence microscopy. We observed that in both types of films the cells proliferated, however, in the films made with microstructured static collector we did not obtain alignment of the cells, so the effect of the alignment of the nanofibers did not affect the cells in the desired way. On the other hand, aligned cells can be observed using the films made with a rotating collector. By using this new mixture of biopolymers, we were able to grow muscle cells in an align manner, which facilitates fusion process and muscle cell development, presenting a new type of film that could be used in the field of muscle tissue engineering.

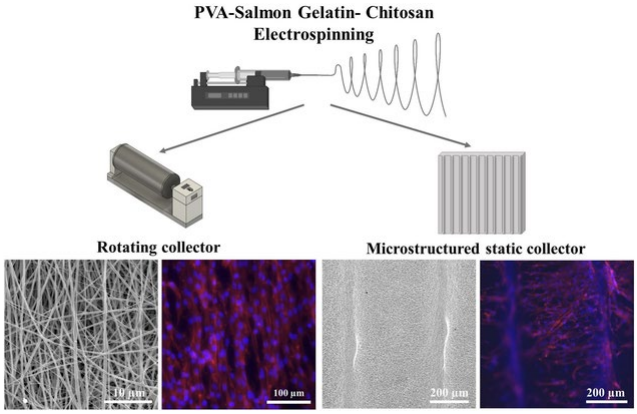


Fig. 1: Effect of films made with electrospinning technique using different types of collectors. SEM images are showing nanofibers and epifluorescence images are showing C2C12 cells.

PoB.10.01

Investigation of polypyrrole-grafted alginate-gelatin hydrogels for cartilage tissue engineering

Lisa Schöbel[1], Alexandra Springer[1], Christian Polley[2], Hermann Seitz[2], Aldo Boccaccini[1]

[1]Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany. [2]Chair of Microfluidics, Faculty of Mechanical Engineering and Marine Technology, University of Rostock, Rostock, Germany

Abstract

Introduction The positive effect of electrical stimulation on various cell types, such as bone, cartilage, and neural cells, has been proven extensively [1]. Hydrogels are suitable for tissue regeneration because of their favorable properties, such as high water content and the ability to be printed or to fabricate individualized three-dimensional (3D) scaffolds. Combining alginate-gelatin (Alg-GEL) hydrogels with conductive polymers, such as polypyrrole (PPy), leads to functional biomaterials for fabricating scaffolds with the capability to electrically stimulate cells. **Materials and Methods** It was previously described how alginate-gelatin hydrogels could be functionalized with PPy [2]. In this work, the electrically conductive PPy was grafted to alginate while varying the dopant using synthetic or natural anionic substances. The synthetic dopant used was polystyrene sulfonate (PSS), whereas chondroitin sulfate (CS) and hyaluronic acid were employed as natural dopants. Several material characteristics, such as mechanical and electrical properties, as well as swelling and degradation behavior, were examined. Further, cell-material interactions of PPy-functionalized hydrogels were investigated using the prechondrogenic cell line ATDC-5. **Results** The investigation of mechanical and electrical properties showed an increase in Young's Modulus and electrical conductivity (EC) when PPy was grafted to Alg-GEL hydrogels. It was observed that the EC was influenceable by the choice of dopant (Fig. 1 A), whereas the Young's Modulus did not change significantly with different dopants, which opens up the possibility of tailoring resulting material characteristics independently. Moreover, the swelling and degradation behavior was not influenced by the incorporation of PPy. The *in vitro* characterization of PPy-alginate-gelatin hydrogels showed no adverse effects of the grafted PPy:dopant on cell viability or proliferation (Fig. 1 B). Moreover, no significant differences between pristine and modified Alg-GEL hydrogels were apparent. **Discussion** In conclusion, PPy:dopant-grafted alginate-gelatin hydrogels were evaluated to understand the effect of different dopants on resulting material properties and cell-material interactions. The results suggested that the grafting of PPy:dopant to alginate represents a promising strategy to tailor the electrical conductivity of resulting alginate-gelatin hydrogels while maintaining their biocompatibility.

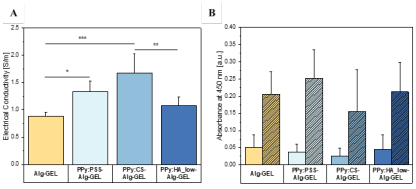


Fig. 1. (A) Electrical conductivity of pristine and PPy:dopant-grafted Alg-GEL. (B) WST-8 results of seeded ATDC-5 cells on different films after 1 d (blank) and 7 d (patterned).

Acknowledgment

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – SFB 1270/2 - 299150580.

References

[1] Chen *et al.*, Biomater. Res. 2019, 23, DOI 10.1186/s40824-019.0176-8 .

[2] Distler *et al.*, Adv. Healthcare Mater. 2021, 10, DOI 10.1002/adhm.202001876 .

PoB.10.02

Alginate-based hydrogels enriched by human platelet lysate and phytotherapeutic agents for tissue engineering approaches

Faina Bider, Artem Klotschan, Sonja Kuth, Aldo Boccaccini

Institute of Biomaterials, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany

Abstract

Tissue Engineering (TE) aims for the development of repairing and replacing tissues or organ functions which are not curable in a traditional way. Sodium alginate is a natural based polymer derived from brown algae and is commonly used as hydrogel material to formulate carrier systems for drug delivery and inks for 3D bioprinting approaches. By an oxidation process of alginate the creation of free aldehyde groups is possible leading to alginate di-aldehyde (ADA) which enables the covalent bonding to free NH2-groups containing in proteins like gelatin (GEL) resulting in ADA-GEL hydrogel via Schiffs base formation [2]. In this study, ADA-GEL hydrogels were used due to their ability to mimic the native extracellular matrix and high biocompatibility. Moreover, the addition of human platelet lysate (HPL) and ferulic acid (FA) was performed to increase the multifunctional properties of ADA-GEL hydrogels which should result in higher antibacterial properties or more cell attractive interaction. Different hydrogel compositions were prepared and crosslinked ionically with CaCl2 and enzymatically with microbial transglutaminase (mTG). The resulting hydrogels and the impact of FA and HPL were then characterized regarding degradation/swelling behavior, mechanical properties, release capability, cell viability, antibacterial properties and printability. The degradation/swelling behavior of the ADA-GEL films showed that the addition of FA and especially HPL decreased the degradation rates. The mechanical compression testing concluded that the Young's modulus increased with the addition of FA and decreased with increasing concentrations of HPL. A FA release study showed that the presence of HPL had no negative influence on the release capability of ADA-GEL samples. Moreover, it could be confirmed, that the addition of FA and HPL increased the cell activity and viability of MC-3T3-E1 cells. Furthermore, antibacterial tests using gram-negative (E. coli) and gram-positive (S. aureus) bacteria showed that FA decreased the bacterial viability, confirming the antibacterial property of FA. Additionally, a 3D bioprinting study was performed to investigate the beneficial environment for cells within 3D printed scaffolds due to the FA and HPL content. Especially for TE, the established results show that the ADA-GEL hydrogels containing FA and HPL represent a promising system for 3D (bio)printing approaches and should gain more attention in the future.

Acknowledgment
The authors thank the "Deutsche Forschungsgemeinschaft" (DFG, German Research Foundation) for financial support (project TRR225/A01).

References
[1] Khademhosseini, et al., Nat. Protoc. 11 (2016) 1775–1781. https://doi.org/10.1038/nprot.2016.123.
[2] Reakasame, et al., Macromol. Biosci. 20 (2020) 1–14. https://doi.org/10.1002/mabi.202000138.

PoB.10.03

The effect of alginate/hyaluronic acid proportion on semi-IPN hydrogel properties for articular cartilage tissue engineering

Izar Gorroñogoitia[1,2], Sheila Olza[2], Ana Alonso-Varona[2], Ane Miren Zaldua[1]

[1]Leartiker S.Coop, Markina-Xemein, Spain. [2]Faculty of Medicine and Dentistry, University of the Basque Country (UPV/EHU), Leioa, Spain

Abstract

In recent years pathologies related to cartilage such as osteoarthritis have become increasingly popular worldwide. Self-healing capabilities of the degenerated articular cartilage are limited due to its avascular and aneural nature. Apart from palliative and reparative treatments already known so far, one of the emergent regenerative treatments is tissue engineering, in which hydrogels can functionally imitate the native tissue and create an optimal microenvironment for the restoration of the defected tissue. It is well known the potential of hyaluronic acid in the field of tissue engineering as a regenerative material for many tissues. It is one of the major components of the articular cartilage extracellular matrix (ECM) contributing to cell proliferation and migration. Among all the glycosaminoglycans (GAGs), hyaluronic acid is the only non-sulphated one and can present long molecular weight. However herein, a hyaluronic acid presenting 50 % of sulphated glycosaminoglycans (sGAGs) is used, completely altering the intrinsic properties of the material particularly in terms of biological response. These sGAGs will act as bioactive molecules providing cell adhesion and interaction. Alginate is another polysaccharide widely used in tissue engineering due to its biocompatibility and rapid gelation that allows the obtention of stiff and stable hydrogels when crosslinked with $CaCl_2$. Taking benefit of the favourable characteristics of each biomaterial for cartilage tissue regeneration, semi-interpenetrating (semi-IPN) hydrogels have been developed by the combination of both materials, in which alginate is gelled and hyaluronic acid remains uncrosslinked within the hydrogel. Varying the concentration of alginate, hyaluronic acid and $CaCl_2$ the final rheological, viscoelastic, and mechanical properties of the hydrogel can be tailored, always seeking a trade-off between biological and physico-mechanical properties. The obtained results demonstrate that all developed hydrogels have good printability and are biocompatible so this study suggests that alginate/hyaluronic acid semi-IPN hydrogels have great potential in 3D bioprinting and tissue engineering applications and can be used as possible candidates for biomedical applications.

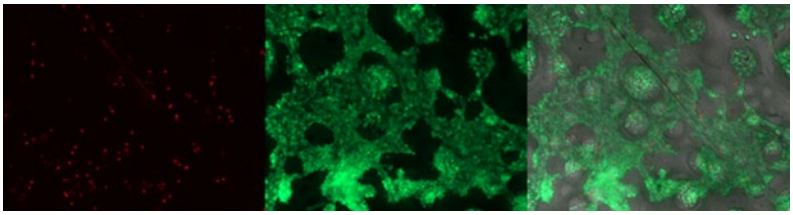


Figure 1. *LIVE/DEAD cell viability staining of L929 cells on hydrogel after 7 days of incubation. Dead cells stained-red and live cells stained-green.*

PoB.10.05

3D printed polysaccharides-based hydrogels as delivery system for plant-derived exosome-like nanovesicles in chronic wound healing application.

Tiziana Fischetti[1], Francesca Perut[1], Laura Roncuzzi[1], Sofia Avnet[2], Nicola Baldini[1,2]

[1]IRCCS Istituto Ortopedico Rizzoli, Biomedical Science and Technology and Nanobiotechnology Laboratory, Bologna, Italy. [2]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

Abstract

Plant-derived exosome-like nanovesicles (EPDENs) have recently been isolated and evaluated as potential bioactive nutraceutical biomolecules. Indeed, we recently isolated EPDENs from *Citrus limon* L. juice and *Fragaria* x *ananassa* extract, and we demonstrated that they carry reliable amount of vitamin C. Moreover, EPDENs entered in mesenchymal stromal cells (MSC) and protect them from oxidative stress. This ability to transfer the antioxidative cargo in MSC may be usefully exploited in regenerative medicine approaches. Diabetic wound complications are a significant challenge in clinical practice, and the healing process can benefit of bioactive medications, able to release specific molecules over time. The aim of this study was the use of 3D bioprinting to develop advanced smart patches as delivery systems for EPDENs release. Thus, we explored the possibility to include plant-derived exosome-like nanovesicles into 3D printed polysaccharides-based hydrogels. We verified EPDENs loading and distribution within the 3D structure, and hydrogel biocompatibility. Then, we studied the swelling ratio and degradation properties of the 3D printed hydrogels, and we tuned and quantified the EPDENs release over time by varying hydrogels concentrations and crosslinking conditions.

PoB.10.06

Injectable hyaluronic acid/ε-polylysine hydrogels loaded with strontium hydroxyapatite nanoparticles for osteoporotic bone fracture healing

Anna Rubina[1], Anastasija Tumilovica[1], Artemijs Sceglovs[1,2], Liga Stipniece[1,2], Kristine Salma-Ancane[1,2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Riga Technical University, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

Abstract

Osteoporosis causes bone fractures and large irregularly shaped bone defects, which are usually treated with bone auto/allografts, inert metallic implants or bioactive ceramic implants. These approaches have numerous limitations, like insufficient availability, disease transmission, infection and brittleness. Therefore, there is a need for novel injectable, antibacterial, bioactive biomaterials for filling osteoporotic bone defects. The aim of this study was to synthesize and characterize composite hydrogels based on natural biopolymers ε-polylysine (ε-PL) and hyaluronic acid (HA), and strontium substituted hydroxyapatite nanoparticles (Sr-nHAp). Sr-nHAp (3 wt% Sr) was chemically precipitated using Ca^{2+}, Sr^{2+} and PO_4^{3-} containing reagents. The phase composition and molecular structure of Sr-nHAp were characterized by XRD and FTIR. ε-PL-HA/Sr-nHAp composite hydrogels were synthesized by chemical crosslinking of ε-PL and HA with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) and by loading Sr-nHAp into ε-PL-HA hydrogel matrix. Initially, eight different ε-PL-HA/Sr-nHAp composite hydrogels were synthesized with ε-PL-HA to Sr-nHAp mass ratios of 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70 and 20:80 wt%. Injectability tests showed that all eight composite hydrogel compositions did not exceed 30 N injection force - the accepted limit of manual injectability. However, syneresis measurements revealed that syneresis of the fabricated composite hydrogels decreases with increasing Sr-HAp concentration. According to these results, ε-PL-HA/Sr-nHAp composite hydrogels with 60:40, 50:50, 40:60 and 30:70 wt% were further evaluated. Rheology, microstructure, molecular structure and phase composition were investigated. Rheology measurements revealed that all selected composite hydrogels are shear-thinning and injectable. The swelling degree decreased with increasing Sr-nHAp mass in the composites. SEM microphotographs showed a highly porous microstructure with Sr-nHAp embedded into the hydrogel matrix. XRD patterns proved the presence of nanosized Sr-nHAp phase. FTIR spectra revealed the formation of amide bonds between HA and ε-PL, forming a stable chemically crosslinked hydrogel. The developed ε-PL-HA/Sr-nHAp composite hydrogel has great potential to be used as an injectable bioactive biomaterial for osteoporotic bone regeneration. Further studies need to be conducted on Ca^{2+} and Sr^{2+} ion release kinetics, cell viability and antibacterial activity to determine the full biomedical potential of this biomaterial. The authors acknowledge financial support from the Latvian Council of Science research project No. lzp-2020/1-0072 “Injectable bioactive biocomposites for osteoporotic bone tissue regeneration (inBioBone)”.

The authors acknowledge the access to the infrastructure and expertise of the BBCE – Baltic Biomaterials Centre of Excellence (European Union’s Horizon 2020 research and innovation programme under the grant agreement No. 857287).

PoB.10.07

Edible microcarriers: From the bench to the bioreactor

Rebeca E Rivero, Ricardo J.A. Oliveira, Francesco Zaccarian, Anitha A Kumar, Panagiota Moutsatsou, Mark J Post

Mosa Meat, Maastricht, Netherlands

Abstract

INTRODUCTION The environmental impact and the controversial animal welfare associated with industrial meat production highlights how sustainable production alternatives are indispensable. In 2013, the world's first laboratory grown hamburger was developed. However, coming at a price of $300.000, and being manually produced, substantial effort is still required to reach sustainable large-scale production.[1] A determining challenge for culture meat production is the scalability of the process. As a solution, microcarriers (MC) provide a strategy to culture a high number of adherent cells in bioreactors. Inert microcarriers, as Cytodex 1, can support cell culture in suspension, although the dissociation of cells from these inedible microcarriers adds complexity to downstream processing. To increase process efficiency edible materials offer a promising approach.[2] We aim towards the development of edible microcarriers, able to support bovine fibro-adipogenic progenitor cells (FAPs) proliferation in serum-free proliferation medium (SFPM) while preserving their stemness for later differentiation. RESULTS AND DISCUSSION Different polymer concentrations and two systems were used for the beads fabrication. For system1, the higher polymer concentration resulted in smooth beads (240±50µm). In the case of system2, the polymer concentration able to form smooth beads was lower (160±20µm). The stability of the beads was studied keeping the beads under mild agitation in SFPM at 37 °C for 60 days. No change in morphology or size distribution was observed.

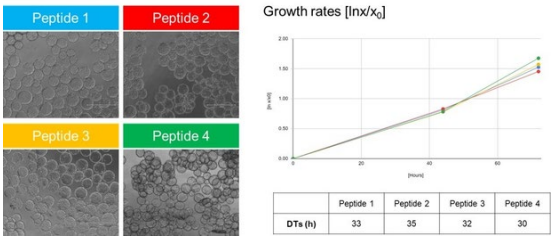


Figure 1. FAPs proliferation.

All the MC modified with different peptides were able to promote good cell attachment and proliferation. Subsequently, the selection of peptides to improve the bead-to-bead transfer was studied. Cells were seeded on MC in spinner flasks and as confluency was achieved, fresh MC were added in a ratio of 1:3. Migration of the cells to the new MC and further proliferation was observed. To verify whether the proliferated FAPs could still retain their stemness, two assays in serum free differentiation media (SFDM) were performed. After 4 weeks, FAPs formed lipid droplets.

CONCLUSION

In this study edible microcarriers were successfully fabricated via vibrating nozzle systems and surface modification. Overall, the microcarriers were shown to support proliferation and to perform bead-to-bead transfer. It is also possible to observe that FAPs proliferated using these microcarriers conserve their stemness.

The steps hereby taken are critical for the scaling up of cell culture.

REFERENCES

[1] Bodiou V. et al., Frontiers in nutrition 7(2020):10.

[2] Norris, Sam CP, et al. Biomaterials 287(2022):121669.

PoB.10.08

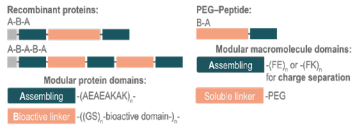
Tailoring supramolecular hydrogels by the design of modular proteins and polymer–peptide conjugates

Dalia Dranseikiene[1], Yusuke Ota[2], Stéphane Bernhard[1], Thomas Edwardson[2], Elia Guzzi[1], Mao Hori[2], Camilla Massoudi[1], Zrinka Raguz Nakic[3], Christin Peters[3], Donald Hilvert[2], Mark Tibbitt[1]

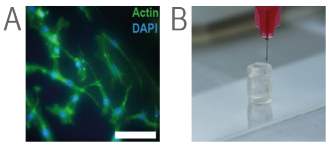
[1]Macromolecular Engineering Laboratory, D-MAVT, ETH Zurich, Zurich, Switzerland. [2]Organic Chemistry Laboratory, D-CHAB, ETH Zurich, Zurich, Switzerland. [3]Biosystems Technology, School of Life Sciences and Facility Management, ZHAW, Wadenswil, Switzerland

Abstract

Background: Supramolecular peptide and protein hydrogels are useful for biomedical applications—biofabrication, neural injury repair, and hemostasis [1-3]. These hydrogels can be formed by utilizing macromolecules designed by linking separate domains for self-assembly, solubility, and bioactivity. Self-assembly can be achieved using domains with amphiphilic peptide sequences (A), whereas specific peptide or polymer sequences can be included for bioactive domains (B) and solubility. In this work, we explored two bottom-up approaches to achieve fibrillar supramolecular hydrogels using designed proteins and polymer–peptide conjugates (*Figure 1*). *Figure 1. Protein and PEG-peptide macromolecules designed by linking separate domains for self-assembly (A), and solubility + bioactivity (B).*



Methods: Modular proteins were designed and expressed in E. coli and purified using His6-Tag. Polymer–peptide conjugates were synthesized using solid-state peptide synthesis. Self-assembly of fibrillar materials was studied using FTIR (Perkin-Elmer SpectrumTwo), TEM (FEI Morgagni 268), and SEM (JOEL JSM 7100F). Gelation differences between different materials was characterized using shear rheometry (Anton-Paar MCR 502). Bioactivity of the hydrogels was evaluated using NIH/3T3 mouse fibroblasts and B6 mouse myoblasts. Biofabrication of hydrogels was tested using direct ink writing (Cellink BioX). **Results:** We used polyethylene glycol (PEG) polymer–peptide conjugates to study the influence of presence and size of the (B) block on peptide solubility and self-assembly. Recombinant modular proteins were used to study the alternation of (A) and (B) domains on the gelation and final hydrogel properties. The increase of assembling domains resulted in faster onset of gelation as well as higher overall storage modulus. Moreover, we confirmed the accessibility of the bioactive (RGD) domains within the supramolecular hydrogels that facilitate integrin binding and cell spreading (*Figure 2 A*). Finally, due to the fibrillar nature, the hydrogels exhibited self-healing and shear-thinning and could, therefore, be used for injection and biofabrication of self-standing structures (*Figure 2 B*). *Figure 2. NIH/3T3 cell spreading on ABA protein hydrogel (A) and biofabrication of ABABA protein hydrogel (B).* **Conclusions and Outlook:** Overall, similar hydrogels were formed using modular recombinant proteins and polymer–peptide conjugates that self-assembled into fibrillar biomaterials.



References:
1 Salinas-Fernández S et al (2019) Appl Mater Today 100500
2 Álvarez Z et al (2021) Science 374(6569) 848-856
3 Sankar S and O'Neill K et al (2021) Front Bioeng Biotechnol 9 679525

Acknowledgements:
The project was funded by Innosuisse Impulse 35545.1 IP-LS.

PoB.10.09

Biomaterial driven 3D *in vitro* spheroid-based lymphangiogenesis model using click crosslinked hydrogels

Dana E. Al-Ansari[1,2], Yangshuo Hu[1], Nicola Contessi Negrini[1], Daniela Pirri[2], Graeme M Birdsey[2], Adam D Celiz[1]

[1]BioEngineering Department, Imperial College London, London, United Kingdom. [2]National Heart and Lung Institute, Imperial College London, London, United Kingdom

Abstract

Introduction: Despite the recent advances in understanding the molecular mechanisms that drive lymphangiogenesis, most studies use conventional 2D monolayer cell culture, which are limited by unconstrained cell migration and x–y plane adhesion. In contrast, 3D cell cultures can replicate the tissue microenvironment and cellular responses by enhancing cell–extra cellular matrix interactions. Herein, we established a 3D *in vitro* spheroid-based lymphangiogenesis assay using biorthogonal click-crosslinked gelatin hydrogels. **Methods:** Gelatin-Tetrazine (Tz)/Norbornene (Nb) hydrogel (6 - 12% w/v) physico-mechanical properties (elastic modulus, cross-linking kinetics, and biodegradation) and cytocompatibility (viability using LIVE/DEAD, and metabolic activity using AlamarBlue) were assessed for optimised encapsulation of lymphatic endothelial cells (LEC). The influence of gelatin hydrogels on the mRNA expression of LEC marker genes was evaluated using real-time qPCR. Using hanging drops method, LEC spheroids were generated and embedded in gelatin-Tz/Nb hydrogel (+/- VEGF-C) to model lymphangiogenesis. Lymphangiogenic activity was assessed by quantifying the sprout number and length emerging from LEC spheroids. After optimising the lymphangiogenesis assay, we tested our model in several applications, including siRNA gene inhibition, modelling patient-derived endothelial colony forming cells (ECFCs), and high-throughput screening assays. **Results:** Gelatin-Tz/Nb demonstrated tuneable mechanical properties (E = 1-6 kPa; Figure 1 A), gelation times (2-15 min), biodegradability (24–96 hr) and swelling ratios. The viability and metabolic activity of LECs were preserved by gelatin-Tz/Nb at concentrations < 12% w/v. An inhibitory influence of matrix stiffness on LEC vascular network (Figure 1 B) and marker gene expression (PROX1, LYVE1 and VEGFR3) was observed at high concentrations (> 8% w/v). Accordingly, the lymphangiogenesis assay was optimised for 6% w/v Gelatin-Tz/Nb. A range of VEGF-C concentrations (0–200 ng/mL) were tested to stimulate LEC sprouting (Figure 1 C). The lymphangiogenic activity of LEC spheroids showed VEGF-C dependent sprouting, evidenced by quantification of sprout number and length. **Discussion and Conclusion:** The tuneable mechanical properties of gelatin-Tz/Nb provide an avenue not only for tissue engineering but also for clinical applications such as cell therapy or growth factor-incorporated hydrogels for targeted therapy. Our data showed the robust nature of the 3D lymphangiogenesis model, which can be used as an in-vitro platform for assays with wide applicability. This will facilitate the understanding of pro- or anti-lymphangiogenic impact of compounds, genes, and pathways on lymphangiogenesis.

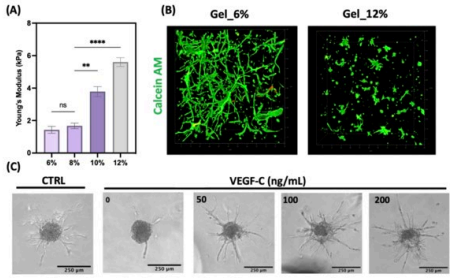


Figure 1. (A) Young's Modulus of Gelatin_Tz/Nb at different concentrations. (B) Top view of Z-stack images of lymphatic endothelial cell (LECs) network formation in 6% vs 12% after 2 weeks of encapsulation. (C) Brightfield images of LECs spheroid – laden Gelatin-Tz/Nb at 6% (+/-) VEGF-C.

Acknowledgments: Graduate Scholarship Research Award; Qatar National Research Fund (GSRA8-I-1-0210-21001); British Heart Foundation Project Grant (PG/20/16/35047); UKRI Future Leaders Fellowship MR/S034757/1

PoB.10.11

Bioadhesives for soft tissue attachment to transcutaneous metallic prosthesis

Sunil Kumar Boda, Vinod Yadav, Sangeeta Shrivas

Department of Biosciences and Biomedical Engineering, Indian Institute of Technology, Indore, 453552, Indore, India

Abstract

INTRODUCTION Intraosseous transcutaneous amputation prostheses are bone-anchored metallic implants protruding through the skin to external fixtures. While titanium osseointegration has seen remarkable success, its soft tissue integration is impeded by epidermal downgrowth [i] mediated peri-implantitis and infection. Previously, we employed dual keratinocyte attachment and anti-inflammatory coatings to promote transmucosal sealing around dental implants [ii]. Currently, we are formulating a bioadhesive to attach transcutaneous metallic implants to interfacing soft tissues. EXPERIMENTAL METHODS Reduced keratin was extracted from human hair using urea, sodium dodecyl sulfate (SDS) and mercaptoethanol, following published protocols [iii]. Extracted keratin was characterized by SDS-PAGE (Polyacrylamide gel electrophoresis) and circular dichroism (CD) spectroscopy. For hydrogel formation, facile Michael addition reaction was employed together with freeze-thaw cycle induced keratin gelation as reported recently [iv]. Hydrogels were characterized by FTIR spectroscopy, swelling behavior and rheological properties. Adhesion strength to titanium by lap shear/ peel tests along with skin compatibility using human dermal fibroblasts and keratinocytes (HaCaT) cells are underway. RESULTS AND DISCUSSION SDS-PAGE of extracted human hair keratin presented two major bands of ~60 and ~40 kDa corresponding to type II (basic) and type I (acidic) sub-units, respectively [v], while CD spectroscopy divulged α-helical secondary structure. While gelation times were significantly longer for Michael addition based gelation of reduced keratin with autoxidized quinone of quercetin/ dopamine, freeze thaw cycles reduced gelation time to ~5 min. Hydrogels swelled in water to ~30 times their weight. Rheological studies and injectability through syringe needles confirmed the thixotropic property of the hydrogel. Mussel-inspired dopamine/ catechol chemistry is expected to confer strong adhesion to titanium, while skin compatibility and anti-inflammatory/ antioxidant activity are envisaged from polyphenol incorporation into hydrogels.

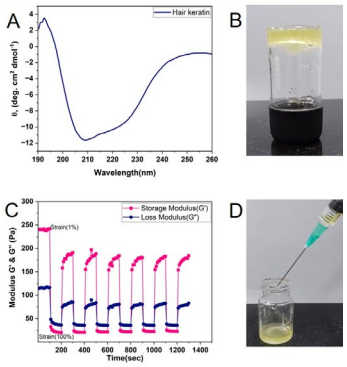


Fig.1. (A) CD spectrum of extracted human hair keratin; (B) Keratin-quercetin hydrogel formation; (C) Thixotropic nature of the hydrogel recorded by cyclic dynamic strain sweep rheology experiment; (D) Injectability of the keratin-quercetin hydrogel

CONCLUSION Our keratin-polyphenol bioadhesive formulation may be a good alternative to titanium implant coatings for promoting soft tissue attachment.

REFERENCES [i] Pendegrass, et al. Biomaterials. 2006. [ii] Boda, et al. Biomater Sci. 2022. [iii] Thirupathi, et al. Biochim Biophys Acta. 2013. [iv] Wang, et al. Macromol. Chem. Phys. 2021 [v] Gao, et al. J Mater Sci: Mater Med. 2019.

ACKNOWLEDGEMENTS

Authors acknowledge funding support from IIT Indore Young Faculty Research Seed Grant, Department of Biotechnology, Govt. of India for Ramalingaswamy Fellowship and Startup Research Grant from Science and Engineering Research Board, Govt. of India.

PoB.10.12

Multiactive hydrogel-based wound dressings

Zdenka Víchová[1], Simona Káčerová[1], Ondřej Vašíček[2], Jan Vícha[1]

[1]Centre of polymer systems, Tomas Bata University in Zlin, Zlin, Czech Republic. [2]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic

Abstract

Open wound belongs to the most common injuries worldwide. The complications caused by insufficient treatment lead to chronic wounds and can have an impact on the quality of life. Moreover, the economic impact on health care is significant. To ensure comprehensive treatment, an ideal wound dressing should support all phases of the wound healing process. I.e. it should act as an anti-inflammatory and antimicrobial agent together with possessing good biocompatibility, moisture, and skin adhesion. Here, we have designed conductive chitosan-based wound dressings containing polypyrrole (PPy) crosslinked using nature-derived 2,3-dialdehyde cellulose (DAC). Water-soluble half N-acetylated chitosan (SCN) was used for the hydrogel preparation. The important advantage is that these wound dressings are possible to prepare by a simple and low-cost process. Prepared wound dressings are shown in Fig. 1a. They offer very good and adaptable skin adhesion (Fig. 1c), appropriate mechanical properties, and specific conductivity comparable to human tissues (Fig. 1b). The polypyrrole particles are homogeneously dispersed in the hydrogel network without the formation of aggregates and their concentration can be controlled (Fig. 1d).

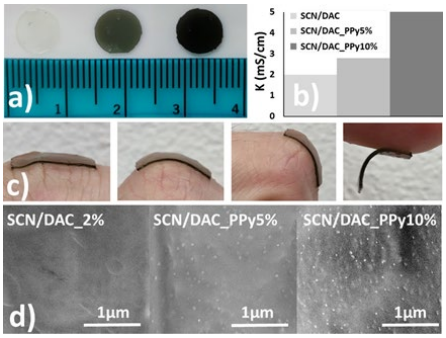


Figure 1: a) Prepared wound dressings containing 0, 5, and 10 wt.% of PPy (left to right); b) specific conductivity of the samples (mS cm-1), c) adhesion of wound dressing to the skin; d) SEM images of dried samples – bright spots are PPy particles dispersed in the hydrogel network.

Moreover, the resulting SCN_DAC_PPy wound dressings possess great biological properties. The non-cytotoxic effect was determined using a fibroblast cell line. Non-irritating nature was established after exposition to the reconstructed human epidermis (RhE) model. The antibacterial activity against S. aureus and E. coli was proved. They show significant free radical scavenging capacity, affect the amount of ROS production by neutrophils, and stimulate NO and IL-6 production of macrophages. Moreover, the synergic effect of PPy and SCN significantly supports the regeneration of wounds. To conclude, the prepared hydrogel-based wound dressing possesses all the essential characteristics required for the next generation of the active wound dressing.

References:

(1) Nourian Dehkordi, A., et al. (2019). Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. Stem cell research & therapy, 10(1), 1-20.

(2) Khan, Z. A., et al. (2019). Chitosan based hybrid materials used for wound healing applications-A short review. International Journal of Polymeric Materials and Polymeric Biomaterials.

Acknowledgment: This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic – DKRVO (RP/CPS/2022/001).

PoB.10.13

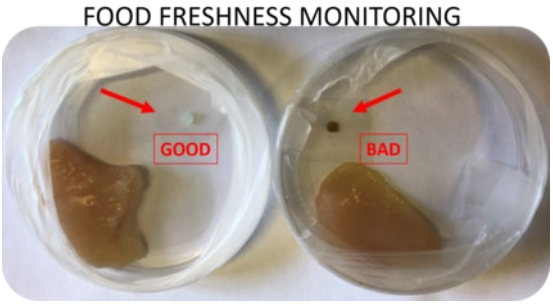
Metal-triggered hydrogels and their applications in water treatment and food spoilage detection

Miriam Mba, Anna Fortunato

Dipartimento di Scienze Chimiche, Università degli Studi di Padova, Padova, Italy

Abstract

In the last few decades, peptide-based biomaterials are playing a vanguard role in different research areas such as drug delivery, regenerative medicine, optoelectronic and sensing fields.[1] The interest in their employment is moved by their ease of preparation and low-cost, but also by their high versatility, biocompatibility and due the fact that their chemistry can be easily manipulated. A particular case of peptide-based biomaterials is the peptide-based supramolecular hydrogel.[2] Hydrogen-bonding, π-π stacking, hydrophobic interaction and Van der Waals forces are the main non-covalent interactions employed to control at the molecular level the hierarchical self-assembly. Recently, metal-ligand interactions have emerged as an important tool to trigger and modulate self-assembly, and to tune the properties of the final supramolecular materials.[3] Herein, we report the metal-cation induced self-assembly of a pyrene-peptide conjugate to form hydrogels. The peptide has been rationally designed to favour the formation of β-sheet 1D assemblies and metal coordination through the glutamic acid side chains. We studied in detail the self-assembly process in the presence of metal salts. We found that the morphology and mechanical properties of the hydrogels are ion-dependent. Moreover, thanks to the presence of the metal, new applications could be explored. Cu^{2+} metallogels could be used for amine sensing and meat freshness monitoring, while Zn^{2+} metallogels showed a good selectivity for cationic dye adsorption and separation.[4]



[1] a) A. Sunna, A. Care, P. L. Bergquist, Peptides and peptide-based biomaterials and their biomedical applications, Springer, 2017; b) S. S. Panda, H. E. Katz, J. D. Tovar, Chemical Society Reviews 2018, 47, 3640-3658.
[2] S. Mondal, S. Das, A. K. Nandi, Soft Matter 2020, 16, 1404-1454.
[3] H. Wu, J. Zheng, A. L. Kjøniksen, W. Wang, Y. Zhang, J. Ma, Advanced Materials 2019, 31, 1806204.
[4] A. Fortunato, M. Mba, Gels 2022, 8, 672

PoB.10.14

Hydrogels based on poly(methacrylic acid) for controlled release of anti-inflammatory drugs

Maja D. Markovic[1], Milica M. Svetozarevic[1], Vukasin Dj. Ugrinovic[1], Rada V. Pjanovic[2], Bojana M. Obradovic[2], Melina T. Kalagasidis Krusic[2]

[1]University of Belgrade, Innovation Center of Faculty of Technology and Metallurgy, Belgrade, Serbia. [2]University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia

Abstract

Everyday struggle of humanity with novel diseases and present once, urge researchers to find novel and improve existing therapies to enhance their efficiency and safety. One of the promising approaches to overcome these challenges is controlled release of drugs. Biomaterials based on poly(methacrylic acid) (PMAA) are excellent drug delivery systems because they can control release rate and released amount of drug. Also, due to their pH sensitivity the PMAA hydrogels can release drug at the site of action. Namely, these nontoxic and biocompatible hydrogels swell in the environment with pH value higher than pKa of PMAA (4.6) and release encapsulated drug during the process. In present study, PMAA hydrogels are synthetized under ambient conditions by simple, cost effective and eco-friendly synthesis. Novel initation system based on hydrogen peroxide, potato peel peroxidase and vitamin C (VC) is used for the first time for free radical polymerization of PMAA hydrogel. In accordance with the principles of circular economy, peroxidase was isolated from potato peel waste by water extraction for 12 h at 4 °C. Four PMAA hydrogels were prepared by using potato peel peroxidase with various enzyme activity (0.4; 0.8; 1.2 and 1.8 IU), whereas the amounts of H2O2 (30 mL) and VC (10 mg) have been kept constant. The composition of the PMAA hydrogels was confirmed by FTIR analysis, whereas their porous structure was revealed by SEM. The swelling of the PMAA hydrogels was monitored in two media: 0.1M HCl (as simulation of human stomach) and phosphate buffer with pH 7.4 (as simulation of human intestines). In order to encapsulate anti-inflammatory drug – dexamethasone into the PMAA hydrogels, the hydrogels were immersed into the dexamethasone aqueous solution (5 mg/ml) and left to swell to the equilibrium, after which they were dried at room temperature. Dexamethasone release from the PMAA hydrogels was monitored in the same environments as was the PMAA swelling. The results showed that around seven times higher amount of dexamethasone was released in the medium with pH 7.4 than in the medium with pH 1. Present study has showed that the PMAA hydrogels, prepared through green and eco-friendly method, have huge potential for encapsulation and controlled release of anti-inflammatory drugs and therefore, for the treatment of rheumatoid arthritis.

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-47/2023-01/200287) and ExcellMater project (European Union's Horizon 2020 No. 952033).

PoB.10.16

In vitro 3D model for monitoring glial cell responses to particles and ions released from spinal implants

Estefanía Echeverri, Paul O'Callaghan, Johan Kreuger, Cecilia Persson

Uppsala University, Uppsala, Sweden

Abstract

Spinal implants have been used for decades to treat different spinal conditions. However, certain implant-related complications have been attributed to the release of particles and ions due to corrosion and wear triggering local immune responses including the release of pro-inflammatory cytokines, leading to local inflammation. The impact of these particles and ions on cells from the central nervous system (CNS) remains largely unknown, with few studies examining the effects on glial cells[1]. Indeed, the particles may migrate to adjacent nervous tissues and increasing our knowledge of the glial cell response is essential since they play a crucial role in maintaining tissue homeostasis and protecting the CNS. Most prior studies have used traditional 2D culture models; however, these lack the 3D spatial arrangement of cells found in tissues where they form important interactions with the extracellular matrix. The aim of this study was to employ an open-source bioprinter[2] to extrude hydrogels containing glial cells into which experimental implant debris can be introduced, enabling monitoring of cell viability and inflammatory responses by fluorescence microscopy. We have previously established that mono-cultures of microglia and astrocytes can be 3D cultured in collagen hydrogels, and their viability monitored using the caspase-3/7 apoptosis reporter and propidium iodide labelling for cell death. Applying a bioprinting strategy to produce these glial-laden constructs increases the reproducibility of these models, and allows the study of a wide range of types and concentrations of particles, resulting in a valuable tool to increase the knowledge about the biological response generated by particles from spinal implants.

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 812765.

References

1. H. Lee et al (2020) Eur Spine J 29:2701–2712
2. A. Engberg et al (2021) Sci Rep 11:21547

PoB.10.17

Silk-based microfiber structures with well-organized geometries and improved elastic properties

Martina Viola[1,2], Gerardo Cedillo-Servin[1,3], Anne Metje van Genderen[4], Isabelle Imhof[1], Paula Vena[1,3], Marko Mihajlovic[2], Jos Malda[1,5], Tina Vermonden[2], Miguel Castilho[3,6,1]

[1]Department of Orthopaedics, University Medical Centre Utrecht, Utrecht, Netherlands. [2]Division of Pharmaceutics, Department of Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht, Netherlands. [3]Department of Biomedical Engineering, Technical University of Eindhoven, Eindhoven, Netherlands. [4]Division of Pharmacology, Department of Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, Netherlands. [5]Department Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands. [6]Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, Netherlands

Abstract

The hierarchical secondary structure of silk fibroin is responsible for its exemplary mechanical properties and which, in turn, has generated great interest for its application in regenerative medicine. At the nanoscale, it combines beta-sheet nanocrystals embedded in a softer semi-amorphous phase, that are assembled into fibrils at microscale and into fibers at millimeter scale. However, with existent silk processing methods, this complex hierarchical structure is destroyed compromising its final mechanical properties. In this work, we hypothesised that electrohydrodynamic writing of SF-based aqueous solutions and their subsequent post-processing in aqueous salt solutions improves hierarchical assembly of the SF and allow fabrication of controllable solid SF microfibers with improved mechanical properties in terms of strength and elasticity. SF was extracted from Bombyx mori cocoons and processed with an in-house built electrowriting printing set-up. Key printing parameters (i.e. speed, voltage and pressure) were investigated to obtain square shape scaffolds with straight fibres. Post-printing fibres were treated with a water solution of NaH_2PO_4 (2M) to induce physical crosslinking, determined by Raman spectroscopy. Post-printing accuracy was evaluated analyzing the diameter and the stacking of the fibers with SEM and mechanical properties of the scaffolds were analyzed with uniaxial tensile test. To confirm, the biological potential of silk scaffolds, conditionally immortalized proximal tubule epithelial cells (ciPTEC) were seeded and cultured *in vitro* on post treated scaffolds. We demonstrated a solvent-free electrowriting process to obtain SF microstructures. By varying the concentration of SF (15-20% w/v) and the concentration of polyethylene oxide (2.5-3% w/v) (Mw: 600-1000 kDa), straight fibres in squared shaped laydown patterns with a fibre diameter between 5 and 20 m and a minimum interfibre distance of 400 micron were successfully produced. We observed that by soaking printed scaffolds in a NaH_2PO_4 part of the beta-sheets and alpha-helixes showing a stiffness of ~250mN and an elastic response at 20% deformation for 10 cycles. Finally, we confirmed that materials used were non-cytotoxic and that ciPTEC cells adhered to fibre scaffolds when coated with L-DOPA and were able to create uniform monolayer characterized by the production of collagen V, typical of healthy epithelial tissue. In summary, we demonstrated that we could achieve the hierarchical structure of SF from the secondary structure to the fibre organization through an electrohydrodynamic writing and a post-printing process, improving the mechanical properties in terms of strength and elasticity.

PoB.10.18

New Photocleavable PEG hydrogels for subtractive two-photon biofabrication

Wanwan Qiu, Ralph Müller, Xiao-Hua Qin

Institute for Biomechanics, Zurich, Switzerland

Abstract

Photocleavable hydrogels (1-3) represent a class of dynamic biomaterials that allow user-dictated control of cell-material interactions with a femtosecond near-infrared (NIR) laser, offering promise for subtractive two-photon biofabrication. However, existing photocleavable hydrogels predominantly rely on the photolabile o-nitrobenzyl (oNB) derivatives with low two-photon absorption cross-section (<0.1 GM) (4). As a result, the low efficiency of these hydrogel systems require high laser dosage to elicit two-photon hydrogel degradation, which may cause cellular damage. Furthermore, the synthesis procedure to introducing photolabile moieties to hydrogel backbones is often laborious. Here, we report the synthesis and characterization of a new photocleavable polyethylene glycol (PEG) hydrogel. First, we synthesized a photolabile PEG linker using the three-component Passerini reaction, involving three different components that bear a carboxylic acid group, an aldehyde group, and two isocyanate groups, respectively. By choosing the photolabile precursor bearing an aldehyde group, the efficiency of the resultant hydrogels for two-photon degradation can be tuned in a modular fashion. Specifically, we synthesized photocleavable PEG acrylates containing different photolabile moieties (Figure 1A). We created photocleavable hydrogels via thiol-Michael addition between photocleavable PEG acrylates and thiol-functionalized hyaluronic acid (Figure 1B). The mechanical properties of these hydrogels were studied by in situ photo-rheology, while two-photon hydrogel degradation was investigated on a confocal laser scanning microscope as well as a commercial two-photon bioprinter (UpNano) (Figure 1C). The print fidelity was investigated as a function of gel mechanics and laser processing parameter (layer height, laser power). The eroded cubes could be observed clearly at a laser power of 70 mW and above. These results demonstrate that the described photocleavable hydrogels in this study show high efficiency for photodegradation under two-photon excitation. Preliminary results of subtractive two-photon biofabrication will be presented in a 3D cell culture.

Reference: 1. Kloxin et al. Science, (2009). 2. Lunzer et al. Angew. Chem. Int. Ed. (2018). 3. Tibbitt et al. Soft Mater. (2010). 4. Aujard et al. Chem. Eur. J. (2006).

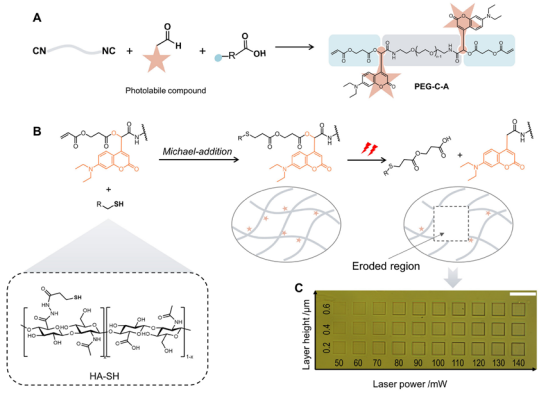


Figure 1. *(A) Schematic of the three-component Passerini reaction and chemical structure of chromophores in this study. (B) Crosslinking by Michael-type thiol-ene addition and subsequent photocleavage of preformed hydrogel under two-photon excitation. (C) Micro-cube image fabricated with varying laser powers in preformed hydrogels. Scale bar, 100 µm.*

PoB.10.19

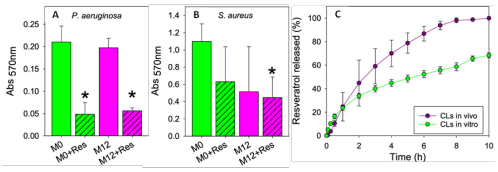
Resveratrol-loaded antibiofilm contact lenses: biomaterials design, characterization and *in vitro-in vivo* behavior

Maria Vivero-Lopez[1], Ana F. Pereira-da-Mota[1], Gonzalo Carracedo[2], Fernando Huete-Toral[2], Ana Parga[1], Ana Otero[1], Angel Concheiro[1], Carmen Alvarez-Lorenzo[1]

[1]University of Santiago de Compostela, Santiago de Compostela, Spain. [2]University Complutense of Madrid, Madrid, Spain

Abstract

Continuous wearing of contact lenses (CLs) is responsible for associated infections, decreasing the number of wearers and making necessary the development of safe and comfortable biomaterials that avoid inflammation and biofilm development. The aim of this work was to design advanced CLs incorporating the antibiofouling monomer 2-methacryloyloxyethyl phosphorylcholine (MPC) and the antioxidant resveratrol to reinforce the antibiofilm capability and manage some oxidative-stress related ocular diseases [DOI:10.1021/acsami.2c18217]. 2-Hydroxyethyl methacrylate-based CLs with different content in MPC (0-12%) were synthesized. All hydrogels were characterized regarding wettability, mechanical properties and MPC distribution into the polymeric network. The capacity of the developed hydrogels to avoid protein adsorption was also measured. Proclear CLs were used for comparative purposes. Resveratrol loading and release profiles were assessed before and after sterilization to fulfill the sterility requirements for commercialization. Then, the antibiofilm, anti-inflammatory and antioxidant properties of most promised formulations were evaluated *in vitro*. Finally, an *in vivo* experiment using New Zealand white rabbits was performed to evaluate the ocular biocompatibility of the CLs and their capability to provide higher and more sustained levels of resveratrol in tear fluid and ocular tissues than previously developed resveratrol-loaded micelles containing the same dose of resveratrol [DOI: 10.1016/j.ijpharm.2022.122281]. CLs were successfully synthesized in one step. The increase in MPC (homogeneously distributed along the polymer network), increased the stiffness of the hydrogels in the dry state, but notably decreased Young's modulus when wet providing greater comfort to the wearers. Moreover, although the CLs became more hydrophilic, the affinity for resveratrol was maintained allowing the loading of therapeutic amounts of resveratrol and providing sustained release *in vitro* and *in vivo* (Figure 1). The adsorption of albumin, although attenuated by MPC, slowed down the release. The addition of resveratrol improved the antibiofilm properties of the CLs against *S. aureus* and *P. aeruginosa* (Figure 1). CLs provided higher and more prolonged levels of resveratrol in tear fluid *in vivo* favoring its biodistribution in the ocular structures. Finally, the developed CLs showed excellent ocular compatibility, and anti-inflammatory and antioxidant properties, being pointed out as comfortable platforms for the sustained release of resveratrol that may help address different ocular diseases. *Figure 1. (A, B) Biofilm growth on the surface of not-loaded and resveratrol-loaded hydrogels, and (C) in vivo-in vitro release profiles of resveratrol from the CLs. *Statistically significant differences (ANOVA, $p < 0.05$)*



Acknowledgments:
MCIN [Grant PID 2020-113881RB-I00/AEI/10.13039/501100011033], FEDER and Xunta de Galicia [Grant ED431C 2020/17; Grant ED481A-2019/120].

PoB.10.21

Injectable elastin-like polypeptide composite hydrogel for soft tissue biofiller application

Mahsa Jamadi[1,2], Mahsa Ghovvati[3,4], Parvin Shokrollahi[5], Nasim Annabi[3,6]

[1]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, USA. [2]Macromolecular Chemistry, Department of Chemistry- Ångström Laboratory, Uppsala University, Uppsala, Sweden. [3]Chemical and Biomolecular Engineering Department, University of California - Los Angeles, Los Angeles, USA. [4]Division of interventional Neuroradiology, Department of Radiological Sciences, David Geffen School of Medicine, University of California – Los Angeles, Los Angeles, USA. [5]Centre for Ocular Research and Education, School of Optometry and Vision Science, University of Waterloo, Waterloo, Canada. [6]Bioengineering Department, University of California - Los Angeles, Los Angeles, USA

Abstract

Introduction. There are still several limitations associated with engineering a soft tissue void filler that is able to closely mimic the host tissue properties, demonstrates desired longevity, provides high chemical and mechanical stability and supports minimally invasive treatment after injection. Elastin-like polypeptides (ELPs) are biopolymers that have been widely investigated for biomedical applications. In this study, a custom-designed ELP sequence, containing thiol residues, was used to engineer stable hydrogels through photo-crosslinking after a short UV exposure. The designed ELP was combined with poly (ethylene glycol) diacrylate (PEGDA) to achieve composite hydrogels with extensibility like natural elastin. The engineered hydrogels could undergo high deformation without rupture under stress, yet exhibited considerable hysteresis and permanent deformation after just one loading cycle. Method. The modified ELP sequence was expressed as described in our previous study [1]. PEGDA was synthesized based on the previously described procedure [2]. A series of solutions were prepared with different volume ratios of PEGDA to ELP ranging from 100:0 to 0:100. Gel distribution was evaluated by visualizing the distribution of auto-fluorescent ELP on a confocal microscope. Viscoelastic properties of the gels were measured using an Anton Paar MCR 301 Rheometer. Tensile and compressive cyclic testing of gels was performed using a mechanical tester (Instron model 5542). The lap-shear strength of hydrogels was measured using 3-(trimethoxysilyl) propyl methacrylate (TMSPMA)-coated glass slides and small pieces of porcine skin and calculated according to ASTM F2255-05 standard. Results. The ability of fabricated hydrogels to withstand mechanical loading and unloading whilst facing external forces was studied by applying compression with a cyclic loading profile at 50% strain. The compressive modulus of the photo-crosslinked ELP/PEGDA composites showed similar values to the soft tissue (8-17 kPa). As expected, the addition of ELP to the PEGDA hydrogel increased the energy loss suggesting the hydrogel's viscoelastic properties. The engineered ELP/PEGDA (50/50) composite hydrogel showed an elongation at break of 322 ± 18 %, low permanent deformation (10.5 ± 1.8 %), and an energy loss of 17.1 ± 2.1% which was comparable to the energy loss of purified native elastin (23 ± 10%). Taken together, results suggest that this composition can be considered for soft tissue reconstruction.

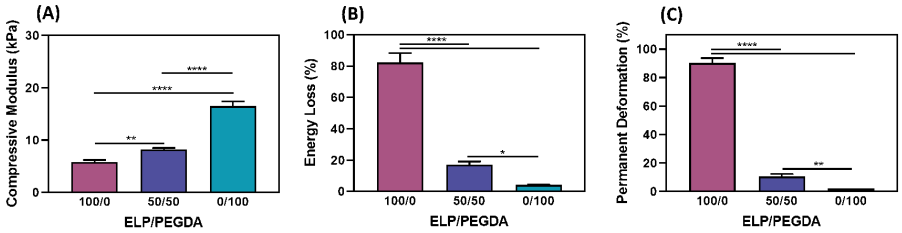


Figure 1. (A) Compressive modulus; (B) Energy loss and (C) Permanent deformation of hydrogels.

[1].Zhang YN, et al., Advanced functional materials 2015, 25:4814-4826.
[2].Jamadi M, et al., Macromolecular bioscience 2017,17:1600479

PoB.10.22

Amniotic membrane-derived anisotropic hydrogels for neural tissue repair

Joana P.M. Sousa[1,2], Inês Deus[2], Catarina Custódio[2], Emmanuel Stratakis[3], João F. Mano[2], Paula A.A.P. Marques[2], Cátia Monteiro[2]

[1]TEMA, Department of Mechanical Engineering, University of Aveiro, Aveiro, Portugal. [2]CICECO, Department of Chemistry, University of Aveiro, Aveiro, Portugal. [3]Institute of Electronic Structure and Laser (IESL), Foundation for Research and Technology-Hellas (FORTH), Aveiro, Portugal

Abstract

Brain-spinal cord electrical communication occurs via longitudinally oriented nerve fibres. Following a spinal cord injury, the neuronal network structure loss, the central nervous system's limited regeneration potential, and the formation of a glial scar seriously hamper patients' sensory and motor capabilities. Therefore, tissue engineering and regenerative medicine researchers are incorporating orientation cues into scaffolds to guide neurons longitudinally and prompt the formation of functional neuronal networks similar to those found in the uninjured spinal cord white matter. Those scaffolds can be classified into three categories according to their micro/nanostructure: fibrous, multichannel, and microporous scaffolds [1]. Here, we present two strategies for the fabrication of 3D scaffolds to promote aligned neural tissue growth: multichannel hydrogels and microporous cryogels. Both scaffolds consist of ECM proteins derived from human amniotic membranes modified with methacryloyl domains (AMMA), the manufacture of which was first reported by our team [2]. AMMA consists primarily of structural proteins, including collagen and fibrillin, but it also contains essential cell-binding proteins such as fibronectin. To fabricate microporous cryogels, a unidirectional temperature gradient was applied to the pre-gel, resulting in the formation of finger-like ice crystals along the longitudinal temperature gradient and the concentration of solutes between them. Following photopolymerization and thawing, cryogels with a microstructure consisting of a negative template of the ice crystals were obtained. On the other hand, multichannel hydrogels were produced using a 3D printed mould. AMMA displayed excellent biocompatibility with neural stem cells (NSC) in *in vitro* tests, promoting cell adhesion and spreading without the use of coatings commonly employed in NSC cultures. These scaffolds supported the differentiation of NSC towards neurons, and their anisotropic microstructure was able to guide neurite extension longitudinally. The interfacing between AMMA and NSC was first reported in this work. The results obtained indicate that microstructured AMMA hydrogels hold great potential for the regeneration of nervous system anisotropic tissues.

[1] Sousa, J. PM. et al. Biomaterials Advances, 148, 213353, 2023.

[2] Deus, I. A., et al. Biomaterials Advances, 134, 112574, 2022.

PoB.10.23

Heparin-binding domains in elastin-like proteins: a way towards tissue integration?

Ken Princen[1], Niels Geysmans[1], Wanda Guedens[1], Peter Adriaensens[1,2], Geert-Jan W Graulus[1]

[1]Biomolecule Design Group, Institute of Materials Research (IMO-IMOMEC), Hasselt University, Diepenbeek, Belgium. [2]Analytical and Circular Chemistry, Institute of Materials Research (IMO-IMOMEC), Hasselt University, Diepenbeek, Belgium

Abstract

A heart attack leads to irreversible damage to the heart muscle. Current treatment methods mainly focus on the prevention of secondary infarcts and do not replace the lost cells. Consequently, a myocardial infarction often initiates a pathway leading to cardiac arrhythmias and heart failure. New stem cell therapies are under development, but are impeded by the limited retention of these cells at the infarct site. Indeed, the majority of injected cells do not have ample time to differentiate into cardiomyocytes. Injectable hydrogels offer an elegant approach to increase stem cell retention. Elastin-like proteins (ELPs) have a long history in this field as a result of their intrinsic lower critical solution temperature (LCST). Dynamic hydrogels based on oxidised hyaluronic acid and hydrazide-functionalised ELPs have been previously demonstrated to increase cell retention without impeding injectability. We used these materials as a benchmark and built on this concept by incorporating heparin-binding domains (HBDs) in the ELP structure. These class of positively charged protein domains has been studied as affinity tag in protein purification and was expected to also interact with negatively charged components in the extracellular matrix (ECM). We studied the interaction of the incorporated HBDs with other ECM components such as hyaluronic acid and collagen in an indirect way by measuring changes in the viscosity of hydrogel formulations. Based on our results, we expect similar interactions to occur in an *in vivo* setting resulting in improved stem cell retention and engraftment.

PoB.10.24

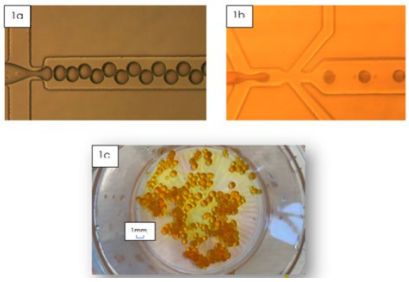
Hydrogel/alginate microspheres: comparison of bulk mixing and microfluidics preparation

Giordana M.S.M. Peregrino[1], Laila Kudsiova[2], Marco Marengo[3], Matteo Santin[1]

[1]University of Brighton, Brighton, United Kingdom. [2]University of Birmingham Dubai, Dubai, UAE. [3]University of Pavia, Pavia, Italy

Abstract

Introduction Microparticles have been advocated as carriers for the controlled release or small therapeutic molecules such as anticancer drugs or nucleic acids. However, their use in clinics depends on the reproducibility, speed and scaling up of their good manufacturing processes (GMP). This study presents the potential of an advanced microfluidic system for alginate microparticles GMP. **Materials and Methods** Microfluidic-based preparation of alginate microparticles were studied by water-in-oil emulsion conditions and by either an off-chip or an on-chip crosslinking process. The off-chip alginate gelation consisted of mixing the alginate solution aqueous phase (2% w/v) with the oil phase made up of mineral oil and Span 80 5% v/v with the final alginate crosslinking occurring in a outlet recipient (Figure 1a). The in-chip system, Figure 1b, was based on a microfluidic system with a channel design enabling the flow of an aqueous phase including 2 % w/v alginate and 0.05 M CaEDTA. This was combined with an oil phase consisting of mineral oil, Span 80 5% and acetic acid (3% v/v). *Figure1: a) off-chip crosslinking. Alginate droplets in the oily phase. B) Alginate is shaped by the first oil phase and crosslinked further inside the chip by the acetic acid. C) bulk preparation.* The latter system allowed a pH decrease to free the calcium ions from the chelator (EDTA) and make them available for the hydrogel crosslinking. All preparations were compared to microparticles prepared by bulk preparation, Figure 1c, where 2% w/v alginate droplets were extruded through a 27 G needle and crosslinked in a 2% w/v $CaCl_2$ solution. Microparticles were filtered through a 0.2 µm filter and rinsed with deionised water to remove excess Ca_2^+ and oil. In all cases, the microparticles were characterised by a dynamic light scattering (Mastersizer 3000, Malvern). **Results and Discussion** Results are reported in Table1: Table 1: Comparison of bulk and microfluidics preparation off- and in-chip gelation. The microfluidic-produced microspheres were highly consistent in shape and the on-chip gelation showed to yield stable microparticles over oil removal procedure by centrifugation, washing and 1h aqueous medium incubation. **Conclusions** The design and testing of the microfluidic system showed its potential to produce drug carriers in the form of highly reproducible, scalable and homogeneous microparticles in GMP-complying conditions while reducing the use of organic media and material waste.



MICROSPHERES	SIZE	PDI
extrusion	1.09 mm	-
Off-chip	307.0±29.1 µm	0.395
In-chip	82.2±0.827 µm	0.172

PoB.10.25

Fiber-reinforced elastin-like recombinamers with biomimetic anisotropic behavior for tissue engineered heart valve application

Federica Sallustio[1,2], Amanda Schmidt[1,3], Ikram El Maachi[1], Martin Frydrych[3], Stefan Jockenhoevel[1], José Carlos Rodríguez-Cabello[2,4], Alicia Fernández-Colino[1]

[1]Department of Biohybrid & Medical Textiles (BioTex), AME-Institute of Applied Medical Engineering, Hemholtz Institute, RWTH Aachen University, Aachen, Germany. [2]Technical Proteins Nanobiotechnology S.L., Valladolid, Spain. [3]Spintex Engineering Ltd., Oxford, United Kingdom. [4]BIOFORGE, CIBER-BBN, University of Valladolid, Edificio LUCIA, Valladolid, Spain

Abstract

Introduction Up to date, many researchers have attempted to create replacements that mimic the native aortic valve, however it is still complex to combine in a single implant the required biological (e.g. hemocompatibility) and mechanical properties (e.g. anisotropy and elasticity), required for optimal functionality. In order to proceed in this direction, here we developed fiber-reinforced Elastin-Like-based tubular scaffolds for the development of an aortic heart valve following the single-point attached commissures technique. Material and Methods The Elastin-Like Recombinamers (ELRs) were bioproduced by recombinant technology, and were chemically modified to bear an azide or cyclooctyne group to enable the formation of ELR-hydrogels, through the catalyst-free click chemistry. The Native Fibroin-Like Protein (NFLP) fibers were kindly provided by Spintex Engineering Ltd., and shaped into fibrillar reinforcement following a winding technique. The fiber-reinforcements were embedded with ELRs by injection molding. Mechanical characerization was performed by tensile testing and the biological behavior was evaluated by culturing primary human endothelial cells (HUVECs). Results and discussion Tubular reinforcements with different fiber angles were successfully obtained, and subsequently embedded by clickable ELRs. The injection moulding resulted in a homogenous Elastin-Like hydrogel, in which the NFLP fiber-reinforcement was completely embedded. Uniaxial tensile tests revealed the capability to adjust the mechanical properties in the radial and circumferential direction, by changing the angle of the NFLP fibers. We established the conditions to obtain scaffolds with a clear anisotropy, comparable to the native tissue. The well-known bioactivity of the ELRs, endowed by the RGD motif, promoted the adhesion of HUVECs on the scaffold. Future steps will focus on fashioning an aortic heart valve following the single-point attached commissures technique.

Acknowledgements
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Skolodowska-Curie grant agreement ID: 956621, and from the Spanish Government PID2019-110709RB-I00. A.F.-C. and I.E.M. acknowledge the financial support within the NanoMatFutur Program of the German Ministry of Education and Research (BMBF, grant number 13XP5136).

PoB.10.26

Facile 3D microgel-based bioprinting using aqueous two-phase emulsion based bioresin

Qingbo Wang, Xiaoju Wang

Åbo Akademi University, Turku, Finland

Abstract

Recently, the development of microgel assembly as void-forming bioinks for 3D bioprinting with microporous hydrogel systems shows promise in supporting the viability and function of cells. To further advance this approach, we have proposed a one-step strategy using a ternary-component aqueous two-phase emulsion (ATPE) that integrates the methacrylated gelatin (GelMA) microgels through vat photopolymerization using digital light processing (DLP)-based bioprinting, as shown in Figure 1. Besides GelMA and dextran as the dispersed and continuous phases of the ATPE, a photo-crosslinkable polysaccharide derived from plant sources, methacrylated galactoglucomannan (GGMMA), was introduced as a multifunctional additive. Attributed to its structural similarity to dextran and chemical similarity of functional groups to GelMA, GGMMA could function as an emulgator that partitioned into both the dispersed phase of GelMA droplets and the continuous phase of dextran to enhance the stability of the aqueous emulsion bioresins. Meanwhile, GGMMA also acts as a photo-adhesive that sufficiently interconnect the GelMA microgels and guarantees the printability of bioresin in DLP printing that allows the conversion of GelMA from emulsion droplets to a microgel and further construction into porous hydrogel through DLP printing.

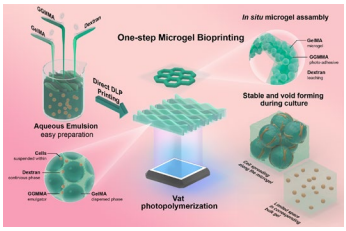


Figure 1. Illustration of the one-step microgel bioprinting of ATPE-based bioresin via vat photopolymerization.

As shown in Figure 2, the printed microgel-based hydrogels showed excellent stability and structural integrity and no swelling was noticeable within 7 days. Benefiting from the spatially interconnected void spaces created by the leaching of dextran during the culturing, the laden murine preosteoblasts (MC3T3-E1) showed great cell activity and spreading within the macroscopic porous hydrogel, compared to the corresponding bulk hydrogel. Moreover, attributed to the facile bioresin preparation, and relatively low shearing associated with this DLP-bioprinting process, the viability of bioprinted MC3T3-E1 and normal human dermal fibroblasts could reach 82.4 ± 5.5% and 92.2 ± 3.7%. Overall, the ATPE-based bioresin of GelMA/GGMMA/dextran amends a functional and facile-to-operate strategy that can realize a one-step workflow of fabricating and assembling GelMA microgels conveniently in the DLP-based cell-laden bioprinting [Wang, Q., et al. Adv. Healthcare Mater. 2023, 2203243. https://doi.org/10.1002/adhm.202203243].

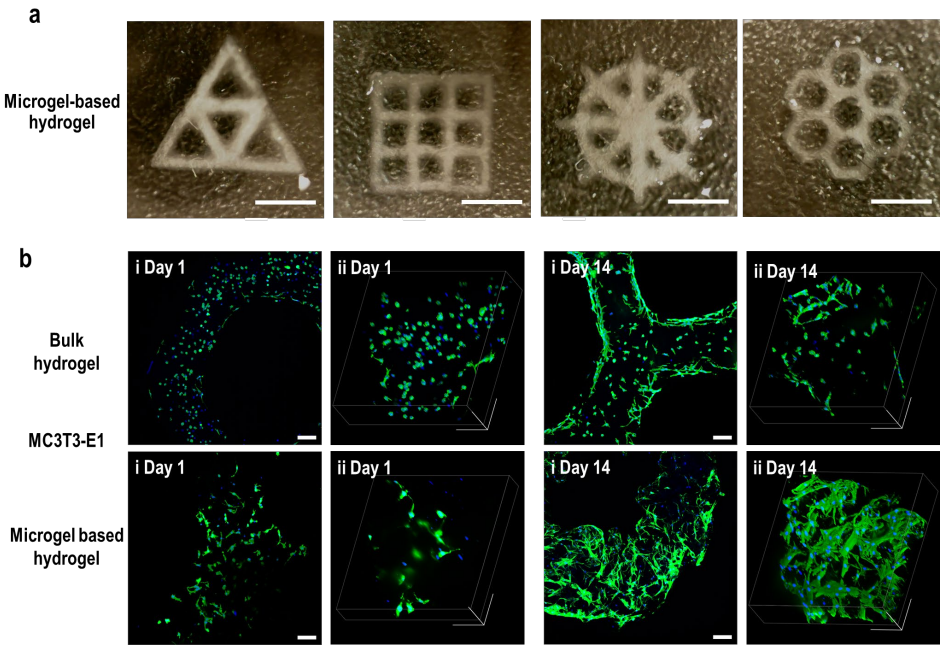


Figure 2. (a) Printed microgel-based hydrogels with different structures showing long-term stability and structural integrity in PBS for 7 days. Scale bars: 2 mm. (b) Fluorescence microscopy images (i) and 3D reconstructed images (ii) of cytoskeleton staining demonstrating MC3T3-E1 spreading in the bioprinted bulk and microgel-based hydrogel on Day 1 and Day 14 (F-actin: green, nuclei: blue). Scale bars: i_100 µm, ii_150 µm.

PoB.10.27

Gentamycin-vancomycin loaded emulsion-based hydrogel to treat methicillin resistant *S. aureus*-orthopedic device-related infection in a single stage revision.

Claudia Siverino[1], Pamela Nylund[1], Andrew L. Foster[1,2,3], Willwmijn Boot[1], Stephan Zeiter[1], Geoff R. Richards[1], Matteo D'Este[1], Fintan T. Moriarty[1]

[1]AO Research Institute Davos, Davos, Switzerland. [2]Queensland University of Technology (QUT), Faculty of Science and Engineering, Brisbane, Australia. [3]Department of Orthopaedic Surgery and Jamieson Trauma Institute, Royal Brisbane and Women's Hospital Australia, Brisbane, Australia

Abstract

The use of biomaterials in the field of orthopedics has positively improved patients' healing outcomes. However, infection complications like orthopedic device-related infection (ODRI) remains a major complication. In this respect, antibiotic-loaded biomaterials can efficiently deliver high local concentrations of the antibiotic directly in target tissues without inducing toxic systemic effects. Antibiotic-loaded cements are often used for infection prevention and treatment. A major limitation of those cements consists in the released subinhibitory antibiotic concentrations which could cause resistance. Moreover, in the context of the intramedullary nail procedure, the use of a bioresorbable and biodegradable, injectable biomaterial like a hydrogel would allow a single-stage surgery with implant removal, debridement, application of the hydrogel and direct exchange nailing without any additional surgical procedures. Therefore, the aim of this study is to develop a gentamycin-vancomycin loaded emulsion-based hydrogel (EBH), named EBH-GV, and prove its antimicrobial activity and biocompatibility *in vitro* and then *in vivo* in a single stage revision ODRI sheep model.

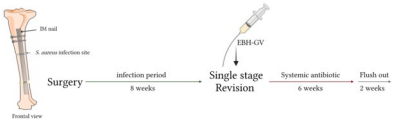


Fig. 1. Schematic representation of the animal study timeline.

EBH revealed storage and loss moduli (G' = 192±8 Pa and G"=168±4 Pa), shear thinning, and elastic recovery indicative of good injectability. Mixing EBH with GV (EBH-GV) led to a granular texture and increased moduli. *In vitro* assays showed no effects of the EBH on viability of telomerase-immortalized human foreskin fibroblasts (hTERT-BJ1) and human bone marrow stromal cells (hBMSCs) or osteogenic differentiation of hBMSCs. The EHB-GV induced a significant decrease in cell viability compared to EBH after 14 days (hTERT-BJ1 p=0.027 and hBMSCs p<0.0001). Similarly, osteogenic differentiation of hBMSCs was reduced with EBH-GV compared to EBH. The *in vitro* antibiotic challenge of methicillin resistant *S. aureus* EDCC-5443 biofilm on pegs showed a significant decrease in colony forming units after exposure to EBH-GV compared to EBH alone (p=0.029) or PBS (p<0.0001). Finally, the EBH-GV was tested in the intramedullary nail-related infection model in the tibia of the sheep. Bacteriology results show complete clearance of the infection in all the tissues retrieved (n=10) from all the animals (n=4) compared to the controls which received only systemic antibiotic treatment (n=3)(p=0.0016 on the total CFU count). The EBH-GV complies with several requirements of hydrogels for local delivery including suitable rheological properties but also *in vitro* effects on MRSA biofilm and *in vivo* results with complete eradication of infection. The EBH shows promising translation potential in clinical application of ODRI.

PoB.10.28

Decellularized extracellular matrix particle-based biomaterials for cartilage repair applications

Jiangyao Xu[1,2], Peng Guo[1], Mauro Alini[1], Andrea Vernengo[1], Sibylle Grad[1], Jeroen Geurts[2], Zhen Li[1]

[1]AO Research Institute, Davos, Switzerland. [2]Lausanne University Hospital, Lausanne, Switzerland

Abstract

Introduction: Decellularized extracellular matrix (dECM) materials derived from cartilage have demonstrated promising regenerative capabilities for cartilage repair due to their chondrogenic bioactivity. However, conventional decellularization processes have limited retention of ECM components and impair the integrity of functional ECM molecules, resulting in compromised biomimetic properties of these materials. Therefore, the current research aims to fabricate biomimetic materials encapsulating cartilage dECM particles with intact molecular structures and natural components for cartilage repair. Methods: A detergent-free decellularization strategy was developed to prepare dECM particles from bovine cartilage tissue which were then incorporated into hyaluronic acid-tyramine (THA) hydrogels . Bovine cartilage tissue fragments were subjected to five cycles of freeze/thaw followed by pulverization in a liquid nitrogen Mixer Mill at 25 Hz for 3 minutes. The resulting material was then rinsed and treated with DNase I and protease inhibitor cocktail for 8 hours to remove cellular DNA. The decellularized tissue was then lyophilized and re-homogenized via pulverization in a liquid nitrogen Mixer Mill at 25 Hz for 15 minutes to generate dECM particles. THA was synthesized by attaching tyramine to hyaluronic acid via amide bond formation in water. After mixing THA with dECM particles at different concentrations, enzymatic cross-linking was initiated by adding H2O2 and incubating at 37°C for 30 minutes. Additional light cross-linking was performed using green light and eosin Y as the photoinitiator. The THA-dECM hydrogels were subsequently subjected to swelling, stability, and compression tests to characterize their properties. Results: The results demonstrated that the decellularization strategy maintained intact proteoglycans and collagens with high retention rate and adequately removed DNA (Fig. 1 A-C). Moreover, the addition of 20% dECM particles enhanced the compressive modulus of THA hydrogels (Fig. 1 E), bringing it closer to the mechanical properties of native cartilage. Conclusions: Our decellularization method successfully preserved functional and intact cartilage components at high yield. Hydrogels containing dECM particles possess long-term stability and enhanced mechanical properties, making them promising biomaterials for cartilage repair.

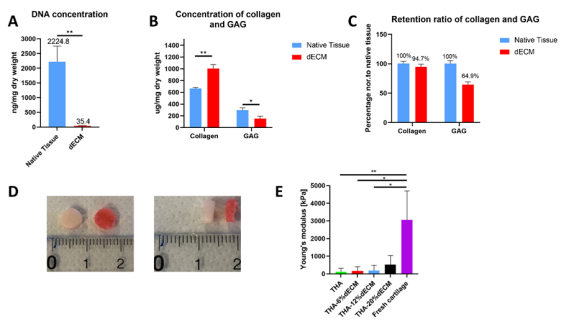


*Fig. 1. Characterization of the dECM materials and THA-dECM hydrogels. (A) DNA, (B) collagen and GAG concentration in native bovine cartilage tissue and dECM particles. (C) Retention ratio of collagen and GAG in native bovine cartilage tissue and dECM particles. (D) Macroscopic images showing the hydrogel biomaterials (red) and fresh bovine cartilage tissue (white). (E) Young's moduli of different dECM hydrogels measured in compression test. Mean ± SD, A-C n=3, E n=9. * $p < 0.05$, ** $p < 0.01$.*

PoB.10.29

Plasmonic control of drug release efficiency in agarose gel loaded with gold-based colloids

Alessandro Molinelli[1], Elisa Lacroce[1], Laura Polito[2], Filippo Rossi[1]

[1]Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Via Mancinelli 7, 20131, Milano, Italy. [2]Consiglio Nazionale delle Ricerche, CNR-SCITEC, Via G. Fantoli 16/15, 20138, Milano, Italy

Abstract

The interest in controlled drug delivery is constantly increasing in the last decades, pointing out the advantages of hybrid nanocomposite containing metal nanoparticles with enhanced properties. Nevertheless, limited experimental research has been carried out on the related performances of these hybrid structures. Among the metal nanoparticles, the ones constituted by noble metals attracted particular attention thanks to the so-called localized surface plasmon resonance (LSPR) when illuminated by a source of light. More specifically, upon the application of external light stimuli, the excited nanoparticles generate a localized increase in temperature in their vicinity.
Based on the current state of art, this project aims to propose the study of a new class of materials, composed of polymeric hydrogels and gold nanoparticles for light induced drug delivery [1]. Indeed, it is possible to promote the molecules delivery from the hydrogel matrix [2], taking advantage of the light induced temperature increase.
In particular, this work focuses on the establishment of a library of nanoparticles containing hydrogel, where the particles are tuned with different architectures, geometries, and functionalization. These parameters would influence the in-situ temperature variation, resulting in a more controlled release of active substances given by the hydrogel network changes. Here a new set of biocompatible hyaluronic acid (HA) based hydrogel and gold nanoparticles (nanospheres, nanorods and nanostars) is presented. The nanoparticles are entrapped in the hydrogel matrix and not released from it; the release of bigger molecules is in this way possible and controlled, when the hydrogel is irradiated by NIR laser source, as schematized in Figure 1. It is expected to select the most promising formulation for the drug delivery, to develop a mathematical model able to predict the temperature increase and therefore the drug release in different conditions.

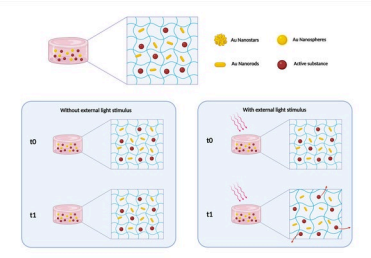


Figure 1. Schematic representation of the substance release from the nanocomposite system. On the left the case without the application of external stimuli, while on the right the case including the external light stimulus

[1] Wang, Y., Kohane, D. "External triggering and triggered targeting strategies for drug delivery". Nat Rev Mater 2, 17020 (2017). https://doi.org/10.1038/natrevmats.2017.20

[2] Moretti, Luca, Mazzanti, Andrea, Rossetti, Arianna, Schirato, Andrea, Polito, Laura, Pizzetti, Fabio, Sacchetti, Alessandro, Cerullo, Giulio, Della Valle, Giuseppe, Rossi, Filippo and Maiuri, Margherita. "Plasmonic control of drug release efficiency in agarose gel loaded with gold nanoparticle assemblies" Nanophotonics, vol. 10, no. 1, 2021, pp. 247-257. https://doi.org/10.1515/nanoph-2020-0418

PoB.10.30

Keratin impacts matrix modulus and cell response in tissue regeneration

A Gnanamani

CSIR-Central Leather Research Institute, Chennai, India

Abstract

Every day thousands of surgical procedures are performed to replace or repair tissue that has been damaged through disease or trauma. The developing field of tissue engineering (TE) is an interdisciplinary field aims to regenerate damaged tissues by combining cells from the body with highly porous scaffold biomaterials. The biomaterials act as templates and guide for tissue regeneration. The biomaterial from natural proteins is well accepted due to its intrinsic advantages. It includes collagen, gelatin, silk, fibrin, keratin etc., Except keratin, all the materials in various forms studied for tissue engineering and received clinical success. Though keratin has superior properties than other protein polymers like low immunogenicity and human origin. It is not much explored as a mainstream biomaterial. Hence, the present study focuses on the utilization of keratin in biomedical and tissue engineering applications. The present study explores extraction of keratin from human hair and fabrication of keratin based composite scaffolds. Since no single polymer or biomaterial could meet the requirements of tissue engineering, denatured fish collagen is is used for the preparation of composites of the present study. Based on the observations and the outcome of the characterization studies, three scaffold materials KFC-5, KFC-7.5 and KFC-9 were chosen for tissue engineering research. It has been observed that apart from several properties played by scaffolds in tissue engineering, the stiffness/matrix modulus found crucial in directing the cell behaviour. All the three chosen scaffolds have wide modulus and the cell behaviour studies assessed using fibroblasts, myoblasts and hematopoietic progenitors. Among the scaffolds chosen, KFC-5 and KFC-7.5 supported the cell behaviour of all the cell types in terms cell adhesion, migration, proliferation and differentiation. The KFC-9 matrix modulus favoured fibroblast behaviour and showed better results on cell storage applications. Thus, keratin may be a suitable natural biological material to impart required modulus to satisfy the requisite properties of biomaterial for tissue engineering applications.

PoB.10.31

Cell migration induces apoptosis of tumor cell via inhibition Wnt-β-catenin signaling pathway

Yao Wang, Jing He, Fang Wu

Sichuan University, Chengdu, China

Abstract

Cancer has becoming a major global public health problem. Surgery, chemotherapy, and surgical removal are the main therapeutic approaches to the treatment of cancer. Nevertheless, there were still some deficiencies in these treatment approach. Such as the destroy of normal cells, and the risk of local recurrence and distant metastasis. To improve the efficiency of tumor therapeutic and further reduce the recurrence rate, attentions are increasingly paid to develop novel anti-cancer biomaterials. Currently, tuning the mechanical properties of the base materials is the mainly approach to design the anti-cancer materials. This this not surprising, because the mechanical property of the tumor microenvironment is critical for regulating the proliferation and apoptosis of cancer cell, which is closely related to the Rho-ROCK signaling pathway. Besides, cell migration is also important for regulating some biological processes, and we hypothesized that tuning the motility is another potential important approach to modifying the tumor microenvironment and inducing tumor apoptosis. To this aim, RGD-modified substrates were prepared to regulate the cell motility through the modification of RGD peptides with different concentrations. The apoptosis of tumor cells had been systematically examined, and the potential involvement of Wnt signaling pathway were further explored. Our study indicated that RGD peptide modification could be used to tune the motility of MG-63 cells effectively, as suggested by single cell trajectory and cell migration distance analysis. The motility study suggested that the high concentration of RGD could effectively suppress the migration of MG-63 cells, which lead to significantly increased apoptosis rate of around 27.5%, about three times of that of the unmodified samples, the similar phenomenon was also observed on the UMR-106 and 4T1 cells cultured on the Col-H group. These results clearly demonstrated that lower motility was much more strongly inclined to undergo apoptosis. Further RNA sequencing was used to exploit the underlying mechanism, and it strongly suggested that the apoptosis of osteosarcoma cells was mediated by mitochondria/caspase dependent apoptosis through inhibiting the canonical Wnt signaling pathway. Activation of the Wnt-β-catenin pathway through HLY-78 significantly suppressed the apoptosis of MG-63 cells, further suggesting the critical role of Wnt pathway in motility-regulated-apoptosis of tumor cells. Our findings provide critical insights to understand the underlying mechanisms that induce the apoptosis of tumor cells, and might provide new strategy for designing the novel anti-tumor materials.

PoB.10.32

3D printed mechanically interlocked PLA-hydrogel interfaces

Lorenzo B Kunkels, Mauricio Cruz Saldívar, Niko E Putra, Carlos Pitta Kruize, Sara Panahkhahi, Sander Leeflang, Lidy E Fratila-Apachitei, Mohammad J Mirzaali, Amir A Zadpoor

Delft University of Technology, Delft, Netherlands

Abstract

In natural systems, hard and soft tissues exhibit remarkable strength at their complex interfaces, as failures are seldom observed at the junctions between them. However, when generating artificial hard-soft material connections, the interface often represents the weakest link, primarily due to stress concentrations arising from disparities in material properties. A unique advantage of nature lies in its capacity to construct interfaces featuring complex gradients in the tissue properties. In this study, we examine the influence of interface geometry on the mechanical properties of hard-soft interfaces, utilizing 3D-printed hydrogel-to-PLA structures designed with the aid of computational analyses. Therefore, we generated several interface designs based on two primary interlocking geometries, i.e., anti-trapezoidal and double-hook. These designs were achieved by altering the opening width, interface depth, trapezoid angle, hook width, and hook depth for the respective geometries. Subsequently, we fabricated these structures by employing an array of techniques, including fused deposition modelling and extrusion-based hydrogel printing. We used finite-element analysis in conjunction with uniaxial tensile tests to characterize and compare the distinct interface geometries and identify the parameters that significantly influence the strength of geometrical interfaces. Furthermore, digital image correlation was utilized to assess the accuracy of computationally predicted interfacial stress distributions. Our preliminary findings indicate that the strength and toughness of hydrogel-to-PLA interfaces are higher in the case of double-hook geometries as compared to anti-trapezoidal ones. Ultimate tensile strengths (UTS) and failure energies (W) ranged between 0.59 ± 0.13 N and 1.14 ± 0.17 N and between 0.63 ± 0.31 mJ and 2.16 ± 0.66 mJ, respectively, resulting in an 190% increase in the ultimate tensile strength and 340% increase in the fracture toughness of double-hook geometries as compared to anti-trapezoidal geometries. The applied finite element models could accurately predict the relative performance of both geometries in terms of ultimate tensile strength, toughness, and stress distribution, up to the yield point. The method used in this work could be used to more rapidly design soft-hard interfaces, identify better performing interface designs, and fabricate hard-soft interfaces with performances close to the predicted values.

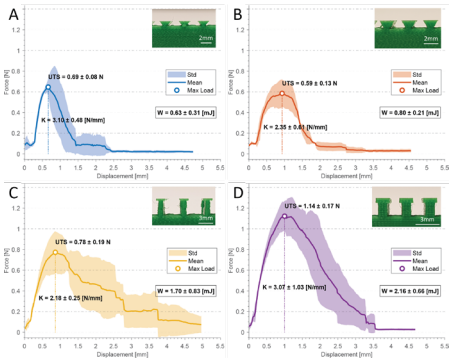


Figure 1. Force-displacement curves for the four fabricated PLA-Hydrogel interfaces, obtained through tensile testing. The ultimate tensile strength (UTS), complete failure energy (W) and stiffness (K) were calculated and marked.

PoB.10.33

Thiol-ene-based inks for standardized tissue models via stereolithography bioprinting

Therese Steudter[1,2], Hamidreza Pirmahboub[3], Joachim von Arnim[3], Samuel Pearson[1], Aránzazu del Campo[1,2]

[1]INM - Leibniz Institute for New Materials, Saarbrücken, Germany. [2]Saarland University, Chemistry Department, Saarbrücken, Germany. [3]Cellbricks GmbH, Berlin, Germany

Abstract

Most bioinks for light-based bioprinting are crosslinked by photoinitiated radical polymerization of acrylates. Despite its widespread use, radical polymerization has some limitations: sensitivity to oxygen, formation of inhomogeneous networks, and it requires light doses which can damage embedded cells. In contrast, thiol-ene polymerization is insensitive to oxygen and forms more homogeneous networks via step growth reaction between vinyl-bearing prepolymers and thiol crosslinkers. The crosslinking of highly activated vinyl groups such as norbornene (Nb) is faster than radical polymerization of (meth)acrylates. This allows reduction of cell exposure to photons and radicals and expediates printing processes. In this work we developed bioinks for stereolithography printing via thiol-ene photocrosslinking based on Nb- and allyl ether-derivatized hyaluronic acid and dextran. The photocrosslinking rate and mechanical properties of the resulting prints were characterized using rheology and benchmarked against radical polymerized methacrylated analogues. The advantages of the thiol-ene based inks for stereolithography printing was proven by obtaining prints with high resolution features at shorter printing times. Mechanical analysis of printed constructs by nanoindentation demonstrated suitable stiffness ranges for free standing tissue models which are currently in development.

PoB.10.34

Synthesis of 4D-printable photocrosslinkable polyurethane inks for creating dynamic humidity sensors and tissue scaffolds using digial light processing

Hossein Goodarzi Hosseinabadi[1,2], Anant Bhusal[3], Ali Yousefinejad[4], Wolfram-Hubertus Zimmermann[5,6,7,8], Leonid Ionov[4], Amir K. Miri[9]

[1]Bayreuth, Bayreuth, Germany. [2]Goettingen Medical Center, Goettingen, Germany. [3]Rowan University, Glassboro, USA. [4]University of Bayreuth, Bayreuth, Germany. [5]University of Göttingen, Göttingen, Germany. [6]Center for Neurodegenative Diseases, Göttingen, Germany. [7]Institute for Translational Medicine and Pharmacology, Göttingen, Germany. [8]Center for Cardiovascular Research, Göttingen, Germany. [9]New Jersey Institute of Technology, Newark, USA

Abstract

A photocrosslinkable polyurethane ink is synthesized for light-assisted 4D printing of smart materials with dynamic shape transformations. The molecular weight distribution of the ink monomers is tuned by adjusting the copolymerization reaction time. Digital light processing technique is used to program a differential swelling response in the printed construct after humidity variations. Bioactive microparticles are embedded into the ink to improve biocompatibility of the printed construct for tissue engineering applications. Self-folding capillary scaffolds, dynamic grippers, and flexible film sensors are made which show reversible shape transformations by changing the humidity level of the atmosphere. The ink can be used for breakthroughs in tissue engineering, actuator, and sensor applications.

PoB.10.35

Magnetic hydrogel activates RAS-dependent signal cascade to induce neuronal differentiation of neural stem cells

Jun Shu, Rongrong Jin, Jing He

National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, China

Abstract

Despite the widespread observations on tissue regeneration of magnetic cue, the pivotal roles of magnetic cues in neural stem cell (NSC) differentiation during nerve repair have not been systematically investigated. Here, we fabricated a biocompatible and magnetic hydrogel composed of chitosan matrices and magnetic nanoparticles (MNPs) with different content, as a magnetic-stimulation platform to apply intrinsically-present magnetic cue and externally-applied magnetic field to NSC grown on the hydrogel. The magnetic chitosan hydrogel facilitated NSC differentiation into neuronal lineage *in vitro* and the subsequent functional recovery in rat hemi-sected spinal cord injury. The MNP content showed a strong regulatory effect on neuronal differentiation and the MNPs-50 samples exhibited the best neuronal potential *in vitro*, as well as accelerated the subsequent neuronal regeneration *in vivo*. Remarkably, the use of proteomics analysis demonstrated the underlying mechanism of magnetic cue-mediated neuronal differentiation form the perspective of protein corona and intracellular signal transduction. Our results indicated that magnetic cues contributed to the activation of intracellular RAS-dependent signal cascades, thus facilitating neuronal differentiation. Magnetic cue-dependent changes in NSCs benefited from the upregulation of adsorbed proteins related to “neuronal differentiation”, “cell-cell interaction”, “receptor”, “protein activation cascade”, and “protein kinase activity” in the protein corona. Additionally, magnetic chitosan hydrogel may act cooperatively with the exterior magnetic field, showing further improving neurogenesis. The synergistic mechanism involving combination of magnetic external magnetic fields would be relatively complicated and involved additional signaling pathway regulating neuronal differentiation, such as chemokine signaling pathway, rather than just coordinated amplification of MNPs. The findings not only provided a simple path to improve magnetic therapies for neuron-related diseases, but also shed light on the underlying mechanism for magnetic cue-mediated neuronal differentiation, coupling protein corona and intracellular signal transduction.

PoB.10.36

Effect of photocuring parameters on viability and activity of mesenchymal stem cells

Masoumeh Jahani Kadousaraei, Shuntaro Yamada, Markus Edward Frøyland Wiik, Samih Mohamed-Ahmed, Kamal Mustafa

University of Bergen, Bergen, Norway

Abstract

Introduction: Many recent biofabrication technologies in the field of tissue engineering involve photocurable biomaterials. As such, photopolymerization parameters including photoinitiator concentration and light exposure time are the critical parameters that require to be optimized to retain cell viability. Objective: This study aimed to investigate the early effect of photocuring parameters including light exposure duration and photoinitiator concentration on the viability and mitochondrial activity of human bone marrow mesenchymal stem cells (hBMSCs) to identify an optimal working range of photocrosslinking parameters. Method: Firstly, various LAP (Lithium acylphosphinate photoinitiator) concentrations (0.25, 0.5, 1, and 2% (w/v)) were introduced to a suspension of hBMSCs. Subsequently, the cell suspensions were directly exposed to visible light with 1200 mW/cm2 intensity for the 60s and 120s. Flow cytometry was used to measure the viability of hBMSCs using propidium iodide (PI) staining immediately after light exposure. Afterward, two optimal parameters were selected for the photocrosslinking of gelatin methacryloyl (GelMA, 5% (w/v)) as the photopolymerized model for embedding hBMSCs. After 1h and 24h, viability and mitochondrial activity of encapsulated hBMSCs were evaluated by live (calcein AM) dead (ethidium homodimer-1) staining and alamarBlue assay, respectively. Results: The flow cytometric analyses showed that the viability of hBMSCs was reduced drastically by irradiating the cells to the light for 120s for all LAP concentrations. The 60s light exposure decreased cell viability to about 40% in the group with 2% (w/v) LAP. However, more than 85% of hBMSCs remained viable in the groups with up to 1 % (w/v) LAP when the cells were exposed to the same photocuring duration. Subsequently, the 60s exposure time was selected to photopolymerize the precursor solution of GelMA-hBMSCs with 0.25 % and 0.50 % (w/v) LAP. Live and dead staining of encapsulated hBMSCs showed comparable cell viability in GelMA with both LAP concentrations after 1h and 24h. However, the mitochondrial activity of cells embedded in the GelMA with 0.5 % (w/v) LAP was significantly lower than the group with GelMA with 0.25 % (w/v) LAP. Conclusion: These findings indicated that the selection of the photocrosslinking parameters such as photoinitiator concentration and photocuring is crucial for the viability of mesenchymal stem cells.

PoB.10.38

Hydrogels for the sustained release of disulfiram to treat tumors and infections

Carmen Alvarez-Lorenzo, Maria Vivero-Lopez, Alex Alcoba-Pacheco, Alejandro Seijo-Rabina, Angel Concheiro

University of Santiago de Compostela, Santiago de Compostela, Spain

Abstract

Disulfiram, a well-known prodrug of diethyldithiocarbamate for the treatment of alcoholism, has been pointed out as a suitable drug for the treatment of several tumor and infectious diseases [1,2]. However, its low aqueous solubility makes necessary the development of new drug-delivery systems capable of increasing its bioavailability. The aim of this work was to design silicone-based hydrogels capable of sustained release disulfiram. Four different types of silicone-based hydrogels were prepared (Table 1) into presilanized glass molds of 0.3 mm thickness at 50 ºC for 12 h and 70 ºC for other 24 h. After polymerization, the hydrogel sheets were demolded, boiled in 1 L of distilled water and cut into 10 and 16 mm-diameter discs. The discs were alternatively washed in water and NaCl 0.9% until the complete removal of unreacted monomers, dried at 70 ºC for 24 h and stored until their further use. The silicone-based hydrogels were extensively characterized regarding swelling in water, transmittance and mechanical properties. Their biocompatibility was also evaluated through an *in ovo* HET-CAM assay. The hydrogels were loaded with disulfiram by soaking in a drug solution containing 2-hydroxypropyl-β-cyclodextrin as solubilizing agent. Immediately after, the loaded-discs were transferred to vials containing saline medium (pH 7.4) at 36 ºC and 180 rpm for 72 h. Aliquots of 300 µL of the loading and release medium were taken at pre-established times and analyzed by HPLC to quantify the amount of disulfiram loaded and released. All developed silicone-based hydrogels were highly transparent and showed adequate mechanical properties and excellent biocompatibility. Moreover, the addition of APMA increased the swelling degree of the hydrogels and their affinity for the drug providing a higher amount of disulfiram released. In summary, the designed silicone-based hydrogels are pointed out as promising delivery systems to increase the bioavailability of disulfiram.

Hydrogel	HEMA (mL)	MCS-MC12 (mL)	NVP (mL)	APMA (mg)	EGDMA (µL)	AIBN (mg)
HS	2.75	0.25	-	-	12.10	4.93
HSA	1.25	0.25	1.5	-	12.10	4.93
HSN	2.75	0.25	-	21.45	12.10	4.93
HSNA	1.25	0.25	1.5	21.45	12.10	4.93

Table 1. Hydrogels composition.

References:
[1] Sauna, Z. E. et al., (2005). Disulfiram, an old drug with new potential therapeutic uses for human cancers and fungal infections. Molecular BioSystems, 1(2), 127-134.
[2] Lajarin-Reinares, M. et al., (2022). Repurposing disulfiram as an antimicrobial agent in topical infections. Antibiotics, 11(12), 1752.
Acknowledgments:
GLIOSILK Project funded by Institute de Salud Carlos III (ISCIII) [AC19/00067] co-financed by FEDER, Spain.

PoB.10.41

Going 3D to understand astrocyte remodeling in ischemic injury: an alginate-based approach

Georgia Athanasopoulou[1,2,3], Miguel R.G. Morais[1,2,3], Eva D. Carvalho[1,2,4], Ana P. Pêgo[1,5,3]

[1]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [2]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [3]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal. [4]Faculdade de Engenharia, Universidade do Porto, Porto, Portugal. [5]i3S - Instituto de Investigação e Inovação em Saúde, Porto, Portugal

Abstract

Astrocytes are the most abundant glial cells in the brain with key roles in CNS homeostasis and response to injury. Upon an insult, astrocytes undergo a process termed reactive astrogliosis, where they alter their phenotype and function. During this process, which is often accompanied with changes in the extracellular microenvironment, astrocytes exist in a range of phenotypes. Here, we aimed at developing a 3D *in vitro* platform to explore astrocyte phenotype in an ischemia-induced injury model and further investigate heterogeneity and mechanosensing in pathology. Alginate hydrogels were produced by combining modified alginate formulations containing the cell adhesive peptide RGD, incorporated via carbodiimide chemistry, and the matrix metalloproteinase sensitive peptide PVGLIG, engrafted through reductive amination, on partially oxidized alginate. To reproduce ischemic conditions an oxygen and glucose deprivation/reperfusion (OGD/R) protocol was implemented. Primary rat astrocytes embedded in alginate hydrogels were cultured in media lacking glucose, and in a hypoxic chamber (<1% O2) for 6h, 24h and 48h. Subsequently, cells were cultured for additional 24h and 48h in complete media and normoxia. Cellular response was assessed by measuring metabolic activity and by immunofluorescence for the glial fibrillary acidic protein (GFAP) and the Ki-67 marker of proliferation. The expression of astrogliosis markers was also evaluated by RT-qPCR. Calcium imaging showed that embedded astrocytes exhibit spontaneous activity indicating maintenance of physiological function within the modified alginate matrices. In the ischemic scenario, astrocytes' metabolic activity tendentially decreased after 6h of OGD, although returning to control levels 24h after re-oxygenation. Image analysis revealed an increased GFAP intensity and volume after 24h of re-oxygenation, and a tendency for increased proliferation after 48h of re-oxygenation. For the first time, an OGD protocol has been successfully implemented in a 3D *in vitro* glial tissue engineered hydrogel-based system. Our preliminary results indicate that 6h of OGD is contributing to an increase in GFAP intensity and cell proliferation, possibly leading to altered phenotype and astrocytic reactivity. By further optimizing the conditions for a 3D ischemia-induced astrocytic model we will be able to explore how matrix alterations impact astrocyte phenotype in the context of ischemia and possibly reveal new avenues to tackle this CNS pathology.

Acknowledgements
Air Force Defense Research Sciences Program, USA (Award FA9550-20-1-0417). G. Athanasopoulou acknowledges ASTROTECH Project for her Ph.D. fellowship (956325 - H2020-MSCA-ITN-2020). M. R. G. Morais and E. D. Carvalho acknowledge FCT from their Ph.D. fellowships (2022.13353.BD and SFRH/BD/140363/2018, respectively).
Keywords: astrocytes, OGD, tissue engineering, hydrogels

PoB.10.42

Beyond the matrix: exploring the mechanobiology of astrocytes in alginate-based hydrogels

Miguel R.G. Morais[1,2,3], Eva D. Carvalho[1,2,4], Georgia Athanasopoulou[1,2,3], Marco Araújo[1,2], Cristina C. Barrias[1,2,3], Ana P. Pêgo[1,2,3]

[1]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [2]INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [3]ICBAS – Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Porto, Portugal. [4]FEUP – Faculdade de Engenharia da Universidade do Porto, Porto, Portugal

Abstract

Neurodegenerative disorders of the central nervous system (CNS) have a tremendous socioeconomic impact, which is expected to escalate with the increasing life expectancy. Although some of these pathologies have been described more than 100 years ago, they remain incurable. There are several known aspects that play a role in these pathological scenarios, including genetic, environmental, and endogenous. Among these, neuroinflammation and astrogliosis are common hallmarks in many neurodegenerative disorders. One of the main players in these processes are astrocytes. Upon an injury these cells provoke extensive alterations in extracellular matrix (ECM) composition and, consequently, in tissue mechanical properties. Here we propose a 3D astroglial tissue engineered model that recreates these key features of astrogliosis and neuroinflammation. Ultrapure high-molecular weight alginate (guluronic acid content 68%) was chemically modified with the cell adhesion peptide GGGGRGDSP by carbodiimide chemistry. The matrix metalloproteinase sensitive peptide PVGLIG (GGYGPVG↓LIGGK) was grafted to alginate by reductive amination on partially oxidized alginates. Primary rat astrocytes were embedded in a 60:40 ALG formulation (ALG-Ox PVGLIG:ALG RGD) to recreate the ECM environment. These modifications to the alginate backbone allowed astrocytes to extend long processes and form a 3D network. A combination of lipopolyssacharide and interferon gamma was used to mimic a pro-inflammatory environment, in which astrocytes acquired an astrogliosis-like phenotype with increased expression of Lcn2, IL-6, C3 and Gbp2 (astrogliosis and pro-inflammatory markers assessed by RT-qPCR), as well as production of nitrite oxide species. Mechanical properties were dynamically tuned using an external chelator (Ba2+) that led to a six-fold increase in the stiffness of the hydrogels, without remarkable alterations on cell metabolic activity, viability and functionality (assessed by calcium imaging). When astrocytes were stimulated via combination of biochemical and mechanical stimuli, Lcn2, C3 and Gbp2 were up-regulated, but not IL-6. This suggests that mechanical properties alterations further impact the astrocytic response to a pro-inflammatory challenge. This model will ultimately be used to explore novel mechanotransduction molecular targets that can later lead to the development of new therapeutic approaches to overcome astrogliosis/neuroinflammation in neurodegenerative disorders.

Acknowledgments

The authors acknowledge the funding from the FCT-UT Austin Portugal Program (project UTAPEXPL/NTec/0057/2017) and the Air Force Defense Research Sciences Program, USA (Award FA9550-20-1-0417). M. R. G. Morais and E. D. Carvalho acknowledge FCT for their Ph.D. fellowships (grants 2022.13353.BD and SFRH/BD/140363/2018, respectively). G. Athanasopoulou acknowledges ASTROTECH Project for her Ph.D. fellowship (Grant Agreement number: 956325 — ASTROTECH — H2020-MSCA-ITN-2020).

PoB.10.44

Rebuilding the anti-angiogenic character of the native IVD microenvironment through the integration of thrombospondin-1 into an ECM-derived hydrogel

Carlos Botelho[1,2], Kaoutar Chattahy[1,2], Raquel M Gonçalves[1,2,3], Mário A Barbosa[1,2,3]

[1]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [2]INEB – Instituto de Engenharia Biomédica, Porto, Portugal. [3]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Abstract

Introduction Intervertebral disc (IVD) degeneration represents a major health problem, with a 37.4% prevalence above the age of 40. As the IVD degenerates, it becomes susceptible to inflammation and herniation, which can cause low back pain. The use of biomaterials, such as hydrogels, has shown promise in providing a pro-regenerative microenvironment for the treatment of degenerated IVDs. However, it is vital to inhibit new blood vessel formation. Therefore, in this work, we have integrated thrombospondin-1 (TSP-1), an anti-angiogenic extracellular matrix (ECM) glycoprotein, into decellularized IVD-based hydrogels. Through the interaction of TSP-1 with key macromolecules present in the tissue (e.g., collagens), we expect to rebuild the anti-angiogenic character of the native IVD and improve the biomodulatory potential of these biomaterials. **Methods** The nucleus pulposus (NP) was isolated from IVDs derived from one-year old bovine tails and decellularized with a 0.1% SDS-based protocol and a vacuum-based system. The decellularized NPs were lyophilized and solubilized in 3% acetic acid/pepsin to generate hydrogels. Following a one-hour incubation with TSP-1 (10 or 20 µg/mL), polymerization was achieved by neutralizing the mixture to pH 7.4 and placing it at 37°C. The mechanical properties of the biomaterials were then analysed by rheology, whereas the presence of TSP-1 was assessed by immunohistochemistry and FTIR. Additionally, the chorioallantoic membrane (CAM) assay was used to evaluate angiogenesis inhibition. **Results** Decellularization removed most cells, nuclei and DNA from the NPs, contributing to the production of a stable and homogeneous hydrogel. Following polymerization, changes in the storage (G') and loss (G'') moduli highlighted the viscoelastic solid-like profile of this platform (G' > G''). The positive immunohistochemistry staining, together with the deconvolution of the FTIR spectrum, allowed for the detection of the TSP-1, suggesting its affinity to the collagenous network of the hydrogel. In the CAM assay, TSP-1 inhibited angiogenesis in a concentration-dependent way, as observed in Figure 1. The assay showed that the hydrogel alone does not impair new blood vessel formation. However, after its integration with the glycoprotein, the desired effect was achieved.

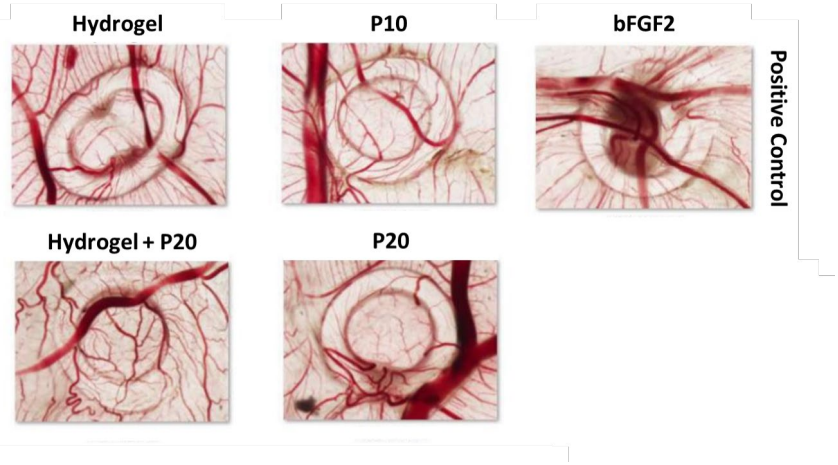


Figure 1 – Representative images of excised CAMs. P10 and P20: TSP-1 at 10 and 20 µg/mL.

Conclusion Our results suggest that the combination of decellularized NP-based hydrogels with TSP-1 may lead to the inhibition of angiogenesis, therefore recapitulating the avascular microenvironment of the native IVD.

Acknowledgments This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through the FETBIO project (FCT-2020-PTDC/BTM-MAT/0438/2020).

PoB.10.47

Uncovering a novel hydrogel based therapeutic approach for osteoarthritis

Nuno M Da Silva Rosa[1,2,3], Morena F Fiordalisi[1,2,3], Susana G Santos[1,2], Mário A Barbosa[1,2,3]

[1]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [2]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [3]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Abstract

Osteoarthritis (OA) is a painful crippling disease that affects various joints throughout the body, and may influence the onset of low back pain(1). OA progression results in articular cartilage erosion with current treatments unable to promote cartilage repair, so a novel approach is needed(2). The aim of this work is to produce a hydrogel that can provide an appropriate microenvironment allowing chondrocytes to produce a de novo matrix. Thus, an approach involving extracellular matrix sourced from decellularized bovine articular cartilage scaffolds (deECM), applied as a hydrogel, may prove suitable to restore the composition of healthy tissues(3). Such gels may incorporate various cell types, especially chondrocytes and/or mesenchymal stem cells (MSC) to aid in cartilage tissue engineering approaches. A decellularization approach requiring the use of hypotonic buffer, detergent and enzymes, was applied with appropriate washing periods. The final product was collected and freeze dried, being then exposed to an acidic pepsin enzyme containing solution and left to stir. The product was neutralized to physiological pH and placed at 37°C, to allow for gelation. Stability at 37°C was verified; rheological analysis was performed while turbidity measurements determined gelation times. Since the decellularization process notably reduces chondroitin sulphate (CS), a procedure to functionalize the soluble pre-gel solution with CS was investigated. This polymer was modified (mCS) with a suitable amine binding complementary functional group and allowed to react with the pre-gel solution for 48 hours at 7°C. Also, human bone marrow MSCs (bmMSCs) were seeded within the gel to test for cell viability, using calcein and propidium iodide staining. Obtained results showed the decellularization strategy removed >99% of DNA. Turbidity measurements showed complete gelation within 30 minutes. The gels were stable for at least 14 days; rheological results showed they are viscoelastic and have solid gel-like properties (elastic modulus: ~220 Pa). Furthermore, the CS functionalized gels appeared to be much softer (0.5 mg/mL mCS mixed with pre-gel solution, elastic modulus: ~170 Pa). Preliminary results for tested gel's cell viability, had approximately over 80% cell survival at 3 days. To conclude, the approaches undertaken appear to successfully allow formation of a gel, along with a functionalisation strategy that has low cytotoxicity to bmMSCs. The chondrogenic potential is being assessed.

This work was supported by Fundação para a Ciência e a Tecnologia through the FETBIO project (FCT-2020-PTDC/BTM-MAT/0438/2020) and the BiotechHealth International PhD Program.

(1)PMID:26117175
(2)PMID:31894891
(3)PMID:36403438

PoB.10.48

Development of hyaluronic acid-based hydrogel for local delivery of itaconic acid to enhance bone regeneration

Maksims Kalinins[1], Jingzhi Fan[1,2], Liva Vita Kaufmane[1,2], Annija Vaska[1,2], Armands Sebris[1], Maris Turks[1], Kristaps Klavins[1,2]

[1]Riga Technical University, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Riga, Latvia

Abstract

Over the past few decades, tissue engineering utilizing biomaterials has emerged as a promising approach for the clinical treatment of bone defects, including fractures. In this regard, the localized delivery of endogenous bioactive metabolites with the aid of biomaterials has been identified as an innovative and potent technique to promote pro-regenerative signaling at the injury site. Itaconate, a conjugated 1,4-dicarboxylate generated by macrophages, has recently been identified as a crucial autocrine regulatory component in inflammation and the development and progression of immunity. It has been shown to stimulate osteoblasts and enhance bone formation by accelerating osteogenic differentiation in mice when it is supplemented. In this study, we aimed to develop novel chemically modified hyaluronic acid (HA)-based hydrogels to facilitate local delivery of this metabolite to promote bone regeneration in tissue engineering. The itaconic succinic anhydride was used to attach the metabolite directly to the hyaluronic acid. The modified HA hydrogel was prepared using a cross-linking method with 1,4-butanediol diglycidyl ether (BDDE). We investigated the modified HA and HA hydrogel samples' molecular structure using techniques such as Fourier transform infrared spectroscopy, NMR, size exclusion chromatography, and mass spectrometry. We also determined the swelling capacity and gel fraction of the HA hydrogels and performed oscillatory rheology measurements using a rheometer. We evaluated hydrogel degradation and metabolite release using PBS, and the concentration of released metabolites was measured using LC-MS. Next, we performed *in vitro* evaluation using MC3T3-E1 cells grown for three weeks in the presence of prepared hydrogels. We assessed ALP production, fibrin deposition, and calcification subsequently. The formation of calcification nodules was evaluated microscopically and quantified spectroscopically. Additionally, we performed metabolomics analysis to characterize hydrogel-induced metabolic perturbations comprehensively. Our results suggest that the modified HA-based hydrogels offer an innovative approach for the localized delivery of itaconate and can promote bone regeneration in tissue engineering.

Funding The authors acknowledge financial support from the European Union's Horizon 2020 research and innovation programme under the grant agreement No. 857287 (BBCE – Baltic Biomaterials Centre of Excellence) and the Ministry of Economics Republic of Latvia) project "State research project in the field of biomedicine, medical technologies and pharmacy", project No. VPP-EM-BIOMEDICĪNA-2022/1-0001

PoB.10.50

New biomimetic matrices for wound dressing

Maria d'Agostino, Antonella D'Agostino, Elisabetta Cassese, Chiara Schiraldi, Annalisa La Gatta

University of Campania "Luigi Vanvitelli", Naples, Italy

Abstract

Hydrogels based on gelatin crosslinked with microbial-transglutaminase (Xgel), in the presence of bioactive polysaccharides such as hyaluronan (HA) and biotechnological chondroitin, (BC) alone or in combination, proved promising scaffolds for bone regeneration (La Gatta et.al 2021). Xgel hydrogels are being increasingly exploited for tissue engineering and proved high potential also in skin regeneration (Anne K Brooks et. Al 2022). Further, the combination HA-BC recently proved more efficient, compared to HA alone, in prompting skin repair (D'Agostino et al., 2022). On these grounds, biomimetic materials (semi-IPN XGel hydrogels with HA and BC) based on an approach similar to La Gatta et al. (2021) were evaluated for skin regeneration purposes. In particular, various processing was exploited to obtain hydrogels with diverse 3D-morphologies (sponges/films). The resulting 3D networks were characterized *in vitro* with respect to Xgel alone in terms of rheological behavior and in relation to the release of HA and BC from the gelatin polymeric network. The materials exhibited lower swelling extent and improved stiffness compared to the gelatin matrix alone, whilst maintaining high stability. Observation at the scanning electron microscope (SEM) revealed physical features promising for skin regeneration. HA and BC release from the scaffold was sustained and supported a prolonged suitable biochemical signaling. The hydrogels were seeded with human dermal fibroblasts and the resulting constructs were cultured under static conditions and characterized to evaluate cell response by proliferation assay and specific biomarkers of tissue integrity. Viability tests indicated higher cell proliferation in the presence of HA and BC. Protein expression of tissue specific biomarkers (collagen, elastin, integrin αV), by western blotting and immunofluorescence, remarked an improvement of functional tissue physiology. Based on these encouraging results, the cell-laden hydrogels are now being evaluated also under perfusion culture conditions using a cell bioreactor. The hydrogel seeding under perfusion conditions as well as the direct incorporation of cells in the material during enzymatic crosslinking are also being evaluated. Data are expected to provide useful information toward the use of these hydrogels for the development of devices intended for topical use aiming at wound dressing applications.

PoB.10.51

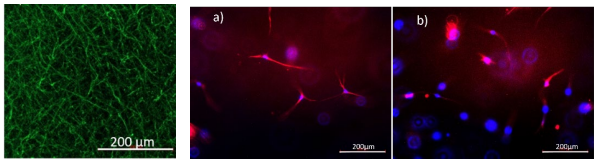
Impact of collagen hydrogel design on embedded smooth muscle cells for optimal 3D mechanobiology study

chloe techens[1,2], Amira Ben Hassine[1,2], Edwin-Joffrey Courtial[3,4], David Eglin[1,2], Stéphane Avril[1,2]

[1]Mines Saint-Etienne, Saint-Etienne, France. [2]University Jean Monet Saint-Etienne, Saint-Etienne, France. [3]3D FabLab, Lyon, France. [4]University of Lyon, Lyon, France

Abstract

Introduction Mechanobiological homeostasis in arteries is normally regulated by the vascular smooth muscle cells (SMCs) contractile phenotype. 3D cell culture models of embedded SMCs offer a realistic model of the in situ cell-matrix interactions for the *in vitro* assessment of contractile forces. Because the matrix itself can induce SMCs phenotypic transitions, the hydrogel selection requires that embedded SMCs keep their contractile phenotype. This study aims to design and characterize a suitable collagen hydrogel, maximizing SMCs viability and the fraction of elongated cells (contractile morphotype) among the cell population. Methods Twelve chemically different type I collagen hydrogels were derived varying the collagen solution dilution (from 2.5 to 10 mg/ml) and the pH (from 7.4 to 8 to allow cell viability). Each hydrogel solution was derived into both acellular and seeded hydrogels. Primary aortic SMCs (AoSMC, Lonza) at passage P10 were mixed with the hydrogel solutions at three cellular densities. After a 5 days differentiation, cell quantity and morphotype were characterized, imaging cell nuclei and actin fibres in fluorescence with confocal microscope (Axio Observer Z1 station, Zeiss). The acellular collagen fiber arrangement was also observed in fluorescence. The acellular hydrogel viscoelastic behaviour was characterized in oscillatory shear using a stress-controlled rheometer (Discovery HR 2, TA Instruments) with 15mm plate-plate geometry at frequencies in the range 0.1-10.0 Hz. Results and Discussion The number of viable cells and the fraction of elongated cells were maximal for the hydrogel with the lowest density (Fig. 1). It exhibited low stiffness (170 Pa) and low viscoelasticity (50 Pa.s). These mechanical properties are well suited for traction force microscopy studies which measures matrix deformation in a time range <5min. A low density mesh also improves diffusion of nutrients and chemicals to the centre of hydrogel. In addition, high fractions of SMC contractile morphotype (Fig. 2 a) were found in low collagen concentration hydrogels (p=0.001) and for sufficient initial cellular densities (p=0.008). However, the study of single cell mechanobiology will require balancing the initial cellular density to enhance the contractile morphotype while avoiding cell superposition (Fig. 2 b). *Figure 1: Collagen fiber structures of the hydrogelFigure 2: SMC populations in different hydrogels. Nuclei (blue) and actin fibers (red) were imaged by fluorescence.*



Conclusion Design characteristics of a hydrogel suitable for studying *in vitro* embedded SMC mechanobiology were identified. Future work will include the quantification of SMCs basal tone and assessment of their mechanobiological response to matrix loading.

PoB.10.52

Tough attachment of hydrogels to solid materials and simultaneous agent-free cross-linking using ion-assisted plasma polymerization technique for biomedical applications

Ghazal Shineh[1], Behnam Akhavan[1,2]

[1]School of Biomedical Engineering, The University of Sydney, Sydney, Australia. [2]School of Engineering, University of Newcastle, Newcastle, Australia

Abstract

Covering the surface of the bioimplants with biocompatible hydrogel materials without changing the bulk properties provides a high level of functionality. The challenge of weak binding between the wet hydrogel materials and solid implants has not yet been resolved to design such hydrogel-solid hybrids. Here, we report the ion-assisted plasma polymerization (IAPP) technique which has dual functionality. The radicals entrapped in IAPP coatings can not only covalently attach hydrogels to the surface of bioimplant, but also can chemically crosslink the hydrogel network without the application of an external agent. This technique strongly binds crosslinked hydrogels to non-polymeric substrates such as titanium (Ti), stainless steel (SS), and glass without changing the bulk properties. The formation of cross-linked Gelatin methacryloyl (GelMA) is confirmed with X-ray photoelectron spectroscopy (XPS) and attenuated total reflection (ATR)-Fourier transform infrared (FTIR) spectrometry. The formation of the covalent bond between different hydrogels and substrates is also confirmed by ATR-FTIR to prove this technique's hydrogel- and substrate-independency. The stability test proved that GelMA is stable on the IAPP coating even after 6 weeks of incubation. 90-degree peel adhesion test demonstrated that the failure occurs at the tape-hydrogel interface. Adhesion strength at the hydrogel-IAPP coating interface is over 380 N/m[GS1]. Cell assays showed that hydrogel-coated substrates improve cell adhesion and spreading. Hence, hydrogel attachment using the IAPP technique can improve the biocompatibility of solid materials.

PoB.10.53

Characterization of the antimicrobial and physicochemical properties of essential oil-loaded collagen type I hydrogels

Caglar Ersanli[1,2], Yves Bayon[3], Ioannis Skoufos[4], Athina Tzora[2], Dimitrios I Zeugolis[5]

[1]Laboratory of Animal Science, Nutrition and Biotechnology, School of Agriculture, University of Ioannina, Arta, Greece. [2]Laboratory of Animal Health, Food Hygiene and Quality, School of Agriculture, University of Ioannina, Arta, Greece. [3]Medtronic, Trevoux, France. [4]Laboratory of Animal Science, Nutrition and Biotechnology, School of Agriculture, University of Ioannina,, Arta, Greece. [5]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Charles Institute of Dermatology, Conway Institute of Biomolecular and Biomedical Research and School of Mechanical and Materials Engineering, University College Dublin, Dublin, Ireland

Abstract

Antimicrobial resistance is a global public health concern, with multidrug (usually antibiotic) resistant microorganism infections being associated with higher hospitalisation rates and healthcare costs. Although the safety and efficiency of essential oils (EOs) over conventional antibiotic-based treatments in battling bacterial infections have been well-documented in the literature, not one product is commercially and clinically available. It has been argued that biomaterial-based delivery of EOs will overcome issues related to low stability, high volatility and off-target toxicity. Herein, using disc diffusion, broth microdilution and microtiter plate biofilm formation assays against different (methicillin-sensitive, methicillin-resistant, ATCC 29213) Staphylococcus aureus strains, we first screened the effectiveness of EOs (Thymus sibthorpii, Origanum vulgare, Salvia fruticosa and Crithmum maritimum) over traditional antibiotics (gentamicin, tetracycline, cefaclor, penicillin and enrofloxacin). We found that Thymus sibthorpii and Origanum vulgare EOs exhibited the highest ($p < 0.05$) antimicrobial activity with 0.091 mg/ml of minimum inhibitory concentration and almost total inhibition of biofilm formation at their half minimum inhibitory concentration. Studies are underway to assess pharmacokinetics (using different concentrations, functional groups, number of arms and molecular weights of star-shaped polyethylene glycol cross-linkers), antimicrobial properties (using disc diffusion, broth microdilution and microtiter plate biofilm formation assays against different Staphylococcus aureus strains) and biological properties (using alamarBlue™, LIVE/DEAD™ and proliferation assays in human dermal fibroblast cultures) of EOs loaded collagen hydrogels.

PoB.10.54

The effect of hepatocyte growth factor with click chemistry based polyethylene glycol in vocal cord palsy

Choung Soo Kim[1], HeeBeom Koo[2]

[1]Department of Otolaryngology-Head and Neck Surgery, College of Medicine, TheCatholic University of Korea, Seoul, Republic of Korea, seoul, Korea, Republic of. [2]Department of MedicalLife Sciences, Department of Biomedicine & Health Sciences, and CatholicPhotomedicine Research Institute, College of Medicine, The Catholic University of Korea, seoul, Korea, Republic of

Abstract

Introduction: Object: In this study, we explored the effects of controlled released hepatocyte growth factor on vocalis muscle regeneration Material & Methods: Twelve rabbits were injected in the lateral aspect of their right thyroarytenoid muscle with o.15cc of polyethylene glycol or hepatocyte growth factor (100 ng)containing polyethylene glycol,1 months after Right recurrent laryngeal nerve section. At. 12weeks after injection. Then larynges were harvested, High speed video camera examination was done for comparing degree of medialization of paralyzed vocal fold between PEG and PEG+HGF we also conduct histological and immunohistochemical examination Results: Histologic examination was performed 3 months after injection. Cross sectional area of vocalis muscle was increased in HGF releasing PEG group Compared to PEG alone group. Glottic gap was decreased in HGF releasing PEG group compared to PEG alone group in High speed video cameraexamination Conclusions: controlled releasing of HGF in PEGshowed vocalis muscle regeneration in vocal fold palsy model

PoB.10.55

Design and characterization of chitosan-sodium alginate hydrogel for enhanced sustained delivery of lenalidomide

Muhammad Hadi Sultan, Ahmad Salawi, Sivakumar S Moni

Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, Saudi Arabia

Abstract

INTRODUCTION: The objective of this research was to develop a chitosan-sodium alginate hydrogel for improved sustained delivery of lenalidomide, an effective drug for the treatment of various hematologic malignancies. The hydrogel was developed as a potential formulation to overcome the limitations of lenalidomide, improve $t_{1/2}$ and achieve sustained release. Chitosan is polycationic, biocompatible, biodegradable, and non-toxic and is ideal for biomedical and drug delivery applications. Sodium alginate is a versatile anionic polysaccharide that gained pharmaceutical attention due to its gelling ability, biocompatibility, and controlled release. **EXPERIMENTAL METHODS:** The chitosan-sodium alginate hydrogel was prepared by ionic crosslinking between chitosan and sodium alginate (5% W/W) as a control. Lenalidomide was loaded in the gel direct mixing. The mechanical characteristics of the GEL samples were determined by using a TA.XT plus texture analyzer equipped with a 50 kg load cell and fitted with a 25 mm flat-faced stainless-steel cylindrical probe. Composite Hardness, compressibility, and adhesiveness were then measured through the two-bites test. It provided valuable information about the impact of drug load on the hardness, compressibility, and adhesiveness of the GEL samples. The (DLS) Analysis was performed using Malvern Nano-ZS Zetasizer (Malvern Panalytical, Malvern, UK).

Compositions	Hardness (N)	Adhesiveness (N)
Hydrogel (Chi-Alg)	10.6 ± 2.3	0.008±0.003
Hydrogel loaded Drug	5.8 ± 1.3	0.004±0.001

Table1: texture profile analysis. Data are expressed as means ± S.D. (n=3).

RESULTS AND DISCUSSION: The dynamic light scattering (DLS) analysis revealed a zeta-potential of +21.6 ± 58.1 mV, a conductivity of 0.416 mS/cm, and an average particle size ranging from 51.62 to 1629 d.nm. The average particle was observed as 1038 r.nm with a PDI of 0.6. The DLS analysis revealed that the developed hydrogel was stable, with high particle concentrations signifying its gel-like nature. The texture analysis results in Table 1 revealed that the positive force was 5.8N, and the negative force (AUC) was 0.004 showing lesser hardness and adhesiveness with hydrogel loaded with a drug which is suitable for the injectable dosage form.

CONCLUSION: The hydrogel exhibited excellent physicochemical properties, including zeta potential, texture characteristics, high water absorption capacity, biocompatibility, and controlled swelling behavior. The hydrogel exhibited good encapsulation and provided a potential platform for efficient and sustained release delivery of lenalidomide, improving therapeutic outcomes in the treatment of hematological malignancies.

ACKNOWLEDGEMENTS: The authors thank the Jazan University Research Deputyship and the Ministry of Education in Saudi Arabia for funding this research work.

PoB.10.56

Optimization strategy for reliable cytotoxicity tests in 3D fibrin gel models

Jessica Petiti[1], Sabrina Caria[2], Laura Revel[1], Mattia Pegoraro[1], Carla Divieto[1]

[1]Istituto Nazionale di Ricerca Metrologica (INRiM), Turin, Italy. [2]University of Turin, Turin, Italy

Abstract

Three-dimensional (3D) *in vitro* model applications include regenerative medicine, toxicity screening, and drug development (DD). Regarding DD, 3D cell culture models can help the selection of promising drug candidates for clinical trials, making more straightforward the translation of new therapeutic solutions into clinical approaches for patients benefit. Although the use of 3D models has recently increased, there is still a lack of standardization of cytotoxicity tests in 3D cell cultures. Among the existing methods for cytotoxicity assessment, resazurin-based assay offers practical advantages: it is more sensitive than other methods and is non-destructive, allowing time-lapse experiments. Nevertheless, literature data underlined a low inter-laboratory reproducibility of cytotoxicity *in vitro* tests highlighting the need to optimize experimental parameters to ensure robust results. To improve reliability of resazurin-based cytotoxicity tests, a standard operating procedure (SOP) to optimize protocols, applicable to any cell line in 2D and 3D *in vitro* models, was developed. The SOP describes: i) how to optimize the key parameters (excitation and emission wavelengths, incubation time, cell concentration, etc.); ii) how to estimate the assay limits (Limit of Blank, Limit of Detection, and Limit of Quantification); iii) how to understand the confidence of the results, by evaluating repeatability, reproducibility, and measurement uncertainty (MU). To assess any cytotoxicity of the resazurin itself in time-lapse experiments, the effects of repeated treatments with resazurin on the same sample (A549 cells cultured in fibrin gel for 11 days) was investigated. Results confirmed that residual resazurin remains trapped in the gel affecting the measurements: this does not interfere with the fluorescence intensity emitted by the metabolized resazurin, however, it affects the cell viability in long-term time-lapse experiments. This SOP was validated on a certified reference material (A549 cell line), comparing 2D and 3D (fibrin gel) results (Fig.1): the SOP, followed step-by-step, allowed to define the optimal parameters ensuring reliable results (MU < 20%) on cell toxicity tests in 3D fibrin gels models, increasing the confidence in pre-clinical drug tests (Fig. 2). In conclusion, this work contributes to filling the lack of reproducibility in drug toxicity pre-clinical research, helping to improve the long-term benefits that come from increased scientific fidelity of cytotoxicity tests.

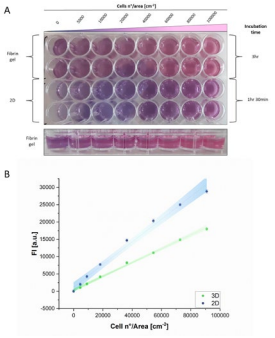


Figure 1: Resazurin assay on A549 cells. A) Qualitative results. B) Fluorescence Intensity (FI) versus cell concentration of calibration curves.

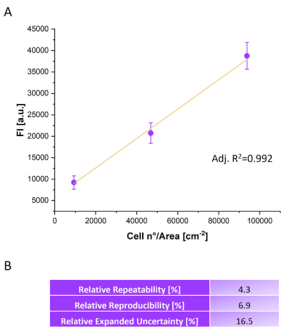


Figure 2: Results obtained for A549 cells in fibrin gel after key parameters optimization A) Fluorescence Intensity (FI) versus cell concentration. B) Data quality estimation.

PoB.10.57

Cell-interactive macroporous PEG hydrogels towards a human bone *in vitro* model

Marion Horrer, Doris Zauchner, Wanwan Qiu, Ralph Müller, Xiao-Hua Qin

Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

Abstract

In vitro tissue models offer a valuable avenue for investigating human biology in health and disease, obviating the need for animal experimentation. However, these models predominantly rely on a three-dimensional (3D) cell culture in poorly defined animal-derived hydrogels (e.g., Matrigel or collagen), which impede mechanistic understanding of cell-matrix interactions.
Herein, we report a class of cell-interactive macroporous hydrogels based on peptide-crosslinked poly(ethylene glycol) (PEG) to study bone development *in vitro*. These hydrogels are formed by polymerization-induced phase separation (PIPS) [1] between 4-arm PEG vinyl sulfones and dextran. A di-cysteine matrix metalloproteinase (MMP)-sensitive peptide [2] was used as the crosslinker to render the matrices biodegradable by cell-secreted proteases. By increasing the dextran concentration, the storage modulus (G') increased from ~60 Pa to ~140 Pa (non-degradable) and from ~90 Pa to ~130 Pa (degradable) as determined by *in situ* rheology (**Fig. 1A-B)**. In addition, increased dextran concentration led to larger pore sizes up to ~10 µm in diameter as shown by confocal imaging (**Fig. 1C**). This effect can be attributed to the strong phase-separating properties of dextran against PEG precursors, which may lead to a localized densification of PEG phase as well as increased gel stiffness and pore size. Upon 3D encapsulation of primary human osteoblasts (hOBs), cell spreading was observed within few hours in both degradable and non-degradable hydrogels (**Fig. 1D**). Enhanced spreading was observed in the degradable hydrogel composition. Importantly, we show that hOBs embedded in the macroporous PEG hydrogels exhibited even faster spreading compared to cells in a collagen hydrogel (**Fig. 1E**), suggesting the benefits of the macroporous structure for cell-cell communication and 3D network formation.
In conclusion, we have developed a class of cell-interactive macroporous PEG hydrogels with defined physicochemical properties that show promises for a human bone *in vitro* model.

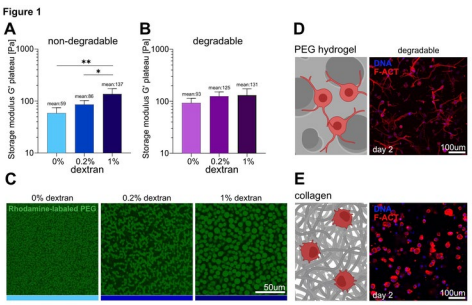


Figure 1 *(**A-B**) Fine-tuning the mechanical stiffness and microstructure of PEG hydrogels: degradable (**A**) and non-degradable (**B**) (n ≥ 3, mean ±SD shown, * P ≤ 0.05, ** P ≤ 0.01). (**C**) Confocal microscopy images showing the effect of dextran concentration on in situ pore formation in non-degradable PEG hydrogels. (**D-E**) Comparisons between hOB morphologies in macroporous PEG hydrogels (**D**) versus a collagen hydrogel (**E**) after 2 days of osteogenic differentiation.*

References

[1] N. Broguiere, A. Husch, G. Palazzolo, F. Bradke, S. Madduri, M. Zenobi-Wong, Biomaterials, 2019, 200, 56.
[2] X.-H. Qin, X. Wang, M. Rottmar, B. J. Nelson, K. Maniura-Weber, Advanced Materials, 2018, 30, 1705564.

PoB.10.58

Mimicking extracellular matrix (ECM) features for meniscal regeneration: from biomolecular signatures to biomaterials design

Maddalena Bracchi[1], Alessandro Panunti[1], Francesca Cadamuro[1], Federica Barbugian[1], Federico Della Torre[2], Marco Crippa[2], Luca Rigamonti[2], Marco Bigoni[2], Giovanni Zatti[2], Marco Turati[2], Francesco Nicotra[1], Laura Russo[1,3]

[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy. [2]Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy. [3]CÚRAM, SFI Research Centre for Medical Devices, National University of Ireland Galway, Galway, Ireland

Abstract

State of the art The menisci are cushions found in the knee joint whose main function is weight distribution. They are composed of three distinct regions with different cell populations, extracellular matrix (ECM) components and morphological features. The menisci have a very small blood supply; thus, they have very limited healing potential. **Materials and methods** The ECM components and the *in vitro* regeneration of the meniscus will be investigated. The ECM profile will be analysed using different stains and SEM images obtained from paediatric and adult menisci. The stress-strain distribution in the articulation will be explored with models from MRI images. The major features (biomolecular, morphological and mechanical) have been analysed and employed for the development of cellularized scaffolds for *in vitro* tissue regeneration. To recreate the ECM composition, two different hydrogels will be used. **Results and discussion** Different pieces of menisci from more patients were analysed to discover the ECM profile and the variations between physiological and pathological matrix. The results (Figure 1) show an increase of mucins and fibrotic tissue in adult menisci, indicators of the tissue degeneration. Moreover, ECM thickness, pores diameter and percentage porosity were calculated from SEM images: the ECM seems to be thicker, less organized and less porous in adult patients.

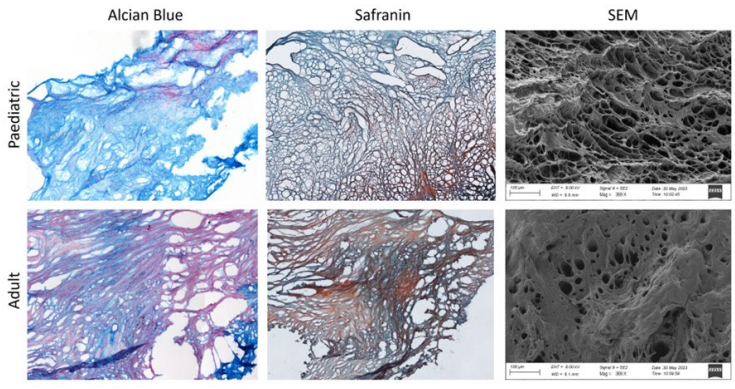


Figure 1 Alcian Blue, Safranin and SEM images on paediatric and adult patients

In Figure 2A, the result of the segmentation of a DICOM file and the FEM simulation on the two menisci are shown: the lateral one is less congruent and more able to increase its circumference than the medial one, which has lower Von Mises stresses. The medial meniscus is damaged more frequently than the lateral one. A preliminary gelatin-HA hydrogel was then formulated to mimic the red-red zone: its formation was confirmed by UV-VIS analysis (Figure 2B), in which the peak of the hydrogel is lower and shifted to right than the blend, used as control. According to ISO 10992-5, this hydrogel can also be considered as non-cytotoxic (Figure 2C).

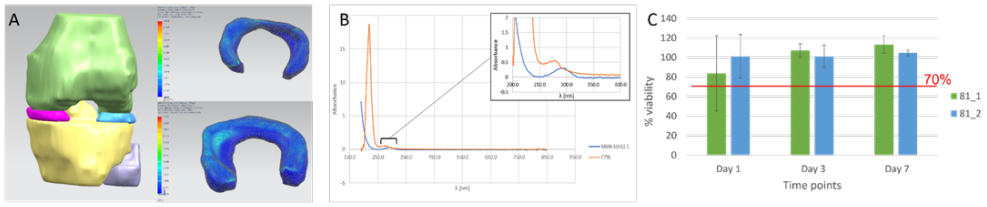


Figure 2 Results of the segmentation and FEM analyses (A), UV-VIS (B) and indirect cytotoxicity (C) tests on Gelatin-HA hydrogel

Conclusions ECM biomolecular and morphological features have been analised in order to generate functional ECM mimics able to resemble the major key cues of meniscal tissue. The obtained results are now employed to generate ECM mimetics with tailored biochemical and mechanical properties employable in 3D printing and bioprinting manufacturing processes.

PoB.10.59

3D PROSTATE CANCER *IN VITRO* MODELS

khalsa alhusaini[1,2], Annalisa Tirella1[3,4], Anna Nicolaou[5], Marco Domingos[6], Esther Chaparro[7]

[1]Division of Pharmacy and Optometry, Medicine and Health, University of Manchester, Manchester, UK, manchester, United Kingdom. [2]Biology Department, College of Science, Sultan Qaboos University, Muscat, Oman, Muscat, Oman. [3]Division of Pharmacy and Optometry, Medicine and Health, manchester, United Kingdom. [4]Biotech Center for Biomedical Technologies, Department of Industrial Engineering , University of Trento, Ternto, Italy. [5]Division of Pharmacy and Optometry, Medicine and Health, Manchester, United Kingdom. [6]3Division of Mechanical Aerospace and Civil Engineering, Science and Engineering, University of Manchester, manchester, United Kingdom. [7]4Cancer Research UK Manchester Institute, Medicine, University of Manchester, manchester, United Kingdom

Abstract

Prostate cancer is one of the most commonly diagnosed cancer in men, and the 5th leading cause of death worldwide. The lack of biologically relevant *in vitro* models that precisely recapitulate the pathophysiology of prostate cancer delays our understanding of the disease, as well as its early-detection and treatment. Bioprinting allows to fabricate 3D *in vitro* models with precise control of constructs' architecture, material dispensing and cell spatial distribution. This work describes the design of new biomaterials and their fabrication to obtain relevant prostate cancer 3D *in vitro* models. This strategy enables to better mimic properties of the native prostate tumour microenvironment *in vitro*, offering new models to study how prostate cancer progresses. This work describes the design and fabrication of prostate cancer 3D *in vitro* models using the combination of both advanced biomaterials and bioprinting. Based on our previous work, oxidised alginate with 50% degree of oxidation was prepared. Laminin peptides (IKVAV, AG73) known to promote cell adhesion were conjugated with OA and hydrogels were prepared with compressive moduli in the range of 1-10 kPa. Human prostate cancer cells (PC-3) and cancer associated fibroblasts (CAFs) were encapsulated at a concentration of 1 million cells/mL in alginate-based hydrogels. From rheological standpoint, all hydrogels showed shear thinning behavior allowing printing. 3D-printed constructs were fabricated using an extrusion-based printer with 20 kPa extrusion pressure and 7.5 mm/s feed rate. Hydrogels were then physically crosslinked with CaCl2. PC-3 cells adaption to the microenvironment, e.g. physico-chemical and mechanical properties, were evaluated by assessing cellular viability, proliferation, morphology, and expression of epithelial to mesenchymal transition markers. Extrusion-based printing technology enables precise spatial control over cellular deposition of PC-3, with approx. 80% cellular viability up to seven-days of cell culture. In conclusion, modified alginate bioinks enables printing 3D prostate constructs with high cell viability. These models have the potential to offer a high-resolution insight into cancer phenotypes, and genotypes thus revolutionize the field of cancer research, drug discovery, and therapeutics.

PoB.10.61

Alginate microbead encapsulation for cell therapy: reduced fibrotic response by intermediate guluronate content and sulfation

Joachim S Kjesbu, Berit L Strand, Anne Mari Rokstad, Abba E Coron

Norwegian University of Science and Technology (NTNU), Trondheim, Norway

Abstract

Cell therapy has emerged as a feasible treatment option for diseases such as type 1 diabetes and acute liver failure, requiring less complex surgery compared to full organ transplants. However, its widespread adoption is partly limited by the necessity for concurrent immunosuppression. Cell encapsulation offers a strategy to eliminate this need by restricting immune cell access. Encapsulation in alginate gel beads is well-tolerated by both the encapsulated cells and patients in clinical trials. A major impediment to long-term efficacy is the foreign body reaction to the materials post-implantation, leading to pericapsular fibrotic overgrowth (PFO) and a subsequent decline in encapsulated cell function.

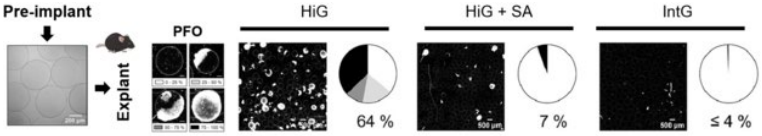


Figure 1. Fibrosis (PFO) on empty alginate microbeads of high guluronate content (HiG), HiG and sulfated alginate (SA), and intermediate guluronate content (IntG) explanted (14 days) from C57BL/6J mice (modified from Coron et al., 2022).

Our research (Coron et al., 2022), demonstrates two strategies to obtain alginate microbeads with minimal PFO, using a fibrosis-responsive mouse (C57BL/6J) model. Firstly, the monomeric composition of alginate significantly impacts PFO. Empty microbeads of intermediate guluronate alginate (47%, IntG) exhibit minimal fibrosis, with no more than 4% showing considerable fibrotic coverage, compared to 64% total PFO for high guluronate alginate (68%, HiG) microbeads (Fig. 1). These PFO-prone HiG beads have previously been preferred in clinical trials, underscoring the importance of alginate composition for implantable microbeads for cell therapy. The second strategy for achieving minimally fibrotic gel beads involves the chemical sulfation of alginate. Incorporating a small fraction of sulfated alginate (SA) in microbeads considerably reduces PFO on HiG alginate beads (Fig. 1), leading to about nine times less fibrotic deposition (7% total PFO). Our collaborative research (Syanda et al., 2022) which is relevant for the treatment of acute liver failure, indicates that encapsulation using SA is tolerable for human induced pluripotent stem cell-derived hepatocytes (hPSC-Heps) *in vitro*. Moreover, SA microbeads containing hPSC-Heps show fibrotic coverage on less than a quarter of all explanted microspheres, compared to all of them for HiG in C57BL/6J mice. This research suggests robust mitigation of PFO by SA in an immunologically challenging xenograft encapsulation. Currently, we are also investigating SA as a potential encapsulation material for the treatment of type 1 diabetes. When transplanted into diabetic C57BL/6J mice, encapsulated rat islets have demonstrated sustained normoglycemia, glucose responsivity, C-peptide levels, and high cell viability over a period of 120 days

PoB.10.62

Engineering naturally based composite hydrogel as flexible bioadhesive for wound healing of internal organs

Ahmet Erdem, Dilara Küçük, Feyzanur Şentürk, Aygün Iseyeva, Gökhan Duruksu

Kocaeli University, Kocaeli, Turkey

Abstract

In recent times, flexible bioadhesives have been introduced in surgical operations for seamless wound closure. Particularly, hydrogels derived from tropoelastin with high flexibility are being used in bioadhesive compositions. However, these materials are quite expensive. As an alternative, methacryloyl-functionalized gelatin (GelMA) based hydrogels have gained significant interest as low-cost bioadhesives for sealing internal leaks. Nonetheless, GelMA bioadhesives exhibit low mechanical strength and weak adhesion properties. To enhance the bioadhesive performance of GelMA, hybrid structures have been developed using various materials. Among the preferred candidates for preparing these hybrid bioadhesives, alginate derivatives are particularly notable. In this study, hybrid bioadhesives were designed by incorporating Fe+3 ions, which possess more dynamic and reversible cross-linking properties compared to the commonly used Ca+2 ions, into GelMA and methacrylated alginate (AlgMA). The tissue adhesion properties, physical characteristics, biocompatibility, and ex vivo performance of the designed hydrogels were examined. The addition of Fe+3 to the hydrogels was found to increase the ex vivo adhesive strength by 200%, reduce swelling, and enhance hemostatic properties. These bioadhesives demonstrated good biocompatibility *in vitro* tests conducted on fibroblast cells. These findings provide an important foundation for improving the adhesive properties of tissue adhesives in future studies.

PoB.10.63

Functional and persistent microvascular networks in a VEGF-decorated matrix: a vascularization module

Adelin Rouchon[1], Priscilla S. Briquez[2], Dirk J. Schaefer[3], Jeffrey A. Hubbel[4], Andrea Banfi[1], Nunzia Di Maggio[1]

[1]University Of Basel, Basel, Switzerland. [2]University of Freiburg, Freiburg, Germany. [3]Basel University Hospital, Basel, Switzerland. [4]University of Chicago, Chicago, USA

Abstract

Objective: Generation of functional vascular networks is an unresolved challenge for 3D engineered tissues, both *in vitro* to produce vascularized organoids and *in vivo* to promote progenitor survival and differentiation. Signaling by the angiogenic master regulator VEGF is physiologically regulated by interaction with the extracellular matrix. Here we decorated fibrin hydrogels with an engineered VEGF protein to generate an optimal matrix-associated angiogenic microenvironment. Methods: Fibrin matrices contained 10mg/ml human fibrinogen, 3U/ml thrombin, 3U/ml factor XIIIa, endothelial cells (HUVEC) and adipose stromal cells (ASC) as support perivascular cells. VEGF164 protein was fused to the transglutaminase substrate peptide NQEQVSPL (TG-VEGF) to enable its covalent cross-linking to fibrin. Results: HUVEC+ASC co-culture rapidly self-assembled into physiologically differentiated vascular networks, with physiological apico-basal polarization and patent lumens. Optimal vessel formation was achieved with 10 mg/ml fibrinogen, containing 5x10E6 cells/ml with 100 ng/ml of TG-VEGF, yielding a 2.5-fold improvement vs no-VEGF controls after both 7 and 14 days. TG-VEGF significantly accelerated endothelial proliferation speed, as shown by pospho-histoneH3. Vessel diameters remained compatible with micro-circulation (median=17 µm). Human-derived vascular structures formed after 7 days of *in vitro* culture could rapidly connect to the host vasculature upon subcutaneous implantation and were efficiently perfused by the systemic circulation. TG-VEGF both accelerated the formation and perfusion of hybrid vessels and improved graft invasion by the host vessels. Notably, human-derived vascular networks rapidly regressed *in vivo* in the absence of TG-VEGF or if it was substituted by wild-type soluble VEGF. Conclusions: Fibrin decoration with 100 ng/ml of TG-VEGF promotes the efficient self-assembly of 3D, perfusable, lumenized and physiologically differentiated micro-vascular networks within 7 days in the absence of flow. Upon implantation *in vivo*, TG-VEGF specifically enabled rapid connection to the host vessels within 3 to 7 days to support blood perfusion and was required for the microvascular network persistence and continued function. This engineered angiogenic microenvironment could be exploited both to provide a vascular component to *in vitro* generated organoids and for the rapid *in vivo* vascularization of 3D tissue engineered grafts.

Acknowledgements: This work was funded by the European Union's Horizon 2020 program (grant agreement 801159).

PoB.12.01

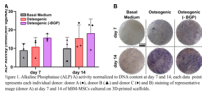
β-TCP from 3D-printed scaffold can act as an effective phosphate source during the osteogenic differentiation of human mesenchymal stromal cell

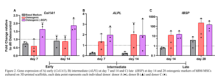
Luan Phelipe Hatt[1,2], Angela Rita Armiento[3], Daphne van der Heide[1,2], Maria Eugenia Pirera[1], Martin James Stoddart[1]

[1]AO Research Institute, Davos, Switzerland. [2]ETH, Zürich, Switzerland. [3]UCB Parma, Slough, United Kingdom

Abstract

Background: Osteogenically driven human bone marrow derived mesenchymal stromal cells (hBM-MSCs) are often combined with calcium-phosphate-based 3D-printed scaffolds with the goal to repair bone defects. *In vitro*, the induction of osteogenic differentiation requires, among other supplements, the addition of organic β-glycerophosphate (BGP), which acts as a phosphate source. Inorganic phosphate as an alternative source has been shown to improve the quality of MSC secreted hydroxyapatite. However, the ability of MSCs to use inorganic phosphate contained within the 3D-printed scaffolds during *in vitro* osteogenesis is poorly understood. The aim of this study is to investigate whether the phosphate contained within 3D-printed scaffolds is sufficient to act as phosphate source during the osteogenesis of MSCs. Methods: hBM-MSCs (obtained will full ethical approval) of three independent donors are seeded on top of 3D-scaffolds at passage 4 composed of poly(lactic-co-glycolic acid) (PLGA) and β-tricalcium phosphate (β-TCP) and kept for 28 days under three different culture conditions: 1) osteocontrol (basal medium), 2) osteogenic (basal medium supplemented with dexamethasone, ascorbic acid and BGP) and 3) osteogenic medium without BGP. Alkaline phosphatase (ALP) staining is performed after 7 and 14 days of culture as well as quantification of the ALP activity. Real-time PCR is performed to assess the gene expression of osteo-relevant markers: *Col1A1* (early marker), *ALPL* (intermediate marker) after 7 and 14 days of culture and *IBSP* (late marker) after 14 and 28 days of culture. Results: ALP activity is upregulated in the osteogenic group compared to the osteocontrol group, and the absence of exogenous BGP from the osteogenic medium shows an even higher upregulation (Figure 1A) at day 7 and 14. ALP staining at day 7 and 14 (Figure 1B) show visibly increased ALP in both the osteogenic groups compared to the osteocontrol group, while no visible differences are observed between the two osteogenic groups. The absence of exogeneous BGP upregulates the gene expression of all analyzed genes (Figure 2) compared to the control group and *Col1A1* and *ALPL* at day 7 compared to the osteogenic group, while maintaining the gene expression of *ALPL* at day 14 and *IBSP*. Conclusions and Outlook: These results suggest that hBM-MSCs are able to utilize β-TCP within the scaffold during osteogenesis, which makes the addition of BGP to the culture medium redundant. β-TCP embedded within personalised 3D-printed scaffolds can potentially act as a local phosphate source, which may improve *in vivo* osteogenesis.





PoB.12.02

Calcium phosphate nanoparticles for intracellular drug delivery

Lea Andrée[1], Rong Wang[1], Fang Yang[1], Merel Adjobo-Hermans[2], Sander C.G. Leeuwenburgh[1]

[1]Department of Dentistry – Regenerative Biomaterials, Radboud University Medical Center, Nijmegen, Netherlands.
[2]Department of Medical BioSciences, Radboud University Medical Center, Nijmegen, Netherlands

Abstract

Calcium phosphates (CaP) are widely used as bone substitutes due to their compositional similarity to bone mineral and resulting favorable osteocompatibility. The pH-dependent solubility of CaP renders them also suitable for application as pH-responsive drug carriers. Consequently, CaP nanoparticles (CaP-NPs) are intensively studied to facilitate intracellular drug delivery. To this end, cellular uptake and subsequent cargo release by dissolution of CaP-NPs without causing cytotoxicity are crucial requirements for successful intracellular delivery. Although (bulk) CaP shows good biocompatibility, enhanced reactivity of nanoscale CaP often leads to higher toxicity. Two important parameters that control CaP-NP reactivity include their aging upon wet-chemical synthesis and surface modification to enhance their colloidal stability. Therefore, we investigated the influence of CaP-NP aging time and surface modification using citrate anions on cellular internalization and cytotoxicity of CaP-NPs. Using a simple wet-chemical one-pot synthesis, we produced spherical CaP-NPs at varying aging times (10 mins, 1 h, and 5 h). Increased aging time resulted in a more crystalline structure as identified by X-ray diffraction (Fig. 1). To increase colloidal stability, we then surface-modified CaP-NPs with citrate anions. We investigated cellular uptake and cytotoxicity of our CaP-NPs *in vitro* using murine pre-osteoblasts (MC-3T3s). We confirmed cellular internalization and lysosomal localization of CaP-NPs (Fig. 2). Interestingly, the assessment of cytotoxicity by Cell Counting Kit-8 assay revealed striking differences between CaP-NPs with and without citrate surface modification as well as between aging times already at low concentrations (25 – 200 µg/mL). Both, aging of CaP-NPs and surface modification with citrate reduced cytotoxicity after 3 days of culture. Nevertheless, cells exposed to CaP-NPs still showed increased metabolic activity compared to the control, indicating metabolic stress. Fundamental biochemical studies on the mechanisms of cell death upon CaP-NP exposure are currently ongoing. In conclusion, CaP-NPs are internalized by murine pre-osteoblasts and localized in lysosomes. Increased aging and citrate modification drastically reduced the cytotoxicity of CaP-NPs.

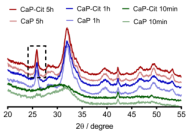


Fig. 1 Structure of calcium phosphate nanoparticles *X-ray diffractograms of calcium phosphate nanoparticles synthesized at different aging times with or without citrate surface modification.*

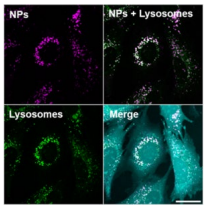


Fig. 2 Internalization of calcium phosphate nanoparticles *Confocal live cell images showing cellular uptake of CaP-NPs (magenta) in murine pre-osteoblasts (cyan) and their lysosomal localization (green) after 24 hours. Co-localization of CaP-NPs and lysosomes appear white. Scale bars represent 25 µm.*

PoB.12.03

3D Printing of alginate dialdehyde-gelatin hydrogels incorporating Ce and Ga-containing mesoporous bioactive glass nanoparticles for bone tissue engineering

Fatih Kurtuldu[1,2], Nurshen Mutlu[1,2], Liliana Liverani[2], Dušan Galusek[1,3], Aldo R. Boccaccini[2]

[1]FunGlass, Alexander Dubček University of Trenčín, Trenčín, Slovakia. [2]Institute of Biomaterials, Department of Material Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany. [3]Joint Glass Centre of the IIC SAS, TnUAD and FChFT STU, FunGlass, Trenčín, Slovakia

Abstract

3D printing of scaffolds is one of the most promising approaches fabricating bone graft substitutes with specific requirements. Recently, there is a growing interest in developing heterogenous inorganic/organic composite 3D printed scaffolds as inspiration from natural bone microstructure. In this study, multifunctional nanocomposite ink is developed based on alginate dialdehyde-gelatin (ADA-GEL), and mesoporous bioactive glass nanoparticles (MBGNPs) which contains therapeutic ions, namely cerium or gallium. 3D scaffolds were fabricated by using an extrusion-based bioprinter and crosslinked with $BaCl_2$ solution. Printed scaffold morphology, mechanical properties, and degradation behavior were determined. The nanoparticles were homogenously distributed in the hydrogels. The MBGNPs release ions such as Ca^{2+}, Ga^{3+}, or Ce^{3+}, which can enhance cell viability, proliferation, and osteogenic differentiation. Divalent Ca^{2+} ions can internally crosslink alginate chains further and affect the degradation of the hydrogels by improving their stability. The incorporation of MBGNPs thus improves the mechanical properties and shape fidelity of scaffold constructs and decreases the degradation rate of the gels. Additionally, MBGNPs induced mineralization which can promote cell adhesion and proliferation. Biocompatibility tests confirmed that the nanocomposites enhanced osteoblast-like cell adhesion, proliferation, and differentiation. The addition of therapeutic ions significantly increased the ALP activity. These features make the obtained nanocomposite ink a potential candidate for multifunctional applications for bone tissue engineering.

PoB.12.06

Antiviral and antibacterial potential of air filters functionalized with Ag_2O, ZnO and CuO particles

Bruno FILIPE GOMES RIBEIRO[1], Antonio Vazquez-Lopez[2,3], Miguel Vazquez-Pufleau[1], Mirella Llamosi[4], Julio Sempere[4], De--Yi Wang[5], José Yuste[4,6], Miriam Domenech[4,6,7], Javier LLorca[5,8], Monica Echeverry Rendón[5]

[1]Imdea materials, Madrid, Spain. [2]Imdea Materials, Madrid, Spain. [3]Universidad Rey Juan Carlos, Madrid, Spain. [4]Instituto de Salud Carlos III, Madrid, Spain. [5]IMDEA materials, Madrid, Spain. [6]CIBER de Enfermedades Respiratorias, Madrid, Spain. [7]Complutense University of Madrid, Madrid, Spain. [8]Polytechnic University of Madrid, Madrid, Spain

Abstract

According to the World Health Organization, 99% of the global population is breathing low quality air, resulting in 3.2 million premature annual deaths of which 21% are attributed to air quality-related respiratory infections. The recent coronavirus (CoVid-19) pandemic, responsible for more than 6.8 million deaths worldwide, has highlighted the issue of airborne pathogens on human health. Air filtration is an established method to ensure a good indoor air quality, being effective at capturing airborne pathogens and preventing diseases such as asthma. Current technologies employ several filtration steps with air filters of increasing efficiency to capture a wide range of air pollutants. Air filter media, is either made of fiberglass or synthetic materials such as polypropylene due to its low cost, without an antimicrobial action. With usage, water from air humidity, dust and dirt, including fungi and bacteria spores, can accumulate on filters creating ideal conditions for the propagation of microorganisms. Thus, the filter can become a secondary source for airborne pathogens. In this work, antiviral and antibacterial filters were prepared by spray coating of Ag_2O, CuO and ZnO particles into commercially available air filters. The reliability of the filters was tested through particle release in air (through a scanning mobility particle sizer (SPMS)). Filtration performance was measured against an aerosol of iron nanoparticles in nitrogen gas produced through a Spark Discharge Generator (SGS). The biosafety of the filters was measured *in vitro* through cytotoxicity tests against L-929 mouse fibroblasts cells. Antibacterial efficiency was measured *in vitro* against two clinically relevant pathogens: S. pneumonieae and P. aeruginosa. Anti-viral performance was measured against respiratory syncytial virus (RSV), a major airborne pathogen implicated in the premature death of 1 in 50 children under 5, worlwide. In general, the results have shown that spray coating is a reliable solution, with no detectable cytotoxicity effects or particle release in air. After functionalization, both Ag2O and CuO compounds have been shown to be effective against RSV, with total antiviral activity (over 99%), while only Ag2O presented a clear antibacterial action. In conclusion, spray coating as post-manufacturing functionalization of air filters can be an efficient, low cost solution against airborne pathogens.

PoB.12.07

Effects of carbonate content on replacement of carbonate apatite bone substitute to new bone

Kunio Ishikawa, Kaai Deguchi, Akira Tsuchiya

Kyushu University, Fukuoka, Japan

Abstract

Although hydroxyapatite has been used as typical bone substitute, carbonate apatite is the inorganic component of bone. Fortunately, carbonate apatite bone substitute was fabricated through dissolution–precipitation reaction using a precursor such as calcium carbonate. One of the key differences of hydroxyapatite and carbonate apatite is the presence of carbonate in apatitic structure. The aim of this study is to shed some light to understand the effects of carbonate content in apatitic structure on replacement of carbonate apatite bone substitute to new bone. Carbonate apatite containing 0.9-8.3 mass% carbonate were fabricated through dissolution–precipitation reaction in phosphate – carbonate solution using calcium sulfate block as a precursor. The carbonate apatite was immersed in pH 5.5 0.08 mol/L acetic acid buffere solution for 12 h to simulate osteoclastic resorption. Ca concentration measured as an index of carbonate apatite dissolution increased with increase in carbonate content in apatitic structure. The results indicated that carbonate content played important role for osteoclastic resorption of carbonate apatite bone substitutes. Then, bone defects at distal condyle of the femur of rabbits were reconstructed with carbonate apatite granules containing 0.9, 4.7, and 8.3 mass% carbonate in apatitic structure. At 4 weeks, new bone was formed even at the center of bone defect regardless of the carbonate content. A larger number of osteoclasts and faster surface resorption of the granules were observed for carbonate apatite containing larger carbonate in apatitic structure. Also amount of remaining bone substitute was smaller for carbonate apatite containing larger amount of carbonate. At 8 weeks, amount of remained carbonate apatite bone substitutes became smaller when compared to 4 weeks regardless of the carbonate content. Similar to 4 weeks results, amount of remaining bone substitute was smaller for carbonate apatite containing larger amount of carbonate. We concluded that carbonate content in apatitic structure influence osteoclastic resorption leading faster replacement to new bone.

PoB.12.08

renacer®-fibers: A non-toxic, fully resorbable and environmentally friendly biomaterial platform

Bastian Christ[1], Tobias Weigel[1], Christina Ziemann[2], Sofia Dembski[1,3], Jörn Probst[1]

[1]Fraunhofer Institute for Silicate Research ISC, Translational Center Regenerative Therapies TLC-RT, Würzburg, Germany. [2]Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany. [3]University Hospital Würzburg (UKW), Chair Tissue Engineering and Regenerative Medicine, Würzburg, Germany

Abstract

Introduction Fibrous materials are used extensively in biomedical applications. Besides nano- and sub-μ-fiber scaffolds that are often used to mimic the extracellular matrix, also μ-fibrous non-wovens, wovens, or knitted fabrics are established, e. g. in wound healing applications. Next to the biocompatibility of fibers, the efficiency and mechanical properties for the intended biomedical indication, environmental aspects are also becoming increasingly important in the development of new biomaterial solutions. Here, naturally occurring organic polymers like proteins or polysaccharides are mostly used to replace non-degrading, synthetic polymers. With the development of renacer® fibers, the authors are pursuing a strategy using a resorbable, inorganic, material – amorphous silica that is fully soluble into natural and bioactive *ortho*-silicic acid (oSA). The renacer® dissolution product oSA is abundantly available on earth. As it is dissolved in sea water, it is incorporated into the shells of diatoms and thus enters the global food chain. Finally, oSA is also ingested by humans through food and is naturally detected in our tissues and body fluids. Meanwhile, oSA is also increasingly attributed bioactive properties as in bone formation or connective tissue formation. **Results** Renacer® fibers were obtained via dry spinning techniques. In detail, electrospinning techniques were used in the fabrication of sub-μ-fibers and pressure spinning techniques for the production of μ-fibers. All of the resulting fibers showed an amorphous structure (powder x-ray diffraction, XRD) and adjustable fiber diameters and mesh sizes, characterized by scanning electron microscopy (SEM) and optical coherence tomography (OCT). Full fiber dissolution was proven using an USP4-dissolution device and oSA was identified subsequently as the degradation product based on DIN ISO 38405-21 for detection of dissolved silicic acids. In-vitro (geno)toxicity assessment of the fibrous materials by cell counts, WST-1, lactate dehydrogenase release and comet assays showed neither (geno)toxic effects in direct material contact nor when testing oSA saturated cell culture media. In ongoing cell culture experiments, potential applications for biodegradable renacer® fibers are assessed with primary human cells: For evaluation purposes, human dermal fibroblasts are used to develop a resorbable wound patch for chronic skin diseases and human chondrocytes to mimic a cell-loaden cartilage implant. **Conclusion** The renacer® fiber platform is promising for the establishment of non-toxic, bioactive and eco-friendly biomaterials in the field of biomedical applications including the complete degradation to natural oSA. Further characterizations will concentrate on the involvement of oSA in physiological processes, to tailor the bioactive properties towards specific biomedical applications.

PoB.12.09

Release products of 3D printed composite scaffolds containing copper-modified mesoporous bioactive glass have different effects on cell viability and differentiation depending on cell type and donor

Vera Guduric, Richard F. Richter, Anne Bernhardt, Anja Lode, Michael Gelinsky

Centre for Translational Bone, Joint and Soft Tissue Research, Faculty of Medicine Carl Gustav Carus, Technical University Dresden, Dresden, Germany

Abstract

Thanks to their osteoconductivity and bioactivity, bioactive glasses are used for dental and orthopedic applications. In addition to mentioned properties, mesoporous bioactive glasses (MBG) have a characteristic highly ordered channel structure and high specific surface area. Besides possibility to load these channels with drugs and growth factors, MBG can act as delivery system by releasing specific bioactive metal ions, assessing desired therapeutic effects. These ions can be easily integrated in the MBG network. With that in mind, ions showing antibacterial effect would be appropriate solution for prevention and/or treatment of implant-associated infections. However, such ions can be cytotoxic towards human cells at concentrations effective against bacteria. Ion release from MBG can be controlled and retarded by integrating the glass in established biomaterial inks. Our aim here was to investigate release of Cu^{2+} from MBG in 3D printed composite scaffolds and to evaluate effects of release products on human pre-osteoblasts (hOB), primary and immortalized mesenchymal stem cells (hMSC) and umbilical vein endothelial cells (HUVEC). Ca^{2+} in MBG was partially substituted with 5 mol% Cu^{2+} (5CuMBG) and completely with 15 mol% Cu^{2+} (15CuMBG). In order to make this particulate material extrudable, we integrated the different MBG variants in alginate-methylcellulose blend to prepare composite biomaterial-inks containing 2 and 7 wt% MBG. Scaffolds were produced using extrusion 3D printing, crosslinked with $CaCl_2$ and incubated in cell culture medium over 21 days. Ion release profiles were determined and the effect of release products on viability of HUVEC, hOB, primary and immortalized hMSC as well as on differentiation towards osteoblastic cells was investigated. Full substitution with Cu disturbed channel structure of the MBG, while it was maintained in 5CuMBG. Release of Cu^{2+} from all composite scaffolds was initially high, but it dropped over time. Initially released concentrations from all 15CuMBG-containing composites as well as from the ones containing 7 wt% of 5CuMBG were highly cytotoxic towards all tested cell types. However, composites containing 2 wt% of 5CuMBG showed different levels of cytotoxicity towards two different donors of hOB. Viability of HUVEC and both types of hMSC was not affected in the presence of release products of the same type of composite scaffolds, while specific ALP activity of osteogenically differentiated MSC increased significantly. Our findings show that the cytotoxic effect of CuMBG in composite scaffolds depends on cell type and is donor-specific. Therefore, it seems that CuMBG can play a promising role in future patient-specific therapies.

PoB.12.10

Therapeutic elements-doped mesoporous bioactive glass nanoparticles with potential to accelerate wound healing

Sara Pourshahrestani, Ehsan Zeimaran, Aldo Boccaccini

Institute of Biomaterials, Department of Material Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany

Abstract

Serious and infected wounds are one of the most common health issues that cannot be healed via the normal wound healing process and would put patient in life-threatening situations. The development of an appropriate biomaterial that promotes wound healing while fights off infection at the wound site is therefore in urgent need. The therapeutic ions-containing mesoporous bioactive glass microparticles (MBG) or nanoparticles (MBGN) featuring high surface area and ordered mesopores have recently proposed as efficient candidates for various biomedical applications.[1] Among numerous therapeutic elements which confer the particles with recognized biological activities, copper (Cu) and zinc (Zn) with antibacterial efficiencies have shown well-documented potential for wound healing applications. Tellurium (Te), as a poorly discovered element, also found to exhibit antibacterial effect and displayed wound healing efficiency in the form of nanoneedle.[2] Herein, we aimed to prepare pure MBGN, Cu-MBGNs, Zn-MBGNs and Te-MBGNs *via* modified Stöber method[3] and compared the effect of different concentrations (1, 2 and 4 mol%) of Cu, Zn and Te doping on physicochemical properties and biocompatibility of MBGN towards normal human dermal fibroblast cells (NHDFs). The FESEM images revealed that all the nanoparticles have sphere-like shapes. Except for 4Cu-MBGN, 2 and 4Te-MBGNs, the XRD patterns of all MBGNs showed only a broad band confirming their amorphous natures. The nanoparticles also illustrated characteristic bands of the silicate glasses in the FTIR spectra. However, with increasing Te concentration, another peak was appeared. The nitrogen sorption analysis was utilized to elucidate the textural properties of the nanoparticles. While MBGNs with 1 mol% of Te and 1- 4 mol% of Zn and Cu showed surface areas in the range of around 300 to 600 m^2/g, MBGNs with higher concentrations of Te showed lower surface areas. More importantly, the biocompatibility of the extracts of nanoparticles towards NHDF cells were assessed using WST-8 assay and the results elucidated the biocompatibility of all the nanoparticles except those doped with high concentrations of Te. Overall, our results revealed that Cu-, Zn-, and Te-MBGNs at proper concentrations of Cu, Zn, and Te ions could offer promising potential for the wound healing applications.

References
1. Vallet-Regi, M., and Salinas, A. J., Materials Today Bio (2021) 11, 100121
2. Huang, L., et al., Materials Today Bio (2022) 15, 100271
3. Westhauser, F., et al., Journal of Biomedical Materials Research Part A (2021) 109 (8), 1457

Acknowledgments
This work was supported by Alexander von Humboldt Foundation.

PoB.12.11

Hydroxyapatite inverse opal on bioactive glass S53P4 for bone tissue engineering

Adriana Augurio[1], Virginia Alessandra Gobbo[2], Riccardo Tognato[1], Mauro Alini[1], Jonathan Massera[2], Tiziano Serra[1]

[1]AO Foundation, Davos, Switzerland. [2]Tampere University, Tampere, Finland

Abstract

Bioactive glasses (BGs) are reported to be able to promote bone tissue regeneration[1]. Their bioactivity and osteoinductivity when placed in physiological fluids make them promising materials for bone implants. This biochemical response involves the ion exchange at the BGs' surface, which induces the formation of hydroxycarbonate apatite (HCA) layer where bone cells adhere to and proliferate. HCA layer on BGs facilitate the interaction with biomolecules (primarily proteins), which are present in the surrounding environment[2]. Proteins are essential in the reaction between living tissue and implanted BGs, determining the fate of the implant in the short or long term. Thus, the surface properties of a material are crucial because they can promote as well as limit the adsorption of proteins, influencing the type of recruited cells in the human body[3]. Herein, we developed a porous HCA layer with an inverse opal structure on S53P4 BG to induce a controlled protein adsorption and cell adhesion. The FDA-approved S53P4 is produced as bulk discs by the standard melt-quench method[4]. A suspension of commercially available polystyrene (PS) microparticles is deposited on S53P4 and the system was incubated at 37 °C for 72h in simulated body fluid (SBF) for inducing the precipitation of HCA. PS microparticles are removed by calcination, and a microstructured HCA layer is obtained with the desired inverse opal porous structure. The morphology and elemental composition of the inverse opal HCA are confirmed by SEM and EDX analysis. The surfaces are characterized by contact angle (CA), demonstrating a significant variation of CA values on the patterned HCA surfaces, showing a superhydrophilic behavior, compared to bare S53P4 and flat HCA, characterized by a lower wettability. After 72h of culture, the human osteoblasts show a good cell viability on inverse opal HCA as well as active colonization of the surface. Moreover, the adsorption of fibrinogen (FGN) as a model protein is implemented and successfully observed on the patterned surface and on the controls (bare S53P4 and flat HCA), with different amount and surface distribution according to morphological structure of the substrates. Thus, this research work shows a facile and low-cost approach for the development of a 3D microporous patterned HCA layer on BGs, which possess a great potential for bone regeneration and ingrowth.

1. J. Biomed. Mater. Res., 1971, 5, 117-141
2. Life Chem. Rep., 1996, 13, 187–241
3. Materials Characterization, 2002, 49, 255-260
4. ACS Biomater. Sci. Eng., 2021, 7, 4483–4493

PoB.12.12

Effect of Sr functionalized hydroxyapatite nanoparticles fabricated using different Sr sources on their physicochemical properties and *in vitro* performance

Liga Stipniece[1], Anna Ramata-Stunda[2], Inta Kreicberga[1], Dora Livkisa[2], Anna Rubina[1], Artemijs Sceglovs[1], Jana Vecstaudza[1], Kristine Salma-Ancane[1]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of Riga Technical University, Riga, Latvia. [2]Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Riga, Latvia

Abstract

It is considered that the main inorganic component of the bone extracellular matrix (ECM) is nanosized carbonated calcium-deficient apatite containing trace elements such as Mg, Zn, Sr, etc. Synthetic hydroxyapatite nanoparticles (nHAp) have certain similarities with bone apatite in terms of chemical composition, structure and size. Ionic substituted nHAp materials are considered advantageous systems for local delivery and sustainable release of the biomimetic bone ECM trace elements. Moreover, such functionalized nHAp can be applied for developing the next-generation biomaterials for therapeutic treatment and reconstruction of bone disorders through a controlled delivery of biologically active inorganic ions or drugs directly to the bone defect site. In this study, the physicochemical properties, *in vitro* Sr^{2+} ion release, and cellular effects of the developed Sr-substituted nHAp (Sr-nHAp) and strontium ranelate (SrRAN)-loaded nHAp (SrRAN-nHAp) systems have been systemically evaluated. In this study, we tried to find an answer to the question – of which of the developed local Sr^{2+} ion delivery system, Sr-nHAp or SrRAn-nHAp, induce favourable effects on cellular activities. The nHAp, Sr-nHAp and SrRAN-nHAp systems were synthesized via the wet chemical precipitation method from Ca^{2+} and PO_4^{3-} ion precursors. Sr^{2+} ion sources, namely, $Sr(OH)_2$ aqueous solution and SrRAN were added to the synthesis medium to obtain the Sr-nHAp and SrRAN-nHAp products with 1 wt%, 3 wt%, and 10 wt% of Sr in respect to the total yield of the synthesis, respectively. The nHAp, Sr-nHAp and SrRAN-nHAp systems were systematically characterized using XRD, FTIR, BET, AAS, SEM. Ca^{2+} and Sr^{2+} ion release profiles were evaluated in TRIS-HCl buffer solution (pH 7.4) for up to 30 days. SrRAN release profile was evaluated in DMEM after 1, 3 and 7 days. *In vitro* biocompatibility was evaluated using MC3T3-E1 preosteoblasts and MG-63 osteoblasts. Also, *in vitro* hemocompatibility was evaluated for all synthesized nHAp systems. We demonstrated that the used Sr^{2+} sources affect the physicochemical properties, *in vitro* Sr^{2+} ion release, and cellular effects of the synthesized Sr-nHAp and SrRAN-nHAp systems. The developed systems exhibited favourable cell viability and hemocompatibility, as well as prolonged supply of Sr^{2+} ions of biological importance.

The authors acknowledge financial support from the Latvian Council of Science research project No. lzp-2020/1-0072 “Injectable bioactive biocomposites for osteoporotic bone tissue regeneration (inBioBone)”.

The authors acknowledge the access to the infrastructure and expertise of the BBCE – Baltic Biomaterials Centre of Excellence (EU Horizon 2020 research and innovation programme under the grant agreement No. 857287).

PoB.12.13

Importance of the solid and liquid phase composition in calcium phosphate bone cement for the development of local drug delivery systems

Estere Oselska[1,2], Athanasia Pylostomou[1,2], Dagnija Loca[1,2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

Abstract

Nowadays, the number of patients who suffer from bone cancer is rapidly increasing. Long-term treatment is needed, which mostly includes combination of surgical procedures and drug chemotherapy. This often adversely affects the health of patients, causing neurological disorders and heart diseases. Furthermore, tumour resection leaves a large void at the affected bone site that preferably must be filled with an appropriate biomaterial, such as calcium phosphate bone cements (CPCs), due to their ability to regenerate the missing tissues and, also, to serve as local drug delivery systems. Thus, the aim of the current study was to evaluate the effect of the drug addition and the variation of different solid and liquid phase compositions on the physico-chemical properties of prepared CPCs, as well as on the drug release kinetics. CPCs were prepared by using α-tricalcium phosphate (α-TCP) as a solid phase (synthesized at different temperatures) and 0.5 M and 1 M sodium salt solutions as the liquid phase. Doxorubicin (DOX) was used as a model anticancer drug and its content in CPCs was kept at 1.5wt% from the solid phase. Obtained cements were characterized using XRD, Archimedes method, helium pycnometry, BET and SEM. The release kinetics of the DOX were determined by UV-VIS at λ=480nm. Obtained results revealed that the release of doxorubicin from the CPCs can be controlled not only by varying the solid phase synthesis temperature, but also by changing the molarity of the liquid phase. It was also found that the addition of DOX affects the setting time and porosity of CPCs, but does not influence the CPC density. Finally, it was established that DOX-loaded CPCs are able to release the active substance for more than 60 days. According to the results, DOX loaded CPCs have a great potential to be used as a porous injectable biomaterial for bone tissue regeneration and drug delivery, however future studies are necessary to demonstrate their efficiency for osteosarcoma treatment. The authors acknowledge financial support from the European Union's Horizon 2020 research and innovation programme under the grant agreement No. 857287 (BBCE – Baltic Biomaterials Centre of Excellence) and the Ministry of Economics Republic of Latvia project "State research project in the field of biomedicine, medical technologies and pharmacy", project No. VPP-EM-BIOMEDICĪNA-2022/1-0001.

PoB.12.14

Calcium silicate/phosphate bioceramics with a multi-scale porosity derived from geopolymer precursor

Tomáš Kovářík[1], Jiří Hájek[1], Michal Pola[1], Jana Dvořáková[2], Lucie Wiesnerová[2], Václav Babuška[2], Jaroslav Kadlec[1], Miloš Svoboda[1], Jan Beneš[1], Zdeněk Jansa[1]

[1]University of West Bohemia, New Technologies – Research Centre, Pilsen, Czech Republic. [2]Department of Medical Chemistry and Biochemistry, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

Abstract

Ceramics, glass-ceramics and ceramic-composites are materials often applied to repair bone tissue, bone defects or joint replacements. Of the many formulations tested, granules, particles or powders based on bioactive glasses/glass-ceramics have been mostly successful[1]. For the production of bone substitutes, it is vital to consider a human's biological characteristics. Hydroxyapatite, known as a bone mineral, is one of the most popular and researched biomaterials. In this context, hydroxyapatite-based composites are now documented for use in maxillofacial surgeries, in bioactive implants as a coating, and for periodontal filling of bone defects[2]. Currently, highly porous geopolymer materials have gained a lot of attention for their favorable and easily designed properties such as tunable microstructure, pore distribution and nanoporous architecture. The extensive variability in achievable porosity, which is mainly due to a set of hybrid foaming techniques and the ability to transform the geopolymer matrix into semi/non-crystalline ceramics, represents a unique direction in the design of a new class of biomaterials[3]. Here we present a novel concept for the preparation of a geopolymer-hydroxyapatite composite based on sintered hierarchically porous spherical beads[4]. The facile synthesis of geopolymer precursor with multi-scale porosity via pipetting and low-temperature foaming is reported. The dynamic viscosity of the mixtures was described using a rotational rheometer. The production of calcium silicate/phosphate bioceramics was investigated by means of simultaneous DSC/TGA and thermomechanical analyzer TMA. Micro-CT revealed heterogeneous macro-porous architecture in the range of 10–20 vol% with an internal porosity of spherical beads reaching 50 vol% and the presence of combined isolated and interconnected pores. Cell adhesion after 24 h as well as cell proliferation after 7, 14 and 21 days from cell seeding were evaluated by using CCK-8 (Figure 1). In this study, three different bioactive composites with adjustable porosity were successfully synthesized. *In vitro* experiments showed an overall increase of human osteoblast-like cell line MG-63 over the period of 14 days. Furthermore, cell membrane integrity was proved by no cytotoxic effects by means of LDH cytotoxicity assays.

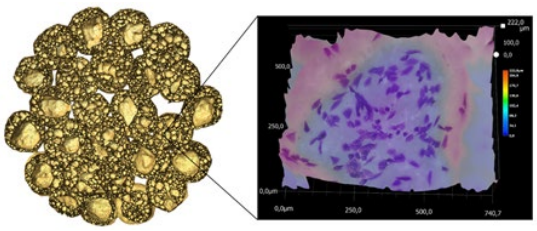


Figure 1. (Left) Micro-CT cross-sectional imagining of porosity architecture. (Right) Cells distribution in the spherical pore visualized by crystal violet staining.

REFERENCES

1. Deshmukh K. et al., RSC Advances, 10, 33782-33835, 2020.
2. Mazumder S. et al., Hydroxyapatite composites for dentistry, Woodhead Publ. 123-143, 2019.
3. Kovářík T. et al. Materials & Design, 198, 109355, 2021.
4. Kovářík T. et al. Cement and Concrete Composites, 124, 104261, 2021.

PoB.13.03

An injectable living hydrogel with encapsulated probiotics to fight against pathogen infections in wounds.

Siyuan Tao, Sixuan Zhang, Kongchang Wei, Katharina Maniura, Qun Ren

Swiss Federal Laboratories for Materials Science and Technology, St. Gallen, Switzerland

Abstract

Wound infections present a significant challenge in healthcare, and traditional treatments involving antibiotics can lead to the emergence of antibiotic-resistant bacteria. Probiotics have been studied widely for their potential antimicrobial effects and use in wound treatment as an alternative to antibiotics. However, an effective and efficient carrier system for probiotics has not yet been developed. This study presents the development of a living hydrogel with probiotics (GeLPro), by entrapping Lactobacillus plantarum into a hydrogel of adipic-acid-dihydrazide (ADH)-modified gelatin, which was crosslinked by Polyethylenglycol difunctionalized with benzaldehyde. The two components were mixed and injected through a double-syringe system, allowing the hydrogel to fit the shape of any wound. GeLPro exhibited great mechanical and self-healing properties due to its Schiff-base bonds. Additionally, the hydrogel provided a favorable environment for the growth and proliferation of Lactobacillus plantarum, with limited escape of the probiotics from the hydrogel. Alamarblue test confirmed that the entrapped Lactobacillus plantarum had great viability comparable with those in planktonic form. GeLPro demonstrated significant antimicrobial efficacy (7 log reductions) against Pseudomonas aeruginosa and Staphylococcus aureus, two common pathogens found in chronic wounds. Cytocompatibility testing with human dermal fibroblasts (HDF) showed no cytotoxicity. Finally, an ex vivo human skin wound model was used to test the therapeutic effect of GeLPro, and a consequent histological study confirmed that the application of GeLPro on the ex vivo wound could effectively prevent Pseudomonas aeruginosa infection. In conclusion, GeLPro shows promise as a candidate for wound infection treatment. Further research is needed to understand the exact mechanism of probiotic treatment and to develop advanced fabrication methods for the living hydrogel, such as 3D printing and microneedles.

PoB.13.05

Harnessing stem cells to deliver TRAIL via magnetic nanomaterial-mediated gene delivery for triple-negative breast cancer treatment

Hsiang-Tzu Lee, Chien-Wen Jeff Chang

National Tsing Hua University, Hsinchu, Taiwan

Abstract

Nearly 15-20% of breast cancers are triple-negative breast cancer (TNBC). Compared with other subtypes of breast cancers, TNBC is prone to metastasis, high recurrence rate, and a low survival rate (<30%). Currently, chemotherapy is still the standard treatment for TNBC in clinical practice. Recent studies have pointed out that TNBC may develop resistance after chemotherapy. Tumor Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL) is a specific anti-cancer protein for various cancers such as TNBC and glioma. However, its uses in cancer therapy are limited by the low stability of TRAIL protein and the lack of efficient delivery technology. To overcome the challenges, a novel biodegradable magnetic gene delivery system, composed of cationic biodegradable polymer and magnetic nanoparticles, was used to construct the TRAIL-expressing human mesenchymal stem cells (^{TRAIL}hMSCs). To enhance the therapeutic efficacy, ^{TRAIL}hMSCs were cultured in the 3D method, which could effectively increase the TRAIL protein secretion while maintaining the tumor tropism of stem cells. The prepared magnetic nanocomplexes were characterized in detail for the particle size, surface potential, magnetism and hysteresis loop, and transmission electron microscopic imaging. The magnetic nanocomplexes exhibited low cytotoxicity and could efficiently deliver nucleic acids into human mesenchymal stem cells. The expression level of TRAIL protein was confirmed from the ^{TRAIL}hMSCs by ELISA assay (Figure 1A). The secreted TRAIL could effectively induce up to 55% of TNBC cells (MDA-MB-231) to apoptosis (Figure 1B). Moreover, a comparison was made between the impact of culture techniques (2D culture and 3D hanging drop culture) on the expression of TRAIL from ^{TRAIL}hMSCs. The results show that TRAIL expression was greatly enhanced by 3D culture over 2D culture (Figure 1C). An injectable and biodegradable hydrogel (GA hydrogel) composed of gelatin and alginate was developed for delivering ^{TRAIL}hMSCs. The encapsulated hMSCs showed high cell viability (>90%), cell spreading, and secretion of TRAIL. The next step involves constructing a mouse model of triple-negative breast cancer (TNBC) to evaluate the therapeutic efficacy of ^{TRAIL}hMSCs delivered through different injection routes (intravenous versus local GA hydrogel delivery). A comparison will be made to determine which method yields better results in the mouse TNBC model.

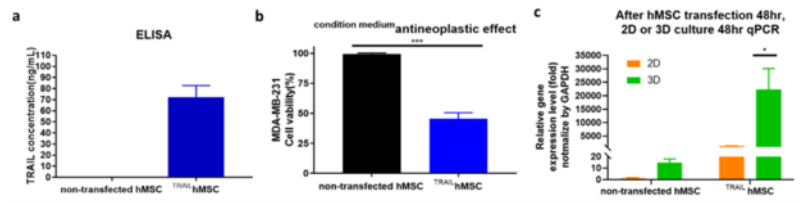


Figure 1. (A) The amount of TRAIL protein from ^{TRAIL}hMSCs conditioned medium was measured by ELISA assay. (B) The anti-neoplastic effect of ^{TRAIL}hMSCs condition medium. (C) The expression level of TRAIL from 2D and 3D-cultured ^{TRAIL}hMSCs was confirmed by real-time PCR.

PoB.13.06

Biofabrication of living materials for carbon sequestration with photosynthesis-based calcification

Dalia Dranseikiene[1], Yifan Cui[1], Andrea Shin Ling[1], Felix Donat[1], Stéphane Bernhard[1], Margherita Bernero[1], John Oakey[1,2], Benjamin Dillenburger[1], Mark William Tibbitt[1]

[1]ETH Zurich, Zurich, Switzerland. [2]University of Wyoming, Wyoming, USA

Abstract

Background: Living materials (LMs) can be fabricated by encapsulating living organisms within a carrier matrix, providing unique abilities such as sensing, adapting, self-healing, and regeneration [1]. LMs with encapsulated photosynthetic microorganisms have also been used for carbon sequestration via on-demand biomolecule synthesis. These LMs can sequester carbon via biomass production under ambient conditions and also by metabolic activities of the microorganism, such as microbially-induced calcium-carbonate precipitation (MICP) [4, 5]. The microbially-produced carbonates serve as an irreversible carbon sink within the LMs. Meanwhile, structuring the LMs with additive manufacturing (AM) has been used to further optimize the carbon sequestration capabilities [2, 3]. **Methods:** The main goal of this project is to use photosynthesis-based MICP as a carbon sink for the generation of inorganic 3D structures via direct ink writing (Cellink BioX). To define the composition and morphology of the precipitates, the composite materials were inspected by SEM (JOEL JSM 7100F) and XRD (PANalytical Empyrean). Thermal decomposition was conducted at 600°C to allow complete combustion of the microorganisms and the carrier matrix. Therefore, the weight that remained after thermal decomposition indicated the extent of carbonate precipitation within the 3D structure. **Results:** PCC 7002 algae were encapsulated in F127-based bioinks with high viability throughout the course of incubation. AM constructed LMs showed enhanced post-thermal decomposition mass compared with abiotic controls, indicating effective carbon sinking within the LMs. Precipitate accumulation in the 3D-printed structures allowed for the manufacture of composite materials wherein the inorganic material was generated by living microorganisms. To further exploit the AM capacity, a more complex scaffold based on unit-based cellular fluidics was modelled and printed to maximize the surface area for more effective light and gas exchange between the printed scaffold and the ambient environment. **Conclusions and Outlook:** LMs with photosynthetic microorganisms have potential in the design of carbon sequestering materials and living composites. The combination of AM and LMs allows structural flexibility, thus potentially maximizing the biomass and inorganic material generated by living organisms and the extent of carbon sequestration.

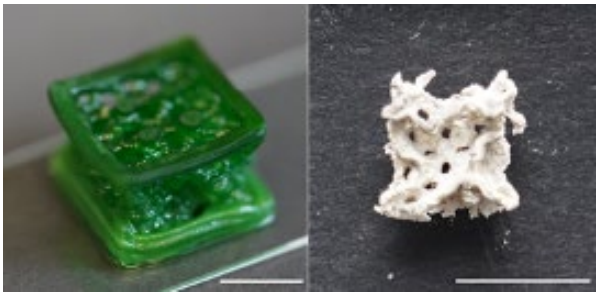


Fig. 1: Left: AM fabricated biotic scaffold with PCC 7002. Right: 30 days incubated biotic scaffold after thermal decomposition. Scale bar: 1 cm.

References:

1. ACS Synth Biol 2019, 8, 1
2. Nat Commun 2020, 11, 563
3. ACS Appl Mat Inter 2018 10, 13373-13380
4. Adv Funct Mater 2021, 31, 2011162
5. Applied Microbiol Biot 2018, 102, 3121

PoB.13.08

Biopolymer-based strategies toward heavy metal removal and neurodegenerative diseases management

Cristina Casadidio[1], Lakshmi Sathi Devi[1], Maria Rosa Gigliobianco[1], Piera Di Martino[2], Roberta Censi[1]

[1]Camerino University, Camerino, Italy. [2]University G. D'Annunzio Chieti e Pescara, Chieti, Italy

Abstract

Introduction: Water bodies deterioration has increased dramatically, mainly due to anthropogenic processes, with heavy metal ions (HMI) being the most persistent and toxic contaminants. These environmental pollutants have been identified as etiological agents for neurodegenerative disorders (Alzheimer and Parkinson diseases) [1]. The design of novel HMI-binding materials based on natural polysaccharides can be a potential strategy to simultaneously apply sorptive matrices for water decontamination and deliver therapeutics for neurodegenerative diseases management. Methods: The system was composed of vinylsulfonated β-cyclodextrin (βCD-VS) and thiol-derivative of hyaluronic acid (HA-SH) exploiting Michael-addition crosslinking reaction (Fig.1). Structural characterizations of the formulated products were performed under NMR, FT-IR, RAMAN spectroscopy and rheological analyses. To confirm the stability of the system, swelling and degradation studies were carried out with the presence of dithiothreitol as reductive agent. Encapsulation efficiency behavior of the system was studied using hydrophobic (docetaxel, DTX) and hydrophilic (gemcitabine, GCB) model molecules. Results: NMR and FT-IR confirmed the structure and degrees of substitution of formulated products. The Michael addition crosslink behind the gelification was proved by rheological measurements and RAMAN spectroscopy. The RAMAN spectrum of the lyophilized gel revealed new peaks around 600-700 cm-1 range that corresponds to -C-S- bond of Michael-addition between HA-SH and βCD-VS groups (Fig. 2a). It was proved that with time under reductive conditions, the HA-βCD hydrogel was stable, while the gel with just HA-SH degraded in 15 minutes (Fig. 2b). Preliminary studies proved that DTX was successfully complexed within the βCD-VS cavity while GCB was entrapped during the gelification process. HA-SH proved to be a good HMI binding material, as also proved before [2].

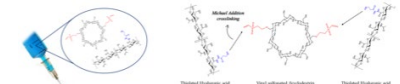


Figure 1. Schematic gel formation via Michael addition crosslinking.

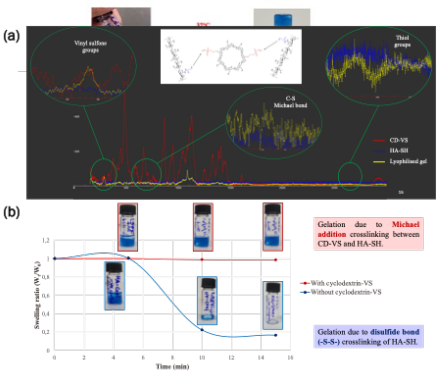


Figure 2. (a) Combined RAMAN spectra of the system; (b) Stability demonstration of Michael addition crosslink under reductive conditions.Conclusion: The HA-βCD hydrogels can be exploited as potential platforms for the treatment of neurodegenerative disorders and as removal systems of metal-based xenobiotics.

Acknowledgements: This work was partially supported by LIFE MERCURY-FREE, project ID: 101074412.

References: [1] Bhattacharjee T. et al., Nat. Comm. (2019): 10; [2] Wang N. et al, Biosensors (2022): 12.

PoB.13.09

Cell-derived extracellular matrix – characterization, application and modification of a promising biomaterial

Svenja Nellinger[1], Petra Juliane Kluger[2]

[1]Reutlingen Research Institute, Reutlingen, Germany. [2]Reutlingen University, Reutlingen, Germany

Abstract

The ECM represents the natural environment of cells. It is a fibrous network of proteins, proteoglycans, and glycosaminoglycans, arranged in a highly tissue-specific manner and is produced and secreted by the resident cells. Due to availability and minimal invasive harvesting human adipose tissue-derived extracellular matrix (dECM) is often used as a biomaterial in tissue engineering applications. Next to dECM, cell-derived ECM (cdECM), can be generated by and isolated from cultured cells. Both types of ECM were investigated extensively for their application in tissue engineering and regenerative medicine. The *in vitro* generated cdECM offers some advantages like tailor-made ECM (stem cells) and reproducible manufacturing processes. In the last years, we have done a systematic characterization and comparison of dECM from human adipose tissue, as well as cdECM from human adipose-derived stem cells (ASCs) in the stem cell stage and the adipogenic differentiated stage. Hereby we analyzed the macromolecular composition, structural characteristics, biological purity, and immunogenic potential of the different types of ECM (1). Furthermore, we were able to demonstrate that adipose stem cell-derived ECM enhanced the spontaneous formation of prevascular-like structures by microvascular endothelial cells (2). For specific modification, we used metabolic oligosaccharide engineering to integrate different modification sites into the cdECM without affecting the native structure of the ECM molecules. We demonstrated the modification with azido groups, which can be addressed by bioorthogonal copper-catalyzed azide-alkyne cycloaddition (CuAAC) and with dienophiles (terminal alkenes, cyclopropene), which can be addressed by an inverse-electron-demand Diels-Alder (IEDDA) reaction (3,4). In summary, we were able to show that cdECM is a promising biomaterial, which can be applied in various applications.

1. Nellinger et al. 2022; Cell-derived and enzyme-based decellularized extracellular matrix exhibit compositional and structural differences that are relevant for its use as a biomaterial; Biotechnol Bioeng; doi: 10.1002/bit.28047
2. Nellinger et al. 2020; Adipose stem cell-derived extracellular matrix represents a promising biomaterial by inducing spontaneous formation of prevascular-like structures by mvECs; Biotechnol Bioeng. doi: 10.1002/bit.27481
3. Nellinger et al. 2019; Generation of an azide-modified extracellular matrix by adipose-derived stem cells using metabolic glycoengineering; CurDirBiomedEng. doi: 10.1515/cdbme-2019-0099
4. Nellinger et al. 2022; An Advanced 'clickECM' That Can be Modified by the Inverse-Electron-Demand Diels-Alder Reaction; Chembiochem; doi: 10.1002/cbic.202100266

PoB.13.10

Cold-induced assembly of non-canonical collagens

Mengjie Shen, Dmitrii Fedorov, Karoliina Elfving, Markus Linder, Sesilja Aranko

Aalto University, Espoo, Finland

Abstract

Collagen is the most abundant protein in the animal kingdom, and maintains the structure and strength of connective tissues, including bones, tendons, skin, and cartilage. Collagen is biocompatible, bioactive, chemically modifiable, biodegradable, and capable to form various shapes and forms, which is why it has been widely studied as a source for biomaterials. The collagen-based products are used in different fields, from food and cosmetics to pharmaceutical applications. Collagen has a characteristic super-coiled triple-helix structure, formed by amino acid repeats of $(G\text{-}X\text{-}Y)_n$. In principle, glycine is necessary to fulfill the spatial requirement of triple helices, while X and Y positions can be any residues. Most commonly X is proline, and Y is hydroxyproline which is hydroxylated proline via post-translational modification *in vivo*. Hydroxyproline is important to the thermal stability of mammalian collagens. Collagen is traditionally obtained from animal sources and due to ethical, economical, and safety reasons, alternatives are needed. Recombinant production of mammalian collagen is challenging, because of the large size of the protein and requirement for hydroxyproline. Instead, a category of non-canonical collagen-like proteins can be recombinantly produced in bacteria. These non-canonical collagen-like proteins are smaller compared to mammalian collagens and have no hydroxyproline. In this work, we show the presence of triple helical structures of the recombinantly produced non-canonical collagen-like proteins by employing circular dichroism spectrometry and analytical ultracentrifugation. The assembly process took place only at sub-zero temperatures. The assembly of the triple helical structures was not dependent on the presence of hydroxyprolines. In addition, the collagen-like proteins can be casted into films with underlaying fiber-like structure. Fourier transform infrared spectroscopy data further indicates that the non-canonical collagens in films are also in triple-helical conformation. The recombinant non-canonical collagens studied in this work show potential for the design of novel biomaterials. The results pave the way towards the design of novel collagen-based biomaterials, that can be obtained recombinantly from micro-organisms.

PoB.13.11

Recombinant production of native-sized spider-silk proteins by engineering a novel catcher/Tag pair

Ruxia Fan[1], Johanna Hakanpää[2,3], Karoliina Elfving[1], Helena Taberman[2], Markus Linder[1], Sesilja Aranko[1]

[1]Department of Bioproducts and Biosystems, School of Chemical Engineering , Aalto University, Espoo, Finland. [2]Deutsches Elektronen Synchrotron (DESY) Photon Science, Hamburg, Germany. [3]Hamburg Unit c/o DESY European Molecular Biology Laboratory (EMBL), Hamburg, Germany

Abstract

Spider silk has an unparalleled combination of mechanical properties, in terms of high strength, modulus, extensibility, and toughness, outperforming most of the known native and synthetic fibers[1]. Despite significant efforts have been made, the fibers spun from recombinant spider silk proteins cannot fully replicate the mechanical properties of natural spider silk fibers. One of the key limitations is the inability to produce highly repetitive native-sized spider-silk proteins (>250 kDa) in an efficient and soluble manner[2]. To overcome this long-standing challenge, we employed Catcher/Tag pairs, known for their ability to spontaneously form an intermolecular covalent isopeptide bond, as a protein ligation strategy. We engineered a novel Catcher/Tag pair, called SilkCatcher/Tag, which is compatible with the widely used SpyCatcher/Tag pair[3] to accomplish controllable protein ligation. We successfully produced recombinant native-sized spider-silk proteins (320 kDa) with >90% purity by a stepwise ligation and purification method. This result lays the foundation for constructing recombinant spider-silk fibers with excellent mechanical properties, potentially enabling broader applications of recombinant spider-silk fibers.

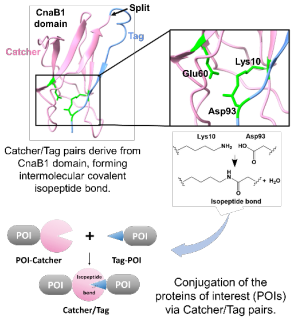


Figure 1. Schematic presentation of designing the Catcher/Tag pairs and ligation strategy based on the Catcher/Tag pairs[4].

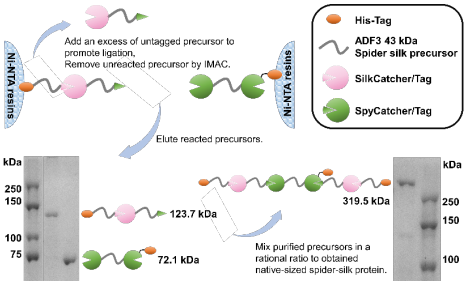


Figure 2. Schematic presentation of the stepwise ligation strategy for native-sized spider-silk protein[4].

1. Bowen, C. H. et al. Biomacromolecules 19, 3853–3860 (2018).
2. Xia, X. X. et al. Proc. Natl. Acad. Sci. USA 107, 14059–14063 (2010).
3. Keeble, A. H. et al. Angewandte Chemie 56, 16521–16525 (2017).
4. Fan, R. et al. Angewandte Chemie 62, e202216371 (2023).

PoB.13.12

In vitro engineering of a growing cartilage using human adult mesenchymal stromal cells to study the growth plate biology

Gangyu Zhang, Adrien Moya, Ivan Martin, Scherberich Arnaud

Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland., Basel, Switzerland

Abstract

Growth plate, or epiphyseal plate, is a highly organized tissue responsible for postnatal bone growth in children and adolescents via endochondral ossification. Engineering models that recapitulate the critical aspects of growth plate will provide a promising avenue to develop novel strategies for cartilage and bone tissue engineering. In addition, such models would offer a valuable platform to study the physiology and pathology of growth plate. The goal of our study was to develop an *in vitro* model of growth plate using bone marrow-derived mesenchymal stromal cells (BM-MSCs) by recapitulating the embryonic and postnatal bone development. BM-MSCs were seeded into collagen sponges and exposed to chondrogenic medium (DMEM high glucose supplemented with transforming growth factor-β3, Acid Ascorbic and Dexamethasone) for up to 4 weeks in order to generate *in vitro* cartilage tissues. The *in vitro* engineered cartilage tissues were analysed throughout the chondrogenic differentiation at 1, 2, 3 and 4 weeks. Safranin-O staining showed clear cartilaginous features starting from the first week that became more mature over time. Immunofluorescent staining revealed the presence of PTHrP+, Col-2+, Ki67+ chondro-progenitors, and Col-2+, Ki67+ proliferating chondrocytes in the engineered cartilage, analogous to the resting zone chondrocytes and proliferative zone chondrocytes present in the growth plate. Flow cytometry analysis showed that, during *in vitro* chondrogenesis, the number and percentage of skeletal stem cells (PDPN+CD146-CD164+CD73+) present in the cartilage engineered tissue were maintained during the first 3 weeks and supplied chondrocytes continuously. However, the skeletal stem cells fraction then decreased dramatically between week 3 and week 4. Single-cell transcriptomic analysis of these growing cartilage revealed distinct cell clusters that express canonical markers involved in the development of the growth plate. In conclusion, the *in vitro* growing cartilage model will provide access to study the growth plate biology and provide a platform to test the effectiveness and safety of the drugs aiming at treating malformation, injury, or diseases of growth plate. Our next steps will be to stimulate the growth of cartilage by supplying specific factors involved in growth plate biology (i.e. IHH, PTH) or applying mechanical stimulation to mimic the physiological force in the growth plate due to gravity.

PoB.13.13

A novel steerable and soft microcatheter actuated by an engineered muscle tissue

Lorenzo Vannozzi, Carlotta Salvatori, Ignazio Niosi, Diego Trucco, Leonardo Ricotti

Scuola Superiore Sant'Anna, Pisa, Italy

Abstract

Introduction: Targeted therapies allow increasing the efficacy of treatments of diseases. Catheters with a steerable tip have been recently proposed to bridge this gap in view of localized treatments in deeper and tortuous regions within the cardiovascular systems [1]. A novel generation of steerable microcatheter, fostered by the unconventional kind of actuation provided by muscle cells, named biohybrid actuator, would represent a scalable actuated system within the human body [2]. Here, a preliminary analytical and simulative analysis of the deflection of a soft catheter, actuated by a contractile biohybrid actuator, is reported. Methods: The deflection of the catheter was investigated according to Euler-Bernoulli's beam theory [3], by assuming the force of the biohybrid actuator equal to 100 µN, applied in the inner wall as a couple of opposite forces. The catheter mechanical properties derived from the analysis of Polydimethylsiloxane (PDMS, Sylgard 184), at the ratios of 1:10, 1:20 and 1:30, Dragonskin 10slow, and Ecoflex 00-10. Tensile tests were performed to evaluate the Young's modulus. Finite Element Model (FEM) simulations in Comsol were used to analyze the dynamic of deformation of the catheter. Results: Material Young's moduli ranged between 1.13 MPa (PDMS 10:1) and 0.04 MPa (Ecoflex 00-10) (Figure 1a). The analytical evaluation of the catheter deflection showed the influence of the Young's modulus, and the dependence on the catheter wall thickness (Figure 1b). Results of FEM analysis highlighted the difference in terms of generated stress due to the actuator configuration, thus catheter deflection (Figure 1c). Conclusion: Preliminary analysis shows interesting potentialities in view of using the biohybrid actuation paradigm for controlling the microcatheter tip. Future works will focus on a deeper investigation of the catheter bendability through simulative analysis, and biological assessment of the engineered constructs.

Acknowledgements:
This project has received funding from the European Union's Horizon Europe research and innovation programme, BioMeld project, Grant Agreement No. 101070328.

References:

[1] - Hu, Xiaohua, et al. Computer Assisted Surgery, 23:1, 2018.

[2] - Ricotti, Leonardo, et al., Science Robotics, 2.12, 2017.

[3] - O. A. Bauchau and James I. Craig, Structural analysis. Springer, pp. 173–221, 2009,

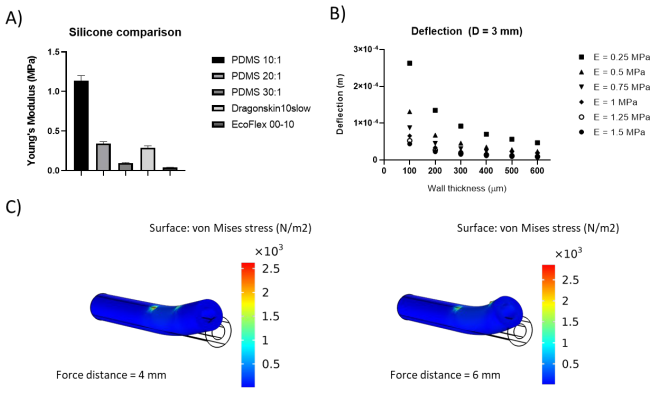


Figure 1: A) Comparison of Young's moduli of the selected elastomers; B) Estimation of the deflection by varying the catheter wall thickness and Young's modulus, fixing the external catheter at 3 mm; C) Examples of the simulative analyses applying the forces at 4 mm (left) and 6 mm (right).

PoB.13.14

Light-responsive living biomaterials based on *Lactococcus lactis*

Aleixandre Rodrigo-Navarro, Manuel Salmeron-Sanchez

University of Glasgow, Glasgow, United Kingdom

Abstract

Lactococcus lactis is a gram-positive bacterium that has been widely studied for its biotechnological and industrial applications due to its ability to produce high-value chemicals and recombinant proteins. To this end, multiple chemically inducible gene and protein expression systems have been developed. Here we present a novel inducible system that relies solely on physical stimulus, specifically blue light, using a combination of small engineered Vivid photoreceptors from Neurospora crassa (enhanced magnets or eMags) and a split T7 RNA polymerase. Our findings demonstrate that the split T7 RNA polymerase is active and non-toxic in *Lactococcus lactis*, and when fused to the eMags, it can drive gene expression in a light intensity- and time-dependent manner. This marks the first time that a split T7 RNA polymerase fused to photoreceptors has been described in a gram-positive bacterium. This system provides an attractive alternative to chemically inducible systems since it can be activated by physical stimulus alone, thus avoiding the need to add inducers externally and improving its reversibility, without the need to remove the inducer from the medium. Furthermore, this system can be applied in various biotechnological applications where precise spatiotemporal control of gene expression is required, especially in the context of living biomaterials where chemical stimuli are diffusion-controlled, difficult to switch off and thus can not be applied locally in a reversible way, a role where light excels. To showcase our system, we expressed GFP as a reporter gene fused to the $III_{7\text{-}10}$ domains of human fibronectin. Our results demonstrate that inducible, variable expression of FN $III_{7\text{-}10}$-GFP on *L. lactis* embedded in a 3D matrix acts as a smart living biomaterial capable of affecting the morphology and cell fate of mammalian cells co-cultured with the bacteria using only light as external stimulus. Our findings provide a promising foundation for further research in the development of novel living biomaterials responsive to external physical stimulus that can simultaneously affect the physico-chemical properties of the biomaterial and the behaviour of cells co-cultured in the same matrix, for novel biotechnological applications in tissue engineering or industrial protein and added value chemical production.

PoB.13.16

Advantages and challenges regarding genus *Vaccinium* fruit extract encapsulation in ethosomes

Ance Bārzdiņa[1,2], Supandeep Singh Hallan[1,2], Dace Bandere[1,2], Agnese Brangule[1,2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Rīga Stradiņš University, Rīga, Latvia. [2]Baltic Biomaterial Centre of Excellence, Headquarters at Rīga Technical University, Rīga, Latvia

Abstract

Although medicinal plants contain hundreds of bioactive compounds, their limited bioavailability, solubility, and stability thus far have prevented their wider use in evidence-based medicine[1]. However, encapsulation in nanoparticles could help to overcome this limitation, but an evaluation of this approach is lacking. As polyphenol-rich medicinal plants possess antioxidant and antibacterial properties, their incorporation in transdermal drug delivery forms, namely ethosomes, could show promising applications in wound healing. This study aimed to incorporate two polyphenol-rich genus *Vaccinium* fruit extracts (*Vaccinium corymbosum* (highbush blueberry) and *Vaccinium myrtillus* (bilberry)) in ethosomes and evaluate the advantages and challenges regarding this approach. A maceration method with subsequent extract lyophilization was applied to obtain fruit extracts. A HPLC-UV method was developed to investigate the chemical composition of extracts and to quantify the chlorogenic acid content in the extracts. Antibacterial and antioxidative properties of extracts were measured, using the well diffusion method and DPPH analysis. Finally, ethosomes containing extracts were prepared using the classical cold method[2] and characterized for physical and chemical parameters. Highbush blueberry extracts contained 7-8 times higher chlorogenic acid content than bilberry extracts. Additional 9 other polyphenols were identified in the prepared extracts. Thanks to the sugars and lipids present in these fruits, lyophilization for 48 hours is necessary to obtain a powder-like substance. For both extracts higher antibacterial activity can be seen against *Staphylococcus aureus* than against *Escherichia coli* reference cultures. The average diameter of empty and loaded ethosomes was around 220 nm, and the polydispersity index was approximately 0.3. The zeta potential values were around -10 mV, indicating good colloidal stability. Chlorogenic acid showed a clear, sharp, intense peak in the HPLC analysis for blueberry extracts due to its' high concentration, making also the HPLC analysis after encapsulation more accurate and replicable. Encapsulation in ethosomes prolonged the stability of analyzed extracts. To sum up, fruit extract encapsulation in ethosomes prolongs the stability of extracts and allows to maintain high concentrations of active substances of interest. Extracts with one dominant compound (blueberries and chlorogenic acid) are more suitable for encapsulation and consequent analysis. With the complex chemical composition of fruits, the main challenge is the preparation of extracts to obtain dry, homogenous samples. Further studies evaluating the biological activity of obtained ethosomes are needed.

1. Barani M. (2021) Int J Nanomedicine 16, 6983–7022
2. Hallan SS. (2020) Pharmaceutics 12, 740

PoB.13.17

Systematic optimisation and scaling-up of cost-effective bacterial cellulose production for biomedical applications

Elliot Amadi[1], Mahendra Raut[1], Syed Mohammad Syed Daniel Mohamed[1,2], David Gregory[1], Gavin Welsh[2], Ipsita Roy[1]

[1]University of Sheffield, Sheffield, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

The design, optimisation and application of biomaterials has uncovered many eco-friendly, sustainable alternative solutions to synthetic polymers, allowing a reduction of the dependency on fossil fuel-based resources. One example is bacterial cellulose (BC), a biopolymer that due to its unique variety of properties, can be used in many biomedical applications. Many BC-based products have already been exploited to produce tissue engineering scaffolds, wound healing patches and medical device development. Due to its chemical structure BC has the inherent ability to be functionalised via both in-situ and ex-situ processes, to obtain specific properties for a particular application. BC also has the advantage of a relatively simple production process, where polymeric fibres are secreted extracellularly by many bacteria. Komagataeibacter xylinus, a model organism, is predominantly exploited for BC production. However, the current production is heavily dependent on pure sugars and is therefore expensive and ends up with low yields. In this work, a novel rotary disc bioreactor was developed to improve the yield of BC. The process was first validated with media containing glucose, for which a yield of 365 g/L (for wet BC) was obtained. The media was further optimised using orange peel extract, a common waste, as a substrate, and a yield of 179 g/L of wet BC was achieved. The BC produced was further oxidised to produce bioresorbable BC. The BC was characterised using SEM, TGA, DSC and FTIR. Further, protein absorption and cell viability assays using kidney (conditionally immortalised human podocytes (CIHP) and conditionally immortalised glomerular endothelial cells (ciGEnC) and skin (HaCaT) cell lines were used to assess the biocompatibility of pure and functionalised BC. Hence, in summary we successfully produced BC using glucose and orange peel extract. This polymer was thoroughly characterised and modified to produce resorbable BC and its biocompatibility was assessed.

PoB.13.18

Effective decellularization of human skin tissue for regenerative medicine by supercritical carbon dioxide technique

Pham Ngoc Chien[1], Trinh Xuan Tung[1], Nguyen Ngan Giang[1,2], Nguyen Van Long[1], Nam Sun Young[1], Heo Chan Yeong[1,2]

[1]Department of Plastic and Reconstructive Surgery, Seoul National University Bundang Hospital, Seongnam, Korea, Republic of. [2]Department of Medical Device Development, College of Medicine, Seoul National University, Seoul, Korea, Republic of

Abstract

Allotransplantation, performed using an acellular dermal matrix (ADM), plays a significant role in the cultivation of constituted and damaged organs in clinical. Herein, we fabricated an innovative ADM for allografting derived from decellularized human skin by utilizing the supercritical fluid of carbon dioxide to eliminate immunogenic components. By using histological staining, the ADM product demonstrated the successful removal of cellular constituents without exerting any harmful influence on the ECM. The results from DNA electrophoresis also supported this phenomenon by showing the complete DNA removal in the product, accompanied by the absence of Major Histocompatibility Complex 1, which suggested the supercritical fluid is an effective method for cellular withdrawal. Moreover, the mechanical property of the ADM products, which showed similarity to that of native skin, displayed great compatibility for using our human-derived ADM as an allograft in clinical treatment. Specifically, the cell viability demonstrated the remarkable biocompatiability of the product to human bio-cellular environment which was noticeably higher than that of other products. Additionally, the ability to regulate cellular processes in human body was manifested widely by the significant increase in the level of growth factor cytokines such as VEGF, CD31, uPAR, GM-CSC, proving the products to be innovative in the field of regeneration. This study provides a thoroughly extensive analysis of the new ADM products, enabling them to be applied in industrial and clinical treatment.

PoB.13.20

Effective decellularization of porcine nerve matrix with supercritical carbon dioxide for nerve regeneration in medicine

Nam Sun Young[1], Pham Ngoc Chien[1], Le Thi Thuy Linh[1,2], Trinh Xuan Tung[1], Nguyen Ngan Giang[1,3], Le Van Long[1], Heo Chan Yeong[1,3]

[1]Department of Plastic and Reconstructive Surgery, Seoul National University Bundang Hospital, Seongnam, Korea, Republic of. [2]Department of Biomedical Science, College of Medicine, Seoul National University, Seoul, Korea, Republic of. [3]Department of Medical Device Development, College of Medicine, Seoul National University, Seoul, Korea, Republic of

Abstract

Tissue engineering scaffolds are often made from the decellularization of tissues. The decellularization of tissues caused by prolonged contact with aqueous detergents might harm the microstructure and leave cytotoxic residues. In this research, we developed a new technique to use supercritical carbon dioxide (Sc-CO2) based decellularization for porcine nerve tissue. The effect of decellularization was analyzed by histological examination, including H&E staining, MT staining and DAPI staining. Moreover, biochemical analysis of the decellularized tissues was also performed through the measurement of DNA content, amount of collagen, and glycosaminoglycans after decellularization. The results showed that the tissue structure was preserved, cells were removed, and the important components of ECM such as collagen fibers, elastin fibers, and glycosaminoglycans fibers remained after decellularization. In addition, the DNA content was decreased compared to native tissue, and the concentration of collagen and glycosaminoglycans in the decellularized nerve tissue was the same as in native tissue. *In vivo* experiment in the rat model showed that after six months of decellularized nerve implantation, the sciatic function index was confirmed to recover in decellularized nerve. Morphological analysis displayed that there were a lot of infiltrated cells in the decellularized nerve, similar to that in native tissue, and the number of Schwann cells that play an important for motor function and sensory in the decellularized nerve was confirmed. These findings indicate that the process of tissue decellularization using Sc-CO2 has been successfully used in tissue engineering.

PoB.13.21

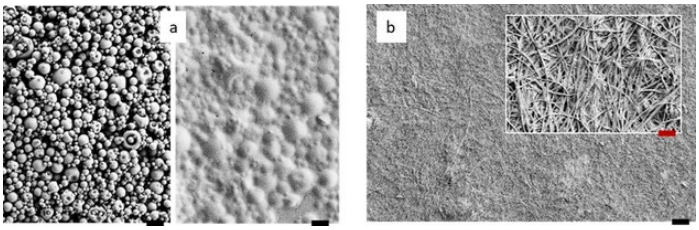
Formulation of nisin-loaded gelatin microparticles for eliciting bioactivity in bacterial nanocellulose

Maša Hren[1], Janja Trček[2], Silvo Hribernik[3], Mateja Erdani Kreft[4], Hristina Obradović[4], Selestina Gorgieva[1]

[1]University of Maribor, Faculty of Mechanical Engineering, Institute of Engineering Materials and Design, Smetanova ulica 17, 2000, Maribor, Slovenia. [2]University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, Koroška cesta 160, 2000, Maribor, Slovenia. [3]University of Maribor, Faculty of Electrical Engineering and Computer Science, Koroška cesta 46, 2000, Marbor, Slovenia. [4]University of Ljubljana, Faculty of Medicine, Institute of Cell Biology, Vrazov trg 2, 1000, Ljubljana, Slovenia

Abstract

Bacterial nanocellulose (BnC), a highly pure "biological nonwoven" material, has garnered significant interest in medicine. It has been utilized in applications ranging from simple bandages to advanced skin substitutes, arterial stent coatings, cartilage and bone implants [1]. BnC does not elicit undesirable reactions when in contact with human tissues, limiting targeted interactions with other biomolecules, and thereby reducing foreign-body reactions. However, the lack of bioactivity can be a limitation in applications where bioactivity is required, such as regenerative medicine. The absence of cell-recognition moieties also means that BnC does not attract human cells for attachment and lacks active functionality against organisms like pathogenic bacteria. In this study, we propose the introduction of gelatin-nisin (GELn) microparticles into BnC to elicit the antimicrobial function. The microparticles were processed by spray-drying, followed by dehydrothermal treatment to stabilize the gelatin. Nisin was added through a solution-adsorption process. The concentration of nisin was determined based on a pre-analysis of its cytotoxic effects on human gingival fibroblast cells. The optimization of the spray-drying process and dehydrothermal treatment aimed to produce smaller microparticles with a higher crosslinking degree capable of retaining their shape and size during the post-modification process of BnC. Gelatin and gelatin-nisin solutions were used as controls to compare the structural effects on the bioactivity profile. The BnC membrane was produced following a previously reported protocol using the new *Komagataeibacter melomenusus* AV436^T bacterial strain and grape-based hydrolysate as a culture medium [2]. The GELn microparticles were introduced into the BnC membrane through a post-synthetic immersion process. A comprehensive examination of the microparticles revealed the formation of round-like particles with a narrow size distribution >2 µm *(Fig. 1)* and a crosslinking degree of 60%, as determined by the TNBS method. The microparticles containing non-cytotoxic dose of nisin (200 µg/mL) exhibited antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853, as reflected in the final BnC-GELn membrane material. *Fig.1. (a) Gelatin microparticles before (left) and after the introduction of nisin (right); (b) BnC produced by acetic acid bacteria strain K. melomenusus AV436^T after inclusion of microparticles. Black and red scale bars correspond to 2 µm and 200 nm, respectively.*



Acknowledgement This research was funded by Slovenian Research Agency, project J2-2487, BI-ME/21-22-002, and researcher program P2-0118 (B). **References** [1] S. Gorgieva and J. Trček, *Nanomaterials*, 2019. [2] S. Gorgieva et al., International Journal of Biological Macromolecules, 2023.

PoB.13.22

Optimization and characterization of enzymatically crosslinked hyaluronic acid microgels for cell encapsulation

Parisa Torabi Rahvar[1,2], Mohammad J. Abdekhodaie[1]

[1]Department of Chemical Engineering, Sharif University of Technology, Tehran, Iran, Islamic Republic of. [2]Tissue Engineering for Orthopaedic & Mechanobiology, Bone & Joint Program, Department for BioMedical Research (DBMR), Medical Faculty, University of Bern, Bern, Switzerland

Abstract

INTRODUCTION Human mesenchymal stromal cells (hMSCs) are potential candidates for regenerative medicine and the challenges that need to be addressed for successful cell-based therapies. Using a microgel system as a cell delivery vehicle is a promising approach because it can mimic the three-dimensional natural environment of cells, allows for effective substance transfer, and is injectable. The extrusion-based method is particularly advantageous due to its ease of operation and the absence of oil in the system. However, the oil-free system requires polymer solutions that rapidly gel on contact with coagulation solutions, which is a challenge since there are limited biopolymers capable of rapid crosslinking. The aim of this study was to design, optimize and characterize horseradish peroxidase cross-linked hyaluronic acid microgels to encapsulate cells using the extrusion-based technique. EXPERIMENTAL METHODS The concentrations of HA-Tyr and the crosslinking agents, horseradish peroxidase (HRP) and H2O2, were chosen as experimental design factors. The Box-Behnken design (BBD) based on the response surface methodology (RSM) was used to analyze and optimize the system based on the minimum microgel size. RESULTS AND DISCUSSION Conditions resulting in a gelation time of less than 4 seconds resulted in spherical microgels. The results from BBD showed that increasing the HA-Tyr concentration led to a decrease in the microgel diameter, while decreasing the HRP concentration resulted in an increase in the diameter. No significant change in microgel diameter was observed at different H2O2 concentrations. The optimized process produced microgels approximately 400 µm in diameter, and live/dead staining confirmed that the optimal concentration for the crosslinker did not damage the encapsulated cells, as there was abundant green staining, indicating live cells (Figure 1).

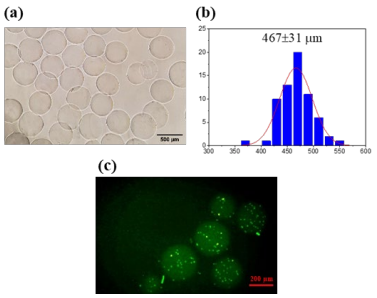


Figure 1. Enzymatically crosslinked HA microgels. (a) light microscopy images, (b) size distribution, and (c) viability of encapsulated MSCs.

CONCLUSION Optimization of the microgels production process revealed that gelation time was the most significant factor influencing microgel diameter. Changing the polymer or crosslinker concentration resulted in smaller microgels if the gelation time was reduced. Additionally, cell encapsulation through enzymatic crosslinking is potentially useful for a variety of polymers and applications involving the preparation of cell-laden microgels.

REFERENCES

1. Feng Q. et al., Acta Biomater. 1;113:393-406, 2020.
2. Hassani A. et al., Carbohydr Polym. 1;277:118807, 2022.

PoB.14.01

Preparation and testing of a pH-sensitive surface based on mixed oxides of titanium and iridium for the detection of inflammation around the implant

Jitrenka Jiru, Vojtech Hybasek, Jaroslav Fojt

University of Chemistry and Technology in Prague, Prague, Czech Republic

Abstract

There is a risk of infection with all surgical procedures, which is increased if an implant is used. In particular, the detection of delayed infection is a difficult task despite current methods, as clinical manifestations must occur. The main agents of implant-associated infections include *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*. All of these microorganisms produce acidic products in their metabolic processes that affect the pH of the surrounding tissue. The possibility of sensing the pH around the implant could be a way of detecting the infection in its early stages and could lead to faster application of the treatment. In this regard, it would be best if the pH sensor was directly part of the implant or in its immediate vicinity. The most common alloys used for the manufacture of implants are titanium alloys which are characterized by a passive TiO_2 surface layer that can be modified. These oxides exhibit a near Nernstian response (0.059 V/pH). Other metal oxides, including IrO_2, can be used to enhance the response. Nanostructured Ti-6Al-4V was chosen as the base material, on the surface of which iridium was deposited by various methods. Further measurements were carried out on samples prepared by the potentiostatic method and underwent an oxidation by cyclic polarization in physiological solution. Energy dispersive spectroscopy was used to confirm the presence of iridium on the surface of the samples, and X-ray photoelectron spectroscopy was used to characterize the surface. To observe the electrochemical response to the change in pH of the physiological solution, the time course of the open circuit potential was evaluated. Reproducible results were obtained using the potentiostatic deposition method. The surface prepared in this way was usable to detect pH changes in the range of 7.6 to 6.5 after 6 weeks of stabilization. After this duration, the sample showed a sensitivity of 69 mV/pH. Samples that underwent oxidation after potentiostatic preparation could be used much earlier, after only 9 days. During the 2 months of measuring pH changes, the prepared surface maintained a sensitivity of 64 mV/pH. At the same time, this surface responded to the change immediately, there was no need to wait for a potential reading.

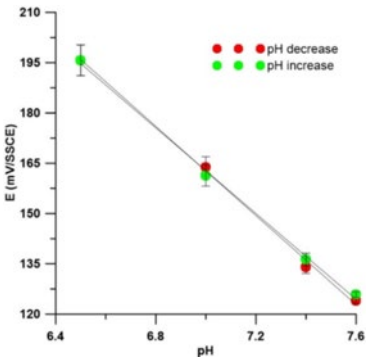


Fig.1: Results of potential change on pH change for PS + CP in physiological solution.

This research was funded by Czech health research council, grant number NU20-06-00424 and by the grant of Specific university research-grants number A1_FCHT_2023_009.

PoB.14.02

Particle load and immune response in peri-implant soft tissue over osteosynthesis plates made of CFR-PEEK and titanium - A study at the proximal humerus

Evi Fleischhacker[1], Christoph Martin Sprecher[2], Stefan Milz[3], Ronny Wirz[4], Robert Zboray[5], Annapaola Parilli[5], Maximillian Saller[1], Tobias Helfen[1], Wolfgang Böcker[1], Ben Ockert[1]

[1]Musculoskeletal University Center Munich, Munich, Germany. [2]AO Research Institute Davos, Davos, Switzerland. [3]University of Munich, Munich, Germany. [4]RMS Foundation, Bettlach, Switzerland. [5]EMPA, Dübendorf, Switzerland

Abstract

Background: Posttraumatic frozen shoulder remains a relevant complication after locking plate osteosynthesis of proximal humerus fractures, however, its pathogenesis remains unclear. It has not yet been identified to what extent capsulitis or an extracapsular inflammatory reaction are responsible for this complication. Materials and methods: The excised peri-implant soft tissue of 16 patients after locked plating of proximal humerus fractures (n=8 titanium, Philos™, DePuy Synthes; n=8 carbon fiber reinforced polyetheretherketone (CFR-PEEK), PEEKPower™, humeral fracture plate, Arthrex) with persistent limited range of motion were examined. The implants with the overlying soft tissue were removed 13.7 ± 5.8 months after implantation. Subsequently, examination for entrapped foreign bodies was performed by high-resolution CT. Characterization of the tissue reaction was subsequently performed by standard histological examination and immunohistochemical labelling with antibodies against CD68, CD163, mature tissue macrophages (25F9), and calprotectin. Results: In the soft tissues over both implant materials, high-resolution CT revealed entrapped foreign bodies with different configurations and sizes. Histological examination with transmitted light microscopy was able to detect and further specify the nature of these foreign bodies. Over plates of CFR-PEEK, roundish objects up to 5 µm in diameter and elongated fibers or wires typically 3 µm in diameter were found most frequently. In comparison, dark appearing particles up to a few micrometers in diameter were identified in soft tissue close to titanium plates. When evaluating the CT-images, it can be seen that the particle volume, the surface area and the mean ferret diameter are larger in the CFR-PEEK group than in the titanium group. However, the particle volume/total soft tissue volume ratio is smaller in the CFR-PEEK group. Semiquantitative analysis of immunohistochemical slides showed clear evidence of CD68 positive macrophages in tissues from both types of implants. Labelling for CD163 and calprotectin (both inflammation related markers) showed a more distinct reaction in soft tissue over CFR-PEEK plates compared to the titanium group, as did staining for mature tissue resident macrophages (25F9). Conclusion: Morphologically, two types of particles are found in the peri-implant soft tissue over CFR-PEEK plates: elongated fibers or wires and smaller roundish foreign bodies, whereas particles up to several micrometers in diameter were identified in the soft tissue over titanium plates. Immunohistochemical examination shows a slightly more pronounced biological reaction to the particles over CFR-PEEK plates compared to those over titanium plates.

PoB.14.03

Effects of stress on corrosion of magnesium

Takamasa Shimogami, Noriyuki Takano

Kanazawa Institute of Technology, Hakusan, Ishikawa, Japan

Abstract

1. Introduction Magnesium is currently attracting attention as a new biomaterial because it has sufficient strength and is safe when decomposed in the body. However, its high activity and low corrosion resistance cause it to decompose before it can fulfill its role. For practical use, it is necessary to control the degradation system. Compressive plastic deformation has been reported as one of the methods to control corrosion1), but there are also reports of accelerated corrosion2). Therefore, we focused on the amount of dissolved oxygen in the test solution, which had not been considered. Polarization tests and immersion tests were conducted under uniform amounts of dissolved oxygen. 2.Experimental procedure A magnesium square (99.0% purity) was used as the specimen. The specimens were ground to #2000 abrasive paper after cutting into approximately 6×6×36 mm, buffed with a mixture of 0.05 µm grain size alumina abrasive and ethanol, ultrasonically cleaned in acetone for 10 minutes, and annealed in a vacuum heat treatment furnace at 623 K for 1 hour to remove stress. Some specimens were plastically bended to stresses at 100 MPa and 150 MPa in 4-point bending. The polarization and immersion tests for the compressed side of the specimens were in 0.9% NaCl solution at 310 K. The amount of dissolved oxygen in the solution prepared ranges from 9.50 to 10.0 mg/L. The corrosion potential was measured by sweeping the potential from -2.0 V to -1.2 V at a rate of 1 mV/s using the potentiostat. A saturated calomel electrode was used as the reference electrode, and platinum as the counter electrode. The corrosion rate was estimated from the amount of the evolved hydrogen gas in the immersion test for 72 hours. 3. Results and discussion In the polarization test, the higher the stress-loaded specimen, the lower the corrosion potential, as shown in Fig.1. In the immersion test, however, there was little dependence of the corrosion rate on loaded stress. However, the initial gas generation was greater for the stressed specimens.

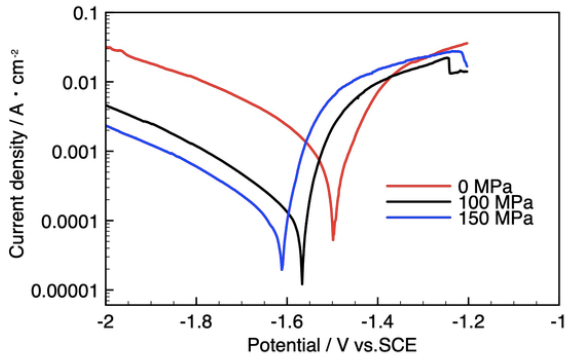


Fig.1 Polarization test results

References
1)T. Kawai and N. Takano, Key Eng. Mater. **713** (2016) 284-287.
2)Y. Zheng and Y. Li, Corrosion Sci. **90** (2015) 445-450.

PoB.14.04

Enhanced corrosion resistance of the AZ31 magnesium alloy with electrochemical oxidised (ECO) ceramic coatings

Berzah Yavuzyegit[1], Katerina Karali[1], Arianna DeMori[1], Roxane Bonithon[1], Nigel Smith[2], Pavel Shashkov[2], Gordon Blunn[1]

[1]School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, United Kingdom. [2]BioCera Medical Limited, Suffolk, United Kingdom

Abstract

Magnesium alloys are candidates for biomaterial implants such as bone plates, intramedullary rods, bone screws, and stents due to their strength and biocompatibility. The resorption of these alloys is beneficial as secondary surgeries to remove metal work may not be required. Corrosion can be uncontrolled leading to a premature decrease in mechanical properties and integrity. One way to optimise their corrosion rate is to coat the alloy. In this study, AZ31 Mg samples were coated by electrochemical oxidation producing ceramic surfaces composed of 1) Phosphate, 2) Phosphate and Silica, 3) Phosphate and Fluoride, 4) Phosphate, Silica, and Fluorine, and 5) a lower Phosphate coating (Type 1-5, respectively) at thicknesses of 5, 10, and 15 µm. To discriminate the effects of these different coatings on corrosion, a 5 M NaCl solution was used. Coated and uncoated AZ31 Mg discs were immersed in the solution for 14 days at 37℃. The corrosion rate of the specimens was calculated from weight loss measurements. The surface corrosion and deposition of corrosion products were investigated by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). In order to investigate coating durability and their ability to protect the underlying magnesium alloy cross-sections through the coating and the alloy before and after corrosion were carried out. Average porosity size of the coating was in the range of 0.5 to 2.5 µm. As the coating thickness increased, the porosity ratio also increased to between 9-16%. It was observed that the corrosion rate decreases with increasing coating thickness. Type 3 and 4, with 15 µm coating thickness, showed very low corrosion rate of 0.2 and 0.3 mm/year compared to the corrosion rate of the uncoated AZ31 Mg sample, which was 7.8 mm/year. SEM and EDX illustrated that corrosion products of the AZ31 were formed by Mg and O, the amount of Oxygen was higher on the surface of the corrosion layer. Type 3 and Type 4 have a fluorine-rich 1 µm thick barrier layer, which prevents corrosion progression (Figure 1). In terms of both mechanical and biocorrosion properties, coating the AZ31 samples with Type 3 or 4 has potential to control the corrosion rate enabling this alloy to be used when control dissolution is required.

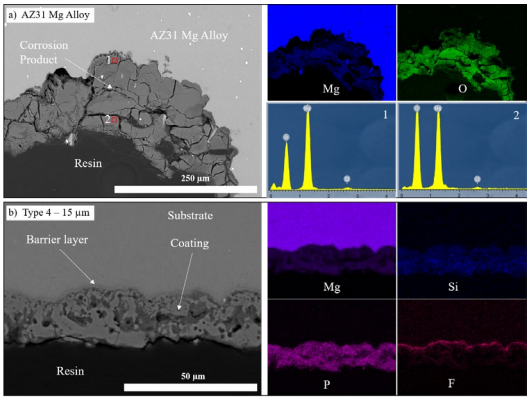


Figure 1. The cross sections of the corroded (a) AZ31 Mg alloy and (b) Type 4 in 5 M NaCl after 14 days and the corresponding elemental and point analysis.

PoB.14.05

Design of high throughput techniques for functional additively manufactured medical devices

Victor M. Villapun, Luke N. Carter, Daisy Rabbitt, Xue Cao, Billy Plant, Sophie C. Cox

University of birmingham, Birmingham, United Kingdom

Abstract

Since the early designs of medical devices, it has become apparent that implants should not simply be bioinert but act as modulators of specific biological processes to ensure short- and long-term benefits[1]. Nevertheless, standard alloys used in orthopaedics have been repurposed from other industries (e.g. aerospace) as a consequence of their mechanical behaviour, corrosion resistance and biocompatibility. With increased life expectancy requiring longer service life of implantable devices and common alloying elements (e.g. aluminium or vanadium) demonstrated to negatively impact biological processes beyond cytotoxicity, it is clear that novel medical alloys should be developed to modulate clinical outcomes[2]. In this work, the difficulties of designing alloys for implantable devices will be contextualised, providing case studies focused on generating high throughput methods for their use in alloy development for biological performance. When metallic elements are considered, there exist a plethora of materials with reported effects on biocompatibility, antimicrobial, angiogenic, osteogenic properties and/or their ability to modulate the innate and adaptive immune response[3]. The first case study will showcase the development of high throughput techniques for material processing and biological evaluation. The use of additive manufacturing and novel Reduce Build Volume designs for Powder Bed fusion coupled with powder blending will be shown as a tool to enable the rapid evaluation of alloy systems for antimicrobial applications. In addition, the power of powder compaction and HIP technologies will be harnessed to enable the rapid analysis of bacterial behaviour to an array of alloys and antibiotic combinations to enable novel databases in the healthcare industry. Besides combinations of different metallic elements, alloy design should consider the effect of microstructure and the manufacturing of complex alloys with significantly different processing parameters. Copper and molybdenum are two elements that have shown promise to tackle antibiotic infection, nevertheless, their disparity in reflectivity or melting point has made their incorporation in titanium alloys a challenge from a manufacturing perspective. Herein, the use of AM, powder compaction and sintering will be used to demonstrate the possibility of providing novel alloys with highly different elemental properties and their use in multicomponent alloys. Similarly, the effect of microstructural variation will be linked with antimicrobial properties and eukaryotic cell behaviour for the rapid optimisation of novel alloys.

[1]Williams, D.F. (2022) Bioactive Materials, 10, 306-322
[2]Brown, R.P. et al. (2022) Toxicology of Metals (pp. 127-136) Academic Press.
[3]Glenske, K. et al. (2018). International journal of molecular sciences, 19(3), 826.

PoB.14.06

Bi-layered coatings for the protection and functionalisation of Mg-based alloys

Jonathan M A Wilson, Colin A Scotchford, David M Grant, Matthew D Wadge

University of Nottingham, Nottingham, United Kingdom

Abstract

Introduction Magnesium (Mg) alloys attract interest due to their similar mechanical properties to bone (Mg:ρ~1.7g/cm^3, E~40-45GPa. Bone:ρ~1.2g/cm^3, E~10-40GPa [1]) and ability to be resorbed by the body. Methods are being explored to decrease corrosion rate or delay onset of degradation so continued structural support can be provided during bone healing [2]. This research investigates a novel bi-layered coating consisting of electrodeposited calcium phosphate (CaP) and radio-frequency magnetron-sputtered (RFMS) phosphate-based glass (PBG). **Materials&Methods** MgWE43 discs (10mmx1mm) were used as substrates to deposit CaP coating by wet electrochemical deposition in electrolyte solution. Applied voltage was -3V with duty cycle, deposition time and electrolyte pH parameter variation. PBG targets were prepared by melt-quench method to prevent crystallisation. Compositions were selected to investigate effects of pyro- and ortho-phosphates [3] as thin-film coatings. The target glass was remelted and annealed in a target mould at 5^{o}C above the glass transition temperature (Tg). PBG coatings were applied via RFMS at 60W, 24h with substrate holder rotation 5rpm. SEM/EDX, XRD, XPS and stylus profilometry were used to detail morphological, chemical and structural properties of the coatings. Influence of these coatings on cell behaviour was investigated via indirect cytocompatibility testing (pH corrected and non-corrected conditioned media) using a neutral red assay and MG63 osteoblast-like cells cultured in supplemented DMEM. **Results and Discussion** Varying duty cycle of CaP deposition showed more crystalline nature at lower duty cycles but more homogenous coatings at higher duty cycles [4] (Fig 1c). Increasing electrolyte pH above 7.5 reduced Ca deposition whilst maintaining similar levels of P, suggesting a significant change in surface charge inhibiting deposition despite potential for preferred HA deposition. Deposited CaP coatings have a similar cytocompatibility to tissue culture plastic (Fig 1b) when pH-corrected although the nature of CaP coatings with cavities suggest further protection is required to compliment this. SEM of PBG depositions has shown promise as a complimentary coating (Fig 1d) with compositional variations being utilised to improve cell performance with higher levels of Q^0 and Q^1 species introduced where P content is reduced and a smooth growth morphology can be achieved on top of a CaP coating [5].

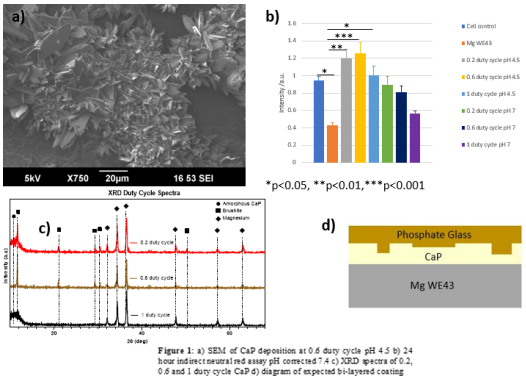


Figure 1: a) SEM of CaP deposition at 0.6 duty cycle pH 4.5 b) 24 hour indirect neutral red assay pH corrected 7.4 c) XRD spectra of 0.2, 0.6 and 1 duty cycle CaP d) diagram of expected bi-layered coating

References
[1] Li et al. Biomaterials 29:2008.
[2] Turvey et al. Int J Oral Maxillofac Surg 40:2011.
[3] De Melo et al. Int J Mol Sci 22:2021.
[4] Saremi et al. Mater Lett 129:2014.
[5] Stuart et al. Bioactive Materials 8:2022.

PoB.14.07

Biocompatibility and hemolytic activity of novel CuO dopped CeO2 nanoparticles

Anastasia Beketova[1], Ioannis Tsamesidis[1], Georgia K. Pouroutzidou[1,2], Chrysanthi Papoulia[2], Maria Bousnaki[1], Eleana Kontonasaki[1]

[1]School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece. [2]School of Physics, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

Introduction According to World Health Organization antibiotics overconsumption and spread of drug-resistant microorganisms has become a global challenge[1]. Therefore, synthesis of novel compositions of antibacterial nanoparticles (NP) as alternative to conventional antimicrobials, that would be non-toxic to human tissues and blood cells is of paramount importance. The aim of this study was to synthesize novel CuO dopped CeO_2 nanoparticles with different CuO content and to evaluate their biocompatibility and hemolytic activity. Experimental methods. To synthesize CuO/CeO_2 composite NPs with different CuO contents (15 and 25 mol%), CeO_2 and CuO NPs, the surfactant-assisted co-precipitation method was used[2]. Copper and cerium nitrate and CTAB were dissolved in water at pH=10 and subsequently sintered at 400°C for 5h. Phase composition was evaluated by XRD, while morphology and size by SEM. For hemolysis testing, nanoparticles suspension was added to 5% diluted red blood cells (RBCs) at different concentrations (0.125-1μg/ml) and evaluated spectrophotometrically at 1 and 24h[3]. For MTT assay, the viability of 24h preconditioned NPs was assessed spectrophotometrically after 1, 3, and 5 days with human periodontal ligament cells (hPDLCs). **Results and discussion** XRD analysis of the CeO_2CuO15 specimen depicts mainly the presence of CeO_2 without noticeable characteristic peaks of CuO, probably indicating copper incorporation into the CeO_2 lattice. In specimen CeO_2CuO25 additional XRD peaks attributed to crystalline CuO can be detected (Figure 1). SEM analysis (Figure 2) revealed spherical morphology and nano-dimensions (<100nm) for the observed materials. *Figure 1. XRD analysis of the obtained powders*

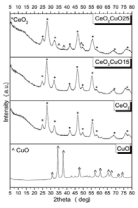
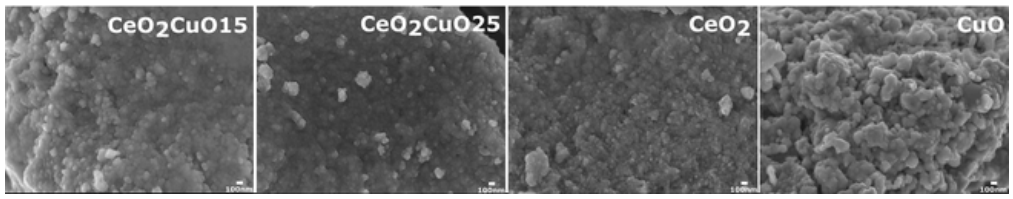


Figure 2. SEM micrographs, magnification x30.000 (bar 100nm)



Larger aggregation was observed for CuO NPs. All NPs were biocompatible except for CuO. The CeO_2CuO15 and CeO_2CuO25 materials were biocompatible and presented no hemolytic activity at concentrations up to 0.5mg/ml. In contrast, CuO nanoparticles were toxic both to RBCs and hPDLCs. CeO_2 NPs presented the best biocompatibility and hemocompatibility. **Conclusions** CuO dopped CeO_2 nanoparticles have good biological properties. Further antibacterial studies should assess their antimicrobial potential.

Acknowledgements

The authors want to thank Prof. G. Vourlias and K. Chrissafis for providing the SEM microscope of the Laboratory of Advanced Materials and Devices. The research has been financed by the Research Committee of the Aristotle University of Thessaloniki, Greece, "Excellence Scholarships in Health Sciences, Physical Education and Sports-2022"(N:50179/630962/β6.γ)

PoB.14.09

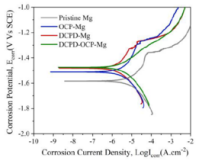
Development of calcium phosphate coated biodegradable magnesium with enhanced corrosion resistance for orthopaedic applications

Tins Sadat Hashemi[1], Mert Celikin[2], Helen O. McCarthy[3], Tanya Levingstone[1], Nicholas Dunne[1]

[1]Centre for Medical Engineering Research, School of Mechanical and Manufacturing Engineering, Dublin City University, Dublin, Ireland. [2]School of Mechanical and Materials Engineering, University College Dublin, Dublin, Ireland. [3]School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom

Abstract

Magnesium (Mg) and its alloys have been identified as a new generation of biodegradable metallic implants, attracting much attention from researchers and clinicians due to their favourable biocompatibility, biodegradability, and osteoconductivity. However, rapid corrosion of Mg alloys leads to loss of mechanical integrity prior to bone healing limiting clinical applications. Surface modification through the application of bioactive coatings has been proposed as an efficient and cost-effective route to reducing the corrosion, and hence the degradation rate, of Mg-based medical implants. In this study, the influence of coating Mg with dicalcium phosphate dehydrate (DCPD), octacalcium phosphate (OCP), and DCPD-OCP coatings on the corrosion resistance of Mg was explored. As-cast pure Mg, i.e. 99.6% purity, was used as the substrate material. Specimens were surface treated, cleaned with ethanol, and dried in air at 25°C for 1 h. The prepared Mg specimens were then immersed in the CaP electrolyte for 24 h to deposit the DCPD and OCP coatings on the substrates at 68ºC with a pH values of 4 and 5, respectively. Microstructural characterisation of CaP coatings indicated the deposition of a dense homogenous coating consisting of well-formed crystals of DCPD and OCP on the substrates. The degradation behaviour of the CaP-coated Mg specimens was evaluated using electrochemical techniques, gravimetric analysis, and quantification of hydrogen evolution. Potentiodynamic polarization (PDP) tests demonstrated an increase in the corrosion potential (Ecorr) of CaP-coated Mg substrates, particularly those coated with DCPD-OCP coatings, compared to uncoated Mg (Ecorr of DCPD-OCP = −1.41 V compared to −1.59 V for pristine Mg). Similarly, the corrosion current density (Icorr) decreased to 2.9 × 10−6 A.cm-2 for DCPD-OCP coated Mg substrate (Figure 1). Electrochemical impedance spectroscopy (EIS) was used to evaluate the anti-corrosion performance of the CaP coatings under accelerated conditions. Results showed that the CaP-coated Mg achieved increased charge transfer resistance compared to pristine Mg, as indicated by the increasing semi-circle diameter (Figure 2). The most significant improvement in charge transfer resistance was observed for DCPD-OCP coated Mg. The PDP measurements indicated that the DCPD-OCP coating reduced the corrosion rate of pristine Mg by eight-fold. The corrosion rate calculated by gravimetric analysis and hydrogen evolution also showed that DCPD-OCP-coated Mg specimens exhibited the lowest tendency towards corrosion. Taken together the results demonstrate that the proposed CaP coating technology could provide many opportunities for the more widespread use of Mg-based medical implants for orthopaedic applications.



(a)

Specimen Type	Corrosion Potential, E_{corr} (V vs SCE)	Corrosion Current Density, I_{corr} (A·cm^{-2})	Corrosion Rate (mm/annum)
Pristine Mg	-1.59±0.003	1.05±0.98 × 10^{-5}	0.578±0.1
OCP-Mg	-1.50±0.05	5.10±0.87 ×10^{-6}	0.155±0.07
DCPD-Mg	-1.45±0.01	3.63±0.64 × 10^{-6}	0.068±0.06
DCPD-OCP-Mg	-1.41±0.02	2.96±0.38 ×10^{-6}	0.060±0.009

(b)

Fig. 1 (a) Typical potentiodynamic polarization curves for pristine Mg, OCP-Mg, DCPD-Mg and DCPD-OCP-Mg specimens when immersed in Hank's solution; and the (b) the relative electrochemical corrosion parameters.

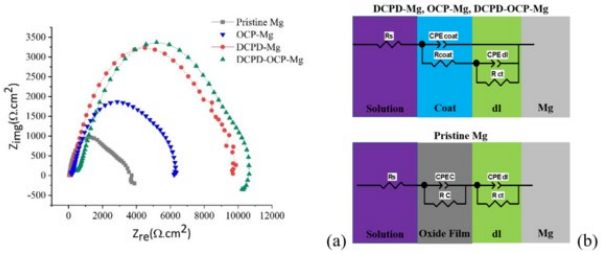


Fig. 2 (a) Typical electrochemical impedance spectroscopy for pristine Mg, OCP-Mg, DCPD-Mg and DCPD-OCP-Mg specimens when immersed in Hank's solution; and the (b) relevant equivalent circuit models.

PoB.14.10

A novel, simple approach to develop hyaluronan-based wound dressings with maximized bioactivity.

Elisabetta Cassese[1], Emiliano Bedini[2], Antonella D'Agostino[1], Marcella Cammarota[1], Livio Luongo[1], Chiara Schiraldi[1], Annalisa La Gatta[1]

[1]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy. [2]University of Naples Federico II, Naples, Italy

Abstract

Biopolymer-based wound-dressings(WDs) are the most promising approach for assisting tissue lesion healing, and hyaluronan(HA) is among the most suitable biopolymers. Although a complete understanding of HA biochemical signalling in relation to its chain length is still to be achieved, the importance of using HA of diverse size to better assist wound repair is well established. On these grounds, highly efficient HA-based WDs should guarantee a long-term release of naturally occurring HA with specific MWs. Self-Esterified-HA derivatives, while hydrolyzing under physiological conditions, progressively release no other molecules than HA, thus representing the safest approach to achieve sustained release of the naturally occurring polymer. However, current protocols for their production are elaborate and hamper control of the MW of the released biopolymer. Other proposed procedures, using 1-ethyl-3-(3-diethylaminopropyl)carbodiimide(EDC), are limited by formation of acyl-urea by-products. Here the EDC-hydroxybenzotriazole(HOBt) carboxyl-activating-system is evaluated for self-esterifying HA in the solid state, expecting to overcome the abovementioned limitations. The system, widely used for the coupling of amines to HA, was never reported for HA-self-esterification. HA(powder/sponge/film) was reacted with increasing EDC/HOBt amounts and the products characterized *in vitro* to assess expected innovation. Results indicated that the set procedure outperforms the conventional/already proposed ones as it is more efficient, avoids side-reactions, allows for an easier processing to diverse clinically-usable 3D-forms, leads to products gradually releasing HA with the avantgarde possibility to tune its MW. The self-esterified-HAs exhibit sound stability to enzymatically-catalyzed-hydrolysis, hydration/mechanical properties suitable for WDs, with improvements over available matrices, and prompt *in vitro* wound-regeneration, comparably to linear-HA. Self-esterified-HA-based devices expected to contemporarily release multiple HA-MWs have been also developed to optimize biochemical stimuli in wound healing. To that aim, two/more HA samples differing for chain length were considered for the reaction. An *in vitro* experimentation is being carried out to demonstrate the release of multiple adjustable MW-distributions as well as matrices potential in skin regeneration (wound-healing assay; tissue specific biomarkers expression). Finally, the approach is being explored for self-esterifying HA while conjugating other low-molecular-weight molecules with known bioactivity in respect to the control of the infection and/or inflammation and/or pain associated to the wound. The experimentation is providing promising data towards the development of HA-based devices meeting all the requirements for rapid and proper healing, combining high safety, optimal biochemical stimuli, anti-microbial, anti-inflammatory and pain-control activity, and availability in diverse clinically-usable 3D-forms.

PoB.14.12

Novel chitosan dermal filler with enhanced moldability and elasticity

Ji-Ung Park[1], Jie Young Kim[1], Su Hee Kim[2], Misun Cha[2]

[1]Department of Plastic and Reconstructive Surgery, Seoul National University College of Medicine, Seoul National University Boramae Medical Center, Seoul, Korea, Republic of. [2]R&D Center, Medifab Co. Ltd, Seoul, Korea, Republic of

Abstract

Dermal fillers are injectable biomaterials that are used for tissue recovery and supplementation. Currently, dermal fillers are largely based on commercialized cross-linked hyaluronic acid (HA) injections, which require a large injection force. Additionally, HA can be easily decomposed by enzymes, and HA-treated tissues present a risk of developing granuloma. In this study, we present a chitosan-based dermal filler consisted of two fomulations: Chitosan dissolved in HCl with sodium phosphate dibasic and anhydrous glycerol. This filler operates on a liquid-to-gel transition and allows the injection force to be kept ~4.7 times lower than that required for HA injections. Evaluation of the physical properties of the chitosan filler indicates high viscoelasticity and recovery rate after gelation at 37 °C. Furthermore, in an in-vivo evaluation, the liquid injection-type chitosan filler transitions to a gel state within 5 min after injection into the body, and exhibits a compressive strength that is ~2.4 times higher than that of cross-linked HA. The filler also exhibits higher moldability and maintains a constant volume in the skin of mouse photoaging model for a longer time than the commercial HA filler, Restylane. Therefore, a novel chitosan filler based on liquid-to-gel transition was prepared, which exhibited superior moldability and elasticity because of its thermosensitivity. The filler could maintain a constant volume in the skin for a longer compared to commercial HA filler.

PoB.15.01

Advanced brain-on-a-chip platforms fabricated by two-photon lithography for drug screening applications: investigations of drug delivery and target selectivity

Attilio Marino, Matteo Battaglini, Omar Tricinci, Alessio Carmignani, Gianni Ciofani

Smart Bio-Interfaces; Istituto Italiano di Tecnologia, Pontedera, Italy

Abstract

Most newly synthesized central nervous system (CNS) drugs fail pre-clinical *in vivo* tests due to their scarce ability to cross the blood-brain barrier (BBB) and reach the CNS. Therefore, intensive research activity has been focused on the realization of *in vitro* platforms reproducing the BBB. To date, commercially available 2D static BBB models are easy-to-use systems that show scarce predictivity: results obtained with these models are difficult to be translated *in vivo*. Moreover, the microfluidic BBB patented or commercially available lack the dimensional scale of *in vivo* brain capillaries due to technological limits. The development of more realistic models of the BBB mimicking as most accurately as possible the brain microcapillaries became crucial in this field. Also, ethical benefits in terms of the reduction of sacrificed animals are expected by using more faithful *in vitro* BBB platforms. In this scenario, our group realized the first real-scale microfluidic BBB model with microtubes inspired by the brain capillaries (Figure 1a,b). The architecture of the device was realized by using the two-photon polymerization (2pp) approach, an innovative real-3D microfabrication technique with unprecedented resolution. The device is interfaced with a microfluidic system imposing a liquid flow in the microtubes with a 1 mm/s speed, the same measured in the brain microcapillaries. Moreover, the system enables triple cell co-cultures, is reusable (3x), and optically transparent. As proof-of-concept, we measured the crossing of the nutlin-3a anticancer drug through the realistic BBB model by high-performance liquid chromatography (Figure 1c). Also, the nutlin-3a anticancer effects and selectivity were tested. To this aim, 3D multicellular organoids with malignant (human GFP-U87 glioblastoma cells) and non-malignant (human neural stem cells-derived neurons and the hCMEC/D3 human brain endothelial cells) cells have been self-assembled and incorporated into our device as described in our patent [1]. The findings showed a significantly higher cell death of malignant compared to non-malignant cells, demonstrating the nutlin-3a selectivity (Figure 1d). Moreover, our brain-on-a-chip device showed high multifunctionality and translational potential, enabling investigations of brain delivery, drug functionality, and selectivity. Our group is currently involved in the NABIS startup project for the lab-to-market transfer of this technology.

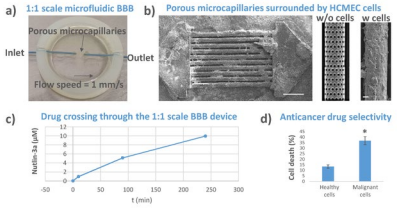


Figure 1. a,b) 1:1 scale microfluidic BBB c) Nutlin-3a crossing. d) Selective anticancer effect after BBB crossing. Scale bars: 250 and 10 μm.

ACKNOWLEDGMENTS

This work was supported by the European Research Council (ERC-2022-MagDock; 101081539).

REFERENCES

[1] Marino A. *et al.* "System and method of cell co-culture"; Patent IT201900018614A1, 20/09/2021.

PoB.15.03

Application of 3D-printed β-TCP scaffolds in skeletal diseases modelling

Ksenia Menshikh[1], Virginia Alessandra Gobbo[2], Jonathan Massera[2], Andrea Cochis[1], Lia Rimondini[1]

[1]Università del Piemonte Orientale, Novara, Italy. [2]Tampere University, Tampere, Finland

Abstract

The field of *in vitro* modelling of skeletal diseases is undergoing rapid development thanks to the elaboration of biomaterials and dynamic culture systems. Still, the reconstruction of complex bone tissue microenvironment, which aims at setting cells into conditions closely mimicking the pathophysiological state, remains a great challenge. The present study proposes an approach to modelling bone tissue pathologies, in this case osteosarcoma (OS), *in vitro* by utilizing a flow-perfused cell-seeded synthetic scaffold. The above-proposed approach was based on using three types of human cells (osteosarcoma U2OS, endothelial EA.hy926, and human bone-marrow derived stem cells MSCs Y201), 3D-printed β-tricalcium phosphate (β-TCP) scaffolds and perfusion bioreactors. The scaffolds were produced by mixing β-TCP powder with Pluronic® 30% wt/wt, 3D-printing the mix in a form of a cylinder with a grid-like structure, and subsequent sintering (1 hour at 500 °C, followed by 3 hours at 1150 °C). OS cell spheroids were obtained by culturing cells on the agarose-coated plates. The spheroids or single cell-suspension of endothelial cells or MSCs were seeded in collagen onto the scaffolds and cultured up to 7 days under static and perfusion conditions. Fluorescent microscopy studies (Fig. 1) and metabolic activity assay revealed sufficient viability of the cells and cell spheroids, as well as their plausible morphology and arrangement on the scaffolds' structures. In comparison to cell-seeded scaffolds cultivated in the absence of flow perfusion, there was a slight loss in cell number within the scaffolds in bioreactors. A comparative analysis of the scaffolds' supremacy in static and dynamic conditions pointed to their good stability independent of the presence of constant fluid flow.

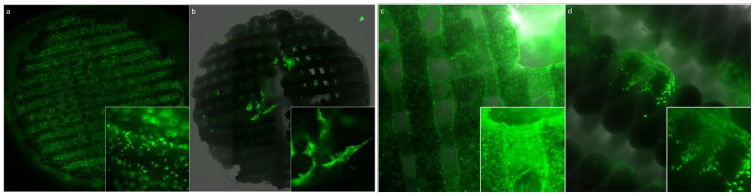


Fig. 1: MSCs (a), OS cell spheroids (b) and endothelial cells (c) after 1 week of cultivation on the β-TCP scaffolds in static conditions, endothelial cells after 1 week in perfusion bioreactor (d). Calcein AM staining.

The proposed application of 3D-printed β-TCP scaffolds for modelling skeletal diseases *in vitro* on the example of OS may be considered promising demonstrating the scaffolds' cytocompatibility and mechanical stability, as well as favourable conditions for reconstructing the microenvironment of bone tissue. Yet, it needs further characterization with the onset of co-culture and prolonged cultivation periods.

This research was funded by the European Union's Horizon 2020 Research and Innovation Programme (GA 860462). We would also like to thank Prof. B. Obradović for kindly providing us perfusion bioreactors "3D Perfuse" (Innovation Center of Faculty of Technology and Metallurgy, University of Belgrade).

PoB.15.04

Peptide-modified biomaterial interfaces to attract and instruct bone forming cells in a non-union bone healing on-a-chip model

Gulistan Kocer[1], Francisco Conceição[2], Dorothee Wasserberg[1], Nathalie Bravenboer[3], Jenneke Klein-Nulend[4], Peter Nolte[4,5], Liliana Moreira Teixeira[2,6], Pascal Jonkheijm[1,6]

[1]Laboratory of Biointerface Chemistry, Department of Molecules and Materials, University of Twente, Enschede, Netherlands. [2]Department of Advanced Organ Bioengineering and Therapeutics, University of Twente, Enschede, Netherlands. [3]Department of Clinical Chemistry, Amsterdam Movement Sciences, Amsterdam University Medical Center, Amsterdam, Netherlands. [4]Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam Movement Sciences, University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, Netherlands. [5]Department of Orthopedics, Spaarne Gasthuis Hoofddorp, Hoofddorp, Netherlands. [6]Organ-on-Chip Center Twente, University of Twente, Enschede, Netherlands

Abstract

Non-union bone fractures present a critical medical condition and are defined as a defect (gap) that shows no healing without further intervention, after a prolonged period (> 9 months). It is highly interesting to develop regenerative strategies and discover cell-instructive factors that can improve the healing potential of non-union fractures. This requires tightly controlled biomimetic *in vitro* models, which allow incorporation of native-tissue/cells, instructive biomaterials, biochemical (e.g., growth factors, cell-adhesive sequences) and physical cues in 3D. Importantly, in such models, incorporation of bone morphogenetic protein 2 (BMP-2) in biomaterial systems is an appealing bone-inducing strategy, but can be hampered due to the lack of specific control over its delivery to cells. Microfluidic platforms (on-chip models) provide very suitable near-physiological microenvironments with high surface to volume ratio, high spatiotemporal control over chemical and physical cues, presence of shear stress and ability to introduce mechanical inputs. In this work, we designed and fabricated an on-chip model that consists of two human-derived bone fragments interspaced by a fibrin hydrogel (bone/hydrogel: bottom compartment), which are separated from the perfusion channel (top compartment) by a biocompatible polyethylene terephthalate (PET) based woven-mesh. The woven-mesh is designed to attract (and instruct) bone cells for gap closure as well as act as a sealing for cells, while allowing media flow to the bottom compartment. In order to render the woven-mesh biomaterial cell-attractive/instructive, we use a chemically defined strategy that we developed for controlled presentation of BMP-2 on the surface of polymeric biomaterials. To this end, peptides that specifically bind to BMP-2 are synthesized and covalently attached to the surface of woven-mesh biomaterial. Peptide modification is characterized by X-ray photoelectron spectroscopy and by immunochemical analysis of BMP-2 binding on the surface. Peptide bioactivity/BMP-2 immobilization on the biomaterial, before on-chip assembly, is characterized by the activation of differentiation in pre-osteogenic cells. The on-chip model is fabricated from cast polydimethylsiloxane (PDMS) using 3D-printed molds, and BMP-2 bound woven-mesh is introduced in the microfluidic assembly via mechanical stabilization. The migration of cells from bone fragments across the hydrogel filled gap and their ability to re-connect the two fragments are assessed via immunocytochemistry and 3D confocal microscopy, while varying the gap size (hydrogel width). Further modification to the woven-mesh is possible by introduction of cell-adhesive peptides that are known to enhance bone-differentiation. This model could reveal defined biochemical and biophysical microenvironmental factors, to design enhanced regenerative strategies for non-union bone healing.

PoB.15.07

Fabrication and advanced characterization of a tumor on a chip platform for cancer therapy

Ionut-Cristian Radu[1], Catalin Zaharia[1], Madalina Necolau[1], Eugenia Tanasa[2], Bianca Galateanu[3]

[1]University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, Romania. [2]University Politehnica of Bucharest, Bucharest, Romania. [3]Department of Biochemistry and Molecular Biology, University of Bucharest, Bucharest, Romania

Abstract

INTRODUCTION Organ on chips (OOCs) are microfluidic devices obtained by various fabrication techniques having perfused chambers of living cells to recapitulate o the organ physiology. Microfluidics is an important field that manipulates microscale fluids (10-9-10-18) through very small channels in the micron range. The combination of microfluidics with OOCs is a very challenging task involving chemistry, biology, and materials science for human physiology. Tumor or cancer on a chip models were developed for the study of tumoral microenvironment, cancer metastasis, drug testing for various types of cancers, nano drug delivery systems etc. [1]. EXPERIMENTAL METHODS We propose here the FDM fabrication and complex characterization of a microfluidic platform with the potential use in drug delivery systems for cancer therapy. The device contains cylindrical microchannels and an external shell which communicate through the microchannel walls. The microchannels are designed with CAD and CAM software and then printed with polyvinyl alcohol (PVA) filament and crosslinked with glutaraldehyde. The microfluidic properties are highlighted by microchannel walls permeability. Various drug tracer molecules are used to study the communication between the external shell and internal microchannels. The platforms were further characterized by SEM, micro-CT, and X-ray diffraction. After sterilization, the microfluidic platform is tested with specific tumor cell lines and drug-loaded polymeric nanoparticles. RESULTS AND DISCUSSION Micro-CT and SEM reveal the complex structure of the entire microfluidic device. The crosslinking ability of PVA microchannels is assessed by FTIR-ATR spectroscopy and swelling measurements. Dynamic mechanical analysis, differential scanning calorimetry and X-Ray diffraction are performed to check the influence of the thermal treatment on the crosslinking process and PVA thermal transitions and crystallinity. The thermal treatment positively influences the crosslinking process and changes the PVA characteristic temperatures. The microfluidic measurements reveal the microchannel walls permeability for several tracers in specific flowing conditions. CONCLUSION We show here the design and 3D printing fabrication of a tumor microfluidic device. The PVA microchannels have good water mechanical stability and permeability for tracers like drug molecules. This new device can be used as an organ-on-a-chip platform for testing various types of polymeric nanoparticle drug delivery formulations in cancer therapies.

REFERENCES

1. Tian, C., Zheng, S., Liu, X. et al. Tumor-on-a-chip model for advancement of anti-cancer nano drug delivery system. J Nanobiotechnol 20, 338 (2022). https://doi.org/10.1186/s12951-022-01552-0

ACKNOWLEDGMENTS

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PN-III-P4-ID-PCE-2020-1448, within PNCDI III.

PoB.15.08

From modular scaffold manufacturing to recapitulating endochondral ossification in perfusion bioreactor: A systematic approach to generate tailored bone-like constructs.

Benjamin Kruppke[1], Sandra Rother[2], Franziska Alt[1], Salman Muhammad Ilyas[1], Ricardo Bernhardt[3], Poh Soo Lee[1]

[1]Max-Bergmann Centre for Biomaterials, TU Dresden, Dresden, Germany. [2]Center for Molecular Signaling (PZMS), Saarland University School of Medicine, Homburg, Germany. [3]Leibniz-Institut für Polymerforschung Dresden e.V., Dresden, Germany

Abstract

The development of biomaterials for bone tissue engineering has expanded tremendously over the last decade. However, most current approaches are at the laboratory scale, and the end products remain less relevant to the native tissue. Some common challenges include 1) Time-consuming manufacturing processes and batch variabilities of biological-based scaffolds with tailored dimensions, 2) Inadequate cell density, 3) simplified cell culture approach, and 4) costly growth factors to specify stem cell differentiation. In this report, we developed a systematic approach to address these current challenges (Figure 1). Firstly, female silicon molds of tailored dimensions were synthesized via 3D-printing and utilized for rapid modular manufacturing of collagen-based scaffolds (Figure 1A). Secondly, spinner flasks were utilized to derive a large number (up to 20 x 10^6 cells per 250 mL flask in 12 days; from 1 x 10^6 at day 1) of undifferentiated human mesenchymal stromal cells (hMSC) shown in Figure 1B. Next, tailored scaffolds containing a cell density of 4.0 x 10^6 hMSC per cm^3 were cultured in perfusion bioreactors for up to 60 days (Figure 1C), which maintained the oxygen tension (pO_2) at 110 ± 17 mmHg over time when a perfusion rate of 5.5 mL/min was applied. Furthermore, chondroitin sulfate A (CSA) was used to support the osteogenic differentiation of hMSC (Figure 1C) at a significantly lower cost. This culture approach was formulated with an aim to stimulate the hMSC via physical cues (pO_2, interstitial shear stress) and have the cells produce the growth factors essential for osteogenesis locally. The CSA was implemented to strengthen osteogenic differentiation further. Our results showed that the modular scaffold manufacturing approach effectively generated large quantities of tailored collagen scaffolds consistently and rapidly. Furthermore, the dynamic cues (pO_2, interstitial shear stress) exerted in the perfusion bioreactors could support scaffolds containing high cell density and osteogenic differentiation over 60 days. Furthermore, CSA is a cheap and potent replacement for growth factors that can generate a diverse osteochondral cell population in the scaffold. A structurally stable and homogeneously mineralized bone construct was achieved after 60 days (Figure 1D). The approach described in this report has provided potential solutions to some of the current challenges to generate consistent tailored bone-like constructs *in vitro* in large quantities. Further exploitations with other cues (mechanical loading, electrical stimulation, pH) could be included to complement the reported approach to generate *in vitro*, bone-like constructs with greater relevance to native bone tissue.

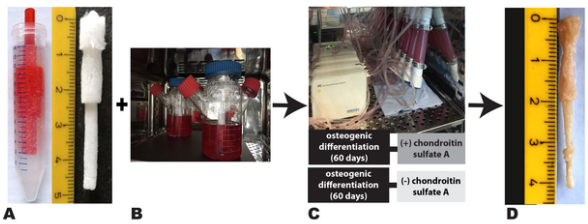


Figure 1: ***(A)*** *3D-printed male mold in silicon (left) and freeze-dried collagen scaffold with tailored dimensions (right).* ***(B)*** *Large scale hMSC expansion in 250 ML spinner flask.* ***(C)*** *The perfusion bioreactors used to implement oxygenated niche, interstitial shear stress, and the induction of osteogenic differentiation with CSA.* ***(D)*** *Mineralized end-product after 60 days.*

PoB.15.09

Enhancing the mineralization capacity of human bone progenitor cells using IL-10 and BMP-7 in a novel model of the fracture hematoma

Matthias G Wiesli[1], Stefanie Kiderlen[2], Arlyng González-Vásquez[3], Katharina Maniura[1], Markus Rottmar[1], William A Lackington[1]

[1]Biointerfaces Lab, Empa, St. Gallen, Switzerland. [2]Prospective Instruments, Dornbirn, Austria. [3]Tissue Engineering Research Group, Royal College of Surgeons in Ireland – University of Medicine and Health Sciences, Dublin, Ireland

Abstract

Introduction: In large bone defects, where classical treatments fail to achieve healing, regenerative biomaterials in combination with immunomodulatory therapies show great promise. Increasing evidence suggests that interleukin-10 (IL-10) and bone morphogenetic protein-7 (BMP-7) have potent immunomodulatory properties. However, their role during fracture hematoma formation and subsequent regulation of bone regeneration remains unknown. This is in part due to the limitations in models of fracture healing, which do not take into account the immunoregulatory role of blood and its interaction with biomaterials. The aims of this study were therefore to (i) establish a model of the fracture hematoma to investigate the interaction between blood and bone mineralization in a regenerative biomaterial, and (ii) functionalize the biomaterial with IL-10 and BMP-7 to enhance mineralization under inflammatory conditions. **Methods**: Collagen-hydroxyapatite (CHA) scaffolds were incubated with blood and the adsorption of fibrinogen and fibrin network formation was assessed using two-photon excitation fluorescence (TPEF). Bone progenitor cells (HBCs) were seeded on CHA scaffolds pre-incubated with blood and its influence on cell migration and mineralization was evaluated using TPEF, µCT reconstructions, and calcium quantification. Cytokine arrays and enzyme linked immunosorbent assays (ELISAs) were used to identify signaling molecules involved in the regulation of mineralization. The effect of blood on the release kinetics of IL-10 and BMP-7 from CHA scaffolds was determined using ELISAs, while the effect of functionalized scaffolds on HBCs was determined using mineralization assays. All experiments were repeated using at least 3 donors of blood and HBCs. **Results and discussion**: Following incubation in blood, fibrin network formation limited the capacity of HBCs to migrate into CHA scaffolds (*Fig*. 1A-B). The mineralization capacity of HBCs was significantly reduced by blood (*Fig*. 1C-D) in conjunction with an up-regulation of leptin, osteopontin, serpin E1, IL-6 and IL-8 signaling. This suggests that blood contributes to the regulation of HBCs differentiation into bone-forming cells via immunoregulatory signaling. Having successfully functionalized CHAs with IL-10 and BMP-7, it was found that blood could stimulate their release and that matrix-metalloproteases were involved in the process. The release of IL-10 and BMP-7 enhanced the mineralization capacity of HBCs, particularly in the presence of blood. Taken together, this suggests that IL-10 and BMP-7 can serve as a co-therapy to stimulate bone regeneration under inflammatory conditions. **Conclusion**: Notably, this study presents both a new model to investigate how hematoma formation can modulate mineralization and a new immunomodulatory strategy to advance bone regeneration.

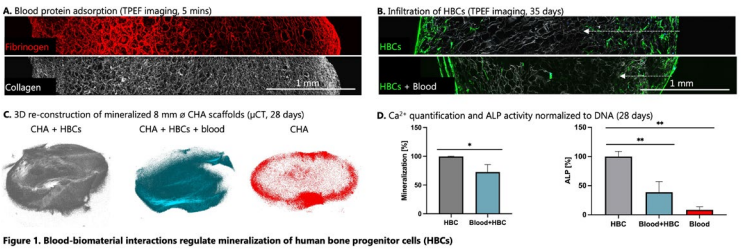


Figure 1. Blood-biomaterial interactions regulate mineralization of human bone progenitor cells (HBCs)

PoB.15.10

Comparison of colloidal probe and single-cell force spectroscopy techniques for the quantification of cell-material interactions

Ngoc Huynh[1], Roberta Teixeira Polez[1], Chris S. Pridgeon[2], Juan José Valle-Delgado[1], Riina Harjumäki[2], Monika Österberg[1]

[1]Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, Espoo, Finland. [2]Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

Abstract

Spheroids have emerged in recent decades as a more physiologically relevant 3D culture model than conventional 2D cultures. Spheroids are thought to form if adherent cells are denied an attachment surface, yet adhesive extracellular matrix (ECM) components are used extensively to aid the spheroid formation process. Previous studies have shown that spheroids exhibit different properties in terms of spheroid morphology and differentiation, when cultured in different matrix materials, but the mechanism remains unclear. We hypothesize that the balance between cell-cell and cell-material interactions is important for spheroid formation. Atomic force microscopy (AFM) is a powerful tool for quantification of both cell-material and cell-cell interactions and previous studies have used different techniques to investigate these aspects, e.g. colloidal probe (CP) and single-cell force spectroscopy (SCFS). However, the methodological differences between two techniques raise concerns about their relevance in accurately reflecting real-world cell cultures and poses challenges in comparing results across the literature and designing new experiments.In this study, CP and SCFS were directly compared as tools for the investigation of the interaction between HepG2 liver cancer cells and several substrates (cellulose nanofibrils, Matrigel® and tissue culture polystyrene). The advantages and limitations of these techniques and how well they replicate the actual events in spheroid formation are described. These data provide crucial guidance on the design of future AFM experiments to best model the interactions occurring during cell culture.

PoB.15.11

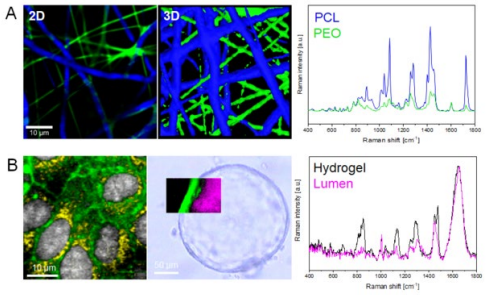
Chemical microscopy of biomaterials and their interaction with cells: A label-free and non-destructive approach

Nathalie Jung[1], Felix Rohde[1], Till Moreth[2], Ernst H. K. Stelzer[2], Francesco Pampaloni[2], Maike Windbergs[1]

[1]Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany. [2]Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany

Abstract

The understanding of the interaction of biomaterials with cells and tissues is critical for their successful implementation for drug delivery systems, cell culture scaffolds, and tissue engineering. However, the complex three-dimensional biological environment often hampers the adequate investigation of the material-cell interface and potential alterations of the material upon contact with living matter. Common analysis procedures such as fluorescence- or scanning electron microscopy are limited due to their inherent requirements for the attachment of fluorescent probes and/or fixation and destructive preparation of the sample. The invasive nature of most analysis approaches inevitably changes the microenvironment and diminishes the usefulness of the acquired data. In this context, confocal Raman microscopy presents an intriguing approach to enable label-free analysis of the chemical profile of the respective sample in a spatially resolved manner. The technique is based on the interaction of photons with molecular bonds, which provides information about the chemical composition of the biomaterial and the surrounding biomass. We implemented confocal Raman microscopy to evaluate its potential for characterising the composition and modification of different biomaterials, as well as their interaction with cells and tissues. Firstly, we investigated electrospun scaffolds with respect to the chemical identity of the different fibres and their alteration during fabrication and incubation in an aqueous environment. Raman microscopy enabled the distinction between different fibre types that were visually indistinguishable (A). Furthermore, the acquired datasets offered a spatially resolved visualisation of crosslinking and degradation events in the scaffold. In a second case study, we used Raman microscopy to investigate organoids within different synthetic or natural hydrogels. The approach allowed for the label-free differentiation between lipids, nucleic acids, and proteins on a sub-cellular level (B). By imaging the cell-material interface, we were able to obtain chemical information about the luminal composition of the organoids, e.g. the presence of cell debris and internalised components of the surrounding hydrogel. We further implemented a one-point analysis approach as a proof-of-concept study to assess the applicability of Raman microspectroscopy for higher throughput analyses of live organoid cultures. Our results demonstrate the potential of confocal Raman microscopy for analysing hydrogels and electrospun scaffolds, providing a better understanding of their properties and interaction with cells. The combination of chemical selectivity and high-resolution imaging without the requirements of additional labels holds great promise for a more comprehensive *in situ* analysis, ultimately improving our understanding of biomaterials and their interactions with cells and tissues.



PoB.15.12

Antimicrobial, antiviral, and anti-inflammatory coatings: implementation of innovative production tools using ultrasonic waves

Yijie Li[1,2], Chloé Guilbaud-Chéreau[1], Cynthia Calligaro[1], Nihal Engin Vrana[1], Philippe Lavalle[1,2]

[1]Spartha Medical, Strasbourg, France. [2]University of Strasbourg, Strasbourg, France

Abstract

Antibiotic resistance is thriving in the current society due to misuse and overuse of antibiotics, patients are suffering from superinfection in many medical treatments.1,2 The world is in need of better antimicrobial and antiviral solutions. Polyelectrolyte biomedical coating made through Layer-by-Layer (LbL) method has proved its ability of both antimicrobial and anti-inflammatory.3 The LbL coating method consists of an alternating deposition of oppositely charged supramolecular materials such as polyelectrolytes, with a buffer wash step in between. At Spartha Medical we have developed an antimicrobial multilayer coating by hyaluronic acid (HA) and poly(arginine) (PAR), which was obtained by the LbL coating process. The PAR/HA coating is biocompatible and antimicrobial and can be applied on a large panel of substrates.4,5 However, low efficiency is the biggest limitation of this coating method. To increase the coating efficiency, we recently designed an ultrasonic coating method. There is a limited number of articles that utilized ultrasonic waves as a coating method despite it being widely used. The idea of applying ultrasonic waves is to transmit ultrasonic wave energy to the drifted polyelectrolyte molecules within the solution, which affects the diffusion coefficient and thus increases the probability of the polymer attachment on the substrate within a shorter time. To test this, polyelectrolyte solutions (HA and PAR) were used to coat glass slides within an ultrasonic bath. We studied the influence of ultrasonic power (W) consumed with 15 seconds of dipping on a different number of bilayers. The antimicrobial activity was tested against *M. luteus*, and the coating was characterized by confocal microscopy. Under weaker ultrasonic power, much less antimicrobial efficacy was found under the same testing protocol. We showed that ultrasonic waves have the potential to increase LbL coating efficiency while keeping antimicrobial properties. This method could be a way to improve the coating efficiency in industrialization. However, more experiments are needed to find the best parameters and settings, such as the ultrasonic wave frequency, coating substrate position, and the concentration of polyelectrolyte solutions.

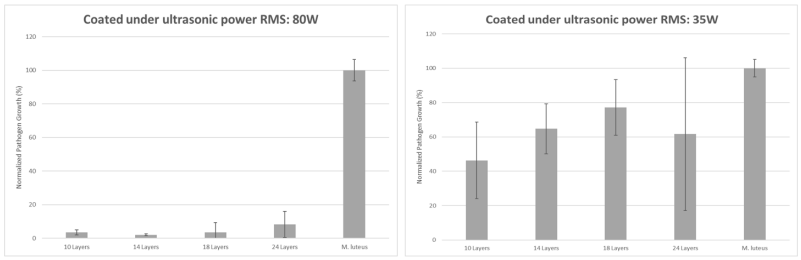


Figure 1: Antimicrobial test results of ultrasonic dip coating against M. luteus

REFERENCES:

1. Abushaheen, M. A. et al. Disease-a-Month 66, 100971 (2020).
2. Aslam, B. et al. Front. Cell. Infect. Microbiol. 11, 771510 (2021).
3. Knopf-Marques, H. et al. Materials Science and Engineering: C 104, 109898 (2019).
4. Mutschler, A. et al. Chem. Mater. 28, 8700–8709 (2016).
5. Mutschler, A. et al. Chem. Mater. 29, 3195–3201 (2017).

PoB.15.13

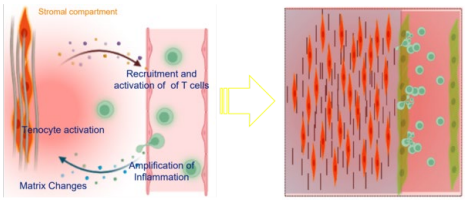
HUMAN 3D TENDON-ON-CHIP MODEL TO UNRAVEL CELLULAR CROSSTALK IN TENDINOPATHY

Syeda Mahwish Bakht[1,2], Alberto Pardo[1,2,3], Manuel Gómez-Florit[1,2], David Caballero[1,2], Subhas C. Kundu[1,2], Rui L. Reis[1,2], Rui M. A. Domingues[1,2], Manuela E. Gomes[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciencia e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco,, Guimarães,, Portugal. [2]ICVS/3B's – PT Government Associate Laboratory,, Braga/Guimarães,, Portugal. [3]Colloids and Polymers Physics Group, Particle Physics Department and Health Research Institute, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain

Abstract

Tendon injuries and diseases are often characterized by inflammation, which can exacerbate the condition and lead to chronicity. The inflammatory phase of tendinopathy is characterized by increased vascularization and influx of immune cells (mast cells, macrophages, T cells) at the healing site. To better understand the complex interplay between these various cell populations and environmental factors, it is important to develop relevant in vitro models. We hereby propose a multicellular 3D model that mimics the inflammatory hallmarks observed in tendinopathy (Figure 1). The model uses magnetically responsive microfibers (MNF@PCL) to recreate the anisotropic fibrillar structure of the tendon extracellular matrix (ECM). The MNF@PCL were produced by incorporating iron oxide nanoparticles into electrospun PCL meshes and then cryo-sectioning them into microfibers. Human tendon-derived cells (hTDCs) were encapsulated in Platelet Lysate (PL) hydrogels along with the MNF@PCL in the central channel of a three-channeled microfluidic chip. The chip was then placed under a uniform magnetic field created by two parallel magnets to align the MNF@PCL in situ. Microvascular cells were co-cultured in the side channel to recreate the open vasculature of the extrinsic tendon compartment, where T cells can be subsequently introduced to evaluate their interactions with stromal tenocytes. Analysis of the hTDCs' cytoskeleton organization within the hydrogel matrices revealed that the topographical cues created by the microfiber alignment strongly dictate the cells' aspect ratio and orientation. The synergy between the PL matrix bioactivity and magnetically aligned MNF@PCL was found to be an effective strategy for inducing cell anisotropic organization within the central compartment and maintaining a tenogenic phenotype. The microvascular cells co-cultured in the side channels organized into a compartmentalized tubular monolayer with an open lumen. The crosstalk between tendon and vascular cells on genes and proteins related to the ECM, tenogenic markers, and inflammatory signaling pathways is currently being assessed. Additionally, the effects of hTDCs on the behavior of circulating T cells (migration and activation) and the impact of these crosstalk mechanisms on the stromal compartment are being studied using this physiomimetic system. Overall, a 3D tendon-on-chip model would provide a powerful platform for studying the complex cellular and molecular interactions that contribute to the development and progression of tendinopathy. It could also serve as a valuable tool for testing new treatments and therapies for this debilitating condition.



PoB.15.14

Using electrospun scaffolds to emulate the inflammatory microenvironment in intestinal *in vitro* models

Nathalie Jung, Felix Rohde, Maike Windbergs

Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany

Abstract

In recent years, there has been a growing recognition of the critical role of the microenvironment in developing more relevant *in vitro* models of human tissues, both in physiological and pathophysiological states. Although Transwell®-based systems are the predominant choice for epithelial *in vitro* models, they poorly replicate the complex, nanofibrous structure of the native extracellular matrix (ECM). While its crucial role for cell-cell communication and tissue function is well established, the ECM and its structural changes within the context of chronic inflammation are generally not acknowledged in intestinal *in vitro* models. To address this limitation, we designed a novel electrospun culture substrate that closely mimics the nanofibrous structure of the human ECM and can be adapted to resemble the inflammation-induced stiffening of the matrix. The scaffold was designed to provide a mechanically stable but bio-mimetic substrate by incorporating the synthetic polymer polycaprolactone (PCL) and the natural polymer gelatine in a hybrid network. By adjusting the fiber diameter, scaffold porosity, and thickness, we closely matched the properties of the ECM of the intestinal submucosa. Further, we fine-tuned the stiffness of the gelatine fibers through the concentration and duration of chemical crosslinking, accounting for the changes in ECM elasticity during chronic inflammation. Our results showed that different scaffold formulations had a notable impact on the initial colonisation of the scaffold by epithelial cells, barrier formation, and cell differentiation, compared to Transwell® models. Further, the stiffness of the underlying culture substrate translated to the elastic properties of the epithelial tissue on top of the PET membranes and fiber scaffolds. The addition of a basolateral cytokine stimulus induced a higher response in scaffold-based *in vitro* models, suggesting a crucial role in the more porous scaffold structure in communication between the apical and basolateral compartments. The electrospun scaffolds presented here constitute a novel approach to mimic the delicate structure of the native basement membrane, while providing a highly adjustable scaffold for modelling a range of epithelial tissues and endothelial-epithelial interfaces. The herein reported modification of scaffold stiffness offers an elegant way of replicating the subtle changes of the human ECM and its elasticity upon inflammation and the respective pathophysiological tissue models *in vitro*. Replacing traditional PET membranes with more physiological scaffolds has the potential to advance our understanding of tissue development, disease progression, and the design of innovative therapies for various pathological conditions, including inflammatory bowel disease (IBD).

PoB.15.15

High efficiency functional elucidation of human bone marrow stromal cells *in vivo*

Matteo Monticelli[1], Bianca Maria Carrara[1], Katharina Gegenschatz-Schmid[1], Queralt Vallmajó Martín[2], Martin Ehrbar[1]

[1]Laboratory for Cell and Tissue Engineering, Department of Obstetrics, University and University Hospital, Zurich, Switzerland. [2]Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, USA

Abstract

Introduction: A huge variety of hematopoietic and non-hematopoietic cells can be found in the human bone marrow (BM). The Bone Marrow Stromal Cell (hBMSC) compartment contains, among others, Hematopoietic Stem Cell (HSC) niches and skeletal stem cell populations. The identity and function of the hBMSC populations remain unclear and require further research, in contrast to the well-characterized HSCs. A better understanding of the BM and the present cells is required since changes in the microenvironment can cause serious diseases including leukemia. Current methods, such as single cell analysis, highlight the heterogeneity of hBMSCs and show that only a tiny subset of cells are able to differentiate into many lineages and sustain long-term self-renewal. 1 We perform cell implantations *in vivo* to conclusively validate the function of hBMSCs in the complex native environment, which will reveal their true fate. Methods: We suggest designing a multiplexed screening device for efficient *in vivo* testing to show the function and hierarchical organization of different hBMSC subpopulations. Additionally, we are creating robust microenvironmental conditions for the osteo-, chondro-, and adipogenic differentiation of hBMSCs utilizing transglutaminase crosslinked poly(ethylene glycol) (PEG) hydrogels. The implantable multiplexing device will then be filled with prospectively isolated hBMSC populations that have been placed in defined microenvironments. Finally, multiplexing devices will be inserted into subcutaneous pouches of immune-deficient mice and utilized to test the ability of putative hBMSC subpopulations to differentiate *in vivo*. Results: Initial studies aimed at reducing the quantity of hBMSCs needed and expanding the range of test settings have shown *in vivo* differentiation in an osteogenic enviroment and development of tiny bone ossicles including a hematopoietic niche within the multiplexing device. Conclusion: In this work, we create a multiplexing platform to more efficiently screen hBMSC behavior *in vivo*. We will develop designs that are optimized for multiplexed testing of low-abundant hBMSC subpopulations associated with health and disease, which requires small hydrogel volumes and few cell counts. The findings of this project will serve as a crucial reference point for the investigation of the human BM stromal hierarchy and the clarification of the functional significance of the various hBMSC subpopulations.

References:
[1] Chan, C. K. et al., Cell, 175(1), 43-56 (2018).

Keyword(s):
Bone marrow stromal cell, bone marrow niche, poly(ethylene glycol), bone organoid

PoB.15.16

Characterization of *in vivo* bone organoid formation unravels developing hematopoietic niches

Bianca Maria Carrara[1], Charles Bataclan[2], Olaia Naveiras[2], Martin Ehrbar[1]

[1]University Hospital Zurich, Zurich, Switzerland. [2]University of Lausanne, Lausanne, Switzerland

Abstract

Introduction Bone Marrow Mesenchymal Stromal Cells (BM-MSCs) have a pivotal role in supporting hematopoietic cells homeostasis in *niches*. When implanted in mice for 8 weeks, BM-MSCs-loaded PEG hydrogels remodel in bone organoids featuring morphology comparable to native bones and marrow. As we assume these organoids contain hematopoietic niches, we are further characterizing them by quantifying the onset of their hematopoietic and stromal compartments within these 8 weeks. Our goal is to track the development of bone organoids over time, creating a real-time model of developing bone and marrow. Methods Human BM-MSCs were encapsulated in 40µl 1.7% (w/v) PEG hydrogels and subcutaneously implanted in Fox1nu mice. Samples were harvested at week 1, 2, 4, 8 and processed via mechanical and collagenase-based dissociation protocols to isolate single-cell suspensions that were pooled from 4 bone organoids per time point. The single-cell suspensions were stained with a flow cytometry optimized marker panel (HLA-ABC, CD90, Ter119, CD45, Lin, Sca-1, c-ckit, CD48, CD150, CD14) to quantify the contribution of human and murine cell populations to the forming stromal and hematopoietic compartments. Results Cells were isolated from week 1, 2, 4, 8 bone organoids with 87.5% average viability. Due to increasing bone matrix deposition, the absolute number of harvested cells decreased from week 1 to 8. Total stromal cells (HLA-ABC+/-, CD90+/-, Ter119-, CD45-) and human BM-MSCs (HLA-ABC+, CD90+,Ter119-, CD45-) constituted 60% and 20% of the organoids at week 1 and 2, respectively. Murine hematopoietic cells (CD45+Ter119+) were 35% of such bone organoids, with the majority being CD14+ macrophages. At weeks 4 and 8, total stromal cells and human BM-MSCs made up 30% and 0.1% of the organoids, respectively. At these later time points, 68% of these cells were murine hematopoietic cells. Interestingly, c-kit+, Lin, Sca-1+ hematopoietic stem and progenitor cells (KLS-HSPCs) accounted for 0.7% of the ossicles, a ratio comparable to marrow from the femur (0.5%) and higher than that in peripheral blood (0.009%). Conclusion We demonstrated that viable cells can be isolated longitudinally from bone organoids. The first weeks of organoid development are dominated by stromal cell components, while murine hematopoietic cells start colonizing the organoids from week 4. This implies that the implanted BM-MSCs, together with the recruited murine cells, create niches in which HSPCs can then accommodate. We are currently attempting to detect rare HSCs-SLAM and performing longitudinal scRNA sequencing experiments in order to understand deeper the development of such bone organoid.

PoB.15.17

Development of innervated 3D cartilage-engineered micro tissues using microfluidic technologies

Emine Kahraman[1,2,3], Estrela Neto[1,2], Meriem Lamghari[1,2]

[1]Instituto de Engenharia Biomédica (INEB), Universidade do Porto, Porto, Portugal. [2]Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal. [3]Faculdade de Engenharia da Universidade do Porto (FEUP), Porto, Portugal

Abstract

Osteoarthritis (OA) is a painful musculoskeletal disorder, with a large impact on the global population. Inflammation in OA mainly results with cartilage extracellular matrix (ECM) degradation and possible growth of neuronal fibers into subchondral bone that triggers pain. Despite the advances have been made in establishing of 3D cartilage models, there is still an urgent need to develop more reliable tissue engineering solutions for cartilage. Nevertheless, neuro-immune response to developed tissue engineered cartilage under pathological conditions might trigger alterations in the sensory innervation of the joint, pointedly articular cartilage and cause chronic pain. Thus, in this work, we developed a 3D model using compartmentalized microfluidic device (CMD) that enables the study of cartilage-engineered micro tissues (CEMT) ability to promote or avoid peripheral tissue innervation under healthy and inflammatory conditions, respectively. In order to provide permeable hydrogel scaffold that can transfer necessary growth factors to the cells inside CMD, diffusion capacity of hydrogel scaffold was tested with and without use of photomask loading FTIC-Dextran in the channel. The use of photomask increased diffusion speed in the channel through spaces between micropatterns. Afterwards, CEMT were developed through the encapsulation of osteoarthritic patients derived chondrocytes in a hydrogel scaffold. The optimal conditions (ex. polymer concentration, cell densities, cell viability and chondrogenic phenotype) to generate bioengineered constructs were determined. Chondrogenic phenotype of engineered cartilage tissues was evaluated through expression of ECM proteins such as Aggrecan, Collagen-II and Sox-9 both at day 14 and day 28 time points using immunohistochemistry. To simulate CEMT under inflammatory conditions, first human primary monocytes were isolated and polarized into proinflammatory M1 macrophages. Afterward, CEMT were exposed to M1 macrophages conditioned medium. CEMT inflammatory status was confirmed by higher IL6 expression levels when compared to those produced by proinflammatory M1 macrophages (M1 conditioned medium). To address the neuronal response to “inflamed engineered cartilage microtissues”, the optimal conditions of coculture of CEMT and dissociated dorsal root ganglions (DRGs) was established in CMD. Neurotrophic versus repulsive capacities were assessed by analysing axonal growth from neuronal compartments towards “cartilage” compartment as well as nerve growth factor (NGF) expression by chondrocytes. In presence of “healthy” microtissues, axonal repulsion was observed while in crosstalk with “inflamed” ones axonal growth towards to cartilage compartment was observed evidencing neurotrophic effect.In this study, we successfully established a 3D compartmentalized microfluidic platform that can recapitulate articular cartilage and its pain-related tissue microenvironment under both healthy and inflamed conditions.

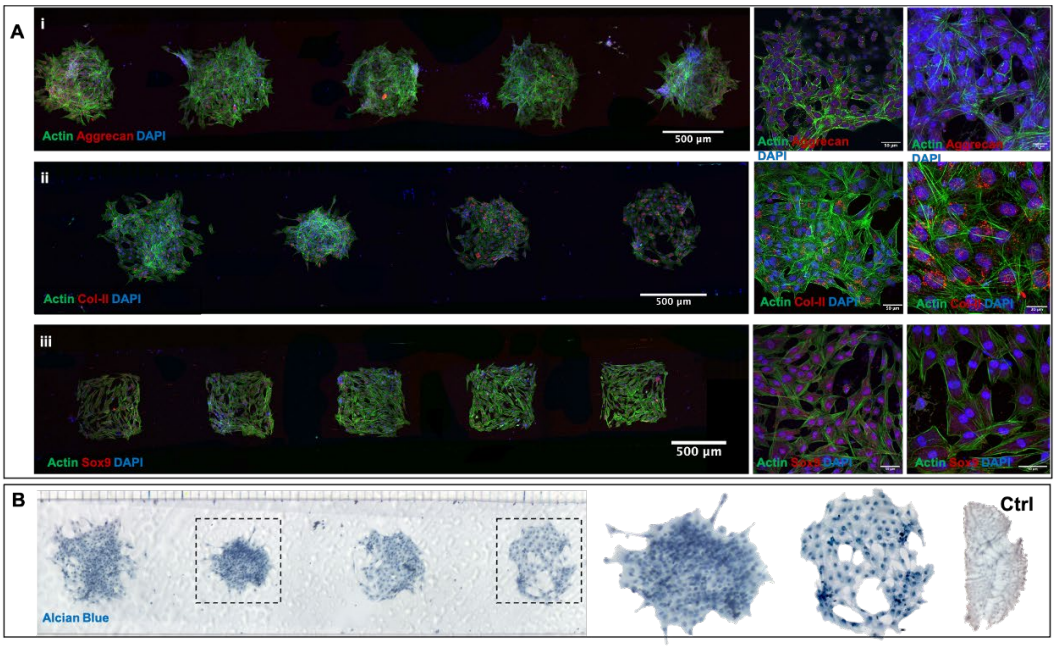


Figure1. *Chondrogenic phenotype of engineered cartilage microtissue at day 28. A. Expression of extracellular matrix proteins i. Aggrecan (red); ii. Collagen-II (red); iii. Sox-9 (red) with Actin (green) and DAPI (blue) respectively. B) Histological Alcian Blue staining for visualization of produced Glycosaminoglycan (GAG). All images were taken using confocal microscopy with 10X; 40X magnification.*

PoB.15.18

BONE-ON-A-CHIP BASED ON A 3D OSTEOCYTIC NETWORK FOR THE SCREENING OF ANABOLIC ANTI-OSTEOPOROTIC DRUG

Sofia Avnet[1], Mariaveronica Lipreri[2], Gemma Di Pompo[2], Gabriela Graziani[2], Elisa Boanini[1], Nicola Baldini[1]
[1]University of Bologna, Bologna, Italy. [2]IRCCS Rizzoli Orthopapedic Institute, Bologna, Italy

Abstract

The osteocyte is recognized as a major orchestrator of osteoblast and osteoclast activity and is the most important key player during bone remodeling processes. Decreased osteocyte viability and activity, that may be caused by hormonal perturbations or alterations in mechanical loading, can cause major imbalances in bone remodeling that, in the long run, can lead to osteoporosis. Because of the limited understanding of the mechanisms underlying this imbalance, current therapies for osteoporosis still fail to be fully effective. In fact, osteocyte studies are based on conventional cell cultures that are unable to recapitulate local microenvironments *in vivo* because of the lack of control over the spatiotemporal distribution of cells and biomolecules. Microfluidics is the science and technology of microscale fluid manipulation and sensing and can help fill this gap. We used a microfluidic device to allow osteocyte-like cells to be cultured in a 3D fashion. Osteocytes were cultured in a perfused, 160-µm-high channel and embedded in a bone-like extracellular matrix. The osteocytes were embedded in a matrigel- and collagen-based hydrogel enriched with nanostructured hydroxyapatite crystals (HA-NC) to mimic bone. To determine the best combination of matrigel enriched with type I collagen, we used fluorescent microspheres and confocal analysis. To assess the viability and expression of osteocyte markers, we used live-dead assay and immunofluorescent staining and confocal analysis combined with automated quantification.

Osteocytes in the organ-on-a-chip model showed high viability and, compared with conventional 2D cell cultures, higher differentiation, as assessed by the live-dead assay and staining of osteocyte markers connexin-43 and alkaline phosphatase. In addition, addition of HA-NP significantly increased the formation of dendrite-like structures spreading through xyz axes, as assessed after G-actin immunofluorescence. Finally, we validated our model for drug screening by evaluating osteocyte protection from apoptosis after estrogen exposure by combined treatment with teriparatide, an antiapoptotic and anabolic drug for osteogenic cells.

In conclusion, using a microfluidic device for MLO-Y4 cell cultures, compared with 2D surfaces, we demonstrated a significant difference in cell differentiation and morphology. In particular, the 3D cultures enabled the formation of 3D cell networks and osteogenic phenotype. As a platform technology, this microfluidic-based 3D in-a-chip osteocytic network can function as a model enabling further osteocyte studies and 3D co-culture with other bone cells for anti-osteoporotic drug screening.

PoB.15.19

Identification of Optimal Proteins and Biomaterial Substrates for Supporting Skeletal Muscle Satellite Cell Adhesion, Growth, and Differentiation

Aryako Rahimi[1], Laila Kudsiova[2], Matteo Santin[1]
[1]Centre for Regenerative Medicine and Devices, School of Applied Sciences, University of Brighton, Brighton, United Kingdom. [2]University of Birmingham, Dubai, UAE

Abstract

Introduction. Skeletal muscle satellite cells (SCs) play a crucial role in muscle regeneration and the extracellular matrix (ECM) provides an essential microenvironment for their function. The selection of appropriate proteins and substrates in the ECM is crucial for the successful handling of the SC during preclinical culturing [1]. In this study, the suitability of collagen type-1 (CT-1), laminin, and fibronectin as ECM proteins and of commercially-available substrates, Poly-L-Lysine (PLL) and PhenoDrive-Y, a basement membrane-mimicking biomaterial, were studied. **Methods.** Human SCs were cultured in PLL-coated T75 flasks. Four 24 well-plates were coated with solutions of 10mg/ml PLL, 0.1mg/ml PhenoDrive-Y (Tissue Click, UK), 1% (w/v) Fibronectin, 0.01% (w/v) Laminin and 0.01% (w/v) CT1. Cells were seeded at a density of $1x10^5$cells/ml for 1, 4, 7 and 14 days in a specialised medium enriched with 10% (v/v) foetal bovine serum. SCs' growth, morphology and motility were observed and analysed by visible light microscopy and related time-lapse microscopy. The expression of biomarkers including Pax7 (pluripotent cells), MyoD1 and Myf5 (cells at different stages of differentiation), Notch1 (proliferating cells) and Integrin-α7 (bioligand-specific cell receptor) was analysed by confocal microscopy of immunostained samples. **Results.** Laminin and PhenoDrive-Y showed the formation of elongated cells aligning into myotube-like structures already within 24 hours of incubation, while PLL led to fast proliferation, the cells reaching an almost confluent status within the same time period (Fig.1). In contrast, CT-1 and fibronectin exhibited relatively slower cell adhesion and proliferation into a monolayer becoming significant only at Day 4. At Day 14, higher MyoD1 and Myf5 expression was observed in aligned cells cultured on Laminin and PhenoDrive-Y (Fig.2) suggesting their differentiation into skeletal muscle cells, while PLL and all the other substrates showed no significant expression of these differentiation markers. **Conclusions**. This study highlights the importance of substrate selection in the pre-clinical handling of SC for future cell and tissue engineering-based therapeutical intervention of

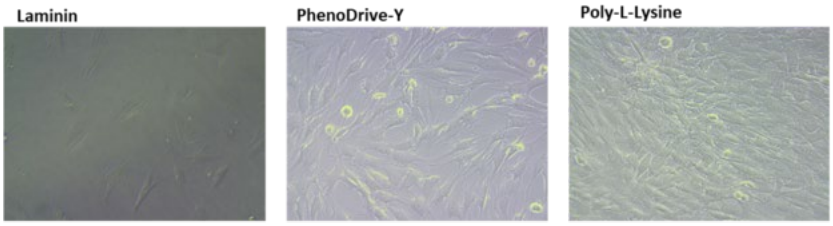


Figure 1. Adhered satellite cells on Poly-L-Lysine, PhenoDrive-Y, and Laminin with serum, on Day1. Visible light microscopy was used to observe the cells.

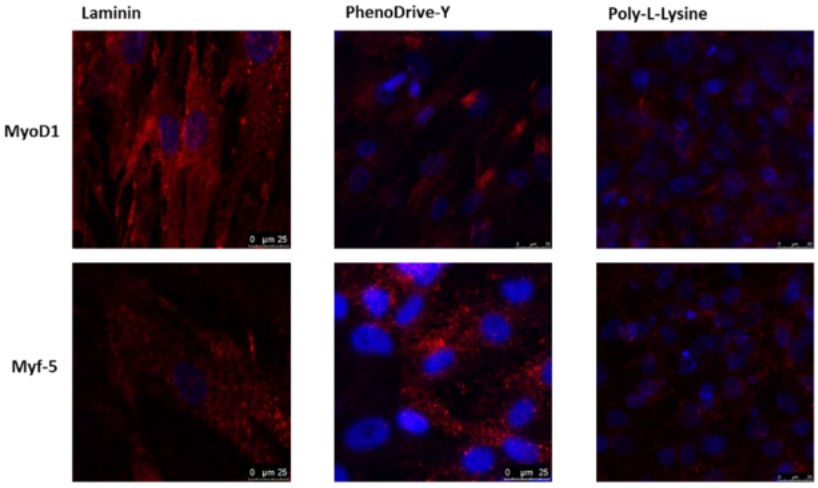


Figure 2. Biomarker expression of MyoD1 and Myf5 on Laminin, PhenoDrive-Y and Poly-L-Lysine with serum, on Day 14.

damaged skeletal muscle tissue. In particular, it is suggested that in regenerative medicine applications Laminin and PhenoDrive-Y may be optimal substrates for promoting SC organisation as myofibers and differentiation into mature skeletal muscle cells, while PLL appeard to be suitable for their preclinical expansion.

References

1. Schmidt, M., et al., Adult stem cells at work: regenerating skeletal muscle. Cellular and molecular life sciences : CMLS, 2019. 76(13): p. 2559-2570.

PoB.15.21

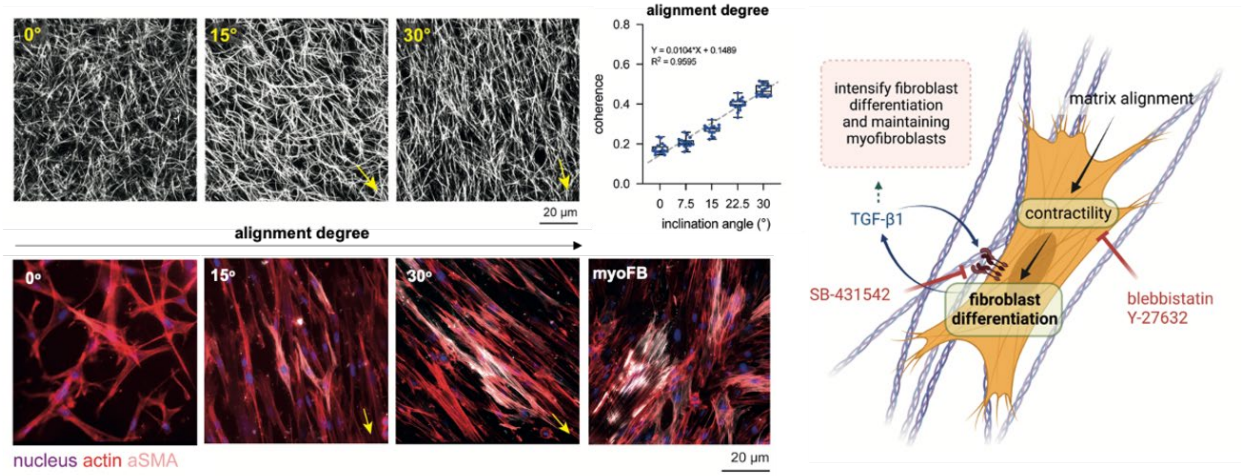
Non-invasive tuning of collagen fibril orientation to recapitulate the progression of fibrosis

Jiranuwat Sapudom[1], Shaza Karaman[1], Brian Chesney Quartey[1], Walaa Kamal Eldin Mohamed[1], Nick Mahtani[2], Anna Garcia-Sabaté[1], Jeremy Teo[1]

[1]New York University Abu Dhabi, Abu Dhabi, UAE. [2]Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

Abstract

Collagen alignment is one of the key microarchitectural signatures of many pathological conditions, including scarring and fibrosis. Investigating how collagen alignment modulates cellular functions will pave the way for understanding tissue scarring and regeneration, as well as new therapeutic strategies. However, current approaches for the fabrication of three-dimensional (3D) aligned collagen matrices are low throughput and require special devices. To overcome these limitations, we developed a simple approach to reconstitute 3D collagen matrices with an adjustable degree of fibril alignment using 3D printed inclined surfaces. By characterizing the mechanical properties of reconstituted matrices, we found that the elastic modulus of collagen matrices is enhanced with an increase in the alignment degree. The reconstituted matrices were used to study fibroblast behavior to reveal the progression of scar formation where a gradual enhancement of collagen alignment can be observed. We found that matrices with aligned fibrils trigger fibroblast differentiation into myofibroblasts via cell contractility, while collagen stiffening through a chemical crosslinker did not. Our results suggest the impact of the microarchitectural organization of collagen fibrils on the regulation of fibroblast differentiation. Overall, our approach to reconstitute 3D collagen matrices with fibril alignment opens opportunities for biomimetic pathological-relevant tissue *in vitro*, which can be applied for other biomedical research.



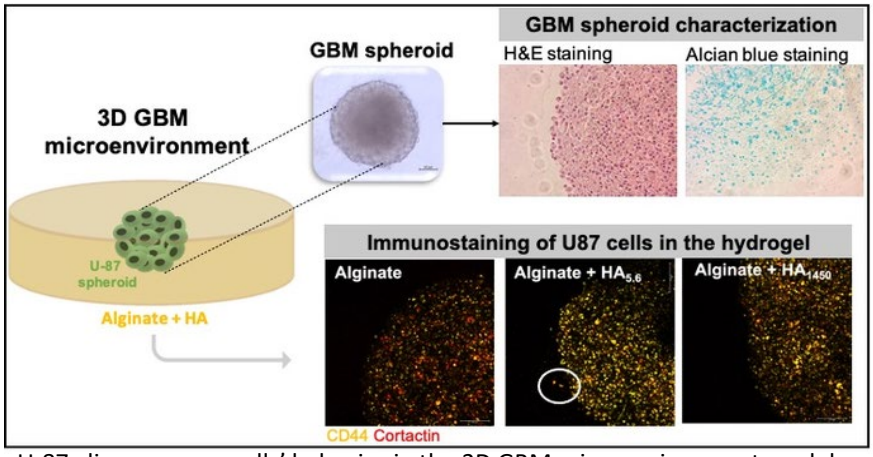
PoB.15.22

IN-VITRO GLIOBLASTOMA MODEL: HYALURONAN AS A KEY PLAYER OF CANCER INVASION AND METASTASIS

Sara Amorim[1,2], Rui L. Reis[1,2], Ricardo A. Pires[1,2]
[1]3B's Research Group, Guimarães, Portugal. [2]ICVS/3B's - PT Government Associate Laboratory, Braga, Portugal

Abstract

Glioblastoma (GBM) is one of the most common and aggressive brain tumors, presenting a 1-year survival rate of only 5%.[1] GBM displays a diffuse invasion pattern, which is strongly correlated to specific components of its microenvironment. An example is the glycosaminoglycan Hyaluronan (HA) found at higher concentration in the GBM's extracellular matrix (ECM), when compared to the healthy brain tissue.[2] Of note, HA of low molecular weight (Mw) has been correlated to the invasive character of cancer cells, while long HA chains are associated with cancer latency.[3] Here, we developed a 3D ECM model, based on a hydrogel generated by the combination of Alginate (Alg) and HA of different Mw, i.e. 5.6 and 1450 kDa. We encapsulated U-87 spheroids into the proposed Alg/Alg-HA hydrogel model and assessed the impact of HA Mw on the migration and invasive character of U-87 glioma cells through live imaging and immunostaining (for CD44, the main receptor for HA, and for cortactin, a key player in the modulation of cellular motility and cancer cell's invasion).



U-87 glioma cancer cells' behavior in the 3D GBM microenvironment model.

Time-lapse live imaging showed a correlation between the hydrogel's composition and the migratory behavior of U-87 cells, namely: the presence of HA of 5.6 kDa induces the invasive character of glioma cells promoting the migration of individual cells from the U-87 spheroids to the surrounding hydrogel. A behavior that is not observed on the hydrogels generated with HA of 1450 kDa. Moreover, immunostaining showed an overexpression of CD44 in the presence of HA, which co-localizes with cortactin. This co-localization is particularly observed in the migratory cells present in the hydrogel that combines Alg and HA of 5.6 kDa. Our data clearly demonstrate that the HA of low Mw influences glioma cells' migration and invasiveness by triggering the CD44 expression that, in turn, activates cortactin promoting its invasive character. The proposed 3D model was able to mimic the GBM microenvironment and allowed to assess the influence of specific biochemical features (e.g. HA and its Mw) on glioma cells' behavior.

Acknowledgements. We acknowledge the financial support from the Portuguese FCT and M-ERA.Net (PTDC/CTM-REF/0022/2020-OncoNeoTreat, CEECINST/0077/2018 and M-ERA-NET3/0007/2021-RePark).

References: 1.Mohiuddin, E. and H. Wakimoto, Am J Cancer Res, 2021. 11(8): p. 3742-3754; 2.Cui, Y., et al., Biomater Sci, 2020. 8(17): p. 4821-4831; 3.Amorim, S., et al.,. Biomater Sci, 2021. 9(1): p. 252-260.

PoB.15.23

Green approaches based on solvent-free methods to prepare nanoparticles and on alternative *in vitro* models for their validation: application in the treatment of metastatic melanoma

Carlotta Mattioda, Clara Mattu, Gianluca Ciardelli
Politecnico di Torino, Torino, Italy

Abstract

Nanoparticles (NPs) are advantageous drug-delivery systems due to their ability to maximize drug efficacy and minimize side effects. However, NPs preparation techniques pose environmental issue, as they require large quantities of organic solvents.

This contribution proposes the use of green methods for the preparation of NPs to deliver protein drugs or therapeutic RNAs. Two green NPs platforms were prepared i) Antibody-loaded Chitosan (CS) NPs obtained by ionic gelation and ii) siRNA-loaded phosphate-poly(allylamine-hydrochloride) (PAH) NPs, obtained through electrostatic self-assembly.

NPs of small size (~200 nm), low polydispersity index, and positive Z potential were obtained. NPs toxicity was investigated against melanoma and fibroblasts cell lines, observing no significant reduction in viability, up to 1 mg/mL. Platelet activation after exposure to NPs was tested as a preliminary assessment of their safety after intravenous injection. NPs were incubated with platelets for 30 min, followed by count of platelet adhesion and SEM analysis on adherent platelets, for a qualitative assessment, and by FACS, for a quantitative measure. PAH NPs did not trigger platelet activation, at any of the tested concentrations, while CS NPs did not induce activation at concentrations below 200 µg/mL.

NPs showed capacity to load model payloads (secondary antibody for CS NPs and mock siRNA or BRAF-silencing siRNA for PAH NPs) and to release it in a controlled fashion. FACS analysis and confocal microscopy showed that PAH NPs were able to significantly enhance siRNA delivery to cells, as compared to free-siRNA administration (Figure 1).

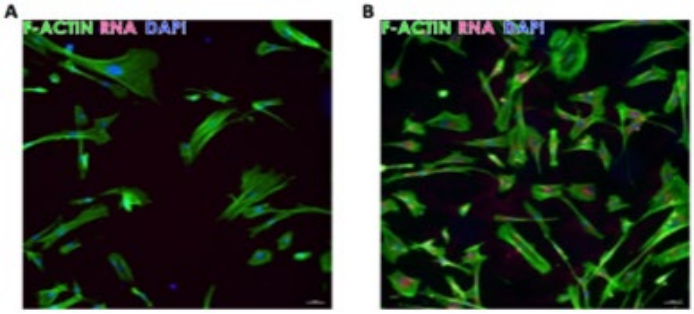


Figure 1: Human fibroblast after free-siRNA administration (a) and siRNA-loaded PAH NPs, (b) at the same siRNA concentration

Cisplatin-loaded CS and BRAF-siRNA loaded PAH NPs were tested against human melanoma spheroids. Since the tumor response to treatment strongly depends on its interactions with the tumor microenvironment (TME), a 3D-printed model of metastatic melanoma is under development as a further NPs testing device, representing an alternative to animal tests. To obtain the model, skin fibroblasts (Hff-1) were embedded in a collagen/hyaluronic acid-based hydrogel, and allowed to grow for one week. To recreate the vasculature, a channel was obtained within the hydrogel and seeded with endothelial cells (hUVECs). The model will be inoculated with melanoma cells and used to investigate NPs extravasation towards the primary tumor and their ability to target metastatic melanoma cells present in the channel.

Carlotta Mattioda acknowledges PON "Ricerca e Innovazione" 2014-2020 Azione IV.R "dottorati su tematiche green" for co-financing her Ph.D scholarship.

PoB.15.24

Development of Customized Bioinks for 3D Printed Dynamic Cancer Models

Paula Vazquez-Aristizabal[1,2], Malou Henriksen-Lacey[1,3], Clara García-Astrain[1,3], Dorleta Jimenez de Aberasturi[1,3,4], Ander Izeta[2], Luiz M Liz Marzán[5,3,4]
[1]CIC biomaGUNE, Donostia, Spain. [2]Biodonostia HRI, Donostia, Spain. [3]CIBER-BBN, Donostia, Spain. [4]Ikerbasque, Bilbao, Spain. [5]CIC biomagune, Donostia, Spain

Abstract

There is an unmet need for monitorable cancer models that better emulate the complex environment found in human tissues. The development of three dimensional (3D) printed solid tumor micromodels based on decellularized extracellular matrices (dECMs) offers a step in this direction, with the biomolecule-rich matrix of dECM allowing cell growth in a naturally-derived 3D environment. To this aim, epidermal and dermal biocompatible inks were prepared via a mechanical and enzymatic digestion process. Printability was confirmed first by rheological assessment and later by printing, and both inks were combined to build a complex 3D cellular model. The melanoma tissue model was characterized using different imaging techniques such as confocal microscopy and surface enhanced Raman spectroscopy (SERS). Our results suggest that such *in vitro* 3D models from printed dECMs are an interesting tool in tissue engineering and disease modelling to better understand cellular behavior and drug responses.

PoB.15.27

Cellulose hydrogels for immune cell *ex vivo* applications

Maria Heilala[1], Rita Turpin[2], Nikolaos Pahimanolis[3], Nonappa Nonappa[4], Olli Ikkala[1], Pauliina M Munne[2]
[1]Aalto University, Espoo, Finland. [2]University of Helsinki, Helsinki, Finland. [3]Betulium Oy, Espoo, Finland. [4]Tampere University, Tampere, Finland

Abstract

Modulating immune system is a promising strategy to treat various diseases, including cancer. While there are several immunotherapies in clinical use, they often lack the efficacy in many cancers and show patient to patient dependent variation. *Ex vivo* models for immune cells would enable to investigate the direct effect of drugs on immune cells, but also provide valuable tools to study mechanisms in immune cells activation. An easily obtainable source of primary human immune cells are the peripheral blood mononuclear cells (PBMCs). However, standard culture of PBMCs in suspension or on 2D substrates does not recapitulate the 3D extracellular matrix (ECM) environment in tumors, which is known to regulate cell behavior. Therefore, hydrogel-based 3D culture systems mimicking physical properties of ECM have been developed. Particularly, nanofibrillar cellulose (NFC) gels are attractive hydrogel materials because cellulose is abundant, contains no animal-derived components, and can easily be modified. In this study, we aim to explore the potential of NFC gels in 3D immune cell culture. For this, we investigate the performance of different types of NFC gels in 3D immune cell culture and focus on the direct effect of the different gels on the immune cell composition. Gels are extensively characterized using rheology, scanning electron microscopy and zeta potential analysis. PBMCs from human donors are cultured inside the gels and viability and resulting immune phenotypes are evaluated with techniques such as flow cytometry and microscopical analysis.

PoB.15.28

Adipogenic Differentiation of Mesenchymal Stem/Stromal Cells in 3D Culture

Anne Wolff[1], Marcus Frank[2,3], Susanne Staehlke[1], Olga Hahn[1], Kirsten Peters[1,4]
[1]Department of Cell Biology, Rostock University Medical Centre, Rostock, Germany. [2]Medical Biology and Electron Microscopy Centre, Rostock University Medical Centre, Rostock, Germany. [3]Department of Life, Light & Matter, University of Rostock, Rostock, Germany. [4]Department Life, Light & Matter, University of Rostock, Rostock, Germany

Abstract

Mesenchymal stem/stromal cells (MSC) are able to renew the progenitor cell fraction or differentiate in a tissue-specific manner. Adipogenic differentiation of adipose-derived MSC (adMSC) is important for several physiological and pathological processes. Adipocytes and their progenitor cells are metabolically active and secrete molecules that have both pro- and anti-inflammatory properties (including the so-called adipokines).
Cell cultures in 2D are commonly used to study cellular responses, but the 2D environment does not reflect the structural situation of most cell types (including adipose-derived cells). Therefore, 3D culture systems have been developed to provide an environment that is considered more physiological. Because knowledge about the effects of 3D spheroid culture on adipogenic differentiation is limited, we examined the effects on adipogenic differentiation and adipokine release of adMSC (up to 28 days) and compared them with the effects in 2D.
We showed that the dimensionality of cultivation was crucial for the MSC behavior: in both 2D and 3D cultures, adipogenic differentiation occurred only after specific stimulation (stimulation medium contained dexamethasone, 3-isobutyl-1-methylxanthine/IBMX), indomethacin, and insulin). While the size and structure of the adipogenically stimulated 3D spheroids remained stable during the experiment, the unstimulated spheroids showed signs of disintegration. The release of adipokines was dependent on the dimensionality of the culture: compared with the 2D culture, we found an increased release of adiponectin and a decreased release of the proinflammatory factors interleukin-6 and monocyte chemotactic protein 1 in the 3D spheroids. Thus, the 3D spheroid culture of adMSC exhibited a higher adipogenic differentiation capacity, whereas its inflammatory status appeared significantly lower compared with the 2D culture.
Our results may be relevant to the success of cell therapeutic applications of adMSC in complex tissues and the 3D culture model used may contribute to a better understanding of adipose tissue related diseases.

PoB.15.29

Modification of a fluidic chamber system to study biomaterials under flow conditions

Manuel Gatzke, Stefan Oschatz, Niels Grabow, Jana Markhoff
Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany

Abstract

The implant-tissue interaction depends on e.g. the type, surface structure and wettability of the biomaterial. Furthermore, the cell-specific adhesion behavior plays a role. Especially in the cardiovascular context, cells are exposed to high mechanical stresses, which, in return has a high impact on cell growth, morphology and differentiation. Thus, flow conditions must be taken into account for evaluation of cell reaction on biomaterials. In the development and characterization of new cardiovascular implants, such as vascular grafts and patches or coatings, ISO standards must be included covering hemocompatibility and implant testing.
Therefore, a commercially available flow system with modular perfusion chambers was modified to optimize leaking behavior, material anchorage and material evaluation with simultaneous calculation and adaptation of flow conditions for specific material and chamber heights. In order to colonize materials in the perfusion system, a corresponding adapter was designed in several iteration cycles and manufactured by stereolithography using a 3D printer. The construction design and adapter material have to resist cultivation conditions (37°C, 95% humidity), cleaning and sterilization processes and allow repeated and parallel usage. A combination of an upper and lower clamp was designed in accordance to the dimensions of the cultivation chamber, exhibiting plug-in modules for parallel connection of several chambers. To increase bending stiffness, reinforcing webs were provided at the long outer edges of the structure. Both clamp parts contain viewing windows for air bubble detection in the flow channel. All edges have been rounded to facilitate cleaning and sterilization of the components. The clamp components are screwed together using hexagon socket screws with standard thread.
A human vascular endothelial cell line and primary human umbilical vein endothelial cells were cultivated under flow for evaluation of optimal seeding density and flow rates. Fluorescence-stained cells were analyzed using CellProfiler software to determine cell numbers and cell orientation depending on static and flow cultivation. Cells were cultivated on coated surfaces under flow conditions in the modular system. Polymer films structured by laser etching were also tested for their adhesion behavior.
Microscopic evaluation was performed to assess cell colonization as a function of material and flow profiles. The modified chamber system could serve as an *in vitro* alternative for the assessment of cardiovascular biomaterials under flow, as well as a supplement to ISO 10993-4 for biological evaluation of medical devices in blood contact.

PoB.15.30

Engineered instructive surfaces to study muscle regeneration-degeneration *in vitro.*

Sarah Willems[1,2], Carlo F. Natale[2], Maurizio Ventre[1,2,3], Paolo A. Netti[1,2,3]
[1]Department of Chemical, Materials and Industrial Production engineering, University of Naples Federico II, Naples, Italy. [2]Centre for Advanced Biomaterials for HealthCare@CRIB, Fondazione Istituto Italiano di Tecnologia, Naples, Italy. [3]Interdisciplinary research centre on biomaterials (CRIB), Naples, Italy

Abstract

Extracellular stimuli from the microenvironment are crucial for cell adhesion, migration, proliferation and differentiation. Therefore, we aimed to design a material to recapitulate the *in vivo* skeletal muscle niche in an *in vitro* environment. This is to guide myoblast growth and organisation, and to induce the formation of skeletal muscle tissue constructs using mechanical and biochemical cues provided by the material.
The engineered niche consists of a polydimethylsiloxane (PDMS) layer with 700 nm wide ridges and grooves of 250 nm depth, that is adapted to mimic the mechanical microenvironment of the muscle cells. Different combinations of plasma treatment and types of extracellular matrix coating provide biochemical cues to better simulate the extracellular matrix and influence the material-cell interface. The uniaxial pattern provided can align C2C12 myoblasts in the corresponding direction. Additionally, our experiments demonstrate the formation of an orthogonal three-dimensional construct after eleven to fifteen days in culture. These skeletal muscle constructs were characterised using immunofluorescence and atomic force microscopy to evaluate the organisation, maturity and mechanical properties.
Starting from single skeletal muscle cells, we engineered an *in vitro* niche that is able to guide the cells towards the formation of three-dimensional muscle constructs. These constructs can be used as an *in vitro* model for healthy and diseased skeletal muscle to represent degeneration and regeneration of the muscle tissue.

PoB.15.31

Directing the fusion, growth and remodeling of cellular microtissues to engineer anisotropic soft tissues

Francesca D Spagnuolo, Gabriela Soares Kronemberger, Kyle Storey, Daniel John Kelly
Trinity College Dublin, Dublin, Ireland

Abstract

Introduction. Regeneration of articular cartilage (AC) requires engineered grafts that mimic the phenotype and functionality of the native tissue. Despite sustained efforts, engineering biomimetic AC remains a challenge. The problems encountered with the use of classical tissue engineering approaches have motivated the development of scaffold-free strategies, where cellular aggregates, microtissues or organoids can be used as building-blocks in the biofabrication of scaled-up constructs. Relatively few studies have explored the capacity of such microtissues to fuse *in vitro* and generate functional AC grafts (1). The aim of this work is to better understand the mechanisms behind cartilage microtissue fusion. **Methods.** Human bone marrow mesenchymal stem cells (MSCs) derived cartilage microtissues were fabricated in micro-moulds as described previously (2). After 2, 4 and 7 days of culture, the microtissues were harvested to investigate their capacity to fuse and form a scaled-up AC graft. After 28 days in chondrogenic induced media (CDM), chondrogenic differentiation in the fused graft was assessed by histology, biochemical assays, and RT-qPCR. Next, the microtissues at day 2 of maturation were fused to regenerate a centimeter-sized (10x1 mm) tissue and cultured for 6 weeks in CDM, at different oxygen tensions (20% and 5 %). **Results.** Contructs generated by the fusion of cartilage microtissues contained higher levels of glycosaminoglycans (GAGs) after 28 days in CDM. Less mature microtissues (removed from micro-moulds at day 2 and 4) possessed a stronger capacity for fusion and ultimately generated a macrotissue containing higher levels of GAGs compared to more mature microtissues (i.e., those removed from micro-moulds at day 7). Next, we investigated the potential of microtissues to form a large-scale tissue that mimics the size of defects typically observed in human AC. In all the four conditions (static and dynamic; 5% and 20% O2) the microtissues were able to fuse and generate a scaled-up tissue over 6 weeks of culture in CDM. **Conclusions.** The final conclusions are: (1) microtissues cultured independently for relatively short periods of time (2-4 days) possess a superior capacity for fusion and generate scaled-up tissues with a more cartilage-like phenotype; (2) it is possible to engineer scaled-up scaffold-free grafts using multiple cartilage microtissues; (3) dynamic conditions don`t negatively affect the capacity of microtissues to fuse. The work supports the use of microtissues to engineer scaled-up tissues for AC regeneration.

References:

(1) Bhumiratana S, et al. (PNAS) 2014
(2) Nulty J, et al. (Frontiers in Biotechnology and Bioengineering), 2021

PoB.15.32

Chondroitin sulfate (CS-A) containing artificial extracellular matrices (aECM) coupled with low pO_2 and slightly acidic conditions enhanced chondrogenic fate of human mesenschymal stem cells (hMSCs).

Bianca de Freitas Machado[1], Vera Hintze[1], Franziska Alt[1], Leif Riemenschneider[1], Gianaurelio Cuniberti[1,2], Poh Soo Lee[1]

[1]Max-Bergmann Centre for Biomaterials, TU Dresden, Dresden, Germany. [2]Center for Advancing Electronics Dresden (cfaed), TU Dresden, Dresden, Germany

Abstract

Bone grafts are widely applied for treating critical-sized defects, but there remain deficiencies in existing tissue-engineered constructs for satisfactory patient outcomes. Generally, the biological steps necessary for fracture repair are often neglected. These encompass the elements of endochondral and intramembranous ossification occurring in a dynamic biophysical niche. In this study, cell density, medium pH, oxygen tension (pO_2), and CS-A are identified as potential modulators of endochondral ossification. The potential for initiating chondrogenic differentiation through the interplay of these biophysical cues was investigated over 30 days. **Methods.** Three-dimensional aECM scaffolds were fabricated by freeze-drying 1 mg/mL collagen (Col) solution ± 1 mg/mL CS-A in silicon molds (Figure 1A), followed by crosslinking with 25% glutaraldehyde vapor and sectioned in 0.5 cm (Figure 1B). These scaffolds were seeded with hMSCs at a density of 0.05- 5 x 10^6 hMSCs/cm^3 and cultivated at 5% or 10% CO_2 in either slightly acidic (DMEM:F12) or slightly basic (DMEM) media (Figure 1C). CS-A, embedded in scaffold and supplemented in media, is employed as a potential continuous driver for chondrogenesis. The potency of CS-A was compared against standard supplements (ITS+1) to induce chondrogenesis *in vitro*. Further, the pO_2 in scaffolds laded with different cell density was also recorded. **Results.** Generally, early bone development occurs in an acidic environment, characterized by local metabolic acidosis and a lack of vascular infiltration. Our results show up-regulated *Sox9* expression, which indicates chondrocyte lineage commitment, in the slightly acidic DMEM:F12/5% CO_2, especially at later time points. *Aggrecan*, one of the major components of cartilage, shows a tendency to be up-regulated towards day 30 in all sample, and is most significant in DMEM:F12/5% CO_2. In contrast to corresponding DMEM, lower pO_2 was also recorded in scaffolds cultured in DMEM:F12 at day 10 and 20. Importantly, similar trends in chondrogenic-primed environments were observed between Col+ITS and Col+CS-A, showing CS-A being equivalent to the standard ITS+1 premix and driving chondrogenic differentiation alike. *RUNX2*, closely associated with osteoblast differentiation and an essential regulator for chondrocyte hypertrophy, was

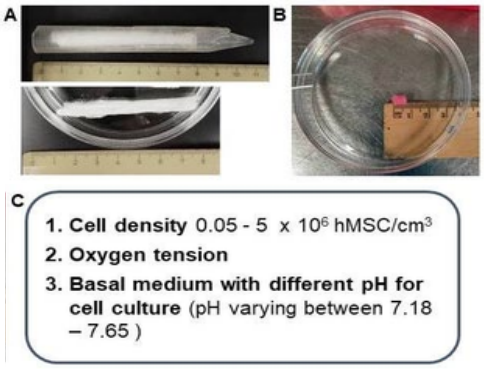


Figure 1. (A) Freeze-dried collagen scaffold. (B) Sectioned scaffold before cell-seeding. (C) Biophysical cues were selected as potential modulators of lineage commitment and outcome of the seed constructs.

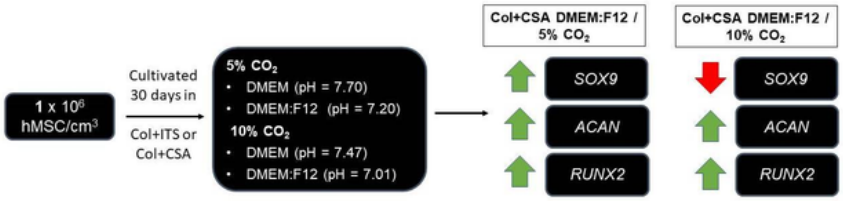


Figure 2. The cell density of $1x10^6$ hMSC/cm^3 was selected based on LDH and DNA assays that indicated that such density was closest to the physiological state ($10x10^6$ hMSC/cm^3). Cell proliferation was still supported when 5% CO_2 was applied and decreased pO_2 was measured in the scaffolds.

expressed after 30 days (Figure 2). Interestingly, osteogenesis supersedes chondrogenesis after 20 days. Therefore, additional enforcement with hypoxia may be necessary to retain the chondrogenic fate past 20 days. It is important to note that these experiments were performed at atmospheric pO_2 (132 mmHg). Further experiments are being conducted at hypoxic conditions (pO_2 = 10 mmHg) to evaluate the effects of hypoxia in enforcing chondrogenesis *in vitro*.

PoB.15.33

LIGHT-SENSITIVE NANOSTRUCTURED DEVICE FOR OPTICAL MODULATION AND CONTROL OF NON-EXCITABLE CELL FUNCTIONS

Anthea Villano[1,2], Vanessa Spagnolo[1], Gabriele Tullii[1], Maria Rosa Antognazza[1]
[1]Istituto Italiano di Tecnologia, Milano, Italy. [2]Politecnico di Milano, Milano, Italy

Abstract

In order to advance diagnosis and treatment of diseases, many studies aim at control cellular functions. Physical stimuli offer important advantages over chemical and pharmacological approaches. In particular, the opportunity to stimulate cells with light provides high spatial and temporal resolution, low invasiveness, high selectivity and low or negligible toxicity. Living cells are transparent to visible light, for this reason a light-sensitive bio-interface is needed.
Conjugated polymers are emerging as optimal candidates for medical applications, because they are highly biocompatible and can combine chemical and mechanical advantages of organic materials with peculiar optoelectronic properties of semiconductors. This work is based on the use of Poly(3-hexylthiophene-2,5-diyl) (P3HT), an organic and photoelectrochemically active conjugated polymer. This material absorbs visible light and support charge photo-generation, sustaining both electronic and ionic charge transport. Several studies show that P3HT acts on cells' membrane potential [G. Onorato, Adv. Healthc. Mater., 2022]. In particular, our study focuses on nanostructured devices based on P3HT, considering that nanostructure formation of conjugated polymers can be useful to enhance effects of optical modulation [S. Higgins, Adv. Mater., 2020]. Active interfaces with nanoscale components can be very useful for matching the complex nanoscale structural features of living tissues.
Here we report on the synthesis as well as optoelectronic and morphological characterization of a biocompatible and light-sensitive nano-structured device. The bio-hybrid interface is capable of modulating different cellular functions, such as photo-electrochemical processes occurring at the interface between the organic semiconductor and living cells, and functions involved in regenerative processes, such as cell migration and proliferation. Efforts are also spent in selectively targeting different kind of non-excitatory cells (e.g. endothelial and epithelial cells) modulating and controlling their functions through the use of light, through both *in vitro* and *in vivo* trials.
Nanostructured P3HT-based devices can pave the way to new frontiers in the field of regenerative medicine.

PoB.15.35

Encapsulation of Mesenchymal Stem Cells with Alginate using Microfluidic Device

Alex Slater[1], Narjes Rashidi[2], Giordana Peregrino[1], matteo Santin[1]

[1]University of Brighton, Brighton, United Kingdom. [2]university of Brighton, Brighton, United Kingdom

Abstract

Alginate microbeads can be adopted for the encapsulation of Mesenchymal Stem Cells (MSCs) with the aim to provide both a delivery carrier and protection from the immune response. However, the formation of stable microbeads depends on the crosslinking of the alginate with divalent calcium ions at concentrations that are toxic for the cells and that make the beads size and a single-cell encapsulation difficult to control. The present work demonstrates a highly efficient and controllable microfluidic on-chip method to produce thin-layer alginate coatings for single MSCs using different concentrations of alginate (1%, 2% and 3% w/v). Dynamic Light Scattering (DLS) measurements of particle size for the encapsulated MSCs in the three different alginate concentrations showed sizes of 69.3 µm ± 0.6, 81.1 µm ± 2.8, and 81.6 µm ± 0.45, respectively (Figure 1). Noticeably, the successful encapsulation of single cells was observed by visible light and confocal microscopy following staining of the nuclei by DAPI (Figure 2A). The viability and behavior of the encapsulated MSCs was demonstrated by the adherence of the beads onto tissue culture plastic and the MSC ability to gradually break out from the microcapsules and proliferate after 24 hours (Figure 2B). This work demonstrates the potential of microfluidic systems to provide precise MSC encapsulation for cell therapy at GMP standards, fast manufacturing and able to preserve cell adherence and proliferation.

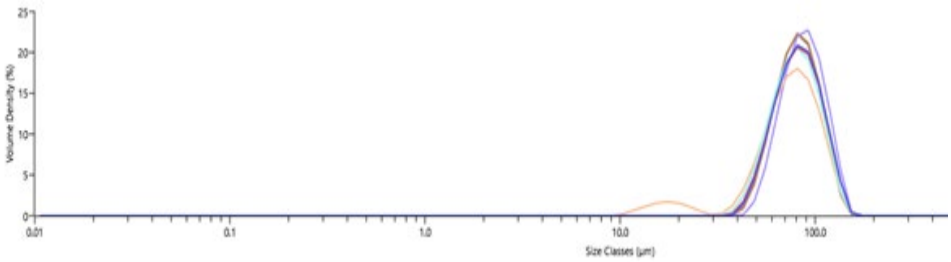


Figure 1. Particle size measurement for MSCs encapsulated in Alginate (2%) obtained by DLS.

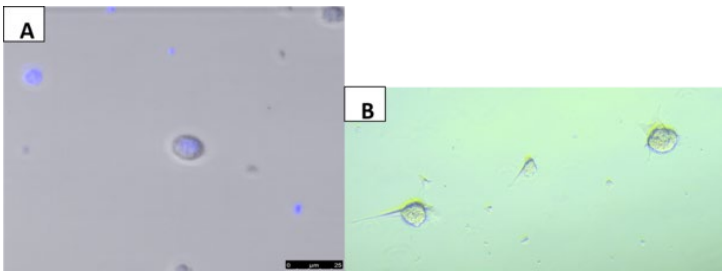


Figure 2. A) Confocal microscopy of single MSC encapsulated in 2% alginate stained with DAPI. B) Microscopy image (x10) of single MSC encapsulated in 2% alginate. Cells had been placed in 24 well plate for 24 hours. MSCs begin adhering to TC plastic and growing outside alginate.

Acknowledgements

This work was supported by the UKRI i3England, project acronym: 3Med.

PoB.15.36

3D Cell Migration Chip a new tool toward breast cancer modelling

Silvia Buonvino[1], Joanna Filippi[2], Eugenio Martinelli[2], Dror Seliktar[3], Sonia Melino[1]
[1]Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Rome, Italy. [2]Department of Electronic Engineering, University of Rome "Tor Vergata", Rome, Italy. [3]Department of Biomedical Engineering, Technion Israel Institute of Technology, Haifa, Israel

Abstract

3D cell cultures are widely used *in vitro* systems for improving our understanding of cell biology, molecular mechanisms of drugs and diseases, hence the optimization of 3D hydrogel-based cellular models is a relevant research field. 3D "gel in gel" cancer cell culture systems (Figure1A) have been developed using the photopolymerizable hybrid hydrogel PEG-fibrinogen (PFHy)[1-2], based on the production of a cell hydrogel microsphere (inner gel) and of an outer gel. In particular, PFHy microspheres with MDA-MB 231 breast cancer cells (MDAPFs) (Figure1B) were used for the analysis of H_2S-donors effects on cancer cell viability and invasion. For our studies we have designed a lab on chip [patent submission number: 102021000025460] named "3D Cell Migration-Chip" (3DCMchip) (Figure1C), which offers many advantages respect to the traditional method and characterized by a removable superhydrophobic nanostructured surface (Figure1D) to optimize the cellular microsphere *in situ* production. 3DCMchip allows to analyse the effects of chemico-physical stimuli on the tumor cells migration/invasion.

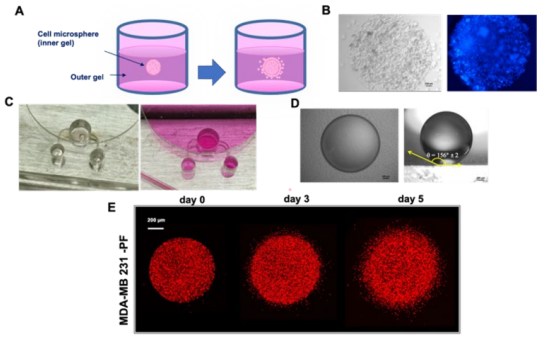


Figure1. A) Representation of the "gel in gel" assay; B) optical and fluorescence micrographs of MDAPFs stained with Hoechst; C) image of 3DCMchip well containing the microsphere on the superhydrophobic surface and filled with culture medium; D) contact angle (θ>150°) of the MDAPF; E) confocal micrographs of mCherry/MDA-MB 231 cells invading the outer gel over time.

The outgrowth of cancer cells was monitored over time and were analysed the effects of GSGa, a glutathionylated garlic extract produced in our laboratory able to slowly release H_2S[3] on MDA cell growth and migration. The treatment with the H_2S-donor showed that, at higher concentration, cell migration was suppressed and the cell viability slightly decreased. 3D CMchip was also used for investigating the effects of human fibroblasts on MDA cells invasiveness in co-culture systems. Our analyses showed an increased migration of tumour cells over time in the presence of fibroblasts, that is related to the activation of the fibroblasts toward Cancer Associated Fibroblasts (CAF). Interestingly, the treatment of the co-culture with GSGa decreases the CAF trans-differentiation, suggesting a potential inhibition of the aforementioned fibroblasts activation by the H_2S-donor. 3D CMchip allows the easy development of more complex co-culture systems using several cell types to improve the reliability of 3D tumour models, toward the realization of *in vitro* systems able to potentially mimic the physiological tumour microenvironment.

References

1. Almany L. and Seliktar D. 2005,Biomaterials-26,15,2467
2. Ivanir E. 2020,Adv. Funct. Mater.-30,1807106
3. Buhyan A. 2015,Molecules-20,1,1731

PoB.15.37

Development of 3D microphysiological systems to study intestinal stem cell fate and model early steps of tumorigenesis

Elise Ponthier[1,2], Lucien Guth[1,2], Julie Foncy[2], Anne Prel[1], Daniel Ferri-Angulo[2], Laurent Malaquin[2], Arnaud Besson[1]

[1]CBI, Toulouse, France.

[2]LAAS, Toulouse, France

Abstract

With 1 million deaths pear year, colorectal cancer is the world's third deadliest cancer. Over-activation of the Wnt signaling pathway (Wnt/Apc/β-Catenin axis) that initiates hyperproliferation of crypts, where intestinal stem cells reside, appears to be the primary driver of this cancer. Conventional therapeutic approaches frequently fail due to tumor heterogeneity and resistance of cancer stem cells to treatments, causing relapse of the disease. Moreover, animal testing is not reliable and over 90% of translation from animal models to clinical trials fail, mainly because of the biological differences between species. Therefore, there is a need for new models that recapitulate human intestinal physiology and complexity more closely. Thus, different *in vitro* models have been developed, some reproducing mechanical cues such as peristalsis-like movements, others recapitulating the villi and/or crypt topography. However, none of the models have combined all aspects. My project aims at reproducing an intestinal epithelium in microphysiological systems (MPS) by using 3D printing and microfluidic devices. The first part of my project, ongoing, is to develop a photosensitive biomaterial allowing the adhesion and proliferation of primary intestinal cells derived from organoids that is compatible for 3D printing and deformable to allow reproducing peristaltic movements. We chose a combination of polyethylene glycol diacrylate and gelatin methacrylate for the mechanical properties of the former and the adhesive properties of the latter that can be printed by high-resolution 3D stereolithography to reproduce the intestinal architecture (Fig. 1). The second step will be to integrate these scaffolds into microfluidic devices to provide mechanical and biochemical cues. Thus, the MPS will reproduce different aspects of the organ *in vivo*, such as topography, peristaltic movements and microenvironmental parameters. Another goal is to reproduce the early steps of tumorigenic transformation in the MPS using cells carrying inducible mutants in the Wnt/Apc pathways. Ultimately, we aim to show that these devices could provide standardized and reproducible *in vitro* models for the study of stem cell fate and cancer etiology and to perform preclinical drug testing.

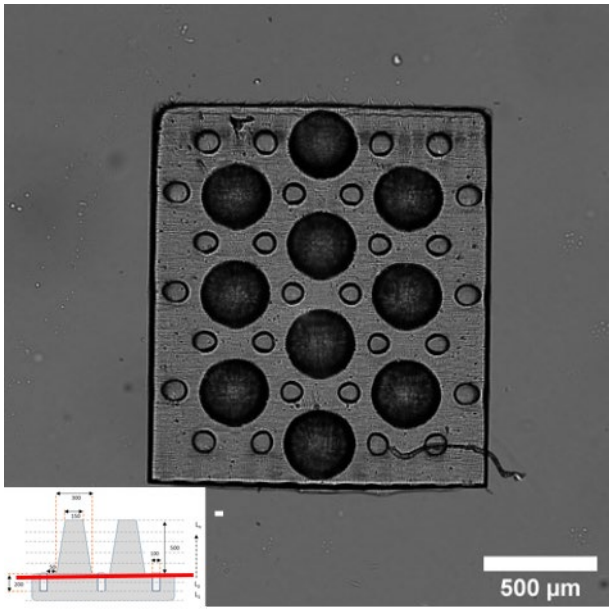


Figure 1. Top view of 3D printed intestinal topography (crypts and villi). Insert: focal plan

PoB.15.38

Feasibility study of injectable intervertebral disc-mimetic cell encapsulated microgels for nucleus pulposus regeneration

Parisa Torabi Rahvar[1,2], Leon Schlagenhof[2], Mohammad J. Abdekhodaie[1], Benjamin Gantenbein[2,3]
[1]Department of Chemical Engineering, Sharif University of Technology, Tehran, Iran, Islamic Republic of. [2]Tissue Engineering for Orthopaedic & Mechanobiology, Bone & Joint Program, Department for BioMedical Research (DBMR), Medical Faculty, University of Bern, Bern, Switzerland. [3]Department of Orthopaedic Surgery & Traumatology, Inselspital, Bern University Hospital, Medical Faculty, University of Bern, Bern, Switzerland

Abstract

Introduction. Low back pain is a common and widespread health problem affecting a significant percentage of adults (up to 80%) worldwide[1]. Mesenchymal stem cells (MSCs) have shown new hope to regenerate the early stages of intervertebral disc degeneration. However, there are debates as to whether transplanted cells in cell suspensions can survive in the harsh environment of degenerated IVDs, which may limit the effectiveness of cell therapy. An appropriate cell delivery system is necessary to ensure the efficacy of MSCs *in vivo*[2]. Herein, hybrid injectable microgels based on hyaluronic acid (HA) and collagen type II (COLII) were developed for cell delivery to the IVD, mimicking the polysaccharide-protein composition of the native NP extracellular matrix. The study also uses an *ex vivo* papain-induced bovine tail disc degeneration model to investigate this cell therapy. **Methods.** Tyramine-grafted HA and COLII were prepared using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and n-hydroxysulfosuccinimide (NHS) chemistry[3]. HA-Tyr and COLII-Tyr were dissolved in phosphate buffered saline (PBS) and mixed at different ratios followed by adding horseradish peroxidase (HPR). The polymer solution was extruded through a syringe pump and microdroplets were generated using a flicking-based vibrating nozzle system. The droplets fell into a H_2O_2 solution and formed microgels. The hydrogels were characterized in terms of their mechanical properties and gelation time. The shape and size distribution of the microgels were analyzed using phase contrast and scanning electron microscopy (SEM). **Results.** With this novel approach, uniform microgels could be fabricated with variable size in the range of 300–500 µm and narrow distribution in all compositions (Figure 1). We are currently optimizing the injection conditions of microgels into NP tissue as a function of microgel composition and needle gauge. **Conclusion.** This study presents the initial optimization process to produce enzymatically crosslinked HA-COLII microgels. The goal is to utilize these microgels as building blocks mimicking the extracellular matrix for NP tissue regeneration.

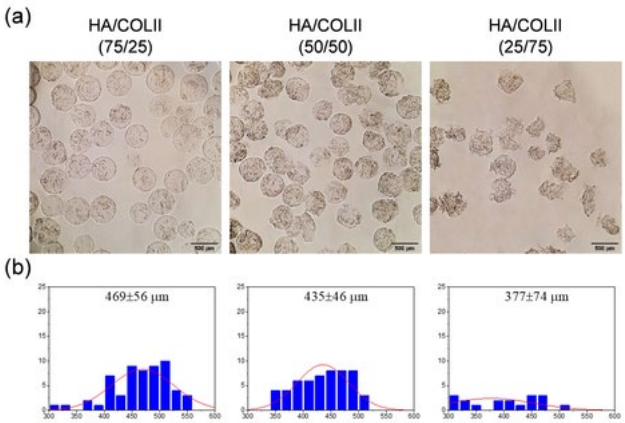


Figure 1. Hybrid HA-Tyr/COLII-Tyr microgels with different compositions produced by flicking-based setup. (a) Optical microscope images and (b) size distribution.

References

1. Wöltje M. et al., Biomimetics. 12;8(2):152, 2023.
2. Xu H. et al., Biofabrication. 31;13(1):015010, 2020.
3. Jooybar E. et al., Acta Biomater. 1;83:233-44, 2019.

Acknowledgement. This research was funded by the Council for Development of Stem Cell Sciences and Technologies (#57940), Iran National Science Foundation: INSF (# 99002288) and an SNF-bridge project # 40B2-0_211510/1.

PoB.15.39

Dual crosslinked glycosaminoglycan-peptide interpenetrating hydrogels with tailored viscoelasticity and degradability for directing induced pluripotent stem cell morphogenesis

Prannoy Seth1, Nicole Fertala1, Yanuar Dwi Putra Limasale1, Uwe Freudenberg1, Carsten Werner1,2

[1]Leibniz-Institut für Polymerforschung Dresden e. V., Max Bergmann Center of Biomaterials, Dresden, Germany.
[2]Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Germany

Abstract

The native extracellular matrix (ECM) consists of a highly hydrated, complex meshwork of proteins, proteoglycans, and glycosaminoglycans (GAGs). Hydrogels, water-swollen crosslinked polymer networks, have been widely applied to mimic and modulate key features of the ECM to create appropriate *in vitro* models and control cell-fate decisions of embedded cells. Specifically, hydrogels that are crosslinked through covalent bonds are made responsive to cell-secreted enzymes, making them highly suitable for cell culture and tissue engineering applications. However, besides enzymatic remodeling, the viscoelastic properties of the ECM are crucial for achieving a well-orchestrated spatiotemporal tissue organization *in vitro*. Addressing this need, within this study we developed GAG-peptide-based dual crosslinked interpenetrating hydrogels with tunable viscoelasticity and degradability to understand how these parameters influence the growth and morphogenesis of embedded human induced pluripotent stem cells (hiPSCs). In particular, these hydrogels were formed with two types of crosslinking units: 1) reversible non-covalent crosslinks between 4-arm starPEG conjugated with heparin interacting peptides (HIP) and the GAG heparin and 2) stable covalent crosslinks via Michael-type addition reaction between thiol terminated starPEG and maleimide functionalized heparin. A wide range of stress relaxation behavior was achieved by adjusting the ratio of non-covalent to covalent crosslinks. Furthermore, the hydrogels were engineered to undergo cell-responsive degradation by incorporating protease-sensitive peptides within the covalent crosslinks, thereby resembling the natural remodeling process of the ECM. The hydrogel design enabled precise control over the viscoelastic behavior and degradability of the matrix, independently. The hiPSCs cultured within non-degradable viscoelastic hydrogels revealed significant growth of the cysts, lumen formation, and maintenance of pluripotency in matrices with high viscoelasticity. Conversely, in viscoelastic hydrogels with degradable covalent crosslinks, regardless of the viscoelasticity of the matrices, cyst growth, lumen formation, and sustained pluripotency were observed, suggesting that both remodeling mechanisms can partially substitute for each other. However, concerning morphology, viscoelasticity plays a crucial role, as highly viscoelastic hydrogels promote an invasive cyst morphology, independent of the presence of enzymatically degradable crosslinks within the materials. Taken together, these results indicate that matrix viscoelasticity and enzymatic remodeling are essential

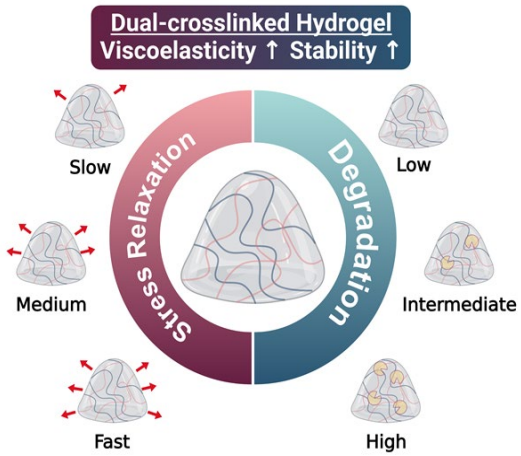


Figure. Dual-crosslinked interpenetrating hydrogel with tunable viscoelasticity and degradability.

regulators of hiPSC morphogenesis and enable the development of biomaterials for controlling cell-fate decisions of embedded hiPSCs.

PoB.15.40

Alginate hydrogel characteristics regulate NHDF biology

Aman S Chahal[1], Daria Zaytseva-Zotova[2], Hanne Haslene-Hox[1], Øystein Arlov[1], Andrea Draget Hoel[1], Robin Mjelle[3], Wenche Iren Strand[3], Anita Akbarzadeh Solbu[3], Geir Klinkenberg[1], Øyvind Halaas[3], Vidar Beisvag[3], Håvard Sletta[1], Pål Sætrom[3], Berit L Strand[3]

[1]SINTEF, Trondheim, Norway. [2]UiO, Oslo, Norway. [3]NTNU, Trondheim, Norway

Abstract

Introduction. Alginate is a marine derived polysaccharide, which is considered an attractive extracellular matrix material for *in vitro* 3-dimensional (3D) cell culture [1,2]. In this work we investigated how varying hydrogel chemical characteristics influenced the viability, morphology and gene expression of normal human dermal fibroblasts (NHDF) within the gel matrices. **Methods.** The tested hydrogels consisted of 1% alginate cross-linked with calcium ions using internal gelation method [3]. Three gel types were tested: (A) UMOD: UPLVG alginate (FG 0.68, MW 237 kDa), (B) OX: a 1:3 mix of UPLVG : POA (periodate oxidized UPLVG, DOX=0.08), and (C) RGD: a 1:3 mix of UPLVG : POA-RGD (POA laterally substituted with a GRGDSP peptide, DS=0.05). NHDF were cultured in the hydrogels for 7 days. Viability and morphology of the cells in gels were examined using confocal laser scanning microscopy (CLSM). For RNA sequencing screening, RNA was isolated from cells growing within the gels at days 1 and 7. NHDF grown in conventional 2D culture were used as a reference. **Results.** It was shown that the hydrogels A, B and C promoted different cell morphologies. Analysis of the RNA sequencing data revealed time and hydrogel dependent clusters in contrast to basic 2D cell culture. Gene enrichment was performed based on gene ontologies, and genes critical to fibroblasts in 3D environments were identified. **Discussion & Conclusions.** Evaluation of cell viability and morphology within the different alginate hydrogels along with analysis of the RNA screening data provide a better understanding of how fibroblasts biology can be modulated by controlled characteristics of the alginate scaffolds.

PoB.15.41

Physiological mechanical stimulation improves the maturation and organization of three-dimensional cardiac bioconstructs *in vitro*

Clotilde Castaldo[1], Anna Maria Sacco[1], Immacolata Belviso[1], Giulia Ricci[2], Veronica Romano[1], Daria Nurzynska[3], Angiolina Catizone[4], Fabrizio Schonauer[1], Stefania Montagnani[1], Franca Di Meglio[1]

[1]University of Naples "Federico II", Naples, Italy. [2]Università della Campania "Luigi Vanvitelli", Naples, Italy. [3]Università degli Studi di Salerno, Baronissi, Italy. [4]University of Rome La Sapienza, Rome, Italy

Abstract

Myocardial damage caused by ischemic necrosis affects both the cellular and extracellular compartments. The extracellular matrix (ECM) makes up the majority of the non-cellular compartment of living tissue and provides a unique combination of biological and mechanical cues. Therefore, any attempt to restore the myocardium morphologically and functionally can only be accomplished by replacing ECM and cells. We have recently developed a fully biological decellularized human skin (d-HuSk) scaffold capable of supporting the engraftment, survival, and differentiation of human resident cardiac progenitor cells (hCPCs) in static conditions. We hypothesize that culturing d-Husk-based cardiac bioconstructs in a cyclic stretch bioreactor further stimulates their maturation due to the intrinsic elasticity of the dermal matrix.
To test our hypothesis, we prepared three-dimensional d-HuSk biological scaffolds, repopulated them with hCPCs, and cultured the cellularized scaffolds in static conditions for one week to allow for cell engraftment and adaptation to the new environment. Then, we transferred the bioconstructs to a bioreactor and applied a cardiac-like cyclic stretch (10% strain at 1Hz) for seven days, and then evaluated the effects of mechanical stimulation on hCPC engraftment, alignment, and differentiation by SEM, histochemistry, immunofluorescence and gene expression analyses. Bioconstructs cultured in static conditions for two weeks were used as a reference.
Histological analysis revealed that hCPCs organized into a multilayered tissue on the surface of d-HuSk under both static and dynamic conditions. Intriguingly, however, cyclic stretch greatly enhanced cell migration to the inner layers of the dermal matrix and the well-ordered arrangement of hCPC that resulted mostly aligned orthogonal to the direction of stretch, but parallel to each other. Finally, gene expression profiling including genes typical of main cardiac cell lineages, like MEF2C, ACTC1, CX43, TBX-3 and -5, GATA6, ACTA2, ETS1, CD90 and CD105, showed up-regulation of transcripts for cardiac myocytes, smooth muscle, endothelial and mesenchymal cells in hCPCs cultured on d-HuSk in dynamic conditions, thus providing evidence of further maturation of stretched cardiac bioconstructs.
Our results support the hypothesis that d-HuSk could be successfully used as a cardiac matrix substitute and that the development of a cardiac bioconstruct based on d-HuSk andhCPC- is significantly improved under a physiological mechanical stimulation favoring its maturation *in vitro*.

PoB.15.42

Organ-on-chip-based *in vitro* approaches for co-culturing 3D human cancer tissues and circulating capillary flow-driven immune cells for more predictive drug testing and human disease modeling

silvia scaglione[1,2], elisabetta Palama[2], Maurizio Aiello[2]

[1]CNR, Genova, Italy. [2]React4life, genova, Italy

Abstract

Introduction. The human disease modeling for basic research and drug testing purposes is currently carried out through 2D cell culture in static conditions, and *in vivo* xenografts or genetically engineered animal models, but predictability, reliability, and complete immune compatibility remain important challenges. For this aim, novel 3D, fully humanized *in vitro* cancer tissue models have been recently optimized by adopting emerging technologies such as microphysiological systems (MPS) and 3D cell laden hydrogels. In particular, a novel Multi-*In vitro* Organ (MIVO) MPS platform has been recently adopted to culture 3D clinically relevant size cancer tissues under proper physiological culture conditions to investigate the efficacy of anticancer treatments. **Methods.** Biologically relevant cancer samples (up to 5 mm), have been developed by using a alginate base structure, resembling the extracellular matrix. The stiffness of such gels has been optimized to support a *in vivo* like tumor cells viability, cluster formation and migration. Ovarian and and ovarian cell laden hydrogels (1 million cells/ml) have been cultured within the MIVO chamber, while either testing molecules (cisplatin) or human immune cells (Natural Killer cells, NK) respectively circulate in the MPS mimicking the blood capillary flow. The tumor cell proliferation and viability were investigated in such dynamic cell culture conditions to assess the cytotoxic efficacy of the treatment. When the systemic administration of cisplatin was simulated within the MPS, the anticancer drug efficacy was also tested and compared to the animal model. When NK cells were placed in circulation, their extravasation through a permeable barrier resembling the vascular barrier, and infiltration within the neuroblastoma cancer tissue were analyzed. **Results.** A human 3D ovarian model was developed and treated with Cisplatin in static conditions, within MIVO, and in the xenograft model. Similar tumor regression was observed in MIVO and in mice, while the static culture displayed an unpredictive chemoresistance, due to unreliable drug diffusion within the 3D matrix. A human 3D neuroblastoma cancer model with proper immunophenotype was optimized to develop a complex tumor/immune cell co-culture as a paradigm of an immune-oncology screening platform. Importantly, a tumor-specific NK cell extravasation was observed under dynamic culture, with NK cells able to infiltration within the 3D tumor where they induced cancer cells apoptosis. **Conclusion.** We generated a relevant human disease model, through the adoption of a MPS system, that can be efficiently employed as a drug screening platform but also for better investigating crosstalk among immune /tumor cells.

PoB.16.01

Biofabrication of microtissue-derived constructs for articular cartilage repair

Gabriela S Kronemberger, Francesca D Spagnuolo, Kyle J Storey, Daniel J Kelly

Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin., Dublin, Ireland

Abstract

Engineering functional and phenotypically stable articular cartilage is considered one of the greatest challenges in tissue engineering. Modular tissue engineering strategies that use cellular aggregates, microtissues or organoids as building-blocks can potentially be used to fabricate complex hierarchical tissues at scale. The aim of this work was to (i) assess the capacity of cartilage microtissues at different levels of maturation to form scaled-up cartilage grafts in the presence or absence of a temporary supporting hydrogel, and (ii) assess if extrusion based bioprinting of such cartilage microtissues could be used for the biofabrication of functional cartilage grafts. Initially, mesenchymal stem cell (MSC) derived microtissues were fabricated as described previously (Nulty, Burdis and Kelly, 2021 and Burdis et al., 2022). These microtissues were harvested after 2 or 4 days of maturation, manually seeded in agarose wells with or without a supporting oxidized alginate and maintained in chondrogenic culture for 6 weeks in either static or dynamic culture conditions. As proof of concept, the microtissues were 3D bioprinted using an oxidized alginate-gelatin-based bioink (Barceló et al. 2022). Initially, it was observed that microtissues that were matured independently for 2 days prior to seeding in oxidised alginate hydrogels generated higher amounts of glycosaminoglycans (GAGs) compared those matured for 4 days. Histological analysis revealed intense staining for GAGs and negative staining for calcium deposits. In contrast, microtissues that were not encapsulated into supporting oxidized alginate stained positive for calcium deposits (Figure 1). These differences in tissue development due to incorporation of a supporting temporary hydrogel were also observed in dynamic culture conditions, where encapsulation in oxidised alginate support a 2 fold increase in sGAG deposition. Furthermore, the microtissues (day 2 maturation level) were 3D bioprinted using an oxidised alginate based bioink. It was observed that the microtissues remained viable after the bioprinting process, were able to fuse after 48h, and generated a cartilage tissue that was rich in GAGs and negative for calcium deposits after 6 weeks in culture. Therefore, the key conclusions from this study are that: (1) less mature cartilage microtissues (day 2 maturation level) generate superior scaled-up cartilage grafts; (2) dynamic culture has a positive effect on the chondrogenic capacity of the constructs when compared to static conditions; (3) microtissues can be successfully used as in extrusion based bioprinting to engineer scaled-up cartilage constructs. This work supports the continued use of cartilage microtissues as biological building blocks in diverse biofabrication platforms.

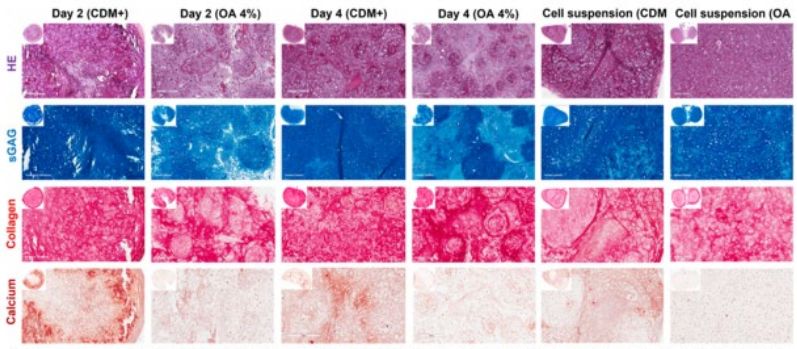


Figure 1: Microtissue-derived constructs show strong staining for sGAG after 6 weeks under static chondrogenic induction. Microtissues at level 2 maturation level cultured in media showed strong staining for sGAG and positivity for calcium deposits. Microtissues at level 2 maturation level encapsulated in OA showed strong staining for sGAG and negativity for calcium deposits. Microtissues at level 4 maturation level cultured in media showed strong staining for sGAG and negativity for calcium deposits. Microtissues at level 4 maturation level encapsulated in OA showed strong staining for sGAG and no positivity for calcium deposits. As a control, cell suspension cultured in media showed strong staining for sGAG and positivity for calcium deposits. Cell suspension encapsulated in OA showed strong staining for sGAG and negativity for calcium deposits.

PoB.16.02

An acellular self-healed trilayer cryogel combined with acupuncture as a new approach to induce osteochondral regeneration

Tsai-Yu Chen[1], Shan-hui Hsu[1], Niann-Tzyy Dai Dai[2], Tsung-Kai Wen[3]

[1]Institute of Polymer Science and Engineering, National Taiwan University, Taipei, Taiwan. [2]Division of Plastic and Reconstructive Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan. [3]School of Post-Baccalaureate Chinese Medicine, Tzu Chi University, Hualien, Taiwan

Abstract

A suitable treatment for osteochondral regeneration remains a formidable challenge in clinical issue despite significant advances in microsurgery. Compressible cryogel provides promising approaches for minimally invasive surgery in regenerative medicine. Herein, an injectable chitosan-based trilayer cryogel as the acellular scaffold was fabricated. The trilayer cryogel with tunable pore sizes ranging from 80 to 200 nm was prepared from self-healing hydrogel with different gelation times. The trilayer cryogel exhibited unique injectability and compressibility with ~2630±238% of water absorption and ~10.8 kPa of compressive modulus. Mesenchymal stem cells (MSCs) grown in the drug-loaded trilayer cryogel for 14 days *in vitro* showed long-term proliferation (150-350%) and osteochondrogenic potential with the sustained release of bioactive molecules. The therapeutic approach combining injectable trilayer cryogel with scalp acupuncture intended for endogenous MSC mobilization was found to regulate the levels of SDF-1 and TGF-b1 in the serum and synovial fluid of the experimental rabbits with osteochondral defects for osteochondrogenic differentiation through the sustained release of bioactive molecules. Moreover, the synergistic effect of immunomodulation on the M2-/M1-macrophage population ratio was verified with a ~7.3-fold enhancement through drug-loaded trilayer cryogel with scalp acupuncture, in comparison to ~1.5-fold increase by acupuncture alone and ~2.2-fold increase by drug-loaded trilayer cryogel alone. This novel strategy using the acellular cryogel scaffold for minimally invasive surgery and accessible acupuncture shows promises to attain a harmonizing healing effect for osteochondral regeneration.

PoB.16.03

Constructing a Cellular Scaffold Using Photo-crosslinkable Polymer Hydrogel For Artificial Cartilage

ryoma Takagi[1], Tadashi H Nakaji[2], Chiaki Yoshikawa[3]

[1]Univ. of Toyama, Toyama, Japan. [2]Univ. of Toyama, Toyam, Japan. [3]WPI-MANA, NIMS, Ibaraki, Japan

Abstract

Currently, existing treatments for osteoarthritis of the knee provide temporary relief of symptoms and do not lead to a complete cure [1]. Therefore, the development of a novel treatment that can produce a complete cure is urgently needed.
Recent research have explore both a cell biology approach using mesenchymal stem cells (hMSCs) and a tissue engineering approach that combines hMSCs with polymer materials as potential new treatments for osteoarthritis of the knee. However, replicating the complex gradient and layered structure of cartilage tissue, which has different structural and compositional properties and is essential for cartilage regeneration, has proven to be extremely challenging. As a result, constructing artificial cartilage tissue with a comparable level of quality and functionality has not yet been achieved [2].
In this study, we aim to create a cell-hydrogel composite artificial tissue with a gradient and layered structure, and thus developed functional polymers for this purpose. To construct the gradient and layered structure of cartilage tissue, we utilized the density gradient based on polymer-specific molecular weight distribution. This method involves producing a gel with a continuously changing hardness by means of centrifugation. It is expected that the gradient structure of the gel hardness will regulate the differentiation behavior of cells, thereby reproducing the complex layered structure of living cartilage tissue. Furthermore, the polymer used as a scaffold for cells is a photo-crosslinkable polymer with both biologically inactive and active properties. This polymer selectively captures hMSCs, enabling the convenient construction of the gel. Specifically, we developed a ternary copolymer consisting of N-(2-hydroxypropyl) acrylamide (HPAm) and carboxymethylbetaine methacrylamide (CMBMAm) with a monomer containing a CD44 binding peptide as a side chain (CD44BPAm), which enabled selective adhesion of hMSCs that specifically interact with CD44 antigens and suppressed non-specific cell adsorption. Furthermore, we induced hMSC differentiation into chondrocytes and performed immunostaining and gene expression analysis of specific marker proteins (aggregan and collagen type II). Using these ternary copolymers (Poly(HPAm-CD44BPAm-BPAm) and Poly(CMBMAm-CD44BPAm-BPAm)), hydrogels could be easily constructed within 5 minutes by UV irradiation (365 nm) (Figure 1).

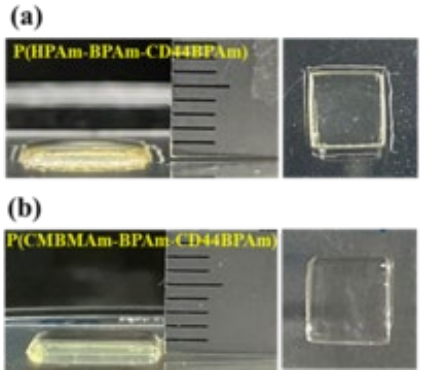


Figure 1. Gel images of the synthesized ternary copolymers. (a) Photocrosslinkable polymer mainly composed of HPAm. (b) Photocrosslinkable polymer mainly composed of CMBMAm.

PoB.16.04

Porous acellular PHB/CHIT based scaffold as a promising biopolymer in treatment of chondral and osteochondral defects

Eva Petrovova[1], Marek Tomco[2], Lenka Luptakova[3], Zuzana Demcisakova[1], Zuzana Tirpakova[3], Lubomir Medvecky[4]

[1]Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovakia. [2]Trauma Surgery and Orthopedics Clinic, Nitra Faculty Hospital, Nitra, Slovakia. [3]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovakia. [4]Institute of Materials Research, The Slovak Academy of Sciences, Kosice, Slovakia

Abstract

Biopolymer composites allow to create an optimal environment for regeneration of chondral and osteochondral defects of articular cartilage, where natural regeneration potential is limited. In recent years, the use of avian chorioallantoic membrane (CAM) has become an integral part of the biocompatibility testing process for developing biomaterials intended for regenerative strategies and tissue engineering applications. We used the chicken ex ovo CAM assay to investigate the angiogenic potential of porous acellular biopolymer polyhydroxybutyrate/chitosan (PHB/CHIT) scaffold, which is intended for the treatment of hard tissue defects. In the next experimental study, the sheep as the large animal model was used for the creation of knee cartilage defects. In the medial part of the trochlea and on the medial condyle of the femur, we created artificial defects (6x3 mm) with microfractures. In four experimental sheep, both defects were subsequently filled with PHB/CHIT based implant. Two sheep had untreated defects. We evaluated quality of the newly formed tissue in the femoral trochlea defect site using imaging (X-ray, CT, MRI), macroscopic and histological methods. The morphological and histochemical analysis showed strong angiogenic potential on the chicken CAM model. The gene expression of pro-angiogenic growth factors, i.e., VEGF-A, ANG-2, and VE-CAD, was upregulated in the PHB/CHIT scaffolds 72 h after the implantation. Using the sheep animal model, macroscopically, the surface of the treated regenerate corresponded to the niveau of the surrounding cartilage. X-ray examination 6 months after the implantation confirmed the restoration of the contour in the subchondral calcified layer and the advanced rate of bone tissue integration. The CT scan revealed a low regenerative potential in the bone zone of defect compared to the cartilage zone. The percentage change in cartilage density at the defect site was not significantly different to the reference area (0.06%-6.4%). MRI examination revealed that the healing osteochondral defect was comparable to the intact cartilage signal on the surface of the defect. Hyaline-like cartilage was observed in the majority of the treated animals, except one where the defect was repaired with fibrocartilage. We conclude that the PHB/CHIT scaffold has a strong endogenous angiogenic potential. The experimental study using the sheep animal model showed that the acellular, chitosan-based biomaterial is a promising biopolymer composite for the treatment of chondral and osteochondral defects of traumatic character. It has the potential for the further clinical testing in the orthopedic field, primarily with the combination of supporting factors.

PoB.16.05

Bioadhesive Microcapsules as a New Cellular Treatment for the Diffuse Cartilage Lesions

Desiré Venegas-Bustos[1], A Vega-Castrillo[1,2], JC Rodríguez-Cabello[1], M Alonso-Rodrigo[1]

[1]University of Valladolid, Valladolid, Spain. [2]Servicio de Traumatología, Hospital Clínico de Valladolid, Valladolid, Spain

Abstract

Diffuse cartilage lesions affect a broad area of the articular cartilage and have a high prevalence as the common type of degenerative processes. Diffuse damage is usually restricted to palliative care or systemic anti-inflammatory treatments up to the point where the majority of the cartilage is lost, when the only option available is the surgical replacement of the arthritic joint with a prosthesis.
Cell-based approaches using autologous chondrocytes or, specially, mesenchymal stem cells (MSCs) have been tested and it can even be found in some clinics. However, the efficacy of such cell-based treatment is controversial where the main problem is the unconfinement of the cells used. In general terms, the applied cells stay in the injection site only for short periods of time, so their regenerative potential is greatly reduced.
In order to resolve the previous task, the general objective of this study is the development of bioadhesive and injectable cell microcarriers with the ability to regenerate the articular cartilage. This aim is materialized in the fabrication of multibiofunctional microcapsules that are able to promote the cell cargo with selective adhesion and location on the articular surface. To do so, special attention is paid to the cell-material interaction on both the inner and outer surface of the microcapsule by means of the inclusion of, on one hand, specific cell adhesion domains and, on the other hand, sequences that promote the adhesiveness to collagen II and chondroitin sulfate, two distinctive components of the extracellular matrix of hyaline cartilage.Liquid overlay technique was used to obtain MSCs spheroids, this method inhibits the attachment of cells to tissue culture plates and promotes cell-cell aggregation. Microcapsules are based on novel advanced and multibiofunctional protein-based polymers, obtained by recombinant DNA technology and named Recombinamers, more specifically a type of them, the Elastin-like Recombinamers (ELRs), designed for this application. In addition, advanced crosslinking chemistry such as ‘catalyst-free click chemistry’ is integrated in MSCs-spheroid encapsulation to produce a reactive layer-by-layer technology. Such coating allows to achieve a nanometer-thin but strong and highly efficient conformational coat on the cell spheroids so a new cell-based therapeutical approach can be developed.

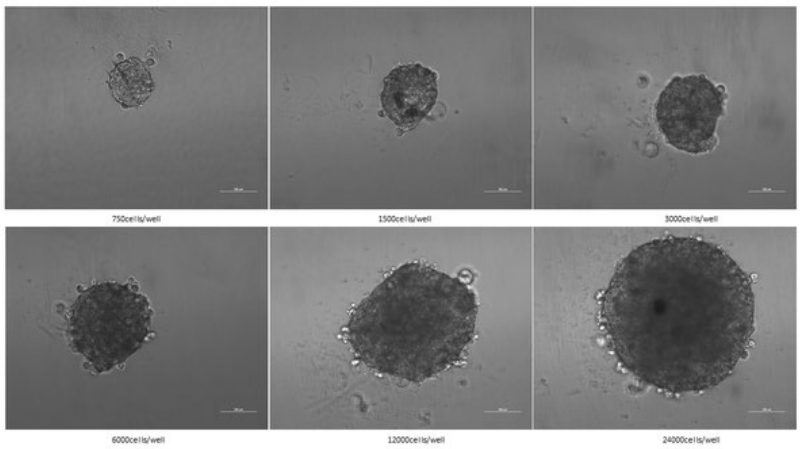


Figure 1. Spheroid formation using hanging drop method and different cell density per well.

PoB.16.06

Braided, Long-Term Biodegradable Scaffolds for Anterior Cruciate Ligament Tissue Engineering: A Biomechanical Investigation

Caroline Emonts, Pauline Riedl, Benedict Bauer, Thomas Gries

Institut für Textiltechnik of RWTH Aachen Univeristy, Aachen, Germany

Abstract

Introduction. Anterior crucaite ligament (ACL) injuries are a common sports injury, with an incidence of 1 in 3500. Tendons and ligaments have limited self-healing capabilities. The current treatment methods rely primarily on autologous implants, which are limited by donor site morbidity, availability, and the need for a second surgery site. Synthetic ligaments are no longer preferred due to their shortcomings in handling long-term mechanical loads, fatigue strength, and friction resistance. Tissue engineering offers a solution by using three-dimensional scaffolds that provide temporary mechanical stability and promote cell ingrowth to regenerate a functional ligament. Among various textile scaffolds, braided scaffolds offer a reproducible and scalable three-dimensional structural design with high strength, efficient loading, and sufficient porosity for cell ingrowth. This study aims to investigate the potential of using long-term degradable scaffolds based on poly-ε-caprolactone (PCL) for ACL replacement, utilizing the round braiding technique. **Methods.** The scaffolds were manufactured using a round braiding machine and melt-spun PCL monofilaments with high-drawing ratio [1]. Five variations of scaffolds, with different combinations of braiding parameters, were examined. To evaluate the effect of various braiding parameters on the maximum tensile load, elongation, and stiffness (linear and toe regions), a uniaxial tensile test in the physiological length of the ACL was conducted (n=10). **Results.** The braided scaffolds exhibit a tensile strength of approximately 4000 N, surpassing the native ACL's tensile strength range of 734-2160 N [2]. The scaffolds are designed to fit within the surgical bone channel diameter of 9 mm. The stiffness and maximum tensile force of the braids are greatly influenced by filament arrangement, braid angle, and braiding pattern. Stiffness is an essential mechanical factor for maintaining the physiological movement of the joint. The linear region stiffness of the braids ranges from 131-236 N/mm, which is comparable to the native human cruciate ligament stiffness range of 180-242 N/mm [2]. **Discussion.** The testing results suggest that the requirements for ACL replacement can be successfully met. Adjusting the braiding parameters could pave the way for developing ligament replacements for medial and lateral knee ligaments or the rotator cuff. Further research will focus on investigating the cell behavior on the scaffolds and the degradation behavior.

PoB.16.07

Development of a Multilayered, Bifunctional Substitute for Osteochondral Tissue Engineering: An *in vitro* study

Deniz Basoz[1,2], Vasif Hasirci[1,3,4], Deniz Yucel[1,3,5]

[1]Acıbadem Mehmet Ali Aydınlar University (ACU), Graduate Department of Biomaterials, Istanbul, Turkey. [2]ACU Biomaterials Center, Istanbul, Turkey. [3]ACU, Biomaterials Center, Istanbul, Turkey. [4]ACU, Faculty of Engineering and Natural Sciences Department of Biomedical Engineering, Istanbul, Turkey. [5]ACU, School of Medicine, Department of Histology and Embryology, Istanbul, Turkey

Abstract

Osteoarthritis, which affects 25% of adults, is characterized by damage to the osteochondral tissue in articulating joints. Osteochondral tissue restoration is difficult due to its complex and heterogeneous structure and lack of vascularization in cartilage. The aim of the study was to develop a multilayered bifunctional osteochondral tissue substitute consisting of bone part with osteogenic cells, calcified cartilage part, cartilage part with chondrogenic cells and a superficial layer. Trilayered PCL scaffold composed of cartilage, calcified cartilage, and bone parts with distinct inner structure was fabricated by 3D-printing. The electrospun mesh was placed over 3D-printed construct as the superficial layer of articular cartilage. Bone part was coated with β-TCP to promote site-specific bone regeneration. SEM results revealed that the different porosity for bone and cartilage parts and non-porous calcified cartilage part was achieved as intended. The compression modulus of bone, cartilage, and entire multilayered scaffold were 61.1±6.2, 38.9±5.3, and 75.7±5.2 MPa, respectively. Osteogenic and chondrogenic cells were differentiated from human dental pulp-derived mesenchymal stem cells (DP-MSCs), which were grown on respective layers of the scaffold. Phalloidin-DAPI staining and MTS results indicated that cells were able to adhere and proliferate on the scaffold. Before co-culture, osteogenic differentiation of the bone part of the scaffold was confirmed by Alizarin Red staining (Fig1). After 21 days of osteogenic differentiation, chondrogenic cells were loaded with collagen-based hydrogel to the cartilage part of the scaffold and then co-cultured for a week. The conservation of cell-specific markers osteopontin and osteonectin by osteogenic cells, and aggrecan and collagen-II by chondrogenic cells within the osteochondral tissue mimic after co-culture was confirmed by immunostaining (Fig2). In addition, both osteogenic and chondrogenic cells proliferated within the scaffold after co-culture. The multilayered osteochondral tissue substitute is expected to meet the desired properties with its heterogenic structure mimicking the native tissue organization with articular cartilage, calcified cartilage, and bone parts. This bifunctional osteochondral tissue substitute has potential to be tested in *in vivo* studies and could be a promising approach to the treatment of osteochondral defects in clinic.

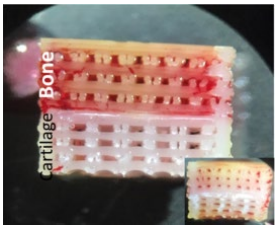


Figure 1. Alizarin Red staining of the multilayer scaffold after DP-MSCs were seeded on bone part and cultured in osteogenic induction medium and in growth medium (inset) for 21 days (control).

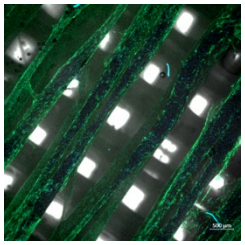


Figure 2. Confocal micrograph of the osteogenic cells differentiated from DP-MSCs on the bone part of the multilayer scaffold after immunostaining against osteonectin (green) and counterstained with DAPI for the nucleus (blue).

PoB.16.08

Analysing the impact of tendon biomimetic scaffold morphology and chemical composition on immunological recognition

Sara Gil Cantero[1], Francesco Iorio[2], Irem Unalan[2], Veronica Pinnaro'[1], Sarojinidevi Künig[1], Claus Wenhardt[1], Peter Steinberger[1], Aldo R Boccaccini[2], Johannes Stöckl[1]

[1]Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Medical University of Vienna, Vienna, Austria. [2]Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nürnberg, Erlangen, Germany

Abstract

Tendinopathies are a major worldwide clinical problem which causes economic, social and quality life burdens. In this context, the development of tendon biomimetic scaffolds is constituted as a promising, therapeutical approach. However, to be clinically effective, scaffolds should avoid immunological recognition.

It has been well described that scaffold composed by aligned fibers lead to a better tenocyte differentiation, vitality, proliferation and motility. However, little has been studied regarding the impact of this fiber spatial distribution on the recognition by immune cells.

Polycaprolactone (PCL) is a versatile, biocompatible and biodegradable synthetic polymer, which shows good mechanical properties for tendon application. However, it is highly hydrophobic, worsening the cellular attachment. Hyaluronic acid (HA) has been described to rise PCL hydrophilicity; to improve tenocyte proliferation, vitality and motility; and to reduce peripheral tissue adhesion, which would lead to loss of tendon mobility.

In this study, both components have been combined and electrospun at different ratios to generate scaffolds with different morphology and hydrophobicity. The impact of these properties on immunological recognition has been assessed.

For that purpose, different concentrations of PCL (12 or 20%) and HA (0 or 0,5 or 0,75%) were used to obtained nano and micro fibers; as well as increasing hydrophilic values, respectively. Random and aligned fibers' distributions were generated. Their morphological, mechanical and hydrophilic properties were tested, as well as the macrophage and dendritic cell-driven biodegradability. Immunological recognition was addressed by culturing Jurkat cells expressing human Toll-like receptors (TLRs) (2/1, 2/6, 6, 4) on the electrospun PCL-HA-based scaffolds. These cells express GFP via NFkß promoter activation when being triggered. GFP fluorescence intensity was measured by flow cytometry.

Our results show that 0,5% of HA leads to a decrease in the fiber diameter, while a higher incorporation of HA results in bigger fibers. The addition of HA increases the hydrophilicity by 20-50%, depending on the alignment of the fibers. Regarding the immunological performance, biodegradability seems not to be affected by scaffold composition or fiber orientation. Besides, TLR 4 signaling pathway seems to be more triggered by higher PCL and HA concentrations, as well as random spatial distribution of the fibers.

In conclusion, the alignment of the fibers not only lead to better tenocyte differentiation, but also prevents immunological recognition of the scaffolds. Additionally, TLR reporter cells have shown to be a useful and effective tool to study the interaction between scaffolds and the immune system.

PoB.16.09

Core-Shell Microgels as a Platform for the Chondrogenic Differentiation of Mesenchymal Stem Cells

Chun-Chi Yang, Chien-Wen Jeff Chang

National Tsing Hua University, Hsinchu, Taiwan

Abstract

Co-culturing stem cells and chondrocytes can promote the chondrogenic differentiation of stem cells and provide a promising approach for cartilage repair. However, there are still challenges to overcome, such as cell separation and distance between the two cell types. In this study, a core-shell microgel co-culture system was proposed and fabricated using a microfluidic device for human mesenchymal stem cells (hMSCs) and porcine chondrocytes (pCHs). The hMSCs formed cell aggregates in the core area of the microgel, enhancing the differentiation ability of the stem cells. Furthermore, the hMSCs and pCHs were spatially separated by the core-shell structure, while maintaining a short distance between them to enhance the exchange of growth factors and the use of allogeneic chondrocytes.

The microgels' core-shell structure was easily regulated by tuning the flow rate ratio of each solution, and the encapsulated hMSCs exhibited high viability in the microgel, forming an aggregate. Co-culturing hMSCs and pCHs in the microgels significantly increased the chondrogenic differentiation efficacy of hMSCs.

The method for preparing the miniaturized stem cell-chondrocyte co-culture system involved injecting the hMSCs and pCHs/alginate solutions into the microfluidic device. The formed microgels were soaked in calcium chloride-containing medium for the gelation of the alginate shell. Morphology of the encapsulated stem cells was observed using Actin/Hoechst staining, showing that the stem cells were well dispersed in the core area initially, forming a single aggregate thereafter and gradually growing to form aggregates.

The proposed core-shell microgels co-culture system was examined using biochemical assay, immunohistology staining, and qPCR, which confirmed that pCHs co-encapsulation significantly enhanced the chondrogenic differentiation of hMSCs. The results demonstrated that the core-shell microgels could be utilized for the generation of stem cell spheroids and their further differentiation, achieving better efficacy of chondrogenic differentiation in animal or clinical applications. This miniaturized stem cell-chondrocyte co-culture system may be further applied for cartilage repair in the future.

PoB.16.10

Novel, biocompatible thermoplastic polyurethanes and their aligned porous scaffolds for uterosacral ligament reconstruction

Charlie Bateman, Biqiong Chen

Queen's University Belfast, Belfast, United Kingdom

Abstract

Pelvic floor dysfunction is a major health issue for older women with over 10% undergoing some form of operation for pelvic organ prolapse. Tissue engineering is an attractive strategy for the reconstruction of uterosacral ligaments (USLs). The overall aim of this research is to synthesize novel, biocompatible and biodegradable polymer with similar mechanical properties to those of USL tissue and use the polymer to prepare aligned porous scaffolds suitable for cell growth in USL tissue reconstruction. A new thermoplastic polyurethane (TPU) was synthesized in one step from polycaprolactone diol, polyethylene glycol and l-lysine diisocyanate (LDI) without the addition of any solvent or catalyst. LDI was used in place of commonly used, aromatic diisocyanates, in order to prepare a TPU with non-toxic degradation products.

Different reaction times, namely 24 (TPU-24) and 48 hours (TPU-48), were used in the synthesis. The formation of TPUs and the complete reaction between hydroxyl and isocyanate groups were confirmed by Fourier transform infrared spectroscopy and nuclear magnetic resonance. The molecular weights of the polymers were characterized by gel permeation chromatography. Differential scanning calorimetry gave the glass transition temperatures of the polymers below 0 °C indicating that the TPUs would be rubbery at room temperature.

The TPUs have a yield strength and yield strain in the range of 5.9 - 6 MPa and 60 - 290% respectively in their dry state. Testing TPU-24, swollen in a phosphate buffered saline (PBS) solution, giving a yield strength of 6.6 MPa, similar to that of USL tissue, and a tensile strength of 12.1 MPa. The strain at break of the swollen sample increased from 54% to 2951%. This is because the water in the PBS solution acted as a plasticiser, allowing the polymer chains to move more freely under load. A biodegradation study showed the polymer degraded by 2.4 % after six months' incubation in a PBS solution containing lipase enzyme at 37 °C and 100 rpm.

TPU scaffolds with aligned porous structure mimicking the aligned fibrous structure in USLs, were successfully produced by using a freeze-drying method and a custom-made mould, and the structure was confirmed by scanning electron microscopy. These new biocompatible and biodegradable TPUs show potential to be used in future USL tissue repair.

PoB.16.11

Designing an immunomodulatory, biomimetic ACL graft based on cellulose nanostructures

Matthew A Graham[1], Julie Gough[1], Leela Biant[1,2], Ahu Dumanli[1]

[1]University of Manchester, Manchester, United Kingdom. [2]Manchester University NHS Foundation Trust, Manchester, United Kingdom

Abstract

The structure of the Anterior Cruciate Ligament (ACL) is largely defined by its hierarchical arrangement of type I collagen: fibrils (tens of nm diameter) are assembled into fibres (tens of µm) which are bundled into fascicles (hundreds of µm). This structure endows the tissue with its unique mechanical properties of high strength, stiffness, toughness, and viscoelasticity. The gold-standard treatment for ACL rupture is an autologous tendon graft, despite a nine-month rehabilitation process with disappointing long-term results, whilst synthetic polymer grafts have been limited by chronic inflammation. Future ACL grafts should therefore offer the following improvements: (1) Confer rapid stability to the knee, (2) Integrate with the body, avoiding an inflammatory immune response. To address these material requirements we aim to fabricate a biomimetic graft based on bacterial cellulose (BC), a crystalline polysaccharide exhibiting a hierarchical structure similar to that of ligament tissue. Chemical and mechanical treatments can liberate the individual nanofibrils, referred to as bacterial cellulose nanofibrils (BCNF). Bacterial cellulose nanofibrils (BCNF) have impressive mechanical properties with similar dimensions to collagen fibrils, and can be functionalised with peptides to modulate specific cellular responses. For example, DGEA has been shown to drive macrophages towards an anti-inflammatory M2 phenotype.

In this work, we functionalised the surface of bacterial cellulose with carboxylate groups via TEMPO-mediated oxidation. The resulting negative charge on the surface of the fibrils allows for their effective mechanical fibrillation, with AFM images confirming their morphological similarity to collagen fibrils (figure 1). We dispersed BCNF into aqueous carboxymethyl-cellulose (CMC) solutions. Rheological analysis confirmed the shear thinning properties of the solutions, with viscosity increasing with concentration of BCNF. To recreate the hierarchical structure of ligament tissue, BCNF-CMC solutions were spun into an acetone coagulation bath, producing hierarchically structured, nano-composite fibres. SAXS, SEM, and tensile testing methods were used to analyse the nanofibrillar alignment, crystallinity, morphology, and mechanical properties of the fibres, with promising alignment of the nanofibrils along the fibre axis. In order to mimic the biological environment of the ACL, the surface of the BCNF were further modified through the conjugation of peptides RGD and DGEA via a 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) cross-linking mechanism, and cell response assessed via macrophage and fibroblast morphology.

Overall, mimicking the hierarchical structure and biological environment of the ACL appears to be a promising approach to replicating its mechanical and biological performance.

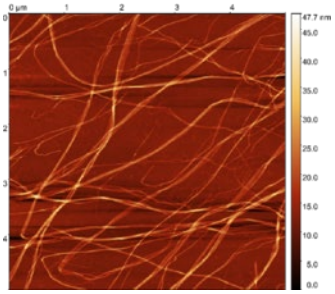


Figure 1: AFM image of isolated BCNF (average diameter 34.60 ± 9.43 nm).

PoB.16.12

Biofabrication of a 3D model of Human Skeletal Muscle to study muscular fibrosis.

Riccardo Francescato[1,2,3], Giuseppe Talò[4], Megi Ishmaku[1,3], Simone Bersini[1,2,5], Matteo Moretti[1,2,4,6]

[1]Regenerative Medicine Technologies Laboratory, Ente Ospedaliero Cantonale (EOC), Bellinzona, Switzerland. [2]Service of Orthopaedics and Traumatology, Department of Surgery (EOC), Lugano, Switzerland. [3]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milano, Italy. [4]Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milano, Italy. [5]Euler Institute, Biomedical Sciences Faculty, Università della Svizzera Italiana, Lugano, Switzerland. [6]Euler Institute, Biomedical Sciences Faculty, Università della Svizzera Italiana, Lugano, Italy

Abstract

Although fibrosis is a relevant disease that can affect all organs, our knowledge of the biological mechanisms regulating the onset and progression of the disease is still limited and just a few therapeutic treatments are currently available. Skeletal muscle fibrosis is a hallmark of physiological muscle aging and incurable muscle diseases including dystrophies. Current pre-clinical *in vitro* models do not faithfully recapitulate the human fibrotic muscle microenvironment, since they neglect key cellular populations (e.g. blood vessels, immune cells, mesenchymal progenitors) that play a central role during disease progression. More in detail, endothelial cells (ECs) are known to contribute to the pathology via the acquisition of fibrogenic traits in a process named Endothelial-Mesenchymal Transition (Endo-MT). Hence, the development of more relevant disease models better mimicking the fibrotic muscle microenvironment could help understanding the complex pathological mechanisms of fibrosis and identify new therapeutic solutions.

Here, we aim at biofabricating a human fibrotic skeletal muscle model where a suspended myobundle is surrounded by a stromal microenvironment. This model is used to investigate the Endo-MT promoting properties of Duchenne Muscle Dystrophy (DMD) fibroblasts and M1-macrophages.

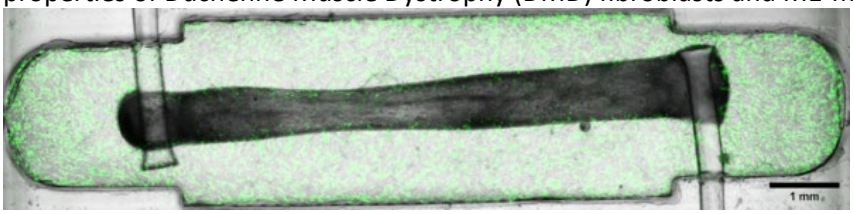


Figure 1. *Muscle bundle (brightfield) co-cultured with GFP-microvascular ECs.*

A 1 cm long, free-standing myobundle was biofabricated using tissue engineering techniques such as Microfluidics and Replica Molding approaches (Fig.1). The myobundle has a diameter of about 1 mm and was cultured for up to one month showing the formation of multinucleated myotubes and the expression of key muscle-specific markers (e.g. dystrophin, desmin, αSMA). An electrical stimulation system was developed and multiple training programs were tested changing parameters such as electrodes distance, voltage, frequency, duration and stimulation pattern. A 3D extracellular matrix containing microvascular ECs combined with muscle-specific fibroblasts (healthy or DMD) or M1-macrophages was cast in the device leading to the formation of microphysiological environments for the study of EndoMT (Fig.2).

Molecular analyses to investigate expression changes of genes and proteins relevant for EndoMT are currently being performed by Flow Cytometry and RT-qPCR to evaluate whether fibrogenic activation is mainly prompted by pro-inflammatory or fibrogenic cells. Once validated, this technology will allow to study the interactions among different cell populations in the context of muscle fibrosis through a

combination of optical, molecular and functional analyses. Importantly, it will allow the screening of candidate compounds targeting fibrosis.

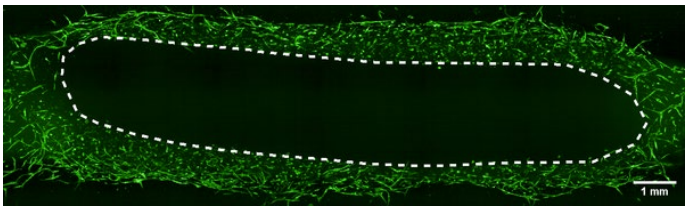


Figure 2. GFP-microvascular ECs forming a vascular network surrounding a muscle bundle (white dashed line).

PoB.16.13

Alginate-based biomaterials for the formation of 3D bovine muscle tissue

Sergio Spaans, Lea Melzener, Guy Wijler, Nicolas Hauck, André Pötgens, Mark Post, Arın Doğan

Mosa Meat B.V., Maastricht, Netherlands

Abstract

At Mosa Meat we aim to produce cruelty-free and tissue-engineered meat from cells obtained from a small biopsy from cows. One of our main processes is the creation of bioartificial muscle (BAM) tissue, which should resemble real muscle to be able to have the same food-related benefits as meat. For the generation of BAMs, satellite cells, which are adult skeletal muscle stem cells, are isolated, proliferated and differentiated within a three-dimensional (3D) synthetic extracellular matrix (ECM). The cellular 3D environment is crucial as it recapitulates certain key aspects of the *in vivo* physiology and improves cell differentiation compared to 2D cell culture. To produce bovine BAMs for cultured meat, the ECM-like biomaterial should be animal-free, inexpensive and edible. Alginate polymers, naturally derived polysaccharides isolated from brown algae, have been extensively studied by us and were found to be a promising candidate for the generation of BAMs. To generate mature BAMs, the physical and mechanical cues of the alginate hydrogel matrix need to be optimized. To achieve this, we modified alginate with cell-adhesive ligands based on well-known RGD peptides and studied 3D differentiation of satellite cells into BAMs. By using different gelation techniques we were able to rapidly crosslink alginate in physiological conditions, which resulted in successful encapsulation of satellite cells. During the 7-day tissue culture, BAMs showed decrease in volume and decrease in transparency, which is the result of compaction and the binding and remodeling of cells to RGD-peptides inside the hydrogel (Figure 1A). An increase in total protein was observed during the 7-day culture (Figure 1B). Normalized for DNA, protein increased from day 1 to day 3 and remained constant up to day 7 (Figure 1B). These findings show an increase of protein per cell during a 7-day culture period, which is mainly due to myogenic differentiation, shown by the increase in slow myosin and α-Actin-1 expression (Figure 1C & D). With this work we show the possibilty to produce 3D differentiated muscle tissue using alginate-based biomaterials.

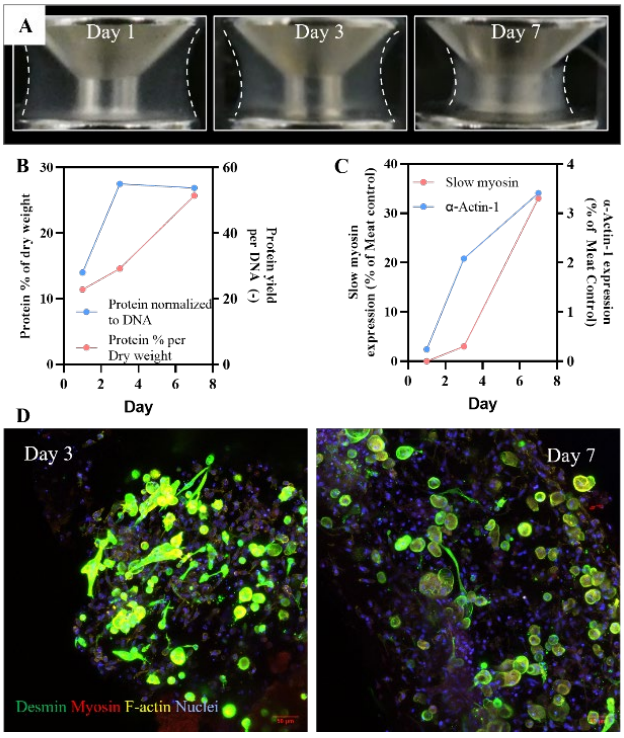


Figure 1. *A) Macroscopic images of BAMs and degree of compaction, which is highlighted by dotted lines. B) Total protein quantification, which is normalized for dry mass (left y-axis) and DNA (right y-axis). C) Slow myosin (left y-axis) and α-Actin-1 (right y-axis) quantification using ELISA and normalization to a meat control) (each point represents a pool of 4 BAMs). D) Confocal images of BAMs on day 3 and 7 showing muscle protein expression.*

PoB.16.14

Resorbable high-strength suture for orthopedic fixation of soft tissue

Benedict Bauer, Caroline Emonts, Louisa Bonten, Thomas Gries

Institut für Textiltechnik, RWTH Aachen University, Aachen, Germany

Abstract

Introduction. Addressing the current deficits of existing solutions (e.g. autografts, allografts or non-resorbable synthetic devices) tissue engineering approaches for tendons and ligaments have received considerable attention. Current fixation systems are either not degradable or not matching the slow degradation rate of tendons/ligaments. Herein, the feasibility of fabricating a respective (slowly) degradable orthopedic suture is investigated based on high-strength polycaprolactone (PCL) fibers which are consequently braided. **Experimental Methods.** PCL filaments were melt-spun from pellets (Capa® 6800, Ingevity, USA) using a single-screw spinning plant (Fourné Maschinenbau, Germany). Monofilaments and multifilaments were fabricated with varying fineness to investigate the impact on the compactness of the resulting braided suture. Fineness was determined according to DIN EN 13392. Mechanical properties were tested in uniaxial tensile tests according to DIN EN 13895 and DIN EN ISO 2062. PCL fibers were multilayer-braided using a 48-carrier circular braiding machine (HS80/48, Steeger, Germany). Suture morphology was examined using light microscopy while mechanical properties were investigated in uniaxial tensile tests. **Results And Discussion.** PCL filaments were melt-spun as monofilaments and multifilaments aiming at maximum suture tensile strength. PCL monofilaments and multifilaments were fabricated in fineness of 100-275dtex and 60-370dtex, respectively. Monofilaments exhibited high tensile strength with 60-73cN/tex (675-840MPa) while multifilaments reached slightly less. Orthopedic sutures were braided from the different filaments by varying filament type, total number of filaments, filament distribution on layers and braiding angle. The resulting structures showed strongly varying diameters and breaking loads. The highest tensile strength (~260MPa) was achieved by a core-sheath combination of 24 monofilaments (~275dtex) as core and sheath consisting of 16 monofilaments (100dtex). The tensile load's ~280N. **Conclusion.** To investigate the feasibility of fabricating a high-strength long-term bioresorbable orthopedic suture, PCL filaments of different yarn structures and fineness were melt-spun achieving excellent mechanical properties and consequently braided using various combinations. Exhibiting ~280N, the resulting orthopedic suture appears sufficiently strong for applications such as rotator cuff repair where failure loads are usually ranging between 200N-300N using two sets of sutures or 300-520N using 4 sets [1]. Using the slowly degradable PCL, the orthopedic suture is ideal for slow-healing tissue such as tendons and ligaments. Exploiting the inherent flexibility of textile processes, it is tailorable to match various applications.

Acknowledgement
We thank the German Federal Ministry for Economic Affairs and Climate Action for funding this research (KK5055951MR3) within the framework of the Central Innovation Program for SMEs.

References

1. Kim, D.H et al. doi:10.1177/0363546505281238.

PoB.16.15

Development of Imaging System of Extracellular Matrix Using Multiple Wavelength Lights for Cultured Cartilage

Shota Minami[1], Koji Yamamoto[2], Yusuke Morita[2]

[1]Graduate School of Life and Medical Scienes, Doshisha University, Kyoto, Japan. [2]Department of Biomedical Engineering, Doshisha University, Kyoto, Japan

Abstract

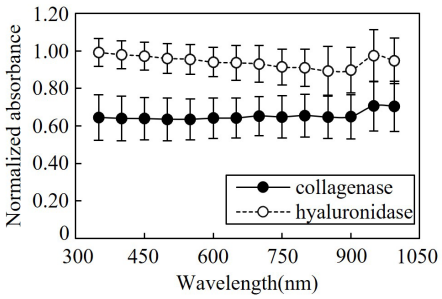


Fig. 1. Result of the normalized absorbance. (mean ± S.D., n = 5).

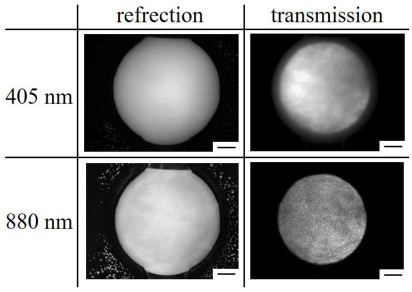


Fig. 2. Acquired image of cartilage (Bar: 1 mm)

Since articular cartilage has superior mechanical function, it is important to evaluate mechanical properties of cultured cartilage. Compression test that generally quantify mechanical properties is destructive method and may damage the cultured cartilage. Therefore, it is necessary to develop a nondestructive evaluation method for cultured cartilage. Mechanical property of cultured cartilage is associated with amount and distribution of extracellular matrix such as collagen and proteoglycan (PG). Since synthesized extracellular matrix might change optical properties of cultured cartilage, it is expected that the synthesized amount and uniformity of extracellular matrix can be detected by acquired images with reflection and transmission of the specific wavelength lights. The purpose of this study was to develop the imaging system by using multiple wavelength lights to evaluate maturity and homogeneity of cultured cartilage nondestructively.

The developed imaging system was composed of the CCD camera, the ring-shaped light and transmittance light source with several LED. Wavelength of LED light was optimized for collagen and PG evaluation by measuring absorbance of cartilages having the different composition of collagen and PG. Disk cartilages (5 mm diameter) cut out from patera glove of a 6-month-old pig were sliced in 100 µm thickness, and digested for 3 hours with collagenase and hyaluronidase solution. Absorbance of sliced cartilage was measured with a spectrophotometer in the range of 350 to 995 nm before and after enzyme treatment.

Normalized absorbance was calculated by dividing the absorbance of the enzyme treated cartilage slice by the absorbance of the cartilage slice before enzyme treatment at each wavelength for collagenase and hyaluronidase treatment. Figure 1 shows normalized absorbance of for collagenase and hyaluronidase treatment. The normalized absorbance for collagenase treatment showed lower value in a range of 400 to 600 nm. The normalized absorbance for hyaluronidase treatment showed lower value in a range of 800 to 900 nm. According to ratio of changes in the normalized absorbances for collagenase and hyaluronidase treatment, LED wavelengths were determined 405 nm and 880 nm to observe change in collagen and PG. Figure 2 shows acquired images of cartilage disk (6 mm diameter) with reflection and transmission lights of the both wavelength. Since different images were acquired using the two wavelengths for same cartilage disk, it is expected that the synthesized amount and uniformity of extracellular matrix can be evaluated by analyzing the acquired images of cartilage with the two different wavelength lights.

PoB.16.16

Effects of proliferation period on myoblast differentiation for tissue-engineered skeletal muscle fiber

Nao Takahashi[1], Koji Yamamoto[2], Yusuke Morita[2]

[1]Graduate School of Life and Medical Sciences, Doshisha University, Kyoto, Japan. [2]Department of Biomedical Engineering, Doshisha University, Kyoto, Japan

Abstract

Many studies on tissue-engineered skeletal muscle have been conducted for regeneration of large-scale muscle damages. Tissue-engineered skeletal muscle with high contractile force and large contraction is required to recover mechanical function of skeletal muscle. Since skeletal muscle has fibrous structure and highly oriented myotube, alignment of differentiated myotube along longitudinal direction is considered to contribute efficient contraction of tissue-engineered skeletal muscle. Therefore, we focused on the fiber-shaped structure to fabricate the tissue-engineered skeletal muscle. It is expected that limitation of myoblast proliferation and cell growth in the tissue-engineered skeletal muscle fiber might promote fusion and differentiation of myoblast continuously and uniformly along longitudinal direction. Moreover, differentiation and fusion of myoblasts associate with differentiation might depend on cell-to-cell adhesion with proliferation to obtain contractile functions. Therefore, it is necessary to clarify effects of the proliferation period on cell differentiation to fabricate the tissue-engineered skeletal muscle with high contraction force.

The spinning solution was prepared by mixing 2.4 w/v% type I collagen solution and C2C12 cell suspension at a cell density of 5.0×106 cells/ml. The spinning solution was extruded from the nozzle with inner diameter of 510 µm into DMEM high glucose at 37°C. After tissue-engineered skeletal muscle fibers were cultured in DMEM high glucose containing 10% FBS for the proliferation period with both ends fixed, they were cultured in DMEM high glucose containing 7% horse serum for 12 days as the differentiation period to enhance differentiation of C2C12 myoblast to C2C12 myotubes. The proliferation periods were 2, 4, and 6days.

Morphological change of the tissue-engineered skeletal muscle fiber was observed by using optical microscope during cultivation. Proliferation and alignment of myoblast along longitudinal direction of the tissue-engineered skeletal muscle fiber were evaluated by histological observation with HE staining after cultivation. In order to evaluate differentiation of myoblast, myosin heavy chain expression was detected by fluorescent observation and calcium influx under electrical pulse stimulation was measured with fluo-4 calcium indicator after cultivation.

PoB.16.17

Effect of electrical stimulation on contractility and myokine secretion from tissue-engineered skeletal muscle

Kaito SASAKI, Tomohiro NAKAMURA, Toshia FUJISATO

Osaka Institute of Technology, Osaka, Japan

Abstract

Skeletal muscle tissue is composed of multinucleated skeletal muscle cells and is often thought of as simply a tissue responsible for physical movement of the body. However, it also has important physiological functions such as protein storage, glucose metabolism, and the maintenance of body temperature. Also, it is highly sensitive to external factors and changes muscle mass, myofiber type (fast- or slow-muscle type), and metabolic parameters. In addition, it has recently been revealed that skeletal muscle secretes various biologically active substances called myokines which contribute to health in response to exercise. We have produced 3D tissue-engineered skeletal muscle (TEM) that can contract and move by electrical stimulation (ES). ES has been generally shown to improve contractility and myokine production of TEM, however long-term continuous ES may decrease contractility. In this study, we measured the contractility and secretion of interleukin-6 (IL-6), one of myokine, during short-term periodical ES.

The TEM was made by ollagcen gel containing C2C12 mouse myoblast cell line. It was cultured in growth medium for 2 days and then in differentiation medium (DM) for 2 weeks. To apply ES to TEM during culture, electrode holding jig was fabricated by 3D printer and Pt electrodes were attached on the jig to be placed both sides of TEM. The jig and wirings were placed at a position where they did not touch the culture medium. After 2 weeks culture by DM, the ES was applied 1 or 3 hours per day for 1 week. After the end of ES, culture medium was collected and the amount of IL-6 were measured using the IL-6 EIA Assay Kit.

The contractility and IL-6 concentration tended to increase with ES compared with control samples without ES. In addition, the contractility with short-term periodical ES showed higher than that with long-term continuous ES. This may be due to the efficient supply of nutrition. Furthermore, IL-6 concentrations tended to increase as contractility increased. This suggests that IL-6 secretion may have some correlation with contractility. In the future study, the most effective ES condition for contractility and IL-6 secretion would be determined.

PoB.16.18

Injectable thermo-sensitive interpenetrated network based on chitosan hydrogel for intervertebral disc repair

Inès Hamouda[1,2], Francesco Touani Kameni[2,3], Atma Adoungotchodo[1,2], Sophie Lerouge[1,2]

[1]Ecole de Technologie Supérieure, Montréal, Canada. [2]CRCHUM, Montréal, Canada. [3]Université de Montréal, Montréal, Canada

Abstract

Chitosan thermosensitive hydrogels are promising candidates as scaffolds for cell therapy, tissue engineering and bioprinting application due to their in situ gelation, high biocompatibility and controlled biodegradability [1,2]. However, while chitosan presents good biocompatibility towards a wide range of cell lines, it has poor cell adhesion and the proliferation is limited over days. Moreover, these physical chitosan hydrogels are not stable in serum, which limits *in vitro* experiments and there is a need to further increase their mechanical properties for some applications. In order to overcome these limitations, in this work, we investigate how gelatin can be added to form either a semi- or an interpenetrated network (IPN) as an injectable thermo-sensitive hydrogel for intervertebral disc repair.

Chitosan-gelatin hydrogels were prepared with a mix of β-glycerol phosphate and sodium hydrogenate carbonate (to form the chitosan physical network) and microbial transglutaminase (to chemically crosslink gelatin). Injectability, kinetics of crosslinking and mechanical properties were studied by rheology and cyclic compression tests. The structure of the hydrogels network was observed in confocal microscopy and histology. Their swelling and stability in various media (PBS and cell culture media) were monitored over time at 37°C. Cell survival, adhesion, proliferation and mechanical properties of cell-loaded scaffolds were evaluated on human Mesenchymal Stem Cells through live/dead and Alamar blue assays over 21 days.

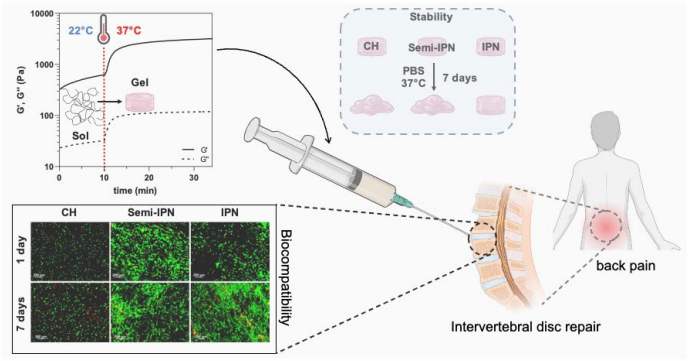


Figure 1: In situ gelling injectable thermo-sensitive hydrogel based on chitosan-gelatin biopolymer for intervertebral disc regeneration

The addition of gelatin to the chitosan hydrogel led to increase twice the storage modulus of the final hydrogel (from 10 kPa for chitosan hydrogel to 20 kPa for IPN). By chemically crosslinking the gelatin, stronger and stiffer hydrogels are obtained with secant young modulus values of 45 kPa compared to 25 kPa for chitosan. Furthermore, the resulting IPN hydrogels were more stable in physiological conditions over 7 days (stable secant young modulus) than chitosan or semi-IPN hydrogel. Moreover, drastic increase in cell adhesion and proliferation was observed within semi and IPN hydrogel than chitosan alone, followed by an increase of the scaffold rigidity over 21 days, which can be related to the production of extracellular matrix by the cells within the hydrogel.

This developed in situ gelling bulk hydrogel based on natural polymer is suitable for the treatment of degenerated intervertebral disc due to its similar mechanical properties and ability to support cell growth without degrading in physiological conditions (Figure 1).

PoB.16.19

Characterization of heparin-conjugated poly(ε-caprolactone)/gelatin aligned nanofibers for tendon tissue engineering

Francesco Iorio[1,2], Mohammad El Khatib[2], Valentina Russo[2], Barbara Barboni[2], Aldo R. Boccaccini[1]

[1]Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany. [2]Faculty of Bioscience and Agro-Food and Environmental Technology, Unit of Basic and Applied Biosciences, University of Teramo, Teramo, Italy

Abstract

Introduction: In the scope of tendon tissue engineering, biomimetic scaffolds mimicking the architecture of native healthy tissue and providing the conjugation of biologically active molecules are required. To this extent, the electrospinning technique offers a versatile approach for the fabrication of aligned fibrous scaffolds with desirable properties for tendon regeneration purposes[1]. Blending poly(ε-caprolactone) (PCL) and gelatin (Gel) results in the production of fibers which can be further functionalized for the conjugation of heparin (Hep), a glycosaminoglycan with high affinity for growth factors and a role in multiple biological processes, among which immunomodulation, response to injury and signaling pathways essentials to cell growth and differentiation[2].
Methods: A 25% (w/v) PCL/Gel solution, in a blend ratio of 2:1, was prepared in a mixture of acetic acid/formic acid and electrospun on a rotating collector. Crosslinking was carried out via EDC/NHS chemistry, and its duration was optimized by testing different incubation times. As previously described, Hep was conjugated on residual amino groups in MES buffer in presence of EDC/NHS[3]. The material was characterized with regards to fibers morphology, size distribution, pores size, surface chemical groups, crosslinking degree, and amount of conjugated Hep. In expectation of performing an *in vitro* biological investigation of the material, the viability of ovine Amniotic Epithelial Cells (oAECs) will be evaluated through MTT assay and live/dead cell counting.
Results: Uniform and free of defects aligned fibers were produced, with a diameter size in the nanoscale. The conjugation of Hep, at all examined crosslinking times, did not alter the morphology of the fibers. A crosslinking time of 15 minutes was identified as the most suitable one, yielding an amount of conjugated Hep equal to 15 µg/mL per mg of material.
Conclusion: Aligned nanofibers from PCL/Gel were successfully fabricated via the electrospinning technique in a benign solvent system. Hep was successfully conjugated onto crosslinked fibers at all investigated crosslinking times, providing a tissue engineering construct for further studies on the biological role of Hep in tendon regeneration and for potential drug delivery applications.
Acknowledgment
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Maria Skłodowska-Curie Grant Agreement N° 955685

References

[1] Sensini, Cristofolini. Materials. 2018, 11(10):1963. DOI 10.3390/ma11101963.
[2] Ling et al. Gene. 2016, 15;576(1 Pt 2):292-303. DOI 10.1016/j.gene.2015.10.039.
[3] Lee et al. Biomaterials. 2012, 33(28):6709-20. DOI 10.1016/j.biomaterials.2012.06.017.

PoB.16.20

A Chitosan-based injectable hydrogel for drug delivery and viscosupplementation in the treatment of temporomandibular joint disorders

Henry Chijcheapaza-Flores[1,2], Florent Barry[1,3], Marie Béret[3], Nicolas Tabary[2], Frédéric Cazaux[2], Feng Chai[1], Nicolas Blanchemain[1], Bernard Martel[2], Romain Nicot[1,3], Maria José Garcia-Fernandez[1]

[1]University of Lille, INSERM, U1008 - Advanced Drug Delivery Systems, F-59000 Lille, France. [2]University of Lille, CNRS, INRAE, Centrale Lille, UMR 8207 – UMET – Unité Matériaux et transformations, F-59000 Lille, France. [3]University of Lille, CHU Lille, Department of Oral and Maxillofacial Surgery, F-59000 Lille, France

Abstract

Temporomandibular joint disorders (TMJD), including disc displacements and degenerative joint disease such as osteoarthritis, affect 31 % of the global population. The main symptoms of osteoarthritis concern the restricted jaw movement and pain, partly due to degradation of hyaluronic acid present inside the articular disc, leading to disc displacement and cartilage degradation. Several treatments have been used to manage the pain and decrease the progression of degenerative joint disease, such as for example viscosupplementation and Non-Steroidal Anti-inflammatory Drugs (NSAID). However, treatments are limited by their short-term therapeutic activity (quick degradation) and side effects. This work aimed to develop an innovative intraarticular drug delivery system, merging the properties of a viscosupplementation hydrogel, based on chitosan (CHT) and a polymer of cyclodextrin (PCD), with the pharmacological activity of naproxen (NX, NSAID drug). Furthermore, the results presented in this work go from the *in vitro* development of the hydrogel up to the proof of concept in an *in vivo* model of TMJ disorder.

First, the viscoelastic and lubricant properties of the injectable hydrogel were evaluated and compared to a 1% sodium hyaluronate solution (Ostenil®, TRB Chemedica, Switzerland). The viscoelastic characterization proved the higher elasticity (tan δ=0.81) and viscosity (η= 200 Pa.s) of the CHT/PCD formulation compared to the control (tan δ=0.95, η =15 Pa.s). Furthermore, the tribological study in a PTFE-PTFE pin-on-disk model proved the lubricant properties of the hydrogel. Subsequently, the hydrogel potential as a drug delivery system was studied, the CHT/PCD/NX hydrogel was tested in a USP I dissolution apparatus (37°C, PBS pH 7.4) and a maximum drug release (94 ± 2%) was observed after 36 h. The *in vivo* test consisted of the pain assessment for 30 days in a monoiodoacetate (MIA)-induced TMJ osteoarthritis model in rats. ANOVA demonstrated a significantly lower sensitivity value ($p<0.05$) for rats randomly treated with the CHT/PCD and CHT/PCD/NX formulation compared to placebo (saline solution) from day 7 to day 21. No toxic effects were observed during the study.

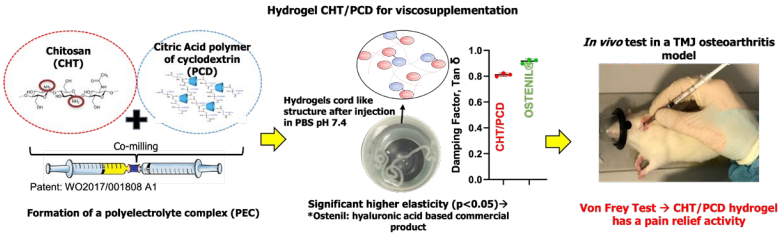


Figure 1: Hydrogel preparation, damping factor values (tan δ: G"/G') comparison to a commercial product, and hydrogel injection in a TMJ osteoarthritis model in rats.

To conclude, a proof of concept was obtained at the end of this work. Results demonstrate the efficacy of this approach by using an injectable hydrogel (polyelectrolyte complex) to lubricate and to control the drug release of NX. Hereafter, the next steps will consist of the validation of its use in other joints and the study of other drug candidates for their use in other osteoarticular disorders.

PoB.16.21

Understanding Soluble Silicate Species (Si) Interactions with Bone Cells

Joel Turner[1,2], Adriana Radu[1,2], Lloyd Zheng[2], Azadeh Rezaei[1], Julian R Jones[2], Gavin Jell[1]

[1]University College London, London, United Kingdom. [2]Imperial College London, London, United Kingdom

Abstract

Introduction. Despite 50 years of silicate bioactive glasses (SBG) research[1,2], there remains a lack of understanding of how silicate species (Si) released from the SBGs influence cellular responses. This includes how Si is internalised, excreted, and the localisation of these ions. With an aim to optimise SBG ion release rates for more precise control of cell behaviour, this study attempts to investigate silicate ion uptake dynamics in osteoblasts whilst examining the roles these ions may play in bone regeneration. **Methods**. Osteoblast-like (SaOS-2) cells were cultured in 2mM Si-conditioned media (Sodium metasilicate). At regular time intervals, cells were lysed, and intracellular Si content quantified by ICP-OES. [Si] in extracellular supernatants were quantified to assess Si secreted from cells. Uptake of different Si species (ortho, di, trisilicates) were also compared to 45S5 BG extracts. Media containing Lysosensor – PDMPO (Thermofisher) was added to SaOS-2 cells and osteoclasts (differentiated from RAW 264.7 cells) following a 4-day culture in media containing 2mM Si. Local changes in pH were then assessed by fluorescence microscopy. For comparison, Si localisation was also assessed by STEM-EDX in 60nm sections of resin embedded cells. 45S5 Bioglass® containing ^{29}Si silica was synthesised by melt-derivation at 1400°C. Si species in ^{29}Si-45S5 were then determined by ^{29}Si NMR following dilution to 1-4mM Si in deuterium water. **Results and Discussion.** An increase in intracellular [Si] was observed up to 96h (whilst the amount of uptake rate per hour decreased over time) (Fig. 1a). Upon replacement with media alone, intracellular [Si] decreased ($p<0.05$), along with Si found in cell supernatants (Fig. 1b). Intracellular [Si] was significantly lower when cultured with 45S5 compared to orthosilicates (Fig. 1c). 29Si-NMR showed, however, that BGs produce only ortho and disilicates up to 2mM Si (Fig. 1d). Concentrations of Si from BGs above 2mM were found to increase the concentration of disilicate species suggesting the initiation of Si polymerisation. Si at 2mM from BGs and silicates were found to be localised within lysosomal vesicles in both osteoblast (Fig. 2a-c) and osteoclast cells (Fig. 2d-e) suggesting uptake of both ion and particulate Si. **Conclusion and Impact.** Bioglass dissolution products stimulate bone production from ostoegenic cells and this is in part due to Si uptake into the cells. Our results imply the importance of tailoring [Si] release from novel BGs to control Si species and thus increase their uptake in cells.

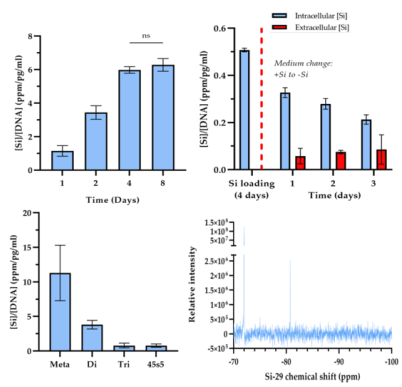


*Figure 1 - a) Intracellular Si concentration over time, b) Si secretion c) Uptake of different Si species from meta, di and tri silicates and 45S5 bioactive glass. d) Si species released from 45S5 bioactive glasses at 3mM [Si] determined by ^{29}Si NMR. ***=p<0.01 +/- SD*

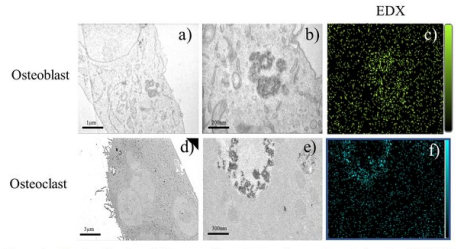


Figure 2 – The localisation of Si in osteoblasts (a-c) and osteoclasts (e-f) using STEM-EDX (e/f). Si was found to be highly localized within intracellular vesicles, whilst low levels were observed throughout the cell cytoplasm.

References

[1]Logroscino G. Materials Science: 2014;
[2]Hench L. Materials Science: 2006;

PoB.16.23

Myokine secretion from tissue-engineered skeletal muscle and its collection by hydrogel encapsulation

Toshia FUJISATO, Manaka SAI, Takaya IWAI, Sho YOKOYAMA, Tomohiro NAKAMURA

Osaka Institute of Technology, Osaka, Japan

Abstract

Skeletal muscles are mainly responsible for maintaining and changing body posture and locomotion. Besides that, they produce various cytokines or peptides, called myokines, such as interleukin 6 (IL-6), brain-derived neurotrophic factor (BDNF), leukemia inhibitory factor (LIF), irisin, and secreted protein acidic and rich in cysteine (SPARC). All of them are associated with metabolic changes, tissue regeneration and repair, and maintenance of healthy bodily functioning. Recent achievements in tissue-engineering research make it viable to construct biological tissue *in vitro* from living cells and a scaffold material. This technology can be applied not only to patients for reconstructing malfunctioning tissue *in vivo* but also to other purposes such as three-dimensional tissue model *in vitro* and also functional foods. In this study, the tissue-engineered skeletal muscle (TEM) was cultured under various electrical and mechanical stimulations and examined its contractility and myokine secretion. In addition, they were encapsulated by hydrogel to collect and concentrate the secreted myokine.

Two artificial tendons were introduced at both ends of our engineered muscle. The two tendons were hold with stainless steel pins placed 12 mm apart before cell seeding. The C2C12 myoblasts embedded in collagen gel was added between and on the surface of the two tendons. After gelation of the cell suspension, the constructs were cultured in growth medium of high-glucose DMEM with 10% FBS for 2 days. Then, they were cultured in differentiation medium of high-glucose DMEM with 7% horse serum with various electrical pulse stimulation or passive mechanical stretch movements. After the stimulation, the contractile force was measured by micro load cell and the amount of secreted IL-6 was determined by ELISA. Also, TEM was encapsulated by alginate calcium by sequential immersion into alginate solution and calcium chloride solution.

The isometric twitch force of the tissue-engineered skeletal muscle cultured with electrical pulse of 5V, 2msec, and 0.5Hz was remarkably higher than that of without stimulation up to 7 days. However, there was no difference when the constructs were cultured more than 14 day with electrical stimulation. In addition, the amount of IL-6 secreted from stimulated skeletal muscle was larger than that of without stimulation. The alginate calcium layer could concentrate IL-6 efficiently inside the TEM-gel construct. These results suggest that tissue-engineered skeletal muscle may be applied to physical exercise model *in vitro* and also functional food including rich myokines.

PoB.16.24

In vitro Comparison of Four Types of Commercially Available Scaffolds for Cartilage Tissue Engineering

Arianna De Mori[1], Ian Graney[2], Marta Roldo[1], Gordon Blunn[1]

[1]School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, United Kingdom. [2]NTL Biologica LTD, Henley-On Thames, United Kingdom

Abstract

Articular cartilage has a limited capacity to self-heal itself, due poor vascularity and lack of abundant progenitor cells. Following trauma, the repair tissue consists of functionally inferior fibrocartilage, that is associated with an increasing risk of osteoarthritis (OA). OA is one of the leading causes of disability worldwide and represents a massive socioeconomic burden. Therefore, the successful treatment of cartilage defects reduces the prevalence of OA. Cartilage tissue engineering techniques, where stem cells and 3D scaffolds are used in combination, have promoted superior repair compared to other more commonly used treatments. Several scaffolds are commercially available which claim to support cartilage regeneration, but no study has simultaneously compared their ability to support chondrogenesis, *in vitro*. In this study, four scaffolds were tested: I) Dehydrated complete placental membrane, II) Benzyl Ester of Hyaluronic acid non-woven scaffold, III) Hyaluronic acid and Polyglycolic acid 3D fibrous implant and IV) Polycaprolactone enriched Hyaluronic acid 3D printed scaffold. In order to discriminate their ability to support chondrogenesis, 60,000 human adipose derived mesenchymal stem cells (hAdMSCs) were seeded on each scaffold and grown in basal (DMEM) and chondrogenic (CH) media, for 21 days, at 5% CO_2. Proliferation was measured by resazurin, cell viability by a live/dead assay, cell morphology by SEM, mRNA expression by RT-qPCR and protein expression by immunofluorescence. All the materials supported cell adhesion, viability and proliferation. It was observed that scaffolds I, II and III had a better cell proliferation than the 3D printed scaffold, but similar cell viabilities. HAdMSCs presented an elongated-shape on scaffold IV, whereas the cells grown on scaffold II spontaneously formed spheroids, in both DMEM and CH media. Markers of chondrogenesis, such as SOX5 and COL2A1, were upregulated at day 21, for all the materials treated with chondrogenic medium, with scaffold III showing the greatest expression of SOX5 and SOX9, at 21 days. RUNX2 - an osteogenic marker- was upregulated on scaffold IV at days 7 and 21. Overall, it appeared that the scaffold III was the best material in terms of supporting chondrogenesis, whereas the membrane I had the lowest relative expression of chondrogenic genes.

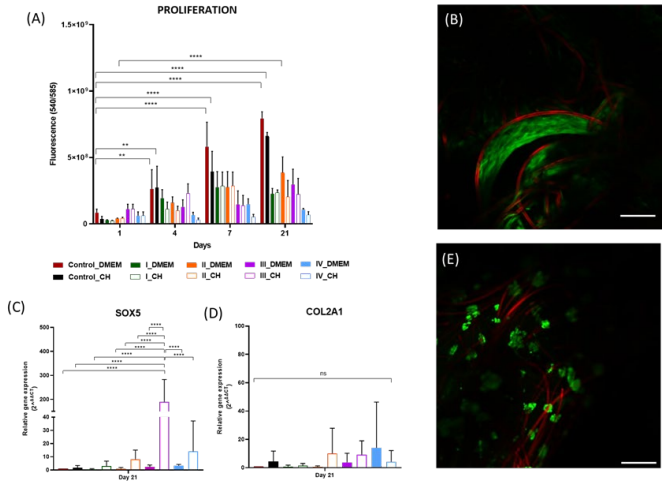


Figure (A) Proliferation, at day 7 and 21, of both controls and scaffolds. (B) and (E) representative live and dead images of hAdMSCs on scaffolds III and II, at day 7. (C) and (D) relative gene expression of SOX5 and COL2A1 for both controls and scaffolds, at day 7 and 21.

PoB.16.25

Engineering freestanding multilayered membranes as delivery vehicles for cell-based therapies in early-osteoarthritis

Sónia G. Patrício[1], Catarina T. Passos[1], Maria P. Sousa[1], Marian Fürsatz[2], Haeshin Lee[3], João F. Mano[1], Sylvia Nürnberger[2]

[1]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal. [2]Department of Orthopedics and Trauma-Surgery, Division of Trauma-Surgery, Medical University of Vienna, Vienna, Austria. [3]Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea, Republic of

Abstract

Articular cartilage injuries caused by aging, trauma or diseases are currently one of the world's top health concerns owing to its limited capacity of self-renewal, thus causing a major economic burden on the healthcare system. Cell implantation strategies resorting to a suitable delivery platform hold a great promising approach to increase cell engraftment at the damaged cartilage. Regarding their immunomodulatory potential, adipose-derived mesenchymal stem cells (ASCs) have been widely explored in cell therapies for the treatment of different inflammatory or autoimmune pathologies. Therefore, we herein propose the design of functional cell carrier membranes with on-demand capability to transport and fix human-derived mesenchymal stem cells into superficially cartilage defect sites (**Figure 1**). Using the bottom-up layer-by-layer (LbL) technology, oppositely charged natural origin biopolymers with structural similarity to glycosaminoglycans (GAGs) were combined in a multilayered fashion with catechol-functionalized hyaluronic acid (HA-DOPA) onto a nanogrooved low surface energy substrate, allowing the multilayered formation of self-adhesive and patternable freestanding membranes in one single platform. The seamless combination of nanotopography and catechol molecular cues has significantly augmented cellular adhesion at the patterned DOPA-HA membrane surface. These highest cell density membranes were further applied onto human chondral discs ex-vivo models to evaluate their capability to act as cell delivery vehicles. Results have shown the successful cell migration and retention at cartilage surface, wherein cells were able to spread, inhabiting both superficial empty lacunae and furrows. Therefore, the present study supplies an important strategy for designing stem cell delivery vehicles to be applied on cell-based therapies in the near-future.

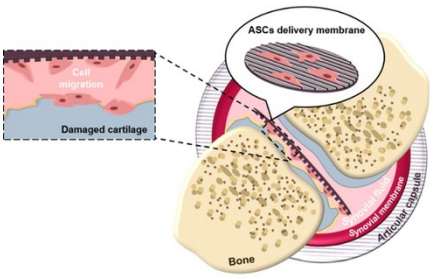


Figure 1. Schematic representation of a joint region (not drawn to scale), highlighting the cell-seeded membrane placed over the cartilage defect area. The combination of topographical and molecular cues maximizes cell adhesion sites to be further delivery at the damaged cartilage surface.

Acknowledgments: This work was developed within the scope of the project CICECO – Aveiro Institute of Materials (UIDB/50011/2020, UIDP/50011/2020 & LA/P/006/2020), financed by national funds through the FCT/MEC (PIDDAC). M.P. Sousa and S. G. Patrício gratefully acknowledge FCT for the PhD grant (SFRH/BD/97606/2013) and for the individual contract (2020.00366.CEECIND), respectively. The authors acknowledge Evercyte (Evercyte, Vienna, Austria, Cat# CHS-001-005) by kindly providing ASC/TERT1 cells.

PoB.16.26

Encapsulation of umbilical cord-derived stem cells toward an *in vitro* model for endochondral ossification

Mariana Carreira, Inês Fernandes, Sónia Patrício, Clara Correia, Sara Nadine, João Mano

Department of Chemistry, CICECO - Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal

Abstract

Most traditional tissue engineering approaches are designed to promote bone repair via intramembranous ossification, by directly inducing the osteogenic differentiation of mesenchymal stem/stromal cells (MSCs). A less explored alternative is engineering bone constructs through a cartilage-mediated approach, resembling the embryological process of endochondral ossification, in which an intermediary hypertrophic cartilaginous template triggers vascular invasion and bone tissue deposition.
Inspired by this mechanism, we proposed an *in vitro* model for endochondral ossification composed of cartilaginous 3D microtemplates, umbilical cord-derived MSCs (UCMSCs), and human umbilical vein endothelial cells (HUVECs) within liquified microcapsules (Fig.1A). For that, cartilaginous and non-primed microtemplates were created in Aggrewell plates using only UCMSCs. Then, the microtemplates were co-cultured with UCMSCs and HUVECs within liquefied microcapsules for 21 days. The liquefied microcapsules are composed of a permselective membrane that allows the diffusion of essential molecules for cell survival while the microtemplates act as cell adhesion sites within the liquefied core, allowing cells to construct their own 3D cell culture assembly system. Four different conditions were prepared (Fig.1B): ECO – replicating the endochondral ossification; ECO control; IMO – mimicking the intramembranous ossification; and negative control.
Results indicate that after 21 days of encapsulation, cell aggregates generated within ECO microcapsules presented higher osteopontin and osteocalcin staining compared to other conditions, suggesting the production of bone extracellular matrix (Fig.2A-B). Furthermore, both ECO and ECO control conditions showed increased endothelial cell recruitment compared to the IMO condition. The elemental analysis by SEM-EDS revealed a higher matrix mineralization in the ECO microcapsules, being the only condition that presented a calcium/phosphorous ratio (1.71) close to the native hydroxyapatite (Fig.2C).
In conclusion, results indicate that the privileged microenvironment of liquefied microcapsules in combination with cartilaginous microtemplates allowed recreating the endochondral native process, leading to the *in vitro* production of vascularized bone-like microtissues.
This work was financed by the European Research Council Advanced Grant ‘‘REBORN’’ (ERC-2019-ADG-883370) and by national funds (OE) through FCT, I.P., in the scope of the project "TETRISSUE" (PTDC/BTM-MAT/3201/2020). This work was developed within the scope of the project CICECO—Aveiro Institute of Materials (UIDB/50011/2020, UIDP/50011/2020 & LA/P/006/2020), financed by national funds through the FCT/MEC (PIDDAC).

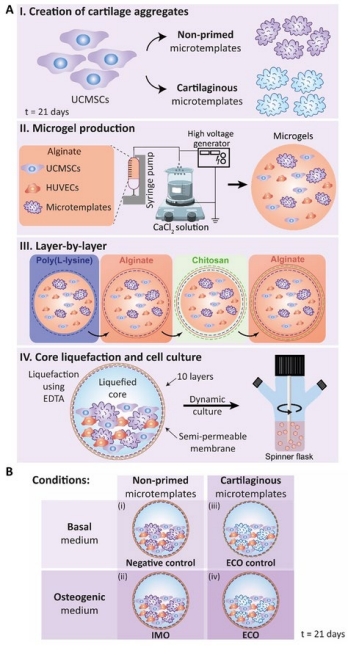


Figure 1. Production of an *in vitro* model for endochondral ossification.

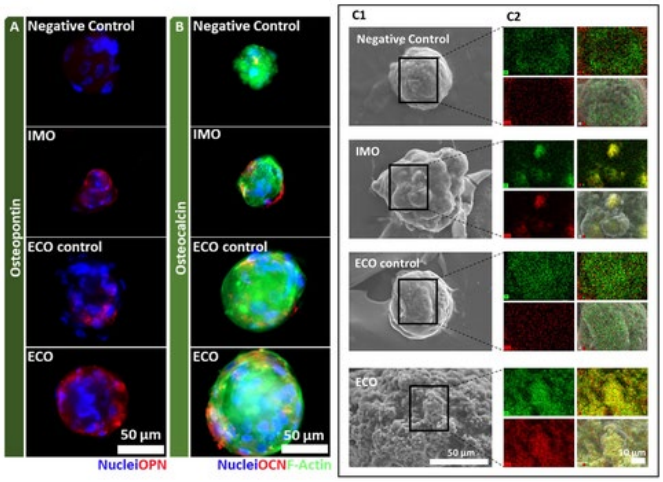


Figure 2. Immunofluorescence staining of osteopontin (A) and osteocalcin (B) of encapsulated cell aggregates. Representative SEM images of encapsulated cell aggregates (C1), followed by (C2) elemental analysis by EDS mapping of phosphorous (red) and calcium (green).

PoB.16.27

Lack of cartilage surface preservation with PMOXA in collagenase-induced osteoarthritis rat model

Sami Kauppinen[1], Lucia Baixauli Marin[1], Gonçalo Barreto[2], David Fercher[3], Patrick Weber[3], Ville-Pauli Karjalainen[1], Salla-Mari Tuutijärvi[1], Giulia Morgese[4], Edmondo Benetti[5], Simo Saarakkala[1], Marcy Zenobi-Wong[3], Mikko AJ Finnilä[1]

[1]University of Oulu, Oulu, Finland. [2]University of Helsinki, Helsinki, Finland. [3]ETH Zurich, Zurich, Switzerland. [4]Zurich University of Applied Sciences, Zurich, Switzerland. [5]University of Padova, Padova, Italy

Abstract

Introduction. Osteoarthritis remains a major medical issue with a lack of accepted drugs or biomaterials to halt cartilage degradation. Articular cartilage has poor healing capacity; once the surface is damaged, it will degrade only further. In this study, we wanted to evaluate possible *in vivo* chondroprotective effects of injectable co-polymer poly(2-methyl-2-oxazoline) (PMOXA). This biopolymer has affinity towards degraded cartilage and decreases friction at damaged cartilage surfaces. **Methods.** In this study, we induced osteoarthritis in Wistar rat's (N=40) knees by injecting collagenase into the synovial space of their left hind limb. We then injected half of the rats with biopolymer PMOXA on day 3, while the other half received a saline placebo. We divided the animals into two groups based on the time elapsed since collagenase injection (10 and 60 days) and further divided each group into four subgroups: CIOA/saline (left leg), CIOA/PMOXA (left leg), Control/saline (right leg), and Control/PMOXA (right leg). Then, we evaluated the extent of cartilage degeneration in each group using contrast-enhanced micro-computed tomography (CEμCT) and a novel surface analysis technique, Local Relative Surface Orientation Angle (LRSOA). In addition, we performed standard histological OARSI grading on two safranin O stained coronal sections from each sample where the average grade was used as the final grade. Finally, we used a linear mixed model to assess differences between groups. **Results.** CIOA/Saline group had more damage in the cartilage surface than Control/Saline in the medial and lateral surfaces of tibias and femurs based on LRSOA ($p<0.01$). Histological analysis showed CIOA/Saline was worse in the tibias (lateral 60 day, medial 10 day, and medial 60 days) ($p<0.01$) and in the femurs (lateral 60 days) ($p<0.05$). No differences were found in other locations. CIOA/Saline and CIOA/PMOXA had no differences in histological or CEμCT analysis at any time point or location (table1). See Figure 1 for average results. **Conclusion.** Cartilage was analyzed using CEμCT and histology. CEμCT detected degeneration in CIOA/Saline compared to Control/Saline, which was not seen in histology. PMOXA did not protect the cartilage *in vivo*, as it continued to degrade after collagenase injection, with or without PMOXA injection.

site	time	(Group1 -Group2)		OARSI FEMURS Mean difference (1-2)	OARSI TIBIAS Mean difference (1-2)	LRSOA FEMURS Mean difference (1-2)	LRSOA TIBIAS Mean difference (1-2)
Lateral	10day	Control / Saline	CIOA / Saline	-0.555	-0.833	**-1.306****	**-1.956****
		CIOA / PMOXA	CIOA / Saline	-0.488	-0.233	-0.294	0.028
	60day	Control / Saline	CIOA / Saline	**-1.150***	**-2.400****	**-1.635****	**-1.922****
		CIOA / PMOXA	CIOA / Saline	-0.550	-0.500	-0.225	0.544
Medial	10day	Control / Saline	CIOA / Saline	0.111	**-5.222****	**-1.916****	**-2.416****
		CIOA / PMOXA	CIOA / Saline	0.127	0.422	-0.359	-0.049
	60day	Control / Saline	CIOA / Saline	0.500	**-5.800****	**-2.760****	**-2.423****

Table1 Group comparison (linear mixed model)

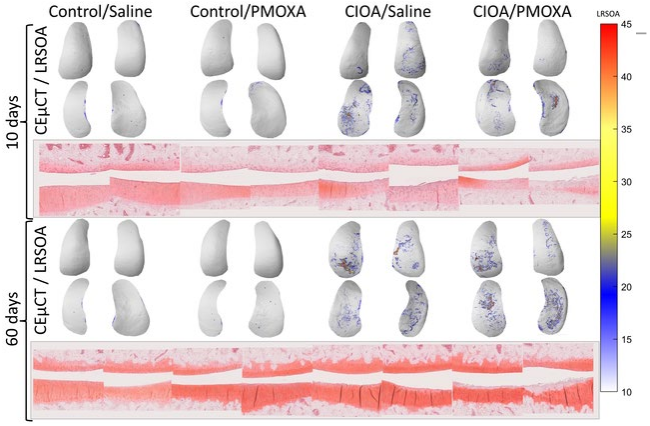


Figure 1. example images of femoral and tibial surfaces using CEμCT and histological sections, with LRSOA values visualized from 10° to 45° to highlight surface damage.

PoB.16.28

A HUMAN-SCALED KNEE BIOMIMETIC BIOREACTOR FOR PATIENT-SPECIFIC OSTEOCHONDRAL TISSUE ENGINEERING

Adrian Djalali-Cuevas[1], Noelia Campillo[2], Juan Antonio Marchal[3,4], José M. Baena[1,2]

[1]REGEMAT 3D S.L., Granada, Spain. [2]BRECA Health Care S.L., Granada, Spain. [3]Instituto de Biopatología y Medicina Regenerativa, Centro de Investigación Biomédica de la Universidad de Granada, Granada, Spain. [4]Terapias Avanzadas: Diferenciación, Regeneración y Cáncer, Instituto de Investigación Sanitaria, Granada, Spain

Abstract

Introduction. Musculoskeletal medical conditions are one of the leading causes of human disability worldwide, accounting the lesions of the osteochondral interface, for a great proportion of these disorders (Hermes *et al*., 2020). The lack of effective regenerative approaches for a growing elderly population accentuates the need to generate new strategies based on cutting-edge technologies for regenerative medicine. In recent years, the emergence of 3D bioprinting has allowed great advances in tissue engineering, making feasible the automatized and controlled ex vivo generation of bioartificial tissue substitutes with a certain degree of complexity (Oberdiek *et al*., 2021). However, the application of biochemical, physical and mechanical stimuli biomimetizing those found in a given tissue, by custom-designed bioreactors, is critical to promote the maturation of the 3D constructs towards clinically relevant products (Selden *et al*., 2018). Despite the need for such devices, the availability of dedicated bioreactors for tissue engineering, with the ability to recreate the complex microenvironment to which cells are subjected *in vivo*, is currently scarce. Therefore, the aim of this work was to develop a novel bioreactor able to mimic the anatomy and physiology of the human knee joint for the maturation of patient-shaped 3D bioprinted cartilage substitutes under a physiologically relevant microenvironment. **Methods And Results.** Functionality testing was performed by monitoring the temperature, CO_2, pH, and relative humidity during the actuation of the device, showing its ability to control these parameters within a range suitable for cell culture. In addition, load was monitored during flex-extension cycles, demonstrating the feasibility of the device to apply loads within the tibiofemoral surfaces of the reconstructed knee joint, suitable for the mechanical stimulation of chondroprogenitor cells. Sterility and cytotoxicity tests were performed following ISO standards for medical devices demonstrating no microbial contamination nor cytotoxic effects throughout the operation of the device. 3D bioprinted polycaprolactone scaffolds loaded with a cell-laden hydrogel containing chondroprogenitor cells were subject to mechanical loading under physiologically relevant regimes and cell viability was monitored by Live-Dead stain and confocal microscopy, demonstrating the viablity of the cells throughout the stimulation period. **Conclusions And Future Work.** In this study, a human-scaled knee joint biomimetic bioreactor was developed for the engineering of patient-shaped 3D printed osteochondral tissue substitutes. The functionality of the device was demonstrated, as well as its ability to support the culture of chondroprogenitor cells under physiologically relevant loading regimes. Future work will adress the chondrogenic effects of physiological-loading within the bioreactor.

PoB.16.29

Decoupling the chemical and structural properties of collagen to study tendon regeneration

Francesca Giacomini, David Barata, Hoon Suk Rho, Zeinab Tahmasebi Birgani, Stefan Giselbrecht, Roman Truckenmüller, Pamela Habibović

Maastricht University, Maastricht, Netherlands

Abstract

High incidence of tendon injuries combined with limited healing capacity of tendon tissue have resulted in a clinical challenge and an increasing need for tendon regeneration therapies. Recapitulating the tendon extracellular matrix (ECM), predominantly consisting of aligned collagen type-I fibers, presents a bottleneck for developing such therapies [1]. Various biofabrication technologies were proposed for creating collagen microstructures with controlled fiber alignment [2]. Here, we developed a user-friendly microfluidic piggyback platform allowing the control of the micropatterned formation and alignment of collagen fibers on the bottom of culture dishes. We identified two configurations of shape and spacing of hexagonally arranged micropillars integrated in the platform's flow chamber the collagen fibers derived of which support an increased expression of tenogenic markers at the gene and protein levels relative to tenocytes cultured on tissue culture plastic. Through nanoimprinting, we replicated the fiber topographies on polystyrene, while a coating-based approach was developed to introduce non-fibrillar collagen. The results indicated that the aligned fibrillar structure of collagen per se affects the tenocytes' morphology, whereas the biochemical nature of collagen plays a prominent role in the expression of tenogenic markers. Beyond the controlled provision of aligned collagen, the microfluidic platform can aid in developing more physiologically relevant *in vitro* models of tendon and its regeneration.

References

1. Beldjilali-Labro M, et al. Materials (Basel). 11, 1116, 2018;
2. Nuhn JAM, et al. Acta Biomater. 10, 1016, 2018;
3. Lee P, et al. Biomed Microdevices. 10, 1007, 2006;

Acknowledgment: This research was financially supported by the Gravitation Program "Materials-Driven Regeneration", funded by the Dutch Research Council (no. 024.003.013) and the Dutch Province of Limburg, project LImburg INvesteert in haar Kenniseconomie (LINK; nos. SAS-2014-00837 and SAS-2018-02477).

PoB.16.30

Enthesis inflammation-on-a chip

Francesca Giacomini, Zeinab Tahmasebi Birgani, Hoon Suk Rho, Maria José Eischen-Loges, Martijn van Griensven, Stefan Giselbrecht, Pamela Habibovic, Roman Truckenmüller

Maastricht University, Maastricht, Netherlands

Abstract

Enthesitis is a common musculoskeletal inflammatory disease characterized by the inflammation of the enthesis, the attachment point of tendon or ligament into bone.[1] The inflammation process often originates from the fibrocartilage region of the enthesis. During enthesitis, waves of inflammatory cytokines propagate in the fibrocartilage region resulting in new bone formation. A collective understanding of enthesitis in the human tissue remains limited, mainly due to the lack of *in vitro* model systems.[2] Here, we first developed an enthesis-on-chip device, consisting of two independent microfluidic compartments separated by a porous membrane. The microfluidic platform enabled selective differentiation of human mesenchymal stromal cells into tenocytes and fibrochondrocytes in the top and bottom chamber, respectively. Then, by introducing an inflammatory cytokine cocktail in the fibrochondrocyte chamber, we could recapitulate key aspects of acute and chronic enthesistis, measured as increased expression of inflammatory markers such as IL-1β and Mmp13. In chronic inflammation conditions, we could observe also hydroxyapatite deposition, enhanced expression of osteogenic markers and reduced expression of tenogenic and chondrogenic extracellular matrix components. Finally, we were able to mitigate the inflammatory state upon addition of the anti-inflammatory drug celecoxib, therefore demonstrating the applicability of our enthesitis-on-chip model for drug screening.

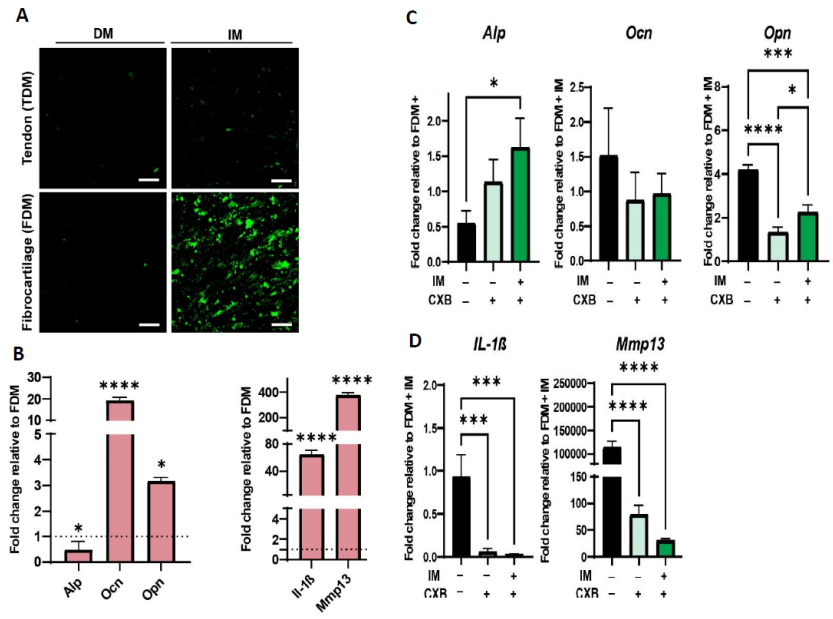


Figure 1. A) Confocal fluorescence images showing the deposition of hydroxyapatite (HA) in the tendon compartment and fibrocartilage compartments, after perfusion of inflammatory medium (IM) for 21 days (green indicates hydroxyapatite, scale bars represent 100 µm). B) Expression of osteogenic and extracellular matrix markers in the fibrocartilage compartment at mRNA level, after perfusion of inflammatory medium. C) Expression of osteogenic and D) extracellular matrix markers in the fibrocartilagecompartment at mRNA level, after perfusion of inflammatory medium and Celecoxib (CXB) . * $p < 0.05$** $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. N = 3.

References:
[1] S. Font Tellado, E. R. Balmayor, M. Van Griensven, Adv. Drug Deliv. Rev. 2015, 94, 126;
[2] G. Schett, R. J. Lories, M. A. D'Agostino, D. Elewaut, B. Kirkham, E. R. Soriano, D. McGonagle, Nat. Rev. Rheumatol. 2017, 13, 731
Acknowledgment: This research was financially supported by the Gravitation Program "Materials-Driven Regeneration", funded by the Dutch Research Council (024.003.013), and the Dutch Province of Limburg, project LINK- LImburg INvesteert in haar Kenniseconomie (grant numbers SAS-2014-00837 and SAS-2018-02477).

PoB.16.31

Cytokine-induced osteoarthritis platform for evaluating miRNA-based therapeutic polyplexes

Annachiara Scalzone[1,2], Xiao Nong Wang[3], Ana Marina Ferreira-Duarte[1], Kenny Dalgarno[1], Piergiorgio Gentile[1]

[1]Newcastle University, Newcastle, United Kingdom. [2]Center for Advanced Biomaterials for Healthcare, Istituto Italiano di tecnologia, Naples, Italy. [3]Translational and Clinical Research Institute, Newcastle, United Kingdom

Abstract

OA is the main chronic form of joint disease. Pro-inflammatory cytokines play a key role in OA pathogenesis by affecting the anabolic activity of chondrocytes in favour of catabolic activity, leading to protease-mediated Articular Cartilage (AC) degradation. This work aims to design and manufacture an AC *in vitro* scaffold-free organoids model in Healthy and OA conditions, with the aim of studying the disease evolution at cellular level and testing therapeutic polyplexes. Spheroids of human articular chondrocytes (HC) and immortalised mesenchymal cells differentiated to chondrocytes (Y201-C) were cultured in three culture conditions: Healthy (DMEM/F12), Low concentration OA (LC-OA) (DMEM/F12 loaded with: IL-1ß:1ng/mL, TNF-α:1ng/mL, IL-6:10ng/mL) and High Concentration OA (HC-OA) (DMEM/F12 loaded with: IL-1ß:5ng/mL, TNF-α:5ng/mL, IL-6:50ng/mL). Spheroid growth kinetics and metabolic activity were evaluated over 10 days and then spheroids were assembled and cultured up to 21 days on gelatin-coated poly(lactic-co-glycolic acid) electrospun membranes, using a protocol inspired by the clinically approved Chondrosphere® (CO.DON AG) technique. HC and Y201-C spheroids showed a diameter decrease over culture in Healthy state compared to OA conditions, where the diameter remained stable. Cell metabolic activity decreased over culture for HC and increased for Y201-Cs in all conditions, but both cell types showed a higher metabolic activity in HC-OA condition at day 10. Gene expression analysis of anabolic (SOX9, COL2A1, ACAN) and catabolic (MMP13 and ADAMTS-5) markers, Histology (H&E, Alcian Blue, Picrosirius red) and Immunohistochemistry (Collagen II, Aggrecan and Ki-67) were performed to assess OA markers in all conditions. At day 21, cells in OA environment showed significant decreased expression of anabolic markers and an upregulation of catabolic markers, as well as a significantly lower production of collagen and glycosaminoglycans. IHC for Collagen II and Aggrecan (Fig. 1A) confirmed this trend. Finally, miRNA-140-5p-based (20 µM) chitosan polyplexes (250 µg/mL) uptake and their therapeutic effect (decrease in the expression of MMP13 catabolic marker and increase the expression of COL2A1) was successfully evaluated using the OA-induced model (Fig.1B). Overall we developed a reliable *in vitro* OA platform where the presence of pro-inflammatory cytokines led to increased cells metabolism, possibly due to the biosynthesis of OA-related inflammatory and degradative enzymes at day 10; decreased expression of anabolic genes in favor of catabolic genes by cells after 21 days culture and lower production of collagen II and aggrecan as effect of the inflammation. Also, miRNA-based polyplexes demonstrated to be potential therapeutics for OA treatment.

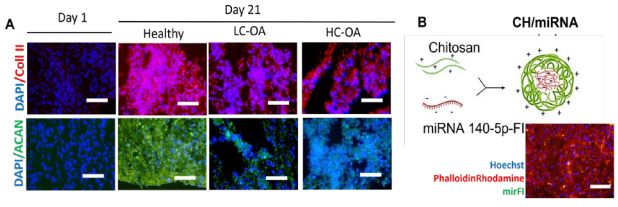


Figure 1: IHC at day 1 and day 21 of Collagen II (Col II) and Aggrecan (ACAN) in healthy LC-OA and HC-OA (A). miRNA/CH polyplexes uptake by HC cells after 48h. Bars: 150µm

PoB.16.32

Characterization of photo-crosslinkable methacrylated gellan gum hydrogels for the *in situ* treatment of chondral defects

Alessia Bacci[1], Diego Trucco[1], Lorenzo Vannozzi[1], Elena Gabusi[2], Enrico Lenzi[2], Cristina Manfredini[2], Liliana Agresti[1], Maria Rosaria Pascale[3], Sandra Cristino[3], Gina Lisignoli[2], Leonardo Ricotti[1]

[1]Scuola Superiore Sant'Anna, The BioRobotics Institute, Pisa, Italy. [2]IRCCS Istituto Ortopedico Rizzoli, SC Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy. [3]De partment of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy

Abstract

Introduction Cartilage tissue engineering (CTE) aims to tackle chondral defects and restore cartilaginous tissue. Hydrogels can drive the recovery process thanks to their similarity with the natural proteomic pattern and guarantee structural support to cells. For clinical translation, injectable hydrogels have been investigated for the localized treatment of defects through a minimally invasive procedure. Gellan gum (GG), a polysaccharide-based polymer, exhibits structural similarity with cartilage glycosaminoglycans [1] and can be functionalized into Methacrylated Gellan Gum (GGMA), to enable in situ photo-crosslinking (Fig. a). Given the controversial safety of UV-based photocrosslinking, visible light-based photopolymerization has been recently investigated as a promising strategy to crosslink cell-laden materials. In this work, GGMA has been sterilized, and its photopolymerization has been optimized in terms of polymer and photo-initiators concentrations and exposure time to ensure the long-term cell encapsulation safety and mechanical stability of the hydrogel. **Methods** Methacrylation was performed according to [2], and lyophilized GGMA was autoclaved before use. Rheological and microbiological tests were conducted on GGMA hydrogel to evaluate physicochemical and bioburden profiles upon treatment with an autoclave. analyses were conducted on GGMA to assess the physicochemical profiles upon treatment with qRT-PCR after 34 days of culture. **Results & Discussion** Microbiological analysis reveals the successful sterilization of GGMA via autoclave. Results show the effects of autoclave on GGMA rheology with a significant reduction in the viscous behaviour upon treatment (Fig. b). In the hypothesis of a lower sol content as an index of a more efficacious crosslinking (Fig. c), two GGMA formulations (GGMA 2%-0.1/1mM-60s and GGMA 2%-0.2/2mM-30s), were screened for their porosity (Fig. d), cell viability and restoration of the collagen type 2 expression in laden chondrocytes (Fig. e). **Conclusion** A sterilized and optimized photo-crosslinked GGMA is proposed as a valid candidate for CTE. This formulation would permit the recapitulation of an instructive milieu for encapsulated chondrocytes toward cartilage restoration, promising a potential future clinical translation.

Acknowledgement The work received funding from the EU's Horizon2020 research program, grant agreement N.814413, project ADMAIORA.

[1] Oliveira J. T. et al., J. Biomed. Mater. Res. A, 93:852-863, 2010
[2] Coutinho D. F. et al., Biomaterials, 31:7494-7502, 2010

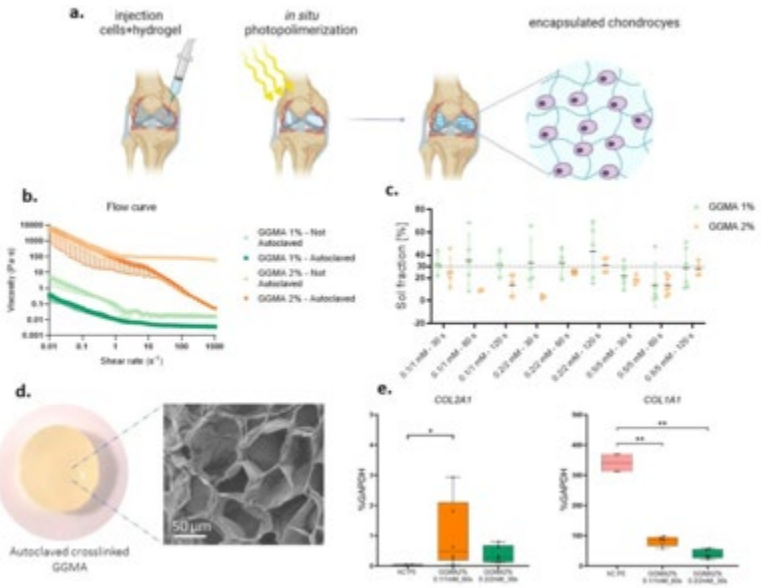


Figure 1. a) Clinical application of GGMA; b) Rheological profile; c) Sol fraction; d) Porosity of crosslinked GGMA; d) Collagen expression

PoB.16.33

Polycarbonate-Urethane Articulation on Cartilage – a Pin-on-Disc *in vitro* Study

Roger Claude von Mentlen[1], Lea Tiziana Dal Fabbro[1], Daniel Baumgartner[1], Viola Mai[2]

[1]Institute of Mechanical Systems, School of Engineering, Zurich University of Applied Sciences, Winterthur, Switzerland. [2]Mathys AG, Bettlach, Switzerland

Abstract

Introduction In case of osteoarthritis, one of the two articular surfaces is more affected by osteoarthritis. A hemiprosthesis may preserve the contralateral healthy side, especially in the shoulder. Nevertheless, the articulation characteristics of promising materials such as Polycarbonate-Urethane (PCU) have not been well studied compared to native cartilage. In previous studies polycarbonate-urethane (PCU) showed a threefold lower coefficient of friction against cartilage compared to traditional orthopaedic materials indicating that PCU might be a promising coating for hemiprostheses [1]. **Methods** Pin-on-disc tests were used to compare cartilage pins taken from bovine knees, against zirconia (ZTA), cobalt-chromium alloy (CoCr), and PCU. Tests were conducted with above-physiological loads (2.5 MPa, test 1) to see differences in wear and surface structure, as well as with physiological loads (0.63 MPa, test 2) to see the reaction of the living tissue (only ZTA and PCU were tested). The surface morphology of the articular cartilage was then analysed, and the loss of cartilage height measured (test 1). A Safranin-o (SAF-O) and lactate dihydrogenase (LDH) staining were performed to show the viability of the chondrocytes post-test (test 2).

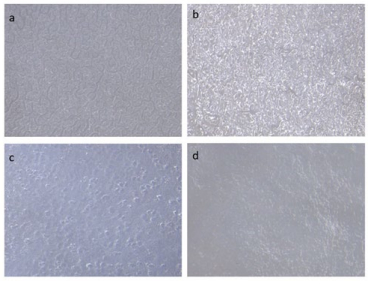


Fig. 1: Surface morphology after testing (test 1) a) PCU, b) ZTA, c) CoCr and d) control group (only soaked, not tested)

Results Microscopic images showed striking differences in the morphology of the cartilage after testing (test 1) with the different biomaterials (Fig. 1). The ZTA group showed a significantly greater decrease in height compared to the PCU group (Fig. 2). There was no significant difference in decreased height between the samples tested with CoCr and PCU. The SAF-O staining (Fig. 3) showed increased fibrillation on the articulating surface in both tested groups compared to the control group. The LDH staining showed a very high viability in all samples. **Conclusions & Outlook** Although articulation of PCU and CoCr showed around three-fold lower cartilage height loss than ZTA, the histological assessment did not find significant differences between treatments. Further analysis will be needed to prove the biological response of cartilage with PCU. In this context, additional pin-on-disc tests are planned to determine the coefficients of friction, which was not measured in previous tests, and to verify with other studies.

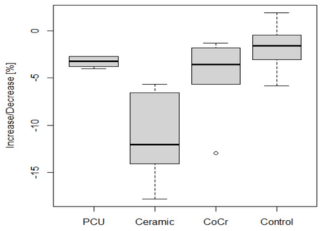


Fig. 2: Boxplot of cartilage height loss after testing (test1, n=6 for each group)

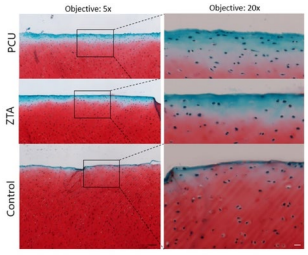


Fig. 3: SAF-O staining after testing (test 2). Scale bar (5x): 100 µm, scale bar (20x): 20 µm. Red staining: GAG, green staining: PG

References

1. J. Sague, et al. (2013) Combined Meeting of Orthopedic Research, Venice.

PoB.16.37

Meeting a clinical need with an advanced biomimetic adhesive material: development of a novel porcine osteochondral fragment fixation model

Alicja J Bojan[1,2], Philip Procter[3]

[1]Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. [2]Department of Orthopaedics, Sahlgrenska University Hospital, Mölndal, Sweden. [3]Div. of Applied Materials Science, Dept. of Engineering Sciences, Uppsala University, Uppsala, Sweden

Abstract

Fixation of osteochondral bone fracture fragments using glue is a holy grail in trauma orthopaedics, the unmet need for it turning up daily in the operating theatre. If small bone fragments could be fixed and subsequently heal, it could make surgery and outcomes more effective. There are currently no approved bone adhesives available to clinicians, and no load-bearing pre-clinical models for evaluating an adhesive candidate. The authors have considered a promising adhesive candidate based on alpha tricalcium phosphate and an amino acid phosphoserine. This adhesive has already been reported to be safe and effective in both a non-load bearing bone core murine model (Procter et al 2021) and a bone to implant porcine model (Tzagiollari et al 2023). The authors have further tested this adhesive's strength ex-vivo in a harvested human fresh femoral head bone core model showing good strength at 2 and 24 hours (Bojan et al. 2022). However, load bearing in an osteochondral setting has not been evaluated in an in-vivo preclinical model. This led the authors to develop the present porcine knee model to determine the biological response where both the glued bone and cartilage will be present in a loaded situation. Figure 1 illustrates the model in which the medial compartment of the knee is exposed during surgery, the patella and tendon retracted and two bone cores are drilled to a depth of 8mm using an 8mm diameter trephine. Each core is removed and core 1 is glued back maintaining the cartilage surface congruity and core original orientation. Core 2 will be replaced and retained using a bioabsorbable SmartNail PLA implant, ConMed. The medial condyle is subsequently harvested and sectioned 6mm below cartilage surface using a diamond saw and then placed in a jig for testing in a shear push-out test, Figure 2. Initial cadaver porcine bone testing shows this method to be feasible and the mean load in the glued core 1 at 2 hours was 34.4N (SD 8.2 N n=5) which is comparable to the 220kPa at 2 hours reported by Bojan et al.

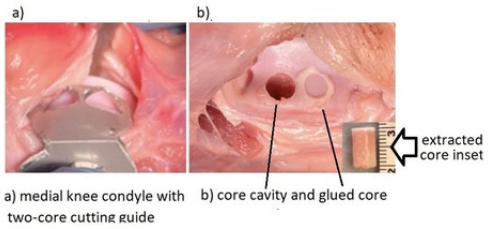


Figure 1 Core model

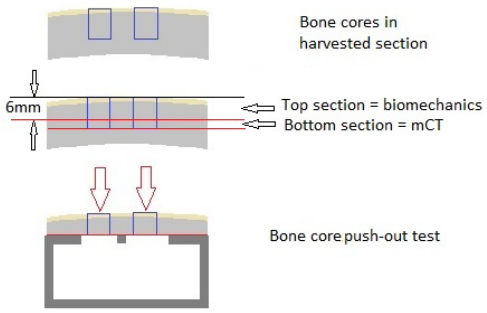


Figure 2: Bone core harvest and testing

A 2-animal pilot study is planned to ensure that the model is technically feasible and with bilateral implantations it will yield 4 test and 4 control samples for microCT and then biomechanical and histological assessment. The adhesive will be removed between the cartilage cut edges to ensure that the adhesive is confined to the bone and subchondral bone layers.

PoB.16.38

Bioengineering an inflammatory model of AF failure in 3D collagen type I hydrogels

Ana Luísa Castro[1,2,3], Mário A Barbosa[1,2,3], Raquel M Gonçalves[1,2,3]

[1]i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [2]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [3]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

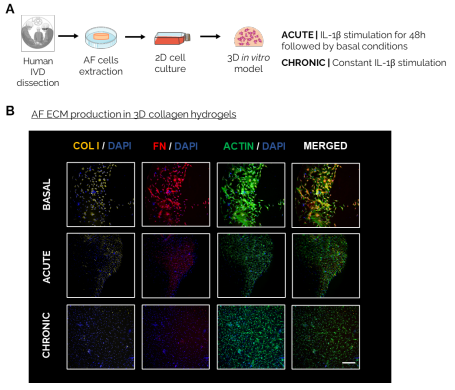
Abstract

Intervertebral disc(IVD) herniation involves a chronic inflammatory process and failure of the annulus fibrosus(AF), in a process still under investigated. Inflammation can lead to disorganization of extracellular matrix(ECM) and fibrotic alterations, among others. Most of the ongoing research in the pathomechanism of AF failure relies on rodent *in vivo* models or on bovine ex vivo organ cultures that do not truly mimic the human IVD microenvironment. Here, we developed a 3D *in vitro* model aimed at recapitulating the inflammation progression in human AF(hAF) to improve the understanding of AF failure.

hAF of herniated samples from low back pain patients were collected and digested as previously established[1]. hAF cells were cultured *in vitro* up to passage 4. hAF cells were cultured in 2D and 3D, embedded in collagen-type 1 hydrogels (3,2mg/mL). Cell seeding in 3D was optimized to guarantee high cell viability. hAF cells in 2D and 3D were then stimulated with IL-1β (10ng/mL), in acute (48h) and chronic (7days) conditions (Fig1A). After 7 days, cell morphology together with extracellular matrix(ECM) (collagen I and fibronectin) were assessed by immunofluorescence. Inflammatory cytokine secretion were analyzed by a protein array.

The results from the 2D experiments showed alteration of cell morphology, with a reduction in cell area upon inflammatory stimulation, that is reversible when inflammation is suppressed. The profile of inflammatory cytokines secreted in AF chronic inflammation showed that: i) IL-1α increases with time in culture, while TNF-α decreases; and ii) IL-1α and MIG/CXCL9 are increased in chronic vs acute inflammation. The ECM analysis showed no differences between control and inflammatory conditions. In 3D collagen I hydrogels, AF cell morphologic alterations with inflammation were also observed. Concerning ECM production, collagen I production was reduced with chronic inflammation, while fibronectin remained unaltered (Fig1B). A higher hydrogel shrinkage was observed upon 7 days in culture in control conditions, compared to inflammatory. Cytokine production from 3D cell culture is currently being assessed. The ECM alterations are being complemented by additional characterization at both, biochemical (collagen II and aggrecan) and biomechanical (nanoindentation) level.

Our data show that the challenges posed by AF regeneration can be overcome by relying on our inflammatory model of AF failure, highlighting the importance of chronic inflammation in *in vitro* studies to better resemble human IVD herniation.



Reference:

[1]:Castro, AL et al., 17Jan.2022, doi:10.1186/s13075-021-02690-w

Fig1: A-Experimental Setup; B-ECM production in 3D collagen hydrogels. Scale bar-300 µm

PoB.16.39

A biofidelic platform for preclinical assessment of hydrogel efficacy in multiaxially loaded intervertebral discs

Amra Šećerović[1], Marcia Mürner[1], Francesco Crivelli[2], Sarah Heub[3], Gilles Weder[3], Stephen J Ferguson[4], Diane Ledroit[3], Sibylle Grad[1]

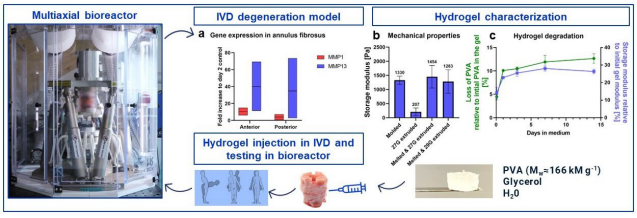
[1]AO Research Institute Davos, Davos, Switzerland. [2]CSEM, Alpnach, Switzerland. [3]CSEM, Neuchatel, Switzerland. [4]ETH Zurich, Zurich, Switzerland

Abstract

Hydrogels are frequently tested biomaterials for intervertebral disc (IVD) repair due to their capacity to mimic the structure of nucleus pulposus (NP) tissue and restore native IVD mechanical properties. Preclinical ex-vivo organ culture assessments of hydrogel efficacy commonly involve tests under uniaxial compressive load in bioreactors. We recently reported an important advance in spine research, demonstrating a new generation of bioreactors that mimic the natural, 6-degree-of-freedom, mechanical load allowing *ex vivo* IVD organ models to be maintained long-term under near-physiological conditions [1].

To establish a representative IVD degeneration model suitable for hydrogel testing, we loaded 4 bovine IVDs from 3 donors in a combination of dynamic compression (0.1-0.25 MPa), shear (6 mm) and flexion (4˚) for 7 days at 50'400 cycles and assessed degenerative changes for which a supportive hydrogel injection would be proposed. The applied protocol resulted in an 18.7±9% IVD height loss and cell death at the NP and annulus fibrosus (AF) interface. The anterior side of AF showed 28-fold and 140-fold upregulation of MMP1 and MMP13 genes (FigA), respectively, indicating a collagen-degrading phenotype. The observed IVD changes reveal a region-specific IVD response to multiaxial load and initiation of a degenerative profile. New protocols are currently being established to optimize conditions required for effecting degeneration in the NP region.

An exemplary PVA (Mw≈166 kM g-1) and glycerol hydrogel with the potential for IVD repair [2] was tested for its suitability for injection and retention in the IVD model. The initial storage modulus of moulded hydrogel (1334.2±118 Pa) was reduced (-84.4±9%) upon hydrogel extrusion in the solid state through a 27G needle (FigB). Mechanical properties could be preserved (+9.3±25%) if the gel was pre-melted at 60℃ before injection. Pre-melting allowed the use of thinner, 29G needle (-11.1±1%), thus reducing inadvertent IVD injury during the injection. The gel lost 4.5% of the initial PVA content 2h after contact with the culture medium and was further reduced by 12.7% after 14 days of incubation in static conditions, resulting in partial gel degradation and a decrease of original modulus by 26.3±1% (FigC).



We demonstrated the ability of our new multiaxial bioreactor to provide complex IVD degeneration models and the necessity of thorough assessment and optimization of a repair biomaterial before injection into an injured IVD. The hydrogel behaviour under multiaxial loading will be the next focus of our investigation.

1.ACSBiomaterSci Eng,2022.8(9):3969-3976.
2.JOrthopTranslat,2022.33:162-173.

PoB.16.41

Pro-Chondrogenic anti-inflammatory biomaterial-based therapy for Articular Cartilage: Ibuprofen vs Diclofenac loaded Chitosan/Poly-gamma-glutamic Acid Nanoparticles

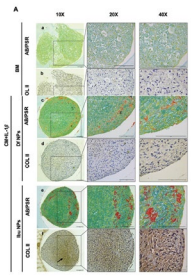
Beatriz Ribeiro[1,2,3,4], Ana Luisa Castro[1,2,3], Cristiana Couto[1,2], Sofia Pilao[1,3,4], Cristina Ribeiro[1,2,5], Mario M Barbosa[1,2,3], Susana G Santos[1,2], Raquel M Goncalves[1,2,3]

[1]i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [2]INEB, Instituto de Engenharia Biomédica, Porto, Portugal. [3]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal. [4]FEUP, Faculdade de Engenharia da Universidade do Porto, Porto, Portugal. [5]ISEP, Instituto Superior de Engenharia do Porto, Porto, Portugal

Abstract

Osteoarthritis(OA) is a highly prevalent disorder characterized by degeneration of articular cartilage and a strong inflammatory reaction. Different drugs have been proposed, namely non-steroidal anti-inflammatory drugs(NSAID), but they carry several drawbacks. Chitosan(Ch) and Poly-γ-glutamic acid(PGA) nanoparticles(NPs) with the NSAID Diclofenac (Df) have shown to be effective in reducing inflammation in intervertebral disc, while promote extracellular matrix(ECM) deposition[1], most probably associated with a pro-chondrogenic potential of PGA[2].

Here we explored Ch/PGA NPs for the delivery of Ibuprofen(Ibuf), another NSAID and compared its performance with Df-Ch/PGA NPs in mesenchymal stromal cells(MSC) chondrogenic differentiation under pro-inflammatory conditions. For that, Df and Ibuf were dissolved in PGA solution and dropped into a Ch solution (pH 5) at constant rate[3]. Physicochemical characterization of Df-Ch/PGA and Ibuf-Ch/PGA NPs was performed by DLS and FTIR. Df and Ibuf release were assessed by UV/VIS. Anti-inflammatory potential of the NPs was evaluated in M1 macrophages[4] and in MSC pellets in chondrogenic medium[2], supplemented with IL-1b(10ng/ml). Cartilage ECM was then evaluated by histology/immunohistochemistry.Df-Ch/PGA NPs and Ibuf-Ch/PGA NPs (200nm, 0.2 PdI, 20mV charge) were obtained. Df and Ibuf were almost totally entrapped in NPs, but only Df was retained upon NPs centrifugation, suggesting weaker electrostatic interactions. Ibuf was totally released from NPs within 1h, while 80% of Df was released in 24h. Both Df- and Ibu-Ch/PGA NPs exhibited their anti-inflammatory action in M1-like macrophages reducing PGE2. In MSC pellets, only Ibu-, and not Df-Ch/PGA NPs neither the free drugs, stimulated sGAGs (10-fold), Aggrecan (2-fold) and Collagen type 2 (4-fold) deposition (Fig.1). Nevertheless, only Df-, and not Ibuf-Ch/PGA NPs, significantly reduced PGE2 produced by MSC. Conditioned medium of MSC treated with both Ibuf- or Df-Ch/PGA NPs significantly reduced CCR7 expression (M1-like phenotype), while increasing CD163 expression (M2-like phenotype), in human macrophages.Overall, these results emphasize the distinctive potential of Ibuf-Ch/PGA NPs to promote MSC chondrogenesis, while Df-Ch/PGA NPs appear to strongly reduce inflammation. This work opens new horizons in NSAID and biomaterial-based synergic therapies to modulate inflammation in the context of OA.



Acknowledgments: RESTORE-User-centred smart nanobiomaterial-based 3D matrices for chondral repair(H2020-NMBP-22-2018)

References: [1]Teixeira(2016) Acta Biomat; [2]Antunes(2015) Tissue Eng Part A; [3]Goncalves(2015) J Mat Sci Mat Med; [4]Bessa-Gonçalves(2022) Acta Biomat

PoB.16.42

Cell-derived extracellular matrix tailoring using CRISPR/dCas9: a novel intervertebral disc regeneration strategy

Catarina Milheiro[1,2], Raquel M. Gonçalves[1,2], Mario Amendola[3,4], Mário A. Barbosa[1,2], Joana Caldeira[2]

[1]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal. [2]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [3]Généthon, Évry, France. [4]Integrare Research Unit UMR_S951, Université Paris-Saclay, Univ Evry, Inserm, Genethon, Évry, France

Abstract

Intervertebral disc (IVD) degeneration is characterized by tissue loss of function and major structural changes, specifically at the extracellular matrix (ECM) level. Depletion of ECM proteins leads to dehydration and mechanical alterations that can culminate into serious conditions namely low back pain. Current treatments fail to tackle the disc's hindered function and degenerative alterations.
Our goal is to promote IVD regeneration by recapitulating a fetal-like microenvironment based on tailored cell-derived matrix (CDM), rich in COLXII and COLXIV. For this, we propose the use of CRISPR/dCas9 to enable the transcriptional activation of two fetal exclusive collagens - COLXII and COLXIV - in mesenchymal stem/stromal cells (MSCs). We hypothesize that this engineered CDM will have a superior pro-regenerative potential and allow IVD functional restoration.
Immortalized mesenchymal stem cells (iMSCs) were transduced with the CRISPR/dCas9-VP64 lentiviral system, targeting *COL12A1* or *COL14A1* promoter regions. Antibiotic selection was used to guarantee engineered population maintenance. Gene expression was evaluated by qRT-PCR. Protein expression was assessed by Western Blot and immunofluorescence. Simultaneously, optimization of ECM deposition and decellularization protocol (based on NH4OH and SDS) was performed. Process efficacy was validated by immunofluorescence for deposited ECM (specifically, fibronectin), presence of cell nuclei (DAPI) and actin cytoskeleton (Phalloidin). DNA content was quantified using the Quant-iT™ PicoGreen™ Kit.
Successful iMSCs engineering was confirmed by an average increase of collagens gene expression levels (2.12-fold for *COL12A1* and 11.7-fold for *COL14A1*). Protein level assessment by western blot and immunofluorescence confirmed increased COLXII and COLXIV, suggesting that the modification in gene expression is translated into protein production. Engineered CDM production was optimized. Cell removal was demonstrated by a reduction of nuclear and actin staining, when compared with the non-decellularized control. DNA content showed a marked reduction in both NH4OH- and SDS-based protocols. Matrix preservation after decellularization was confirmed by fibronectin staining only in the NH4OH-based decellularization. Interestingly, preliminary proteomic characterization data of engineered MSCs as shown some functional impact namely on Sonic Hedgehog (Shh) pathway and repression of pain by DREAM (Downstream regulator element antagonist modulator).
Overall, our results indicate we were able to establish an activating CRISPR/dCas9 system for COL14A1 and COL12A1, which allowed us to use iMSC as biofactories of IVD fetal-tailored ECM. Altogether, the use of a customized ECM and the recapitulation of a fetal microenvironment constitute a pioneer strategy for IVD functional restoration, opening new avenues in the tissue regeneration field.

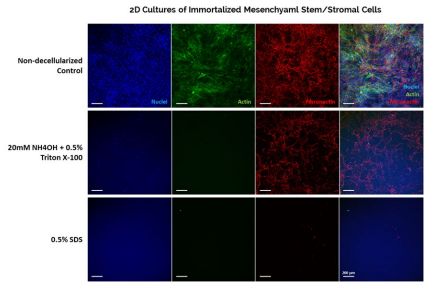


Figure 1. Optimization of the Decellularization Protocol in Immortalized MSCs.

PoB.16.43

Incorporation of Decellularised Anterior Cruciate Ligament Extracellular Matrix in an Aligned Fibre Tissue-Polymer Hybrid Scaffold to Promote an Enhanced Cellular Response and Ligament Protein Deposition

Dominic Andrew Williams[1,2], Michael McNicholas[3], Jonny Blaker[1,2,4], Julie Elizabeth Gough[1,2]

[1]University of Manchester, Manchester, United Kingdom. [2]Henry Royce Institute, Henry Royce Institute Hub, University of Manchester, Manchester, United Kingdom. [3]Orthopaedic Surgery Department, Liverpool University Hospital, Liverpool, United Kingdom. [4]Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo, Oslo, Norway

Abstract

Autologous hamstring and patella-tendon grafts are current gold standards for anterior cruciate ligament (ACL) reconstruction [1], however, donor-site morbidities from harvesting often limit patient recovery [2]. Bioengineering approaches have utilised non-/biodegradable polymers and decellularised tendon grafts to promote a regenerative response, yielding varied results and often revised with a gold standard approach due to poor graft integration and mechanical failure [2]. Bioengineered electrospun polycaprolactone (PCL)-ligament extracellular matrix (ECM) scaffolds may enhance the biological response, omitting co-morbidities associated with autologous grafting. ACL tissue harvested from juvenile porcine stifle joints was decellularised [3,4,5] and cryomilled to form a dECM powder. Changes in ligament-specific ECM components were determined using immunohistochemistry and biochemical assays. PCL:dECM solutions were prepared in hexafluoroisopropanol and electrospun onto a rotating mandrel to produce aligned fibre scaffolds. Chemical characterisation determined the presence of dECM with fibre morphology evaluated against murine fibroblasts for initial cytotoxicity and adhesion. Human fibroblasts were cultured for 7-10 days to assess the longer-term proliferation, cellular interaction and protein deposition. DNA content decreased significantly following decellularisation with cell nuclei absent versus fresh ACL. dECM exhibited a significant reduction in collagen I, elastin and sulphonated-GAG content, with similar results mirrored with histology. The addition of dECM to PCL reduced fibre diameter versus PCL controls collected using the same parameters, however, increased the elastic modulus although failure strain was reduced. FTIR indicated the presence of the Amide I/II bands in dECM [6] as a shoulder to the carbonyl peak in PCL:dECM fibres [7], with nitrogen confirmed using CHN elemental and energy dispersive X-Ray analyses. Murine fibroblasts were stable on PCL:dECM and control fibres after 3 days and showed alignment with fibre orientation. Whole tissue decellularisation has shown particular promise in allogenic tendon grafting for ACL reconstruction, offering larger grafts for easier fixation and closer mechanical matching of the native ligament. Incorporating decellularised ACL ECM into a polymer matrix may offer tailorable mechanical properties of synthetic approaches with the bio-induction of native tissue.

1) Gabr A. et al. NLR, 2019
2) Legnani C, et al. Int Orthop. 2010, 34, 465-71
3) Grant, R. et al. Sci Rep. 9, 6293, 2019
4) Reid, J., Callanan, A. Applied Polymer Science. 136, 44, 48181, 2019
5) Vavken, P. et al. Orthop Res. 27, 12, 1612-18, 2009
6) Cortizas, A M., Lopez-Costas, O. Sci Rep. 10, 1788, 2020
7) Huang, A. et al. RSC Advances. 7, 43432, 2017

PoB.16.44

Multi-layer hybrid scaffolds for articular cartilage repair

Rachel Cordeiro[1,2,3], Nuno Alves[1,4,5], Ana C. Maurício[2,6,7], Carla Moura[3,1,5]

[1]Centre for Rapid and Sustainable Product Development, Polytechnic of Leiria, 2430-028, Marinha Grande, Portugal. [2]Veterinary Clinics Department, Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto (UP), Rua de Jorge Viterbo Ferreira, No. 228, 4050-313, Porto, Portugal. [3]Polytechnic Institute of Coimbra, Applied Research Institute, Rua da Misericórdia, Lagar dos Cortiços – S. Martinho do Bispo, 3045-093, Coimbra, Portugal. [4]Department of Mechanical Engineering, School of Technology and Management, Polytechnic of Leiria, 2411-901, Leira, Portugal. [5]Associate Laboratory for Advanced Production and Intelligent Systems (ARISE), 4050-313, Porto, Portugal. [6]Animal Science Studies Centre (CECA), Agroenvironment, Technologies and Sciences Institute (ICETA), University of Porto, Rua D. Manuel II, Apartado 55142, 4051-401, Porto, Portugal. [7]Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), 1300-477, Lisbon, Poland

Abstract

Osteoarthritis is a joint disease that affects 250 million people worldwide and involves progressive articular cartilage (AC) loss, a problem aggravated by AC's intrinsic incapacity for self-repair.
This study aims to develop an AC replacement that closely mimics the complexity of the native tissue, recreating the fibre alignment and extracellular matrix composition. This region-specific customisation will be manufactured layer-by-layer to produce a hybrid scaffold (figure in attachment). Chondrocytes and osteoblasts from human dental pulp stem cells (hDPSCs) will be seeded on this scaffold using a multiphase cell seeding process. After seeding, the scaffold will be placed in a two-chamber perfusion bioreactor to support the individualised cell proliferation but simultaneous tissue growth.
To mimic the structure of the deep zone (DZone), we considered its native structural parameters, a columnar alignment and 80% porosity. DZone was 3D printed with poly(lactic-co-glycolic acid) (PLGA) (85:15), a medical-certified polymer, by using a custom GCode written to manufacture an orthogonal arrangement followed by 2 column layers. This structure was repeated in height, resulting in a 1.8 mm layer, about 30% of the total AC thickness. So far, a columnar structure with 64% porosity was achieved. Optimisation work is still in progress on 3D printing parameters to increase porosity. Mechanical and *in vitro* cellular tests will be performed.Another layer under active work is the calcified zone (CZone). To mimic this layer, we hypothesise the creation of a PLGA-hydroxyapatite (HAp) composite that will give rise to a structure with CZone native porosity (around 70%). Incorporating HAp, a naturally present bioceramic in this zone, it is intended to promote mineralised matrix production. To achieve this, composites with 60% and 70% HAp are being developed with different reaction times to obtain proper bonding. TGA, DSC and FTIR analyses were carried out. DSC results showed that longer reaction time leads to lower composites' melting point and glass transition. FTIR and TGA proved that the two materials are bonded through the peak at 1170 cm^{-1} and that this bond represents about 10-15% of the composite. Further characterisation with SEM and NMR will be performed to detail each composite's structure and bonding type. The most suitable composite will be extruded into a 3D printing filament to manufacture the CZone structure with its native porosity. By closely mimicking AC structure, composition and cell positioning, we believe the proposed approach will improve the treatment of type IV lesions and overcome previous implant limitations.

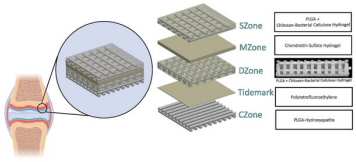


Figure 1 - Schematic diagram of the multi-layer hybrid scaffolds for articular cartilage repair

PoB.16.45

Bioprinted Nano-Zirconia composite scaffolds for Intervertebral disc restoration

Julia Ce de Andrade[1], Philipi Cavalcante Ricardo[2], Frank Jorg Clemens[1], Bruno de Castro Henriques[2], Marcio Celso Fredel[2]

[1]Empa, Dübendorf, Switzerland. [2]UFSC, Florianópolis, Brazil

Abstract

The stability of the spinal column is principally determined by the structural integrity of the intervertebral disc (IVD), composed of an internal 'gel-like' nucleus pulposus (NP) and an external fibrous structure, the annulus fibrosus (AF). Chronic low back pain often originates from IVD degeneration, a condition that can considerably compromise spinal biomechanics and can potentially lead to serious health repercussions. Unfortunately, current therapeutic interventions fall short of fully regaining the native biological functions of a healthy IVD. Surgical procedures, although potentially beneficial, frequently yield reduced spinal flexibility. Non-invasive treatments such as analgesics temporarily relieve pain symptoms without addressing the underlying cause, leaving the core issue untreated.

Inspired by the nature, the objective of this research is to augment the life quality of individuals suffering from IVD pathologies. This is achieved by generating biomimetic IVD substitutes using bioprinting technology, with the intention of reinstating the mechanical and biological properties of the native disc. Given the hydrogel-like consistency of the NP, bioprinting emerges as a potentially effective solution for its reconstruction. This cutting-edge application of additive manufacturing (AM) uses cells and various biological substances to generate precise replications of live tissues and organs.

To accomplish the bioprinting, this research engineered bioinks (hydrogels) from a combination of biomaterials, including alginate gel, chitosan, type I collagen, and zirconia nanoparticles. Zirconia acts as a bioinert ceramic filler, bolstering the bioink's mechanical properties, chitosan contributes to cell proliferation and bioscaffold production, in conjunction with alginate. Collagen mimicking native biological properties is proposed to facilitate differentiation, morphogenesis, and tissue repair. The bioinks were synthesized via a process of ultra-turrax blending and centrifugation, then printed using a UV-light activated BioX bioplotter, culminating in the creation of NP scaffolds that exhibit both mechanical and biological properties suitable for IVD reconstruction applications.

PoB.16.46

An investigation of the fluid structure interaction in articular cartilage across disparate scales

Emily J Butler, David Head, Mark Walkley, Michael Bryant, Greg de Boer

The University of Leeds, Leeds, United Kingdom

Abstract

Articular cartilage (AC) is found at opposing surfaces in mammalian joints. It provides a smooth bearing surface, promoting low friction articulation and facilitating continuous operation under relative motion. A lack of cells within AC renders a low capacity for intrinsic healing or repair. This leaves it prone to degeneration and disease, resulting in a high clinical demand for cartilage repair [1]. To streamline treatment, an accurate computational model of the tissue is essential to inform rapid pre-screening of therapeutic interventions. However current models generally use a single-scale approach, which fails to capture the complex multi-scale features of the tissue, including its intrinsic inhomogeneity and depth dependant properties [2, 3]. Instead, this project aims to couple an immersed fibrous network (micro-scale) model with a continuum (macro-scale) model, using the Heterogeneous Multi-scale Method [4] to create a multi-scale poroelastic model that can capture the fluid-structure interaction arising within AC across disparate scales. The novelty of the approach lies with using homogenized stress, strain, and velocity from the micro-scale, alongside superposition, to populate the macro-scale elasticity and permeability tensors.

A continuum-continuum model has been developed for validation of the multi-scale poroelastic model. Currently, a fibrous network representation of the micro-scale behaviour is being integrated into the multi-scale model to include anisotropy and depth dependent properties. Once this continuum-fibre coupling has been established, zonal structuring will be introduced, and the resulting model will be validated against experimental data.

The micro-scale fibre model is based on a regular spring lattice in which lattice bond occupation and spring stiffness can be freely varied, making it an advantageous choice for damage modelling, as fibre anisotropy and damaged tissue can be incorporated by selective modification of the micro-scale elastic elements. Modelling weakening areas of tissue and eventual lesions that arise in damaged cartilage tissue will inform an understanding of the change of behaviour and mechanical properties of the damaged tissue, which could be a critical step to inform clinical interventions for cartilage repair and replacement in the future.

References:

[1] Dhinsa, B S et al., Current stem cell research & therapy 7.2 (2012).
[2] Kilka, V et al., Mechanical Behaviour of Biomedical Materials 62 (2016).
[3] Halloran, J P et al., Annals of Biomedical Engineering 40.11 (2012).
[4] Weinan, E et al., Communications in Mathematical Sciences 1.1 (2003).

PoB.16.47

Chitosan biomineralized with ions-doped nanohydroxyapatite as a cytocompatible and anti-bacterial tool for bone regeneration

Franco Furlani[1,2,3], Alfredo Rondinella[1], Matilde Clarissa Malfatti[2], Matteo Zanocco[1], Elisabetta Campodoni[3], Barbara Skerlavaj[2], Monica Sandri[3], Gianluca Tell[2], Lorenzo Fedrizzi[1]

[1]University of Udine, Polytechnic Department of Engineering and Architecture, Udine, Italy. [2]University of Udine, Department of Medicine, Udine, Italy. [3]National Research Council of Italy - Institute of Science, Technology and Sustainability for Ceramics (ISSMC-CNR, former ISTEC-CNR), Faenza, Italy

Abstract

Bone is a load-bearing material consisting mainly of hydroxyapatite – bioceramic material based essentially on calcium and phosphate – and collagen. This tissue is frequently damaged by trauma and diseases and in some cases needs to be replaced, nevertheless, most of the currently proposed materials are not able to promote regeneration and integration with native tissue due to the lack of biomimetic behavior, *i.e.* the ability to reproduce the properties including the mechanics and composition of the native tissue. Consequently, there is a need to develop new biomaterials to achieve these goals. Chitosan is a low-toxic, biodegradable, and versatile polymer that shares a peculiar mechanical performance with collagen.

In the present contribution, we report an original method for chitosan biomineralization – a naturally occurring process – with ions-doped nanohydroxyapatite. The hydroxyapatite lattice was doped with different ions able to partially replace calcium – including magnesium, iron, and copper – to tune its biological activity, and the same procedure was also performed without any polymer. According to this process, it was possible to produce nanohydroxyapatite-based nanoparticles endowed with low crystallinity and with different percentages of ions substitution and of the organic component.

The biological activity of the resulting nanoparticles was then investigated towards osteoblasts-like cells, including HOBIT (Human OsteoBlast-like Initial Transfectant) and MG63 cells. Specifically, cell metabolic assays confirmed the good cell viability and proliferation of cells, whereas the oxidative stress in cells was investigated by using a General Oxidative Stress Indicator. Simultaneously, high-resolution confocal microscopy images were acquired in order to analyze cell morphology and detect possible H2A.X foci corresponding to DNA damage response. These aspects were also deepened by Western blot analyses of H2A.X and PRXSO3 expression. These latter tests indicated that the presence of iron and copper ions within the hydroxyapatite lattice stimulates the production of Reactive Oxygen Species (ROS) promoting the activation of the DNA damage response and this activity is enhanced by the presence of chitosan suggesting that chitosan favors the internalization of nanoparticles by cells and modulate the biological activity of resulting nanocomposites. Finally, the antibacterial activity of resulting nanoparticles was assessed after the incubation with *S. aureus* and *E. coli* incubation confirming the good antibacterial activity of some ions-doped nanoparticles.

Considering the overall results, the present nanoparticles can be proposed for the development of bone fillers for bone regeneration and to devise materials and coatings for bone replacement.

PoB.16.48

BMP-2 along with ERK inhibitor and Phenamil effectively enhance osteogenesis of human adipose-derived stem cells cultured on SrO- or ZnO-modified bioactive glass-PLGA composites in static and dynamic cultures

Karolina Truchan[1], Barbara Zagrajczuk[2], Katarzyna Cholewa-Kowalska[2], Anna Maria Osyczka[1]

[1]Jagiellonian University, Faculty of Biology, Institute of Zoology and Biomedical Research, Department of Biology and Cell Imaging, Kraków, Poland. [2]AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Glass Technology and Amorphous Coatings, Kraków, Poland

Abstract

Strategies to improve bone regeneration therapies with adult stem cells remain in high demand. Adipose-derived mesenchymal stem cells (ASCs) offer an alternative source of multipotent cells to bone marrow stem cells (BMSCs), capable of differentiating into osteoblasts. However, it has been reported that the osteogenic potential of ASCs is lower than that of BMSCs.

In this study, we present novel strategies for the effective osteogenic differentiation of human ASCs *in vitro*. Our approach combines culturing ASCs on bioactive composites, treatment with specific chemical compounds, and the application of dynamic culture conditions to further enhance the osteogenic progression. We cultured human ASCs line (ASC52telo, ATCC) on poly(lactic-co-glycolic acid) (PLGA)-based composite sheets containing 50% wt. sol-gel bioactive glasses in the SiO_2-CaO-P_2O_5 system, additionally modified with either ZnO or SrO. The culture media were supplemented with bone morphogenetic protein 2 (BMP-2), ERK kinase inhibitor PD98059 and Smurf1 inhibitor Phenamil. Dynamic culture conditions were applied using a standard laboratory horizontal rocker to subject the cells to fluid shear stress.

Our results demonstrate that the treatment with the above-mentioned chemical compounds significantly promotes and supports osteogenesis of ASCs in both short-term and long-term cultures on the SiO_2-CaO-P_2O_5/PLGA bioactive composites. Furthermore, the modification of the composites with SrO or ZnO, along with the application of fluid shear stress at 3-day intervals, markedly enhances the expression of key markers associated with bone formation in ASCs, such as osteocalcin, osteoprotegerin, and osteonectin. Importantly, bioactive growth surfaces are required to promote osteogenesis in ASCs. Notably, pre-culturing ASCs for 7 days under the combined conditions, followed by their transfer to typical polystyrene cell culture dishes, leads to robust extracellular matrix mineralization within 14 days.

We believe that these novel strategies for osteogenic differentiation of ASCs hold great potential in various bone regeneration therapies. Both the composites and chemically/mechanically treated ASCs, either in combination or separately, demonstrate suitability for *in vivo* applications.

This work was financed in part by the Polish Ministry of Science and Higher Education 'Diamond Grant' No. 0114/DIA/2020/49 (KT), Centre for Technology Transfer of the Jagiellonian University grant (AMO), and the Polish National Science Centre grants Nos. 2019/32/T/ST5/00453 (BZ) and 2017/27/B/ST8/00195 (KCK).

PoB.17.01

Osteoinductive properties of SAPO zeolites and metal organic frameworks synthesized with lithium and calcium.

Cristian Covarrubias[1], Camila Torres[2], Daniel Vargas[2], Amaru Agüero[2], Miguel Neira[2], David Beltrán[2], Rolando Vernal[2], Benjamin Le Monnier[3], Michael Tsapatsis[3], Emma Whitehead[3], Warren Grayson[3]

[1]Universiity of Chile, Santiago, Chile. [2]University of Chile, Santiago, Chile. [3]Johns Hopkins University, Baltimore, USA

Abstract

Introduction Zeolites and metal organic frameworks (MOFs) are crystalline materials characterized by their ordered nanoporous architecture, high surface area, and excellent surface properties. However, these materials have been few explored for bone tissue regeneration applications. Lithium and calcium cations are elements with already demonstrated properties to promote bone tissue formation [1]. So, we explored the synthesis of silicoaluminophosphate (SAPO) zeolites and MOFs containing calcium and lithium and demonstrated their osteoinductive properties. **Methodology** SAPO-34 and SAPO-5 zeolite types were hydrothermally synthesized at 200 ºC using Li or Ca as intra/extraframework cations. MOFs were synthesized at room temperature using 1,4-benzendicarboxylic acid (BDC) as organic linker bonded to Li or Ca cations. Structural characterization was performed by ATR-FTIR, XRD, SEM/EDX, solid-state NMR, and N2 sorptometry. Apatite formation was assessed in simulated body fluid (SBF) and ion release in buffer TRIS pH 7.4. Viability of preostoblastic MC3T3 cells incubated with the particles was assessed with the MTS assay. Cell adhesion was examined by fluorescence and SEM microscopies. Osteogenic cell differentiation was evaluated by measuring the alkaline phosphatase (ALP) activity. **Results And Discussion** SAPO and MOFs crystals were produced with nanometric dimensions and lamellar morphology (Fig. 1). XRD confirmed the crystalline structure of the synthesized SAPO-5 and SAPO-34 zeolites, which is formed by nanoporous of 0.73 and 0.43 nm, respectively. MOF containing calcium as metal framework metal nodes exhibited a stable crystalline structure, however, lithium- based MOF was not water-stable. FTIR analysis demonstrated that both SAPO and MOF particles promoted the formation of apatite in SBF as judged by the characteristics P-O apatite vibrations. This effect was favored by the calcium release capacity and high surface area of the materials (~300 m2/g). The SAPO and MOF particles did not alter the viability of MC3T3 cells and offered a cytocompatible substrate to cell spreading and adhesion. More interestingly, SAPO and MOF were able to promote the cell differentiation toward mineralizing lineage as assessed by an increasing in the ALP activity, particularly, those materials impregnated with calcium and lithium cations. **Conclusions** The SAPO and MOF nanosized particles promote the formation of apatite, are cytocompatible and stimulate the osteogenic cell differentiation, making of them excellent candidates for *in vivo* bone tissue regeneration studies.

Acknowledgments FONDECYT Grant 1211314, National Agency for Research and Development (ANID), Chile.

References

[1] C. Covarrubias, J. P. Durán, M. Maureira, Mater. Letters, 219, 205, 2018.

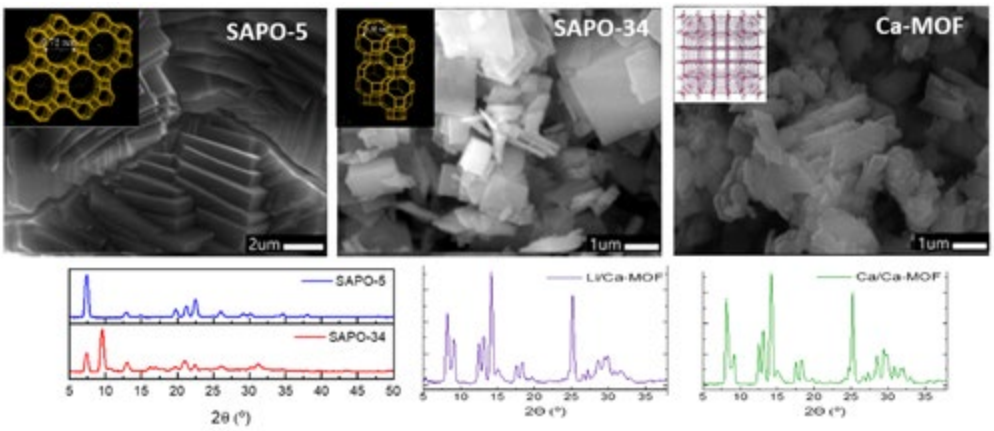


Fig. 1 SEM images and XRD patterns of synthesized SAPO and MOF particles.

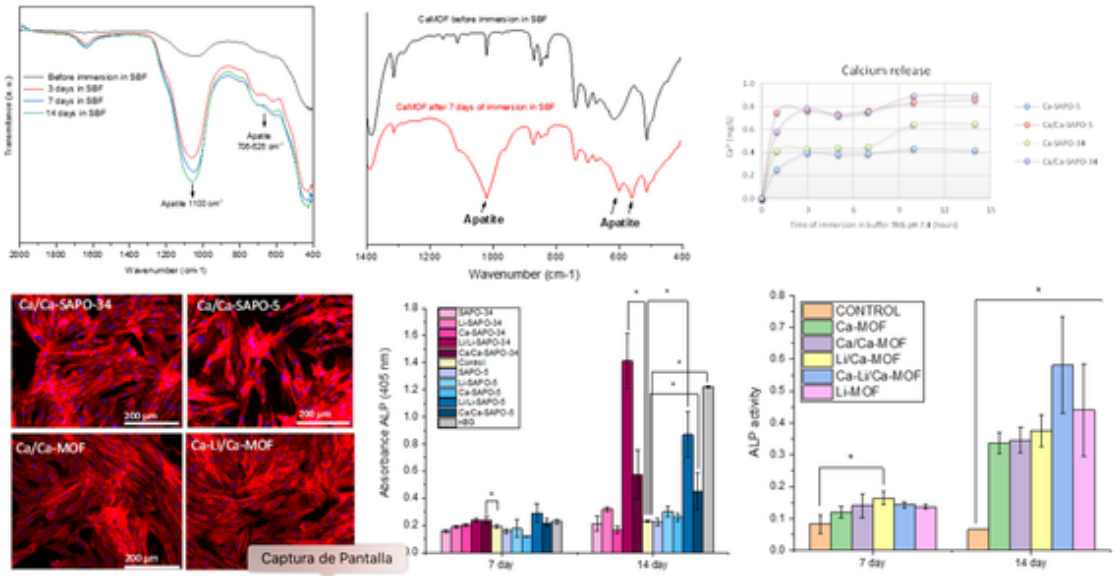


Fig. 2. FTIR spectra showing apatite formation on SAPO-5 zeolite and Ca-MOF, calcium release curves of MOFs, fluorescence microscopy images and ALP activity of MC3T3 cells cultured with the studied particles.

PoB.17.02

Effect of Cerium Precursor to Gelatin Ratio on the Development of Cerium Oxide Nanoparticles

Georgia K. Pouroutzidou[1,2], Konstantinos Tsachouridis[3], Ioannis Tsamesidis[2], Anastasia Beketova[2], George Vourlias[1], Antonios D. Anastasiou[3], Eleana Kontonasaki[2]

[1]School of Physics, Aristotle University of Thessaloniki, Thessaloniki, Greece. [2]School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece. [3]Department of Chemical Engineering and Analytical Science, University of Manchester, Manchester, Greece

Abstract

Introduction Nanomaterials can be used as antibacterial agents to overcome bacterial drug resistance caused by systemic or local administration of antibiotics[1]. Numerous metal- and metal oxide-based nanomaterials, such as cerium oxide nanoparticles (NPs) have recently been fully integrated into antibacterial applications and achieved excellent performance. Surprisingly, less toxic cerium oxide nanoparticles function as potent antibacterial agents by reversibly converting their oxidation state from Ce(III) to Ce(IV), which is an effective functional mechanism against pathogens[1,2]. In this study, different amounts of gelatin acting as a stabilizer and Ce-precursor were investigated in order to optimize the size, crystallinity and hemocompatibility of CeO_2-NPs. **Experimental Methods** The synthesis of CeO_2-NPs was performed via the sol-gel method. As precursor, $Ce(NO_3)_36H_2O$ at 1, 3, or 5g was used, while CeO_2-NPs were synthesized with (0.4 g) or without gelatin (stabilizer), all dissolved in d.d.H_2O (Table1)[1]. The appropriate amount of ammonia solution was added until the pH reached 10. Following this, all materials were kept under mechanical stirring for 4 h and inserted into the freeze-drier until the excess of the solvent was removed. Finally, each sample was heated at 550ºC for 1h.

Sample	Reactants			
	Gelatin	H_2O	$Ce(NO_3)_36H_2O$	Gelatin/Ce precursor
CeG1	0.4g	40 ml	2g	0.20
CeG3	0.4g	40 ml	6g	0.07
CeG5	0.4g	40 ml	10g	0.04
CeWG1	-	40 ml	2g	-
CeWG3	-	40 ml	6g	-
CeWG5	-	40 ml	10g	-

Table1. *Amounts of gelatin and Ce precursor.*

The physiochemical characterization of CeO_2-NPs, cell viability of human periodontal ligament fibroblasts and their hemolytic activity in contact with human red blood cells were investigated. **Results And Discussion.** The XRD patterns revealed the presence of 100% CeO_2 crystalline phase, while the particle size of all samples was under 100nm (Fig.1). The increase in Gelatin/Ce-precursor ratio led to a decrease in particle size, formation of well-shaped particles, and improved hemocompatibility. Higher ratios presented a slight hemolytic effect (3-5%) after 24h of incubation only at the highest tested concentration (1mg/ml). However, CeG1 and all the NPs synthesized without gelatin were hemocompatible at all the tested concentrations (0.125–1mg/ml). **Conclusion** CeO_2-NPs were successfully synthesized by the sol-gel method. The Gelatin/Ce-precursor ratio affected the morphology, particle distribution and hemocompatibility. The increase in this ratio improved hemocompatibility, decreased particle size and led to the formation of more uniform-shaped particles.

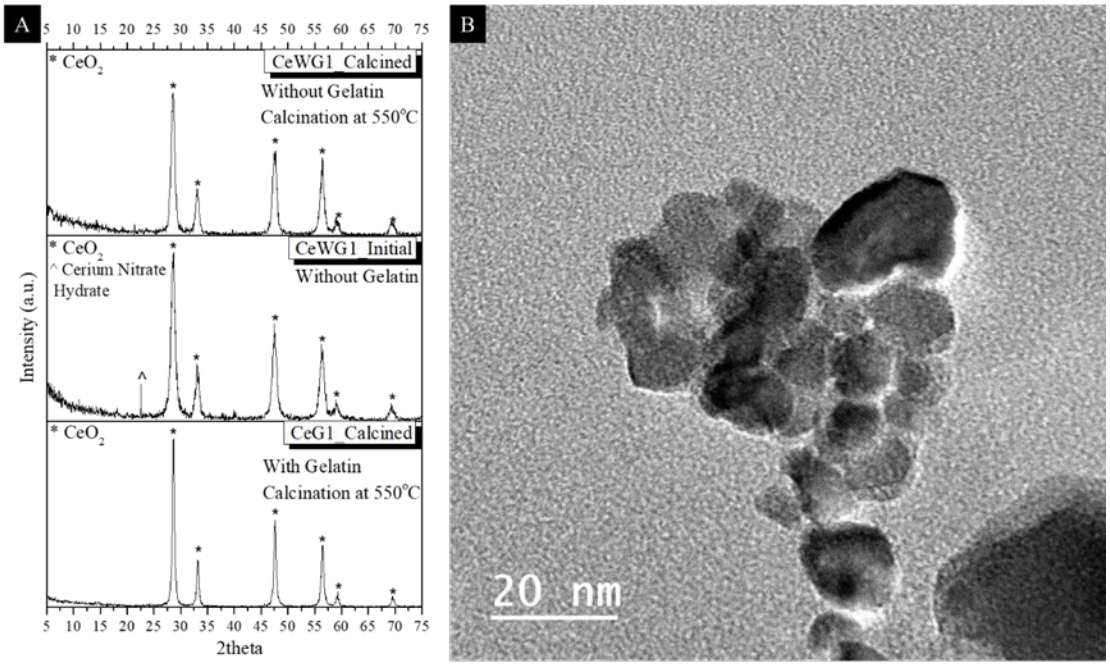


Fig.1: *FTIR spectra (A) and TEM micrographs (B) of sample CeG1.*

References

1. *Darroudi M. et al., Ceram Int.39:6917-6921,2013*
2. *Ioannou M. et al., Appl. Sci.2023,13(4),2676*

Acknowledgments

This work is supported by European Union's Horizon2020 research and innovation programme under grant agreement No953128, project: Smart, Multifunctional Dental Implants: A solution for peri-implantitis and bone loss.

PoB.17.04

Effect of synthesis modifications on the biological behavior of cerium oxide NPs

Ioannis Tsamesidis[1], Dimitrios Gkiliopoulos[2], Anna Theocharidou[1], Diana Samara[3], Athanasios Lettas[3], Eleana Kontonasaki[1]

[1]School of Dentistry, Aristotle University of thessaloniki, Thessaloniki, Greece. [2]Department of Chemistry, Aristotle Uinveristy of Thessaloniki, Thessaloniki, Greece. [3]General Hospital of Naousa, Naousa, Greece

Abstract

Introduction Cerium oxide nanoparticles (Ce-NPs) present antioxidant properties and improved biological properties due to the bivalent [Ce(III) and Ce(IV)] state of CeO_2[1]. Under CeO_2 reduction to various defective phases, CeO_{2-x}, oxygen vacancies are formed in the lattice structure, while the reversible process of reduction and oxidation in the CeO_2 structure modulates the formation and migration of these oxygen vacancies, creating oxygen storage capacity. Oxygen vacancies after materials synthesis may differ while Ce(III)/Ce(IV) ratio is negatively correlated to the particle size of CeO_2 NPs[2]. The aim of this study was to investigate the effect of modifications in Ce-NPs synthesis on their biological behavior and correlate it with cerium oxidative states. **Experimental Methods** Cerium oxide nanoparticles CeNPs were synthesized via solution precipitation using $Ce(NO_3)3{\cdot}6H_2O$ dissolved in aqueous ammonia solution [molar ratio $Ce(1):NH_3(12)$] of pH 10. Modifications in a standard synthesis method were applied regarding i) sonication, ii) oxidative environment, iii) organic co-factor, and iv) calcination during synthesis. Biological behavior was evaluated through hemolysis assays, while antioxidant properties were analyzed using Reactive oxygen species [ROS] fluorescence analysis (2-hydroxyethidium (2OH-E+))[3]. Ethical approval was obtained from the General Hospital of Naousa, Greece (ID_233205920) and the Ethics Committee of the School of Dentistry, AUTH (#110/10-2-2021). All the biological assays were performed according to ISO 10993-4:2017. **Results And Discussion** All materials presented biocompatible behavior. None of the Ce-NPs induced hemolysis at all tested concentrations after 60 minutes of incubation. Ce-NPs synthesized in the oxidizing environment presented better hemocompatibility compared with Ce-NPs produced via ammonia precipitation. Concerning antioxidant properties, ROS levels presented an overproduction after RBCs' exposure to NPs for 24 hours (Figure 1). **Conclusion** All tested concentrations of Ce-NPs proved to be hemocompatible without inducing any hemolytic effect or disruption of cells' membranes. Although the Ce(III) to Ce(IV) ratio presented variations leading to different ROS levels among the synthesized NPs, they all presented high biocompatibility.

Acknowledgments

This work is supported by European Union's Horizon 2020 research and innovation program under grant agreement No 953128, project: Smart, Multifunctional Dental Implants: A solution for peri-implantitis and bone loss

References

1. Zhang M. et al. 2019. https://doi.org/10.1007/s40843-019-9471-7
2. Deshpande et al. 2005. https://doi.org/10.1063/1.2061873
3. Tsamesidis I. et al. 2020. https:// doi:10.1007/s12195-020-00614-3

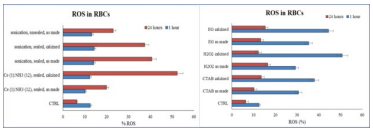


Figure 1. ROS production after 1 and 24 hours of incubation with RBCs in contact with NPs synthesized with various modifications.

PoB.17.05

Sustainable synthesis of nano- and micro- particles in microfluidic reactors.

Konstantinos Tsachouridis, Antonios D Anastasiou

The University of Manchester, Manchester, United Kingdom

Abstract

Introduction: Although most reports regarding biomaterials focus on biological response and characterization of physicochemical properties, there is an urgent need to develop new efficient methods for their synthesis. In this work, we examine microfluidic reactors (mrs) for sustainable synthesis of common nano- and micro-particles used in tissue engineering. Compared to traditional batch reactors, mrs offer precise control of reaction parameters, high surface-to-volume ratio, energy efficiency and ability to handle small volumes of reagents which result in stricter control of particle size, shape, and morphology. Two different systems are investigated. Firstly, the synthesis of cerium oxide nanoparticles (cons), known for antioxidant properties, antibacterial potential and regenerative properties. Secondly, a calcium phosphate mineral (cap), which's crystals size might range to several microns. Caps have been extensively investigated for tissue engineering applications due to structural and chemical similarity with the natural mineral of human hard tissues. **Experimental methods:** mrs with channel diameter of 250μm are fabricated using a resin 3D-printer and PDMS elastomers. A high viscosity oil is the continuous phase, keeping particles from fouling, and two aqueous based reactants merge in a droplet where the reaction is taking place. Droplet formation experiments were conducted using a high-speed camera AOS S-MIZE and an inverted microscope KERN OCM-161 for visualization. Caps and cons were also synthesized at different flow conditions and were compared to standard batch synthesis products. The particles were characterized using XRD, DLS, SEM and TEM. **Results And Discussion:** The droplet formation was investigated for Re numbers 0.03 ± 0.02 with a droplet diameter 70 to 150μm [Fig.1a], and adjustments on geometry and flowrates were required to facilitate droplet formation, which was optimized for 10ml/h of oil and 2ml/h of reagent (aq.) Flowrates. Small quantities of caps and cons in the MR synthesis were acquired and optimizations are currently in progress.

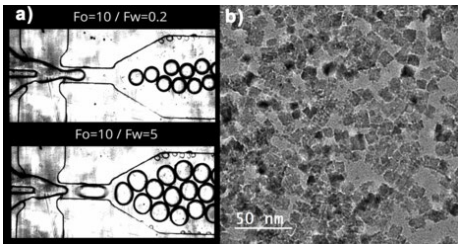


Fig.1a) Droplet formation in high-speed camera. b) TEM imaging of CONs with square-shaped morphology.

On TEM imaging, the particle size of CONs is quite consistent at 6 ± 2nm with slight agglomeration in both batch and MR syntheses [Fig.1b]. Both CONs and Caps XRD peaks from international database's reference (ICDD) are matching, thus confirming the syntheses [Fig.2].

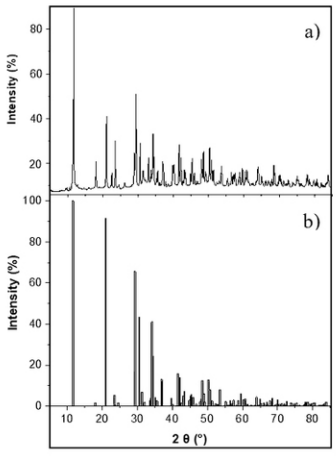


Fig.2a) XRD of CaPs crystals, b) Reference pattern from ICDD.

Conclusion. MRs have been fabricated, and synthesis of CaPs and CONs has been monitored. Resulting products have been characterized for their size, shape, and morphology, and compared to those produced through conventional synthesis routes.

PoB.17.06

Ciprofloxacin-loaded mucosomes outperform the free drug in the treatment of *S. aureus* and *P. aeruginosa* lung-resident *in vitro* infections

Giuseppe Guagliano[1], Cosmin S Butnarasu[2], Elisa Restivo[3], Paola Petrini[1], Livia Visai[3], Sonja Visentin[2]

[1]Department of Chemistry, Materials, and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy. [2]Department of Molecular Biotechnology and Health Science, University of Turin, Turin, Italy. [3]Molecular Medicine Department (DMM), Centre for Health Technologies (CHT), UdR INSTM, University of Pavia, Pavia, Italy

Abstract

Introduction. The lack of therapies to fight life-threatening infections is progressively alarming societies and, to date, the costly quest for new drugs runs in parallel with the rapid increase in drug-resistant bacteria. New methods are needed to tackle pathogens from multiple angles. Among the different nanocarriers, mucosomes -mucin based nanoparticles- [1] may possess multiple advantageous properties. Here, the efficacy of ciprofloxacin-loaded mucosomes was studied in complex *in vitro* mucus models (Mu^3Gel) of *P. aeruginosa* and *S. aureus* lung-resident infection. **Materials and methods** Mucosomes were synthesized and loaded with ciprofloxacin through a one-pot synthesis, as previously described for other drugs. [1] *S. aureus* (ATCC_25923) and *P. aeruginosa* (PA01) were grown in Mueller-Hinton broth. Bacterial suspensions at the concentration of 10^4 cells/ml were seeded and treated with equal volumes of free ciprofloxacin or ciprofloxacin-loaded mucosomes (both native and rehydrated), with concentrations of drug in the interval [0-128] µg/ml. Viability was measured after 24 hours. Three-dimensional bacterial cultures were produced by exploiting Mu^3Gel. [2] Mucus models were infected with 100 µl of 10^5 cells/ml, incubated for 24 hours, and then treated in the same way as planktonic cultures. Viability was measured 24 hours after the treatment. Interactions between bacteria and mucosomes within 3D models were studied through confocal microscopy (CLSM), exploiting red fluorescent *S. aureus* and *P. aeruginosa*, and FITC-loaded mucosomes. **Results and discussion** In planktonic cultures no changes of efficacy were reported between different treatments. This indicates that ciprofloxacin activity is preserved after its inclusion within mucosomes and after the freeze-drying followed by rehydration of the nanoparticles. Within 3D models, ciprofloxacin-loaded mucosomes were active against both bacteria at significantly lower concentrations than ciprofloxacin. This may be explained by the nanocarriers-mucus interaction, resulting in improved drug-delivery. [1] CLSM observations revealed that mucosomes are preferentially distributed in the close proximity of pathogens. The exact dynamics driving the interactions between nanoparticles and specific bacterial strains still need to be unveiled, but the observed “trap-and-kill” mechanism might be caused by the interactions between the mucosomes glycans and the surface proteins of bacteria. **Conclusions** Mucosomes represent a promising technology in the treatment of pathogens colonizing the mucosal district of the organism. Results obtained in this study encourage the further exploration of ciprofloxacin-loaded mucosomes activity in complex scenarios, like *in vivo* models of chronic pulmonary infections.

References

[1] *Adv. Healthc. Mater. 11 2200340*

[2] *J. Mater. Chem. B 7 4940–52*

PoB.17.09

Covalent organic framework nanosheet anchored with highly dispersed Au nanoparticles as a novel nanoprobe for DNA methylation detection

Yao Wu

Sichuan University, Chengdu, China

Abstract

Covalent-organic framework nanosheets (CONs) have received increasing attention in various fields. However, large-scale and high-yield preparation and flexible functionalization of CONs remain still a challenging task. Herein, we designed and fabricated a novel ultrathin (<5 nm) vinyl-functionalized CON in large scale (>80 mg) and high yield (>60 %) via an imine-exchange synthesis strategy (Figure 1A) . Through post-modification strategy, the vinyl-functionalized CONs were sequentially modified with thiols and Au nanoparticles with high distribution density (mass ratio of CONs to Au was about 4 : 1) and narrow size distribution (3.5 ± 0.5 nm). The synthesized hybrid nanosheets were employed as platform to design a novel biosensor for ultrasensitive detection towards cancer-associated methylated DNA (Figure 1B). Integrating site-specific base oxidation damage strategy and target cyclic amplification effect, the well-designed CONs-based biosensor achieved high sensitivity for detecting methylated DNA as low as 10 fM and ultrahigh specificity for distinguishing 0.0001% methylation level. Noteworthy, the proposed biosensor was successfully applied to the analysis of DNA methylation in human colon cancer cells, expanding the application scope of CONs and opening a new horizon to develop new CONs-based biosensors.

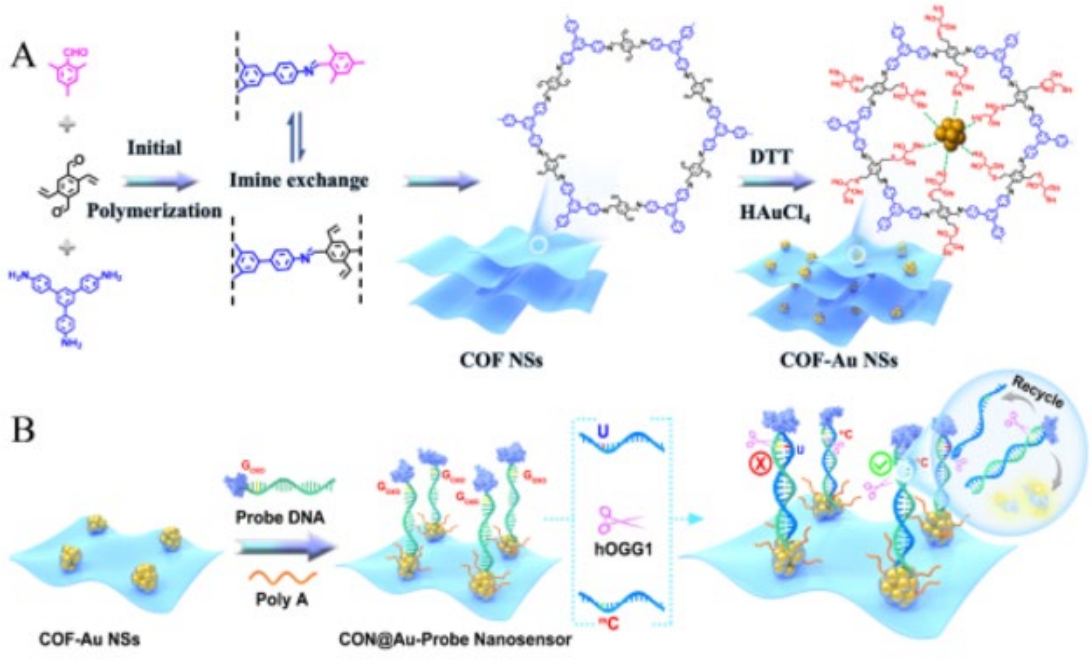


Figure 1. *Schematic illustration for (A) the fabrication process of COF-Au NSs and (B) the fabrication process of CON@Au-Probe biosensor and mechanism of the proposed biosensor for target methylated DNA detection.*

PoB.17.10

Hydrophilic magnetic covalent organic frameworks for highly integrated pre-enrichment and analysis of colorectal cancer differential glycoproteomics

Zhiyu Li, Zhiyu Li, Zhiyu Li

Sichuan University, Chengdu, China

Abstract

According to the global cancer burden data released by the World Health Organization's International Agency for Research on Cancer (IARC) in 2020, colorectal cancer (CRC) has become the third most common cancer in the world, with a high mortality rate. Early diagnosis and treatment of CRC was the critical for the survival rate of patients, it was necessary to develop more accurate biomarkers, therapeutic targets and understand cancer progression. However, the current research on CRC was uncomprehensive. New way and methods were needed to expand CRC research more urgently. Glycoproteins were related to inflammation, cancer metastasis and apoptosis. With the development of proteomics, more and more glycoproteins were used as biomarkers for cancer or other diseases in early diagnosis of diseases. Finding glycoproteins related to both biomarkers and therapeutic targets was essential prerequisite. Unfortunately, the glycoproteins were hard to directly detect by mass spectrometry due to their low abundance and abundant of interferents. Herein, we report a systematic integration of enrichment and comparative analysis strategy using the hydrophilic magnetic covalent organic frameworks (COFs) as the platform to fish the glycopeptides from normal and colorectal cancer (CRC) cell lysates by bottom-up methods. The layer of COF was controlled growth on the surface of Fe_3O_4 nanoparticles and the hydrophilicity increased with L-cysteine (L-Cys) was modified. The Fe_3O_4@PVP/PEI@COF-L-Cys nanoparticles would be an ideal material for glycopeptide enrichment, due to porous COF layer and L-Cys. Sure enough, the Fe_3O_4@PVP/PEI@COF-L-Cys nanoparticles have exhibited the excellent selectivity (IgG/BSA, 1:2000) and low detection limit (0.1 fmol) for glycopeptide enrichment. In addition, the Fe_3O_4@PVP/PEI@COF-L-Cys nanoparticles were used for in-depth comparative exploration of human normal colorectal epithelial cells (FHC) and human CRC cells (HT-29) successfully. A total of 214 glycopeptides and 597 glycosites belonged to 289 glycoproteins were enriched in FHC cell lysate by Fe_3O_4@PVP/PEI@COF-L-Cys nanoparticles. And 711 glycopeptides, 824 glycosites belonged to 440 N-glycoproteins were enriched in HT-29 cell lysate. Subsequently, the differential glycoproteins were in-depth explored through statistical analysis and gene ontology (GO) etc. calculation. Furthermore, we have initially verified the reported therapeutic targets (TM9SF3, etc.) and biomarkers (LGALS3BP, PDIA3, etc.), and explored the relationship of differential glycoproteins with the tumor growth, metastasis processes, endoplasmic reticulum stress response and immune escape, etc. This method bridges the enrichment technology and clinical requirements, and in-depth explored differential glycoproteins for helping established differential glycoproteomic mapping and identified novel biomarkers and therapeutic targets of CRC.

PoB.17.11

Processable 1D Conductive nanocomposite of Polypyrrole Structured by Cellulose Nanofibril for Constructing Biointerfaces and Bioscaffolds

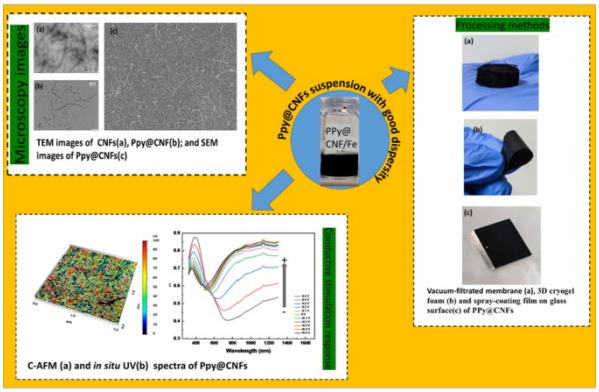
Xiaoju Wang, Shujun liang

Åbo Akademi University, Turku, Finland

Abstract

Conductive polypyrrole(PPy) are smart biomaterials for building bioelectronics to interface with biological systems, i.e., biointerfaces or bioscaffolds. However, for the obtained powdered form of the PPy with distinct globular morphology during the chemical oxidation, the poor water dispersity and processability are the main challenges in the biomaterial processing for functional device. Cellulose nanofibrils (CNFs) are considered a good structural template in constructing 1D nanocomposite of PPy, owing to the strong mechanics and flexibility. In this study, we obtained a core-shell nanocomposite of PPy@CNFs with good dispersity through ferric chloride (FeCl3) oxidation. Intriguingly, the PPy@CNFs present each individual fibril surface coated with a thin PPy layer on nanoscale and the highly positive surface charge that is originated from the protonated PPy renders durable colloidal stability of the nanocomposite PPy@CNF in aqueous suspension. More importantly, the fibril-fibril entanglement among the PPy@CNFs facilely supports downstream processing to fabricate versatile dry forms, e.g., spray thin-coating, flexible and mechanically robust membranes, or three-dimensional cryogels.

In transmission electron microscopic (TEM) imaging, the ultrathin coating of PPy that tightly conforms to the fibril surface of the template CNF was verified. Under the scanning electron microscope (SEM), well-interconnect fibril-fibril network is prominent in the spray-coating sample. The continuous and evenly distributed PPy coating on the fibril endows the nanocomposite with conductivity. Imaging with a conductive atomic force microscope showed the high-density conductive paths (50 ~ ≥100 nA) of the spray-coating PPy@CNFs at the nanoscale. In other words, a concentrated and homogeneous conducting network is created. High electrical conductivity up to 12 S·cm-1 of the vacuum-filtrated membrane was also confirmed by 4- probe conductivity. The transition state of PPy@CNFs from the neutral to cation radical form (conductive) was recorded by UV spectroscopy when the potential was applied in situ and progressively shifted from – 0.5 to 0.5 V in 0.1 M KCl. This further confirms the electrochemical activity of the PPy@CNFs and its properties can be dynamically controlled by applying an electric field (Figure 1).



We also analyzed the cytotoxicity of the material by extract method and non-direct contact method. The results showed that the extract solutions and the presence of the membrane did not have adverse effects on human dermal fibroblast culture. All these properties underpin the promises for PPy@CNFs as a conductive element in the fabrication of flexible and metal-free electrodes interacting with biological systems.

Figure 1. Processing methods, microscopic images, and conductive stimulation responses of PPy@CNFs.

PoB.17.12

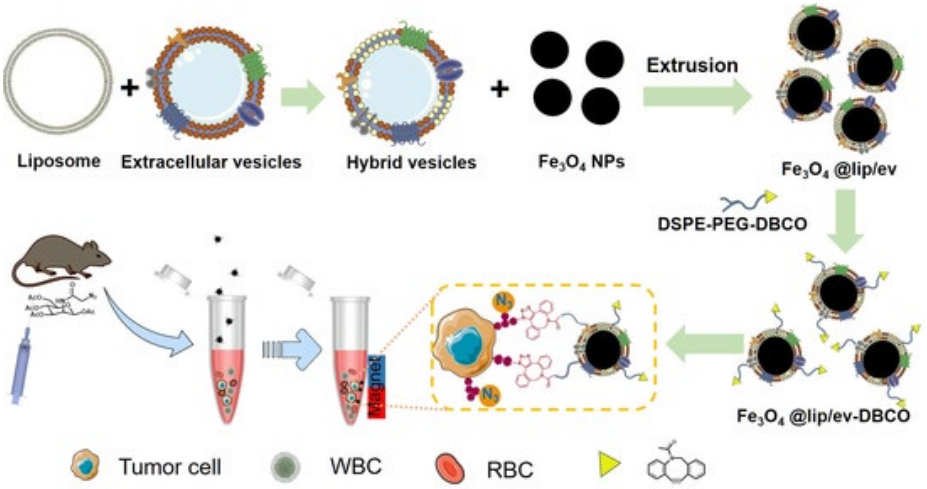
Hybrid Extracellular Vesicles-Liposomes Camouflaged Magnetic Vesicles Cooperating with Bioorthogonal Click Chemistry for High-Efficient Melanoma Circulating Tumor Cells Enrichment

Ke Kang, Yujia Zhang, Xiaoxi Zhou, Yue Yu, Nanhang Zhu, Jia Cheng, Qiangying Yi, Yao Wu

Sichuan University, Chengdu, China

Abstract

The capture of melanoma circulating tumor cells (melanoma CTCs, MelCTCs) is of great significance for the early diagnosis and personalized treatment of melanoma. The rarity and heterogeneity of MelCTCs have greatly limited the development of MelCTCs capture methods, especially those based on immune/aptamer-affinity. Herein, an extracellular vesicles-camouflaged strategy was designed to functionalize the magnetic nanoparticles (Fe_3O_4) and to generate magnetic vesicles (Fe_3O_4@lip/ev) with excellent antifouling and active tumor cell targeting properties. Combined with the bioorthogonal click chemistry, the engineered magnetic vesicles with dibenzocyclooctyne (DBCO) can be widely used to target and separate all the metabolically labeled CTCs with varied phenotypes, organ origin and even the biological species. The capture efficiency exceeded 80% with an extremely low detection limitation of 10 cells. Most importantly, the strategy proposed can be directly applied to enrich MelCTCs from 0.5 mL blood samples of melanoma-bearing mice, with a greatly minimized residue of white blood cells (only 21-568) while ignoring the fluctuations of MelCTC phenotype.



https://doi.org/10.1002/adhm.202202825

PoB.17.13

Flame-Spray-Synthesis of ultrabright, nanoscale near-infrared fluorescent copper silicates for *in vivo* bioimaging

Robert Nißler[1,2], Quanyu Zhou[3,4], Björn Hill[5], Benjamin Mächler[6], Xosé Luís Deán-Ben[3,4], Sebastian Kruss[5], Daniel Razansky[3,4], Inge Katrin Herrmann[1,2]

[1]Nanoparticle Systems Engineering Laboratory, ETH Zurich, Zürich, Switzerland. [2]Particles-Biology Interactions, Department of Materials Meet Life, Swiss Federal Laboratories for Materials Science and Technology (Empa), St. Gallen, Switzerland. [3]Institute for Biomedical Engineering and Institute of Pharmacology and Toxicology, Faculty of Medicine, University of Zurich, Zürich, Switzerland. [4]Institute for Biomedical Engineering, Department of Information Technology and Electrical Engineering, ETH Zurich, Zürich, Switzerland. [5]Department of Chemistry, Bochum University, Bochum, Germany. [6]Nanoparticle Systems Engineering Laboratory, ETH Zürich, Zürich, Switzerland

Abstract

Two-dimensional (2D) copper silicates of the family XCuSi4O10 with X=Ca,Ba,Sr are well known for their stabile and bright fluorescence emission in the near-infrared (NIR) spectrum around 950 nm. Recent advances in dispersing and exfoliating these materials into nanosheets (NS) render them useful as novel nanoscale imaging and labeling agents, as well as for chemical sensing applications. So far, various synthesis routes are available for the production of bulk copper silicates, however, the scalable synthesis of nanosheets remains challenging.

Here, we introduce the method of flame-spray-pyrolysis (FSP) for the production of various copper silicates. This approach yields nanoscale metal oxide particles, which, following annealing, transform into the desired 2D crystal lattice. The obtained copper silicates are ultrabright with a photoluminescence quantum yield of up to 32%, allowing rapid imaging of exfoliated nanosheets at video rates >178 frames per second (fps) in a stand-off approach (>20 cm). These promising luminescence properties offer a route to next generation bioimaging in the second NIR (NIR-II) spectral window, e.g. for *in vivo* localization-based tracking of luminescent nanosheets in the mouse brain for super-resolution structural imaging of the vasculature and measuring blood flow velocity. Overall, the FSP-synthesis of 2D copper-silicates gives access to precise control over chemical composition, crystal phase and fluorescence quantum yield and fluorescence lifetime, creating highly luminescent nanosheets for NIR bioimaging.

PoB.17.16

Nanofibrous cellulose hydrogel as release reservoir for light sensitive liposomes

Zahra gounani, puja Gangurde, Vili-Veli Auvinen, Patrick Lauren, Tatu Lajunen, Timo Laaksonen

University of Helsinki, Helsinki, Finland

Abstract

Introduction: Nanofibrous cellulose hydrogels are natural material with excellent mechanical properties, tuneable surface chemistry, high surface area and good biocompatibility, allowing for vast range of applications in biomedicine and pharmaceuticals1. Here we combine these unique hydrogel matrices with light responsive liposomes to produce a light-responsive loco-regional drug delivery system. The nanoparticles embedded in nanofibrous cellulose hydrogel perform as stationary drug “nano”-reservoir. We have developed anionic and cationic liposomes to study the effect of surface charge of liposome on their interaction with nanofibrous cellulose hydorgel2. Cationic nanoparticles were shown to be retained almost indefinitely inside the hydrogel. The interaction of nanofibrous cellulose and cationic and anionic liposomes was analysed by surface plasmon resonance (SPR). The data showed strong adsorption of cationic nanoparticles to nanofibrous cellulose hydrogel in contrast with low adsorption of anionic liposomes. The mixture of liposomes, loaded with calcein with excitation/emission wavelengths of 495/515 nm, and nanofibrous cellulose hydrogel, stained with Nile red with excitation/emission wavelength of 552/636 nm, was analysed with confocal laser microscopy. The results showed that liposomes tend to surround heterogeneous gel clumps rather diffusing inside gel fibers. Further microscopy analysis is ongoing to investigate the movement of liposomes inside hydrogel. The liposomes are developed from thermosensitive lipids and loaded with calcein and indocyanine green (ICG). ICG is a photothermal photosensitizer that can elevate the local temperature by absorbing energy from the light at 800 nm. Thermos and light-triggered calcein release from nanoparticles were analyzed at different temperature and light irradiation times. Data confirmed light exposure result in release, and it was consistent with heat stimulated release. Release rate varies depending on the thickness of hydrogel. Different light intensity didn’t affect the release rate. We believe the data from this project contribute greatly to advancing cellulose hydrogel applications in drag delivery.

(1) De France, K. J.; Hoare, T.; Cranston, E. D. Review of Hydrogels and Aerogels Containing Nanocellulose. Chem. Mater. 2017, 29 (11), 4609–4631. https://doi.org/10.1021/ACS.CHEMMATER.7B00531/ASSET/IMAGES/LARGE/CM-2017-00531A_0012.JPEG.

(2) Lajunen, T.; Kontturi, L. S.; Viitala, L.; Manna, M.; Cramariuc, O.; Róg, T.; Bunker, A.; Laaksonen, T.; Viitala, T.; Murtomäki, L.; Urtti, A. Indocyanine Green-Loaded Liposomes for Light-Triggered Drug Release. Mol. Pharm. 2016, 13 (6), 2095–2107. https://doi.org/10.1021/acs.molpharmaceut.6b00207.

PoB.17.18

Tumor pH-triggered Vanadium carbide nanosheets degradation enabled NIR-II photothermal cascade catalysis treatment against breast cancer

Jun Cao, Hai Zhu, Bin He

National Engineering Research Center for Biomaterials, College of Biomedical Engineering, Sichuan University, Chengdu, China

Abstract

Photothermal therapy has been employed extensively in the treatment of tumors due to its great selectivity and non-invasive benefits. However, single photothermal therapies face the heat resistance of tumors. Moreover, the majority of the currently available photothermal agents frequently experience issues like low absorption rate, low photothermal conversion efficiency, and insufficient depth of tumor penetration, which results in unsatisfactory therapeutic effects. Hence, in this study, Vanadium carbide (V2C) nanosheets were created as the photothermal agents with NIR-II absorption and biodegradability to achieve a photothermal cascade catalytic treatment against breast cancer. V2C nanosheets were synthesized through acid etching plus ion exchange and modified with hyaluronic acid (V2C @HA) on their surface, which had a particle size of about 250 nm and a potential of -19 mV and could be degraded in a pH-responsive manner. Additionally, V2C @HA showed improved NIR-II absorption and had a 49.92% photothermal conversion efficiency. *In vitro* catalytic and cellular experiments showed that the degraded product, VOx, had nanoenzyme properties and could consume glutathione and catalyze hydrogen peroxide to produce reactive oxygen species, thus achieving photothermal cascade catalytic therapy to inhibit 4T1 growth.

PoB.17.19

Antibacterial activity of functionalized carbon nanoparticles

Alicja K. Olejniki[1], Radoslaw A. Wach[1], Bożena Rokita[1], Justyna Fraczyk[2], Anna Gajda[2], Beata Kolesinska[2], Anna Karczemska[3], Ahmed El-Mallul[3], Dariusz Witkowski[3]

[1]Lodz University of Technology, Institute of Applied Radiation Chemistry, Lodz, Poland. [2]Lodz University of Technology, Institute of Organic Chemistry, Lodz, Poland. [3]Lodz University of Technology, Institute of Turbomachinery, Lodz, Poland

Abstract

In the era of an increasing number of antibiotic-resistant pathogens, the research is in pursuit of new methods and compounds of antibacterial activity. Nanoparticles have been in focus for several decades, as the antimicrobial activity of silver or gold nanoparticles was explored, whereas less information is available on carbon nanoparticles (CNP) as antibacterial materials.

The antibacterial effect of carbon nanoparticles of various allotropic forms was assessed towards standard gram-negative (Escherichia coli ATCC 8739) and gram-positive (Staphylococcus aureus ATCC 6538) bacteria strains. The potential of CNP was tested by three methods: 1/ agar gel diffusion method (modified Kirby-Bauer disc diffusion method), 2/ in solutions/suspensions and 3/ in layers, adapting the ISO 22196 method: 'Measurement of antibacterial activity on plastics and other non-porous surfaces'. Chemically functionalized nanodiamonds (ND), carbon nanotubes (CNT), fullerenes (Fu) and reduced graphene oxide (rGO) were examined. Nanoparticles were prepared in the form of suspensions at various concentrations. The results showed excellent and good activity against test microorganisms, depending on the type of the core CNP and surface functionalization.

The obtained results allow to assume the possibility of wide applications of modified carbon nanoparticles as antibacterial agents. For the time being, they are not anticipated to be used as medicines, but as additives to materials for everyday use and commodity objects, such as handrails, handles, countertops or doors, i.e. objects in public space used by many people. The use of carbon nanoparticles with antibacterial properties can be particularly attractive in medical facilities cultural centers, offices or public transportation, where enormous amount of pathogens is spreading.

Acknowledgement. The research has been financed by M-ERA.NET2/2019/3/2020 project 'Antibacterial Coatings Containing Carbon Nanoparticles Obtained by Sol-Gel Method'.

PoB.17.20

Biological characterization of Zn containing SiCa mesoporous nanoparticles for periodontal regeneration

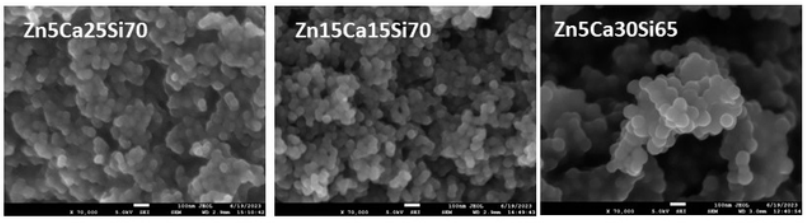
Anastasia Beketova[1,2], Ioannis Tsamesidis[1], Georgia K. Pouroutzidou[1], Chrysanthi Papoulia[3], Maria Bousnaki[1], Petros Koidis[1], Dagnija Loca[2], Eleana Kontonasaki[1], Janis Locs[2]

[1]Aristotle University of Thessaloniki, School of Dentstry, Thessaloniki, Greece. [2]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia. [3]School of Physics, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

Introduction Ion doped mesoporous calcium silicate nanoparticles (MSNs) have been widely investigated for applications in bone and soft tissue regeneration. Specifically, Zn doped Ca-Si MSNs may be potentially used for periodontal regeneration as Zn is intensively involved in regulating inflammatory responses and immune functions, controlling bacterial infection, and reducing oxidative stress, associated with the pathogenesis of the periodontal disease [1], while the release of Ca and Si ions can further induce osteogenesis and regeneration of periodontal bone defects. The aim of this investigation was to synthesize Zn MSNs with different Zn/Ca/Si ratios and to evaluate their bioactivity, hemocompatibility, and biocompatibility with human periodontal ligament fibroblasts (hPDLFs). **Experimental methods** Mesoporous zinc doped calcium silicate nanopowders with the composition of $70SiO_2$-25CaO-5ZnO, $70SiO_2$-15CaO-15ZnO and $65SiO_2$-5CaO-30ZnO (in % mol) were synthesized by a surfactant-assisted cooperative self-assembly process (pH 12) and calcinated at 550 °C, for 6 h before being observed by SEM and XRD. The bioactivity of the NPs was evaluated by FTIR and SEM/EDS analysis after immersion in SBF for 14 days To determine the hemolytic activity on human red blood cells (hRBCs), nanoparticles suspension was added to 5% diluted hRBCs at different concentrations (0.125, 0.25, 0.5 mg/ ml) and evaluated spectrophotometrically at 540 nm after 24h of incubation. Biocompatibilty of the nanopowders was assessed by MTT assay in direct contact of the materials with hPDLFs (10^3 cells/well). **Results and discussion**XRD analysis of the obtained powders showed that the materials were composed mainly of amorphous phase, with minor quantities of Zn-rich crystalline phases in the material $70SiO_2$-15CaO-15ZnO.

Figure 1. SEM images of the obtained powders



SEM images presented in Figure 1 revealed spherical morphology and nano-dimensions (50-100nm) of the synthesized NPs. As expected, Zn retarded the formation of hydroxyapatite (HA) in SBF [2], while indications of HA precipitation in material $65SiO_2$-5CaO-30ZnO were detected by FTIR and SEM/EDS. All materials presented no hemolysis at the tested concentrations, while decreased hPDLFs viability was observed at the highest concentration of the materials (0.5 mg/ml). **Conclusions.** Zn containing SiCa MSNs with tunable degradability and biological properties were synthesized. The optimal Zn/Ca/Si ratios should be further

investigated to develop MSNs with tailored properties such as high biocompatibility and enhancced regenerative capacity to be applicable for periodontal regeneration.

Acknowledgements. The research has been funded by RegrOssBio (HORIZON-MSCA-2022-PF-01-01) GA 101106882 project.

1. Aziz J et al.. 2021, https://doi.org/10.1016/j.jtemb.2021.126754
2. Neščáková et al., 2019, https://doi.org/10.1016/j.bioactmat.2019.10.002.

PoB.17.22

Cytotoxicity studies of functionalized carbon nanoparticles

Bozena H. Rokita[1], Radoslaw A. Wach[1], Alicja K. Olejnik[1], Anna Gajda[2], Beata Kolesinska[2], Anna Karczemska[3], Ahmed El-Mallul[3], Dariusz Witkowski[3]

[1]Lodz University of Technology, Institute of Applied Radiation Chemistry, Lodz, Poland. [2]Lodz University of Technology, Institute of Organic Chemistry, Lodz, Poland. [3]Lodz University of Technology, Institute of Turbomachinery, Lodz, Poland

Abstract

Nanomaterials are widely used in medicine and various areas of industry. We have developed a way to chemically functionalize carbon nanoparticles (CNP), such as nanodiamonds, reduced graphene oxide, fullerenes and carbon nanotubes, which have an antibacterial effect on gram-negative and gram-positive bacteria. These functionalized CNP were developed as a response for demand for new solutions of antimicrobial materials due to the growing number of antibiotic-resistant pathogens. Yet, nanomaterials, including CNP may be toxic or non-biocompatible, thus in general, the guidance of ISO 10993-22 should be followed for safety assessment.

The aim of this study was to evaluate potential cytotoxicity of CNP with antimicrobial functionality. Normal Human Primary Dermal Fibroblast (cell line ATCC-PCS-201-012) and Normal Primary Human Umbilical Vein Endothelial Cells (HUVEC, cell line ATCC-PCS-100-010) were used. Cytotoxicity/viability test using assays of XTT and Live/DeadTM Viability/ Cytotoxicity were used. The effect of functionalized CNP, particularly nanodiamond, concentration and contact time on cells were investigated.

It has been shown that nanodiamonds are non-toxic, unless in very high concentration that physically hinders cells development. However, surface-functionalized nanodiamonds may have a toxic effect on cells at concentrations above a certain level. Therefore, applications of CNP should be designed with particular concern, taking under account primarily size, surface chemistry and concentration factors. For that the data presented in this report should be considered.

Acknowledgement. The research has been financed by M-ERA.NET2/2019/3/2020 project ‘Antibacterial Coatings Containing Carbon Nanoparticles Obtained by Sol-Gel Method’.

PoB.17.23

Challenges of the preparation and biocompatibility of the cannabidiol-containing liposomes

Margarita Brante[1,2], Andra Grava[1,2], Arita Dubnika[1,2]

[1]Riga Technical University, Riga, Latvia. [2]Baltic Biomaterials Centre of Excellence, Riga, Latvia

Abstract

Cannabidiol (CBD) is offered as a medication for the treatment of neurodegenerative and inflammatory diseases. However, cannabinoids exhibit a short half-life, and encapsulating CBD in liposomes as biocompatible drug carriers could prevent its degradation and premature release. Key characteristics determining liposomal preparations' effectiveness include their size, zeta potential (surface charge), encapsulation efficiency, drug release, stability, and cytotoxicity. Extrusion is the most effective method for reducing liposome size, but it is influenced by various parameters related to liposome composition and preparation techniques. As CBD is a hydrophobic substance it can alter the lipid phase transition temperature and the efficiency of extrusion.

Liposomes of different lipid (DSPC, DPPC, DSPE-PEG, cholesterol) compositions were prepared with and without CBD by thin film hydration method. 5-41 extrusion cycles were performed on liposomes using membrane filters with 100 and 400 nm pore sizes and analysed using FT-IR, DLS, SEM, and light microscopy. CBD release was determined with UPLC for 21 days, and *in vitro* cell viability was studied on gingiva-derived mesenchymal stem cells with CCK-8.

Our findings reveal that neither the manufacturer's instructions nor the existing literature provide a complete procedure for obtaining 100 nm particles. By performing 15-41 extrusion cycles of DSPE-PEG liposomes without CBD through a 100 nm pore filter, we observed a decrease in particle size from 15 to 25 extrusions. However, increasing the cycles to 31-41 maintained a particle size of 165 ± 2 nm. The extrusion efficiency is affected by the length of the alkyl chain of the lipids in their composition but the inclusion of CBD in the lipid bilayer does not affect the size of the liposomes, but significantly increases the absolute zeta potential of the suspension, for example in the case of 400 nm filters from 7.5 ± 2.5 to 39.1 ± 6.5, and reduces the agglomeration rate. CBD release kinetics was observed for 21 day and a reduction of cell viability was observed for the CBD-containing liposome concentration exceeding 4 to 10 µg/mL after 24 h and 1 to 4 µg/mL after 48h.

The study highlights the need for comprehensive procedures to achieve desired particle sizes and provides insights into the influence of CBD on liposome characteristics and therapeutic potential.

Acknowledgements

The authors thank the M-era.Net 2 project INJECT-BIO, No. ES RTD/2020/14 and the EU Horizon 2020 research and innovation program under the GA No. 857287 (BBCE) for support.

PoB.18.01

Self-supporting films from hyaluronan derivatives for wound healing applications

Josef Chmelař[1], Vojtěch Pavlík[1], Jana Bažantová[1], Jiří Mrázek[1], Lenka Součková[1], František Ondreáš[1], Eva Kriváková[2], Lukáš Kubala[2], Luboš Sobotka[3], Vladimír Velebný[1]

[1]Contipro a.s., Dolní Dobrouč, Czech Republic. [2]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic. [3]University Hospital Hradec Králové, Hradec Králové, Czech Republic

Abstract

Hyaluronan (HA) is a natural polysaccharide well known for its positive effects on wound healing. However, the fast dissolution and resorption of topically applied HA limit its use to short term treatment or require repeated applications to the wound site. Also, sufficient protection of the wound is not provided, so it may be necessary to combine HA based products with further wound dressings.
To overcome these shortcomings, we aimed to develop a HA-based wound dressing that would provide long term protection of the wound while creating a moist environment enriched in HA that is optimal for healing. The resulting wound dressing is in the form of a self-supporting film from a mixture of two unique HA derivatives: lauroyl HA [1] and chloramide HA [2]. This dressing combines the biological effect of HA, which actively promotes the healing process, with advantageous physical properties. Once in contact with the wound, the film absorbs exudate, swells, and forms a thin hydrogel layer that provides the required moist healing environment, while also enabling some water transport through the film to prevent maceration (exudate management). Importantly, the swollen film retains sufficient toughness and thus provides mechanical protection against infection. The film composition was optimized with respect to tissue adhesion, mechanical resilience, and barrier properties.
To verify safety, extensive in-vitro testing including cytotoxicity, pyrogenicity, skin sensitization and skin irritation assays was carried out, followed by several in-vivo studies on mice and rats. Where applicable, the tests were carried out in accordance with ISO 10993. Overall, our results suggest that the developed HA-based films are a promising material for the treatment of large, slow healing wounds and chronic wounds.

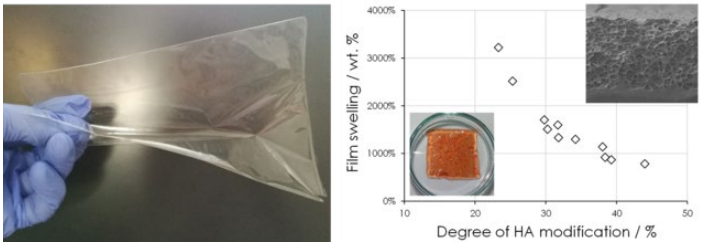


Figure: manipulation with the HA-based film (left) and dependence of film swelling on the degree of HA modification (right); inserts show a film swollen in diluted blood (simulation of a bleeding wound) and the internal structure of a swollen film (SEM).

[1] Chmelař J., Mrázek J., Hermannová M., et al. Biodegradable free-standing films from lauroyl derivatives of hyaluronan. Carbohydrate Polymers 2019, 224, 115162.
[2] Buffa R., Hermannová M., Sojka M., et al. Hyaluronic acid chloramide - Synthesis, chemical structure, stability and analysis of antimicrobials, Carbohydrate Polymers 2020, 250, 116928.

PoB.18.02

Biochemical and Biomechanical clues to promote angiogenesis for tissue engineering application

Jéssica Pinto[1], Belén Arteaga[2,1], Ana Guerra[3], Renato Natal[4,5], Fernando Monteiro[1,4,6], Christiane Salgado[1,6]

[1]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [2]University of Granada, Granada, Spain. [3]INEGI - Instituto de Ciência e Inovação em Engenharia Mecânica e Engenharia Industrial, Porto, Portugal. [4]FEUP - Faculdade de Engenharia, Universidade do Porto, Porto, Portugal. [5]LAETA - Laboratório Associado em Energia, Transportes e Aeronáutica, Porto, Portugal. [6]INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Porto, Portugal

Abstract

The success of the engineered tissue is only achieved if, after implantation, the construct is able to connect to the patient vasculature in order to ensure cells' viability. However, this process takes a substantial time starting cell death and, to overcome this problem, a functional vascular network should be added in the scaffold before implantation [1]. The aim of this work was to bring new insights concerning 3D vascularized bioconstructs by describing which are the cell culture conditions that shall improve angiogenesis.
Human umbilical vein endothelial cells (HUVEC) were seeded ($0.2x10^6$ cells) into fibrin hydrogel with VEGF (5, 10 ng/mL) under normoxia or hypoxia. Cells viability and morphology were evaluated (24, 36h) using AngioTool. Human dental follicle mesenchymal cells (DFMSC) were seeded into biocomposite scaffolds as described at previous work [2], and filled with HUVEC-loaded hydrogel ($0.2x10^6$). Microscope images, secretome and DMA analysis (Frequency scan) were performed to evaluate cell response.

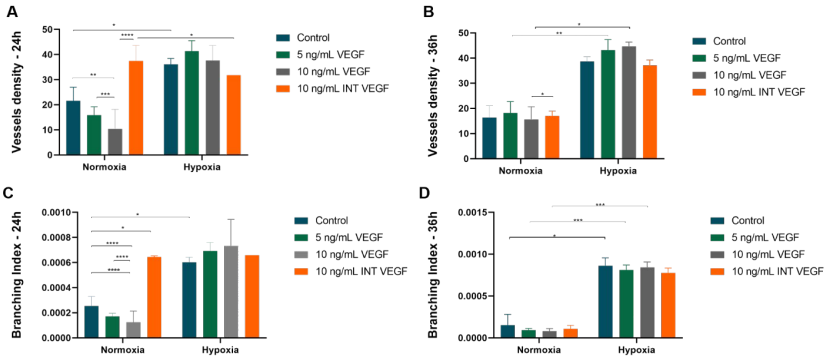


Figure 1: AngioTool analysis of endothelial cell response with VEGF under normoxia or hypoxia for 24h (A, C) and 36h (B, D).

There is no statistical difference on cellular viability after both time points with different culture conditions. Angiotool showed that all VEGF concentrations had higher vessel density under hypoxia, but there was a decrease in the cell response after 36h. The branching index was higher with hypoxia and lower VEGF (5 ng/mL) or within the hydrogel (10 ng/mL) (Figure 1). Bioconstructs (DFSC+HUVEC) subjected to dynamic culture showed higher Tan Delta, but there were no differences in terms of elastic properties (storage modulus) between all the samples (Figure 2). HUVEC response (tubular formation) was higher in the presence of DFMSC for both conditions. This effect was promoted by the release of important pro-angiogenic molecules (VEGF ang Ang-1) after 24h. Concluding, DFMSC+HUVEC under dynamic conditions, we were able to promote optimal endothelial sprouting and tube potential, which will produce mature vascular beds. Future studies shall address their *in vitro* performance in a bioreactor under biomechanical solicitation that shall generate functional perfused bioconstructs.

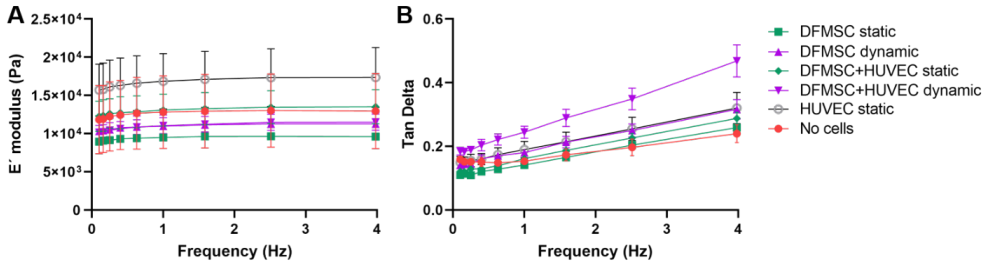


Figure 2: (A) Storage modulus (E') and (B) Tan Delta versus increasing frequencies (from 0.1 to 5 Hz) under dynamic compression for cell-loaded scaffolds with different culture conditions.

Acknowledgement: The authors acknowledge FCT for financial support (PTDC/EME-APL/3058/2021; CEEC-INST/00091/2018/CP1500/CT0019).

References: [1] Jaklenec A, et al. (2012). Tissue Eng Part B Rev, 18(3),155-66.
[2] Salgado CL, et al. (2020). Front. Bioeng. Biotechnol, 8:724.

PoB.18.03

Use of freeze-dried human amniotic membrane of umbilical cord (hAM-UC) as scleral patch graft for treatment of scleral thinning

Laurence Barnouin, Justine Bosc, Florine Grossetete

TBF, Mions, France

Abstract

Statement of the Problem: In ocular oncology, scleral thinning is a complication that can occur after conjunctival tumor excision surgery. Scleral thinning depends on the tumor invasion of the sclera and on individual anatomical variations. Ocular surface repair surgery can use autologous, allogeneic or even xenogeneic graft. A monocenter, open and prospective clinical trial was conducted to evaluate the safety of a freeze-dried hAM-UC used as a patch to reinforce the sclera thinned by tumor excision surgery. Methodology & Theoretical Orientation: Patients aged 18 years and older with a risk of scleral thinning after conjunctival tumor excision surgery were included. The main objective of this study was to assess the tectonic effect of the implant including clinical tolerance using a composite score. Secondary objectives were the evaluation of the scleral thickness recovery by swept-source optical coherence tomography (OCT) at 15 days, 1 month, 3 months and 6 months. Findings: Twelve patients were implanted during surgical management of conjunctival tumor. Clinical tolerance was good throughout the study. Tectonic recovery was achieved with a minimal score for local reactions after 3 months. OCT images showed good integration of the graft and recovery of the ocular surface. At 6 months, all patients, including the 5 patients with invasive malignant conjunctival tumor treated by proton-therapy one month after implantation, had sclera thickness at physiological level. Conclusion & Significance: The use of a freeze-dried human amniotic membrane of umbilical cord is a promising option as sclera graft substitute in the management of scleral thinning in ocular oncology.

Figure 1: Macroscopical structure of the inverted vessel product

PoB.18.04

Scaffold properties aside from stiffness modulate myofibroblast activation without using biochemical signals

Anna DY Rhodes[1], Claire Higgins[1], Nuria Oliva[2,1]

[1]Imperial College London, London, United Kingdom. [2]IQS-URL, Barcelona, Spain

Abstract

Introduction. Fibrosis is characterised by the activation of fibroblasts into myofibroblasts, leading to excessive deposition of extracellular matrix (ECM) elements[1]. This impairs organ function resulting in 45% of all deaths in the developed world[1]. In the skin, pathological fibrosis is a feature of untreatable keloids and hypertrophic scarring[2]. To understand the mechanisms of fibrosis and develop efficient therapies, human-derived models of fibrosis that recapitulate both the altered cellular and extracellular components are needed. Existing models rely only on the cellular component, overlooking the extracellular elements that comprise the fibrotic dermis and their properties. We have developed 3D *ex vivo* models of healthy and fibrotic skin made of fibroblasts seeded within a silk fibroin-collagen (SF-COL) matrix and demonstrated that the biochemical and mechanical properties of the scaffold dictate fibroblast fate and identity.

Methods. SF-COL scaffolds were fabricated[3] varying SF molecular weight (MW; High, Medium & Low), and solid content (SC; 7, 11 and 15%). Storage moduli were measured in the linear viscoelastic region with a rheometer, using a cross-hatched Peltier plate with a 20-mm parallel plate. Expression of ACTA2 (fibrosis marker) in dermal fibroblasts (HDF) seeded in SF-COL scaffolds was measured after 48 hours by RT-qPCR.

Results and discussion. For equal SC, silk MW is directly proportional to G' (Figure 1A) and ACTA2 expression (Figure 1B), with higher molecular weight SF scaffolds exhibiting the higher storage moduli and pro-fibrotic gene expression. However, within the same MW SF, ACTA2 expression appeared to be independent of G' values: 11% and 15% SF scaffolds presented the same G' (Figure 1C), but a significant difference in ACTA2 expression (Figure 1D). Contrarily, 11% and 7% scaffolds had significantly different G' values, but equal ACTA2 expression. Further studies revealed that stress-relaxation is also independent of myofibroblast activation (data not shown).

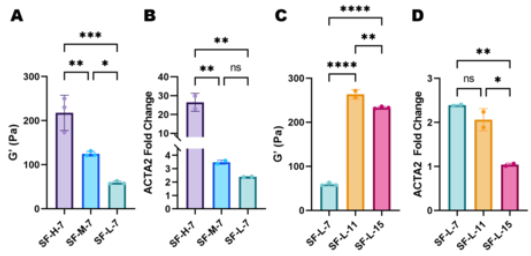


Figure 1. Storage moduli (A&C) and ACTA2 expression (B&D) of SF-COL formulations.

Conclusions. This work demonstrates that myofibroblast activation is mediated by several factors beyond the widely accepted substrate stiffness (G'), and further investigation is required to elucidate the mechanisms governing fibroblast-myofibroblast transition. Nonetheless, SF-COL scaffolds present themselves as robust candidates for human-derived 3D *ex vivo* models of healthy and fibrotic dermis, an enabling tool for the investigation of the mechanisms of fibrosis and the high-throughput screening of drugs.

References. 1. Henderson, N. C., *et al.* Nature (2020). 2. Eming, S. A., *et al.* Science Translational Medicine (2014). 3. Lightfoot-Vidal, S. E. *et al.* Biomaterials (2019).

PoB.18.05

Effect of resection line geometries on the stress distribution near the resection line

Hitomi Sakai[1], Sumiko Maeda[2], Noriyuki Takano[3]

[1]Kanazawa Institute of Technology, Nonoichi, Japan. [2]Dokkyo Medical Univercity, Mibu, Japan. [3]Kanazawa Institute of Technology, Hakusan, Japan

Abstract

Introduction. Primary spontaneous pneumothorax is a disease caused by the breakdown of lung cysts on the lung surface. The treatment is surgical resection of the lung, including the failed lung cyst. However, pneumothorax may recur after surgery due to new lung cysts that develop near the resection line. In contrast, it is believed that a change in the resection method of the affected area can prevent recurrence of pneumothorax. It may be due to the stresses applied by the different shapes of the resection. In this study, a finite elements analysis was performed on a simple model of a lung after resection surgery to confirm the difference in the stress generated near the resection line due to the difference in the shape of the resection line. **Analytical Model.** Figure 1 shows a simplified model of the lungs, assuming a simple dome shape. Model A is a normal lung, and models B and C are postoperative lungs; model B has a I-shaped resection line used in pneumothorax resection, and model C has a V-shaped resection line used in lung tumor resection. Each model comprises a plate with 0.1 mm thickness on the surface and solid elements inside the model. **Analysis Conditions.** Table 1 shows the analytical conditions used in this analysis. The stress-strain diagram of the pulmonary pleura was obtained from the pleura of a porcine lung. The lung expansion was reproduced by thermal-expanding solid elements virtually. **Results And Discussion.** The analysis results under the above conditions showed that Model A, which assumes healthy lungs, was loaded evenly across the entire model and that the load was applied to the entire model. In contrast, Model B showed high loading near the resection line. Model C, which has a V-shaped resection line, did not show the high loading near the resection line that was observed in Model B. It is due to the difference in the shape of the resection line. Because the I-shaped line cannot follow the deformation of the lung when the lung is inflated by inspiration, and a high load is applied near the resection line, while the V-shaped resection line itself is less deformed.

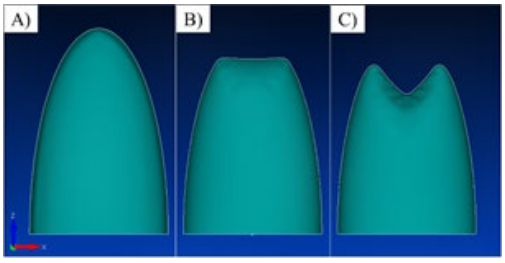


Figure 1. Analysis models A) Normal model, B) I-shaped resection model, C) V-shaped resection model

Table 1. Analysis conditions

Model	Simplified model
Solver	FEMAP with NX Nastran SOL 601 non-linear static analysis
Young's modulus	Non-linear stress-strain curve
Load	Thermal expansion
Pleural thickness	0.1mm

PoB.18.06

Human skin equivalent epidermis/derma and endothelial barrier with immune cell components: a case report for skin sensitization

Edith Filaire[1], Dario Fassini[2], Marina Simon[1], Pierre gaudriault[2], Christian Poinsot[1]

[1]ICARE, Saint Beauzire, France. [2]Cherry Biotech, Montreuil, France

Abstract

Introduction. Before a chemical product is placed on the market, evaluation of its safety profile, including the assessment of skin sensitization hazards and potency is mandatory. Currently, three technical Test Guidelines (OECD 442 C, D and E) describe a total of seven such methods, including the Direct Peptide Reactivity Assay (DPRA), KeratinoSensTM and LuSens, h-CLAT, U-SENSTM, GARDskin and the IL-8 Luc assay. These methods should be considered in the context of a tiered testing strategy, a so-called defined approach (DA), where a fixed data integration procedure is used to arrive at a final classification, based on the readout from several New Approach Methods (NAMs) . Despite the many advancements and capabilities with skin models to do some of these tests, none of them, to our knowledge, recapitulate cutaneous immune responses, and fail to acknowledge the influence of non-immune cells on immune cells. Therefore, the aim of our study was to realize a human skin equivalent epidermis/derma and endothelial barrier with simplest immune cells components for skin sensitization assay. **Methods:** The model integrated human epidermal keratinocytes, normal human dermal fibroblasts (NHDF), Human Umbilical Vein Endothelial cells (HUVECS), and THP-1 (a human monocytic cell line). Structure and cell-specific markers were thoroughly characterized. To compare the developed model with the IL-8 Luc assy, it was exposed to molecules with properties determined *in vivo* and *in vitro* (sensitizing substances and negative controls, cf OECD 442) such as Ethylene glycol dimethacrylate; Phenylenediamine and formaldehyde. **Results:** Skin model reveals good dermis/epidermis development. A multilayered, differentiated epidermis gradually developed on a rich dermis, as revealed by the histology analysis. Specific markers of epidermal (Involucrin), dermal (CD26), endothelial (CD54) cells and differentiation of macrophages (M1, TNF-alpha; M2: Dectin-1) were evaluated. **Conclusion:** We have developed an innovative mature macrophage and vascularized-based full-skin *in vitro* testing system for the evaluation of the safety of chemical products designed to skin sensitization. Moreover, this 3D skin model should allow investigations on screening molecules to prevent inflammation.

PoB.18.07

Effect of bioactive glass with spherical gold nanoparticles in diabetic wound healing

Klara Magyari[1], Sorin Marza[2], Mirela Moldovan[3], Zsejke-Reka Toth[1,4], Ionel Papuc[2], Lucian Baia[1,5]

[1]Interdisciplinary Research Institute on Bio-Nano-Sciences,Babes-Bolyai University, Cluj-Napoca, Romania. [2]Faculty of Veterinary Medicine,University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Romania. [3]Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania. [4]Doctoral School of Physics, Babes-Bolyai University, Cluj-Napoca, Romania. [5]Faculty of Physics, Babeș-Bolyai University, Cluj-Napoca, Romania

Abstract

Diabetes mellitus is a metabolic disorder that can cause significant harmful effects regarding tissue healing. So-called impaired wound healing is a serious complication leading to pathologies like chronic open wounds, gangrene, sepsis, and in several cases even death.
Bioactive glasses with gold nanoparticles can induce the acceleration of wound healing including tissue regeneration, connective tissue regeneration, and angiogenesis. The aim of the present work was to evaluate the impact of using bioactive glass with spherical gold nanoparticles (BGAuSP) embedded in the oil-water cream on skin wound healing in experimental diabetic rat models for 14 days. Prior to *in vivo* assay, the obtained samples were structurally characterized and tested for *in vitro* cell viability.
The BGAuSP was prepared via the sol-gel method, inducing the spherical gold nanoparticles in the sol. The BGAuSP, which represented the active ingredient of the preparation, was incorporated into an oil-in-water (O/W) cream. The other ingredients selected for the cream preparation were: caprylic/capric triglycerides, paraffin oil, cetearyl alcohol, Sepigel 305®, Euxyl PE 9010®, glycerin, and rice powder.
FT-IR spectrum of BGAuSP presents characteristic bands of silicate network, and the XRD pattern indicates the presence of both amorphous structures of the glass, as well as crystalline features originating from gold nanocrystals. The TEM micrographs reveal nanoparticles sizes of about 30 nm in the BGAuSP composites. The viability of human keratinocytes cells after 24 h interaction with samples indicates good *in vitro* tolerance.
In vivo experiment was performed on Wistar-Levis rats. For the positive control silver sulfadiazine (Regen-Ag) cream was used, which promotes the wound-healing process of diabetic foot ulcers. The wound healing potential was evaluated using the optical imaging method followed by histopathology and immunohistochemistry assays. After 14 days the wound treated with BGAuSP-oil-water cream is completely healed, compared with Regen-Ag case, where the wounds present a small crust even at 14 days. The obtained results suggest the BGAuSP-oil-water cream wound healing potential in diabetic patients.
Acknowledgments: This work was supported by a grant of the Ministry of Research, Innovation and Digitalization, CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2021-2176, within PNCDI III.

PoB.18.08

Antithrombotic modification of hair-derived keratin

Soki SONOHARA[1], Kazuya SAWADA[2], Toshia FUJISATO[1]

[1]Osaka Institute of Technology, osaka, Japan. [2]Osaka seikei college, osaka, Japan

Abstract

Keratin is water insoluble fibrillar protein that constitutes the intermediate diameter filaments of vertebrate epithelial cells. Since the keratin derived from hair and wool are non-vascular origin, it may be used as biosafety and biocompatible biomaterials. We have been developing scaffolds for cell implantation using extracted keratin from animal hair and have demonstrated the excellent biocompatibility. In this study, heparin has been immobilized onto the keratin film surface to have anti-coagulant property in order to develop artificial blood vessels made from human hair-derived keratin.
The keratin was extracted from human hair using a reducing agent and a protein denaturant. Then, the extracted keratin was formed and processed into a film. Before heparin immobilization, heparin, N-Ethyl-N'-[3-(dimethylamino)propyl]carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) were dissolved in MES Buffer as heparin solution at pH 5.6. Then, the keratin film was immersed in the heparin solution of 0.005 mg/mL. To determine the amount of heparin immobilized, the unreacted heparin was mixed with toluidine blue (TB) and the absorbance was measured with spectrophotometer. The difference of absorbance before and after the immobilization reaction was compared, and the absorbance after the reaction was higher than that before the reaction. TB is a blue dye that specifically binds to heparin through electrostatic interaction and changes color. This indicates that the reaction reduced the amount of unreacted heparin remaining in the solution, i.e., heparin was immobilized on the film. Also, the absorbance increased as the surface area of film increased. These results show heparin can be immobilized onto the keratin film surface and may be applicable as anticoagulant material.

PoB.18.10

Tunable patterning of vascular networks for hierarchical tissue engineering

Silvia Pravato[1,2], Lisa A. Krattiger[1,2], Dhananjay V. Deshmukh[3], Jürg Dual[3], Silvia Farè[4], Mark W. Tibbitt[3], Martin Ehrbar[1,2]

[1]University of Zurich, Zurich, Switzerland. [2]University Hospital Zurich, Zurich, Switzerland. [3]ETH Zurich, Zurich, Switzerland. [4]Polytechnic University of Milan, Milan, Italy

Abstract

Introduction: If a vascular scaffold with cells is implanted in a living organism, it must quickly integrate into the host's vascular system for the cells to survive. Prevascularization of the implant can improve this integration. In this work we make us of acoustofluidics, a non-contact and tunable technique, to develop vascular networks within Gelatin-Methacryloyl (GelMA) hydrogels. Bone marrow-derived mesenchymal stromal cells (BM-MSCs) and human umbilical vein endothelial cells (HUVECs, GFP and RFP expressing) are patterned into parallel lines, resulting in increased interaction between cells. The effect of this localized cell enrichment on vasculogenic and angiogenic processes is evaluated. **Methods:** As scaffold to embed cells, GelMA was employed. To pattern GFP-HUVECs, RFP-HUVECs and BM-MSCs into lines, an acoustofluidic glass device (fig. 1a) consisting of a 1 mm wide square capillary was used. A piezoelectric transducer was used to generate an ultrasonic standing wave within the device, with the signal being controlled externally. The cells moved towards the pressure nodes, which caused them to form parallel lines. To encourage the formation of vessel-like structures, the cells were cultured in the presence of FGF-2 and VEGF-A. The cultures were observed microscopically for 3 or 7 days and then fixed and stained for f-actin, DAPI, and ECM-proteins. To assess the vessel formation, the length of sprouts in samples with varying cell densities is quantified by using the "Angiogenesis Analyzer" tool from ImageJ. **Results And Discussions**: Cells could be patterned without damage using acoustofluidic devices. The existence of a pattern resulted in the formation of vascular-like networks at pre-defined locations. Over several days of culture, initially patterned cells were observed to migrate from their original location. By using HUVECs that express two different fluorescent proteins, it was possible to trace the formation and development of sprouts. Higher local cell densities facilitated the growth of long, continuous and parallel vessels (Fig. 1b). **Conclusions:** In this study, an *in vitro* model for vessel formation was developed. Acoustofluidics allowed us to improve the cell-cell interaction, resulting in the formation of well structured vascular networks. Next, our system will be interfaced with bone simulating hydrogels, to simulate the *in vivo* environment and demonstrate the modularity of the model.

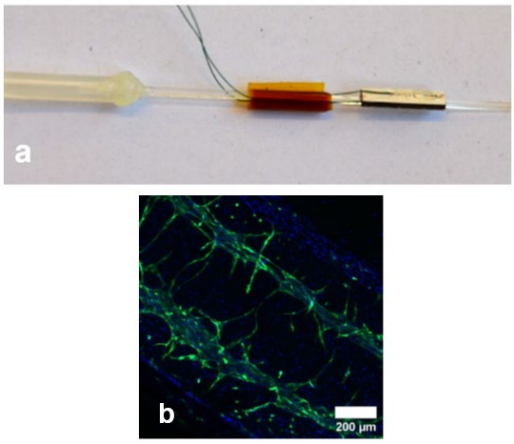


Fig 1: a) acoustofluidic glass device for patterning; b) GFP signal (green) and nuclei (blue) of a sample with a coculture of patterned GFP-HUVECs and BM-MSCs after 7 days in culture

PoB.18.11

Lung organoids formed from mouse primary cells

Kouhei ADACHI, Toshia FUJISATO

Osaka Institute of Technology, Osaka, Japan

Abstract

Unlike other organs, the lungs are the only internal organs that are directly exposed to the outside air. Since the air contains various toxic substances, bacteria, and viruses such as COVID-19, the lungs have the highest potential for infection and inflammation and are sometimes very difficult to repair. It may be needed to develop the *in vitro* lung model to well understand the pathology of lung diseases. In addition, animal experiments are being avoided all over the world these days. The *in vitro* lung model will also be useful as alternative to animal testing. In this study, lung organoids are developed *in vitro* from mouse primary cells.

Lungs were isolated from ICR mice. All procedures were approved by the ethical committee of the institution. The isolated lungs were rinsed by PBS to remove blood inside and then trypsinised. After predetermined time, they were filtered to remove undigested tissue and the isolated cells were seeded in 100 mm culture dishes with DMEM for 3 weeks. They were seeded on polymeric surfaces with different hydrophilicities for organoid formation and subjected to lung protein assay and SEM observation.

From the lung protein assay, alveolar epithelial cells were confirmed not lost in the 3-week culture. Also, lung organoids were found by inverted phase contrast microscopy. They were compared with living mouse lungs by SEM observation and confirmed that the organoids and the living lung had a similar structure although not exactly the same. These results suggest that the lung organoids could be formed from animal primary cells and may be used as alternative to animal tests.

PoB.18.12

Evaluation of *in vitro* self-assembly of microvessels-like structures induced by co-culture of human osteoblasts and human dermal microvascular endothelial cells on silicate, zinc co-substituted hydroxyapatite

Paula Milena Giraldo-Osorno[1], Tim Kreuz[2], Daniel V Bax[2], Farah Asa'ad[1], Margarita Trobos[1], Serena M Best[2], Anders Palmquist[1]

[1]Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. [2]Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, United Kingdom

Abstract

Synthetic hydroxyapatite (HA) closely resembles natural bone mineral. This chemical similarity endows it with unique properties that make it an ideal material for bone tissue regeneration and repair. However, while HA has shown potential, there remains scope to optimise its bioactivity and immunomodulatory properties to achieve successful osteoinduction. One obstacle to clinical translation of implants is their inability to be efficiently vascularised by the host. This leads to a limited supply of oxygen and nutrients and insufficient waste elimination, causing low cell viability and apoptosis. One strategy for tuning HA bioactivity and stimulating vascularisation is the incorporation of small amounts of physiologically relevant ions such as silicate and zinc.

Using an *in vitro* co-culture model of human osteoblasts (hOB) and dermal microvascular endothelial cells (HDMEC), this study aimed to evaluate the comparative cytocompatibility of HA and HA containing Si and Zn substitutions and whether the ionic additions could promote self-assembly of endothelial cells into microvessels-like structures.

Stoichiometric HAp was synthesised using a wet precipitation method with up to 1.5 wt% Si and 0.8 wt% Zn additions. Dense ceramic discs were pressed and sintered at up to 1200 °C and characterised using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and X-ray fluorescence (XRF).

Angiogenic potential was evaluated by analysing cell viability, immunofluorescence, gene expression, cytokine secretion and scanning electron microscopy after 7 and 14 days of direct co-culture. Further immunostaining of platelet endothelial cell adhesion molecule-1 (PECAM-1) and Von-Willebrand Factor (vWF) was investigated to quantify endothelial cell-cell network organisation into microvessels. Image J was used to quantify the vessel density and parameters of the microvascular network formation such as the number of vessels, junctions and nodes, and total vessel length.

Densities of >89 % were achieved for the ceramic discs and XRD results confirmed phase purity after sintering. The presence of Si and Zn was identified through refining lattice parameters, characteristic FTIR bands, and quantitative XRF results. Co-culture of hOBs and HDMECs revealed excellent cytocompatibility for up to 14 days. In addition, the SiZn-HA co-substituted material was found to promote vessel density and vessel-like network complexity (increased number of nodes and junctions) after culture with HDMECs and hOBs.

In conclusion, we have found that a novel co-substituted hydroxyapatite ceramic (SiZn-HA) enhances the ability of HDMECs to form vessel-like structures in comparison to the HA control and demonstrate the strategy of tuning HA bioactivity by ion substitution.

PoB.18.13

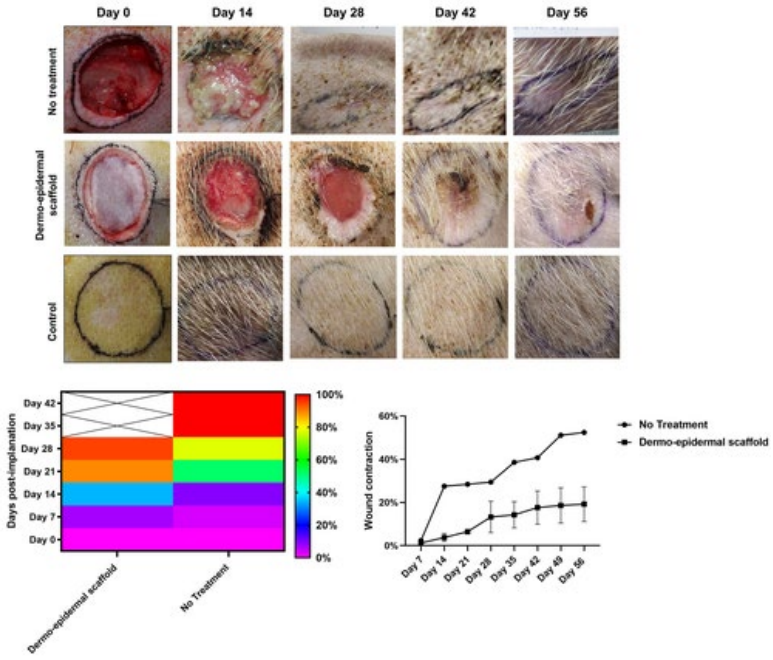
Development of dermo-epidermal skin substitute scaffold using reverse bioengineering

Prasad Sawadkar[1,2], Jia Hua[2,1], Karin Greco[1], Ferdinand Lali[2,1]

[1]Division of Surgery and Interventional Science, University College London, London, United Kingdom. [2]Department of Surgical Research Northwick Park Institute for Medical Research, Northwick Park & Saint Mark's Hospital, London, United Kingdom

Abstract

Skin is the largest and outermost organ in the human body; hence it is prone to injury and a significant cause of death worldwide. As per WHO, 10% of total deaths globally are caused by injuries. In some severe injuries like burns and non-healing wounds. The impaired skin repair mechanism can lead to life-threatening conditions; hence skin grafts are required. Autologous skin grafts are the current gold standard, but their availability is limited and causes donor site morbidity (1) To address this issue we have developed an off-the-shelf dermo-epidermal scaffold fabricated by reverse bioengineering. A porcine skin using a dermatome at the setting of 50 µm sheet was trimmed and decellularised using our patented technology, and scaffolds were fabricated. Material and material-cell characterisation was performed using microCT, degradation assay, porosity and mechanical testing to understand the scaffold's physical and biological properties were studied using CAM assays, cell differentiation and viability essays. *In vivo*, these scaffolds were implanted in 6 porcine models by creating split-thickness wounds of 2 cm diameter. The wound-healing analysis results showed that there was a normal wound-healing mechanism. Still, there was significant wound contraction in the no treatment (Fig.1). The results showed that a re-epithelialisation in the dermo-epidermal scaffold was achieved at day 28. In contrast, in no treatment, it was at day 42. Wound contraction is a significant problem with the present-day dermo-epidermal scaffold, but in our scaffold, the wound contraction was 19.20% at day 56, whereas in no treatment, it was 52.42%. Hence it was proved that our scaffold was mimicking native tissue biochemical and physical parameters. Has the potential of replacing split-thickness grafts and avoiding donor site morbidity.



PoB.18.14

Antibacterial Bacteriocin-Modified Nanocellulose Wound Dressing for Treatment of Wound Infections

Elisa Zattarin[1], Emanuel Wiman[2], Zeljana Sotra[3], Yagmur Bas[4], Kristina Hanna[3], Jonathan Rakar[3], Linn Berglund[4], Robert Selegård[1], Emma M. Björk[5], Hazem Khalaf[2], Kristiina Oksman[4], Johan P. E. Junker[3], Torbjörn Bengtsson[2], Daniel Aili[1]

[1]Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden. [2]Department of Microbiology, Immunology and Reproductive science, School of Medical Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden. [3]Center for Disaster Medicine and Traumatology, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden. [4]Division of Materials Science, Department of Engineering Sciences and Mathematics, Luleå University of Technology, Luleå, Sweden. [5]Division of Nanostructured Materials, Department of Physics, Chemistry and Biology (IFM), Linköping University, Linköping, Sweden

Abstract

Wounds that take longer than 6 weeks to heal are considered "chronic" and are highly susceptible to infections. If not treated properly, wound infections can spread to other tissues, leading to life-threatening conditions such as gangrene and sepsis. Wound infections significantly affect the physical and mental well-being of patients and impose a considerable financial burden on healthcare systems. Therefore, timely and effective treatment of infections is crucial for wound healing and preventing severe outcomes.

In this study, we present novel wound dressing materials based on nanocellulose that combine excellent wound dressing properties with potent antimicrobial activity. We employed two nanocellulose hydrogels, bacterial cellulose (BC) and TEMPO-oxidized nanocellulose derived from hardwood (TC). Both materials are nanoporous and nanofibrillar, exhibiting high exudate adsorption capacity, gas permeability, transparency, and mechanical strength, which make them excellent for wound dressings. Additionally, they conform to the wound bed, preventing exudate accumulation, and reducing pain perception during the healing process. However, they are unable to eradicate bacteria already present in the wound. To confer antimicrobial activity to the dressings, we functionalized them with the two peptide bacteriocin PLNC8 α/β. PLNC8 α/β shows potent antimicrobial activity against gram-positive bacteria and low cytotoxicity against human fibroblasts and keratinocytes. The functionalization of the dressings with PLNC8 α/β was achieved by direct physisorption peptides on the nanofibrils or by using immobilized mesoporous silica nanoparticles (MSNs) as a carrier for the peptides.

Whereas physisorption did not affect the physical properties of the dressings, and the released concentrations of PLNC8 α/β were sufficient to inhibit proliferation and biofilm formation of S. Aureus *in vitro*. However, the use of MSNs resulted in more than a 5-fold increase in specific surface area, leading to PLNC8 α/β enhanced loading capacity, which is expected to further improve the antimicrobial properties of the dressings. MSN functionalization had minimal impact on the dressing properties but resulted in an increase in dressing thickness (+30%) and moisture transmission (+9%) and a slight decrease in exudate retention capacity.

The functionalization of advanced nanocellulose wound dressings with antimicrobial peptides will further improve the applicability and performance of these materials in facilitating the healing of infected wounds.

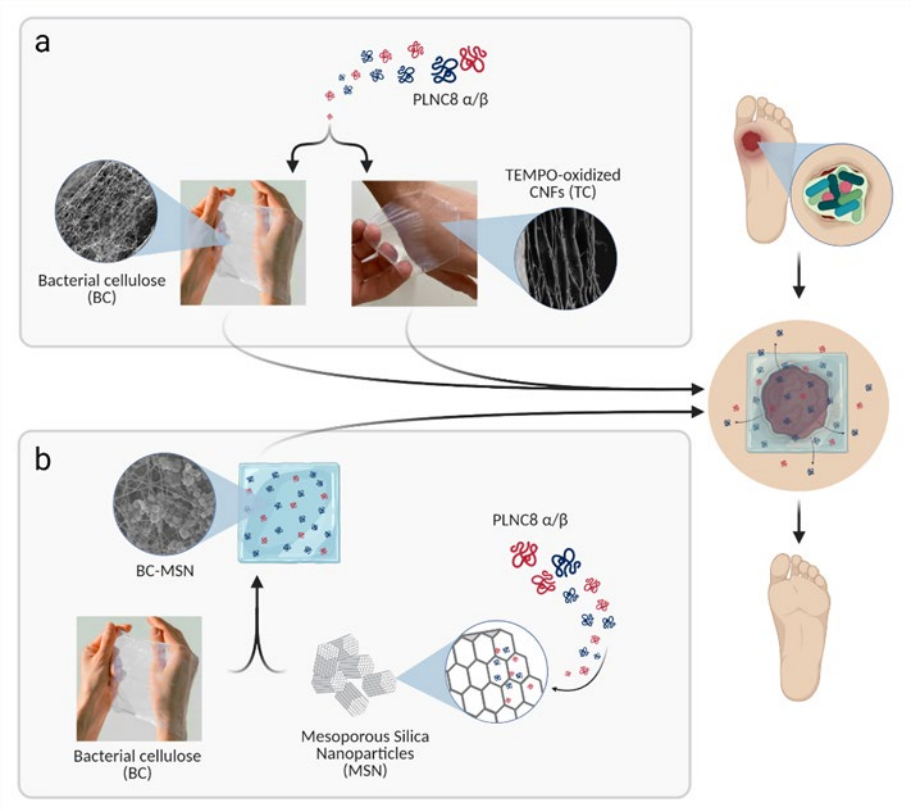


Figure 1. Antimicrobial wound dressings were obtained by physisorption of antimicrobial peptides PLNC8 α/β on a) bacterial cellulose (BC) and TEMPO-oxidised nanocellulose hydrogels (TC) and b) in mesoporous silica nanoparticles immobilized in the BC dressings. Figure created with BioRender.com

PoB.18.15

Nanofibrous proteolytic mats for wounds and burns sensitive debridement

Miroslava Rysova, Hana Tomankova, Milena Maryskova, Marketa Schaabova, Pavel Kejzlar, Alena Sevcu

Technical Universiy of Liberec, Liberec, Czech Republic

Abstract

Over the last decades, drug delivery has undergone a significant evolution as novel nano-based dosage forms for application in specific areas such as tissue engineering, active wound management, or cancer therapy have been developed. The major reason for applying nanosized dosage forms in drug delivery is precise targeting, designed drug release profile in situ and increased bioavailability of hydrophobic drugs leading to dose optimisation and reduced overall body load.
Recently, electrospun nanofibres have attracted attention as potential matrices for targeted drug delivery and controlled release in wound healing due to their possible barrier effect and unique characteristics – especially small fibre diameter and high porosity as characteristics leading to the extreme surface-to-weight ratio. One of the major advantages of the electrospun nanofibres is their wide variability in surface morphology and composition, enabling the precise design of their bioactivity, degradation kinetics and surface functionality. The last parameter is then applicable for biomolecule conjugation on the surface of nanofibres. Conjugation of drugs on the surface leads to their increased stability, prolonged functional half-life and minimal release into the bloodstream. The minimal release is crucial in the long-term treatment of extended wounds and defects, minimizing the risk of overloading the patient´s system. In this study, we present the results of the development of nanofibrous proteolytic mats with different degradation rates for wound debridement with increased enzyme stability leading to expanding the frequency of the bandage change. Three different types of nanofibres were studied as carrier matrixes providing a comparison of natural, synthetic and inorganic materials in terms of their stability, biocompatibility, conjugation efficiency, biocompatibility and proteolytic activity of the conjugated enzyme.

PoB.18.16

Using the Chicken *Ex Ovo* Chorioallantoic Membrane Model as a Pre-Screening Method for Evaluation of Angiogenesis in an Acellular Porous Biomaterial Based on Polyhydroxybutyrate and Chitosan

Zuzana Demcisakova[1], Lenka Luptakova[2], Zuzana Tirpakova[2], Alena Kvasilova[3], Lubomir Medvecky[1,4], Ward De Spiegelaere[5], Eva Petrovova[1]

[1]Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovakia. [2]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovakia. [3]Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic. [4]Institute of Materials Research, The Slovak Academy of Sciences, Kosice, Slovakia. [5]Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Abstract

The chorioallantoic membrane (CAM) is a highly vascularized avian extraembryonic membrane widely used as an experimental assay to study angiogenesis and its inhibition in response to tissues, cells, or soluble factors. In recent years, the use of CAM has become an integral part of the biocompatibility testing process for developing biomaterials intended for regenerative strategies and tissue engineering applications. In this study, we used the chicken *ex ovo* CAM assay to investigate the angiogenic potential of innovative acellular biopolymer polyhydroxybutyrate/chitosan (PHB/CHIT) scaffold, which is intended for the treatment of hard tissue defects, depending on treatment with pro- and anti-angiogenic substances. On embryonic day 7, the experimental biomaterials were placed on the CAM alone or soaked in vascular endothelial growth factor (VEGF-A, 25 ng), saline (PHY), or tyrosine kinase inhibitor (SU5402, 5 mM). After 72h, the formation of vessels was analyzed in the surrounding area of the scaffold and inside the pores of the implants, using markers of embryonic endothelium (WGA, SNA), myofibroblasts (α-SMA), and macrophages (KUL-01). We further evaluated the number and diameter of vessels and thickness of CAM layers. The morphological and histochemical analysis showed strong angiogenic potential in untreated scaffold (77.51 ± 3.31%) compared to soaked scaffolds with VEGF-A (74.70 ± 4.06%) and PHY (51.24 ± 8.04%). The lowest angiogenic potential was observed in scaffolds soaked with SU5402 (19.69 ± 6.83%). In newly formed CAM tissue as well as inside the scaffold pores, the formation of the CAM villi and the presence of endothelial cells in blood vessels were observed. The presence of myofibroblasts on a border between CAM tissue and the biomaterial as well as the presence of macrophages inside the pores of scaffold was a sign of repair process of the CAM tissue and ongoing angiogenesis. Gene expression of proangiogenic factors, i.e., VEGF-A, ANG-2, and VE-CAD, was upregulated in untreated scaffolds after 72h, indicating a proangiogenic environment. We concluded that the PHB/CHIT has a strong endogenous angiogenic potential and could be promising biomaterial for the treatment of hard tissue defects. The ability to detect angiogenic changes both on surface and in the pores of material makes this alternative animal model a suitable system for pre-clinical testing of biocompatibility, functionality as well as the tissue reaction of potential materials that can be used in regenerative medicine with respect to the 3R´s.
This research was supported by the Slovak Research and Development Agency APVV-20-0073 and VEGA 1/0413/23.

PoB.18.17

FRESH PRINTING OF SACRIFICIAL 3D NETWORK FOR 3D *IN VITRO* MODELS DEVELOPMENT

Matteo Pitton[1], Chiara Liguori[1], Silvia Farè[1,2]

[1]Politecnico di Milano, Milan, Italy. [2]INSTM, Milan, Italy

Abstract

Introduction. Adipose tissue (AT) is involved in complex interactions with human systems [1], and *in vitro* AT 3D models could be useful to study tissue dysfunctions (e.g., breast cancer). Anyway, lack of a functional vascular network in the 3D scaffold-based model remains thrilling. Herein, FRESH printing technique was selected to develop a 3D scaffold-based model with an internal 2D and 3D hollow channel network. **Materials and Methods.** GelMA was synthetized, dissolved (10% w/V) in DPBS, and Irgacure 2959 (0.05% v/V) was added. Pluronic F127 was dissolved (23% w/V) in distilled water at 4°C. Shear rate ramp (0.1-1000 s-1) tests were performed on both. Pluronic F127 was FRESH printed in a 2D serpentine shape in GelMA bath and samples were post-crosslinked (150s, 535nm). 3D helix shaped channel was printed into the GelMA matrix. Obtained samples, after Pluronic dissolution (GelMA_ch), were observed to check the channel dimension; *in vitro* stability in physiological-like environment, and compressive mechanical tests were performed. Preadipocytes 3T3-L1 were encapsulated in GelMA bath, differentiated, and cultured after seeding endothelial cells EA.hy 926 in the channel (GelMA_ch vs GelMA_bulk). **Results and Discussion.** Capability of GelMA bath to retain channel morphology and accommodate nozzle movement was evaluated. Different pre-crosslinking time was tested to couple ink and bath viscosity (**Fig. 1**), and t = 120s was selected. Once 2D serpentine was printed (v = 6 mm s-1, EM = 4, 25G), stable GelMA_ch hydrogels were obtained up to 4 weeks in physiological-like fluid. Channel diameter resulted in the 400-900 µm range, comparable to physiological vasculature dimensions [2]. 3D helix shaped channel was printed (**Fig. 2**), and its dimension resulted about 600 µm in diameter. GelMA_ch mechanical properties resulted comparable ($p>0.05$) to GelMA_bulk and its elastic modulus (8.57 kPa) fitted in the range of breast cancer condition [3]. Preadipocytes proliferated better ($p<0.05$) in GelMA_ch than GelMA_bulk samples, since faced a direct access to nutrients and oxygen, while both formulations allowed adipocytes differentiation (Nile Red). Co-culture was successfully performed and checked by LiveDead (7 days). **Conclusions.** We optimized a FRESH printing of Pluronic F127 vasculature-like channel in a GelMA bath to recapitulate human vasculature. We obtained GelMA_ch samples which resulted suitable to mimic pathological AT, and we optimized printing of a 3D helix shaped network, to allow a better internal perfusion.

References

[1] Unnikrishnan, Front. Onc., 2021
[2] Wu, Adv. Mater., 2011
[3] Samani, Physics in Medicine & Biology, 2007

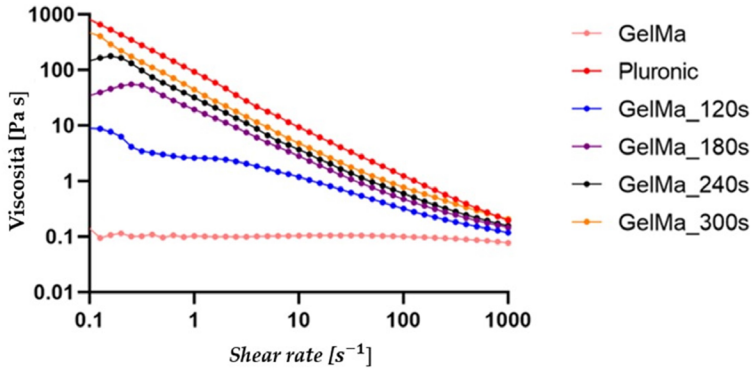


Fig. 1: Rheological tests. Shear rate ramp tests performed on sacrificial ink (Pluronic) and GelMA supportive bath pre-crosslinked at different times (120, 180, 240, 300 s). Rheometer was equipped with a plate-plate geometry (PP25, gap = 0.5 mm), and shear rate sweep (0.1-1000 s^{-1}) was performed after a pre-conditioning of 0.1 s^{-1} for 120 s and a rest phase for 60 s. Pre-crosslinking time equal to 300 s was selected for further characterization since allowed to obtain comparable viscosities between sacrificial ink and supportive bath.

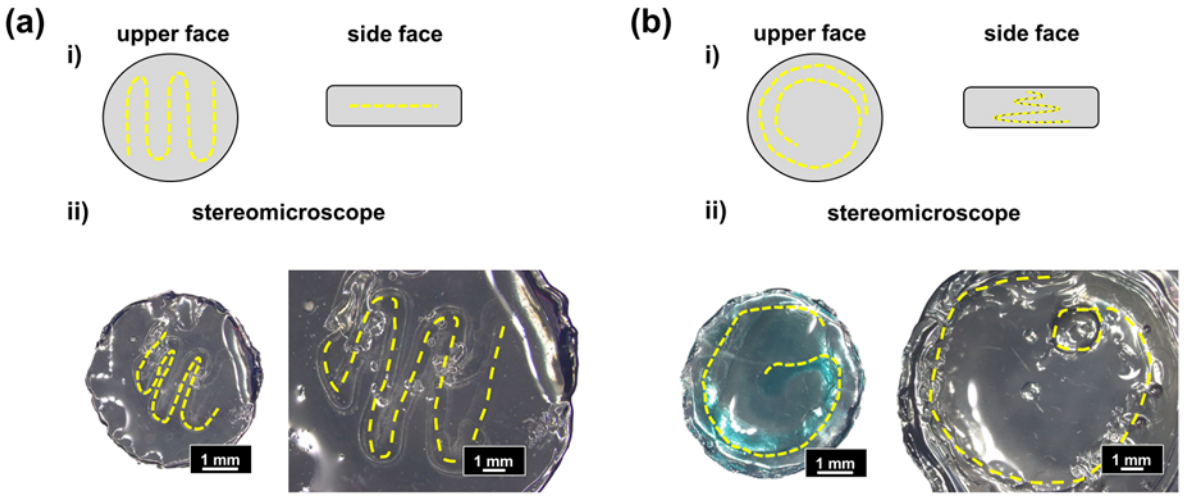


Fig. 2: FRESH printing of sacrificial Pluronic F127 ink into pre-crosslinked GelMA supportive bath. (a) i) Representation of upper (left) and side (right) face of models with embedded 2D serpentine shaped channel. ii) Images of 2D channel network acquired via stereomicroscope (magnification 1X and 2X). (b) i) Representation of upper (left) and side (right) face of models with embedded 3D helix shaped channel. ii) Images of 3D channel network acquired via stereomicroscope (magnification 1X and 2X).

PoB.18.18

Epidermis-on-a-chip for toxicological evaluation of cosmetic nanoformulations

Ana Rosa Ribeiro, Samantha Costa, Ana Beatriz Carneiro

International Iberian Nanotechnology Laboratory, Braga, Portugal

Abstract

The cosmetic industry is experiencing a significant increase in the utilization of nanomaterials, primarily due to their distinctive physicochemical properties. Sunscreen products that incorporate UV nano-filters, nano-TiO2, and nano-ZnO particles offer notable advantages over conventional alternatives, including broad UV protection and minimal side effects on the skin [1]. Presently, safety evaluations of cosmetic nanoformulations rely on 2D skin cell monolayers and commercially available 3D skin models cultivated under static conditions, which fail to accurately represent normal human physiology. Additionally, recent European legislation has prohibited the use of animal testing for cosmetic ingredients [2]. As a result, there is a pressing need for reliable, reproducible, and high-throughput alternative approaches within the fields of cosmetic, pharmacological, and toxicological sciences. Organ-on-a-chip technology presents a promising avenue, despite the engineering and biological challenges it entails, with the potential to revolutionize the risk assessment of nanomaterials in the next generation. This study aimed to establish an epidermis-on-a-chip model that mimics the complex microenvironment of the outer skin layers, serving as an ideal tool for screening cosmetic nanoformulations and assessing their toxicity. The barrier integrity of the developed models and the viability of the cells were evaluated using TEER measurements and the PrestoBlueTM assay, respectively. After culturing the epidermis-on-a-chip at the air-liquid interface, it exhibited histological features comparable to those observed in normal human epidermis, including a proliferating basal layer and differentiating spinous, granular, and cornified layers. Furthermore, immunofluorescence analysis confirmed the expression of typical keratins such as keratin-14, keratin-10, and loricrin. These results demonstrate the successful construction of a viable epidermis model that can be reliably employed in future safety assessments of nanomaterials utilized in cosmetic formulations.

References:
1. P. L. Sanches, L. R. O. Geaquinto, R. Cruz, D. C. Schuck, M. Lourencini, J. M. Granjeiro and A. R. L. Ribeiro, "Toxicity Evaluation of TiO2 Nanoparticles on the 3D Skin Model: A Systematic Review," Frontiers in Bioengineering and Biotechnology, 2020.
2. M. P. Vinardell and M. Mitjans, "Alternative Methods to Animal Testing for the Safety Evaluation of Cosmetic Ingredients: An Overview," Cosmetics, 2017.

PoB.18.19

Diverging nano-fiber architectures and their influences on an immortalized motoneuronal cell line as a model system to study neurodegenerative diseases.

Maya Kasteleiner[1], Nicoletta Murenu[1], Zan Lamberger[2], Gregor Lang[2], Natascha Schäfer[1]

[1]Institute for Clinical Neurobiology, Würzburg, Germany. [2]Department for Functional Materials in Medicine and Dentistry, Würzburg, Germany

Abstract

Neurodegenerative diseases can cause severe disability or even fatality and therefor represent a significant and challenging area of research within the field of Biofabrication. Malfunction of motoneurons are in most cases the underlying pathomechanism in neurodegenerative diseases. Effective treatments or cures for these conditions are currently lacking for some, especially rare diseases, like Hyperekplexia or Stiff-Man-Syndrome. Therefore, our study primarily focuses on investigating motoneuronal cells in a precise 3D spinal cord model system to further elucidate molecular pathomechanisms.

As the spinal cord represent a very soft tissue, the use of reinforcements is necessary to handle the established constructs. Particularly, nano-fibers offer here promising and modifiable prospects to reinforce the matrix used to develop 3D *in vitro* neuronal spinal cord tissue. Whit this aim, we employed Poly-ε-caprolactone (PCL) and collagen fibers to generate diverse types of fibers: one very stiff and one more gel-like. PCL is a stiff, but biocompatible polymer commonly employed as scaffolds in cell culture applications. Collagen is the most abundant protein in mammals and a crucial component of the extracellular matrix, resembling a soft material. Both fiber-types were produced in different architectures. The influence of these fiber-types with respect to fiber architectures and stiffness's, was studied using a moto neuronal cell line, NSC-34 cells. These cells resemble a hybrid cell line, which is a fusion of mouse motor neurons and mouse neuroblastoma cells. This cell line exhibits characteristics similar to primary motor neurons, making it suitable for *in vitro* studies.

Throughout the experiments, we monitored cell behaviour caused by confrontation of NSC-34 cells with stiff or soft nano-fiber-types of different architectures. We examined in detail cell viability, metabolic activity, as well as cell migration along the fibers and their precise interaction with the different fiber-types.

By investigating the impact of different fiber-types on the behaviour of this motor neuronal cell line, we aim to gain a better understanding regarding cell survival, migration, and interaction. This knowledge can contribute to the development of novel *in vitro* model to study neurodegenerative diseases.

PoB.18.20

Hydrogel platform for modeling retinal pigment epithelium interaction with retina

Sanna Korpela, Heli Skottman, Soile Nymark, Teemu O Ihalainen

Tampere University, Tampere, Finland

Abstract

Retina is a light-sensory tissue that initiates the sense of vision. At the back of the eye in close contact with retina resides its essential maintenance tissue, retinal pigment epithelium (RPE). The RPE-retina interaction is both structural and functional. Apical side microvilli of the RPE cells protrude between retinal photoreceptor outer segments, forming a tight interlocked structure. In addition, RPE cells are responsible of the daily renewal of retinal photoreceptor outer segments by phagocytosis. Thus, complications in the RPE-retina interface can lead to the degeneration of retina and vision loss. However, current *In vitro* RPE cell culture systems lack these contacts between RPE and retina, possibly limiting not only RPE maturation but also hindering studies of the RPE-retina interface. Here we show that a biomimetic retina platform can be constructed from soft polyacrylamide hydrogel coated with Matrigel. Hydrogels were placed on the apical side of near-mature human embryonic stem cell (hESC) and induced pluripotent stem cell (hiPSC) derived RPE cells cultured on Transwell inserts. RPE cells adhered to the hydrogels during culture, enabling further studies of the physical properties of RPE with methods developed from rheology and traction force microscopy. RPE-hydrogel adhesion force was quantifiable with a rheometric tack adhesion test. Interestingly, degradation of cellular actin cytoskeleton with Cytochalasin D detached the hydrogel from all RPE cells, demonstrating that the adhesion is reversible and depends on intact actin cytoskeleton. hiPSC-derived cells with retinitis pigmentosa mutation in gene PRPF8 resulted in more frequent detachment of the hydrogel compared to control hESC-derived RPE cells. Furthermore, traction force microscopy with fluorescent beads embedded in the hydrogel showed that RPE cells actively generate forces and transmit them to the hydrogel. In addition, particles of porcine photoreceptor outer segments were incorporated into the hydrogel surface, and the tractions that RPE cells exerted on the hydrogel during phagocytosis were followed. In conclusion, these tunable polyacrylamide hydrogels enable specific measurements of the RPE-hydrogel interaction. Therefore, this biomimetic retina platform offers an inexpensive, reproducible tool for further biophysical studies of the RPE-retina interface.

PoB.18.22

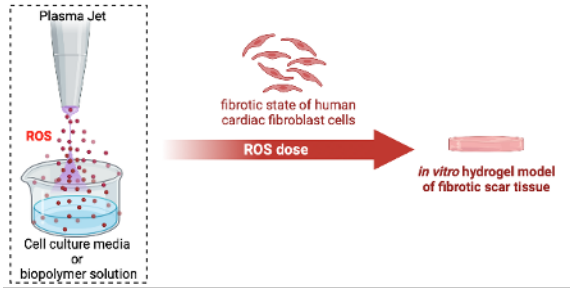
Plasma-activated hydrogels for *in vitro* fibrotic cardiac tissue modelling

Inès Hamouda, Alice Zoso, Elena Marcello, Valeria Chiono

Politecnico di Torino, Turin, Italy

Abstract

Ischemic heart disease is caused by cardiomyocyte death under hypoxic conditions, followed by the formation of a dysfunctional fibrotic scar. Reactive oxygen species (ROS) play an important role in this process. Physical atmospheric pressure plasmas (APP), generate unique mixtures of ROS and started to be considered for medical therapy. Due to the many potentials of this blooming new field ahead, there is a need to exploit the main concepts derived from plasma research in chemistry and biology as a mechanistic link between the ROS production from APP and their medical benefit (1). Indeed, different types and doses of *in vivo*-like ROS can be generated by APP treatment of physiological solutions, and their effects on different cell types have been studied, including ROS-induced cell apoptosis (2). On the other hand, the exploitation of APP treatment to model *in vitro* diseased tissues has not been reported up to now. The aim of this work was to develop an *in vitro* cardiac fibrotic tissue model for preclinical studies of drugs. An APP was used to generate ROS at a proper amount to induce oxidative stresses into human adult cardiac fibroblasts (AHCFs) without affecting their viability. Cells were exposed to plasma-activated medium for 24 h and their viability was evaluated after 1, 3 and 7 days. AHCF activation into myofibroblasts was also analyzed versus control conditions. Then, ROS-loaded hydrogels were prepared with proper concentration and crosslinking degree to match the stiffness of healthy and fibrotic cardiac tissues. ROS stability into the starting polymer solutions and their release kinetics from freshly-prepared hydrogels were studied. ROS-loaded hydrogels were used for AHCF culture. In the future, an *in vitro* model of the fibrotic cardiac tissue will be prepared using the selected ROS-loaded hydrogel and its structural and functional properties will be analyzed.



ROS tuning dose from APP-jet into media inducing fibrotic state of AHCF.

References: (1) Privat-Maldonado, A. et al. (2019) / (2) Hamouda, I. et al. (2021).

Acknowledgements: PACGEL project was funded by the European Union under HORIZON-MSCA-2021-PF-01 Project: 101067882. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the granting authority can be held responsible for them. BIORECAR project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No 772168.

PoB.19.01

New device for tissue adhesion using heat produced by ultra-vibration of polymeric material

Megumi Higuchi, Toshia Fujisato

Osaka Institution of Technology, Osaka, Japan

Abstract

Tissue adhesion is a technology used in hemostasis and surgery and includes suturing, adhesives, and surgical instruments. Suturing may require skill and time, adhesives may have cytotoxicity, and surgical instruments may be too hot and cause tissue damage. So, it is necessary to develop device to be used more easily for surgeons with less damage to living tissue. In the case of tissue adhesion using heat by surgical instruments, tissue damage and adhesion have a difficult relationship. For example, less tissue damage may lose adhesion and strong adhesion may increase tissue damage. Since it is reported that this boundary is around 105℃, we focused to develop an adhesive device using polymeric material with micro vibration which can keep the temperature near 105℃ in this study.
Polytetrafluoroethylene (PTFE) film was chosen as polymeric material since it has low tissue and cell adhesion by its high hydrophobicity. The PTFE film was driven by piezoelectric element with various frequencies for making heat. Porcine blood vessels were adhered by the device for 60 seconds under the weight of 1.0 kg. Then, the tissue damage was verified by H&E staining.
The heat temperature increased as the induced frequency increased and did not reach 105℃. In addition, this device showed less damage to the tissue and no adhesion boundaries could be seen (Fig.1). However, electrocautery showed significant damage to the tissue (Fig.2). These results show this new device may be useful for tissue adhesion with less thermal damage and more tissue reorganization compared to the current electrical scalpels.

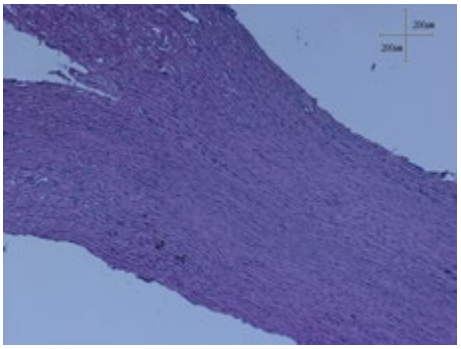


Fig1

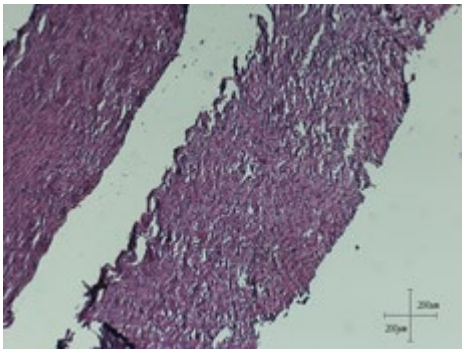


fig2

PoB.19.02

Anisamide-targeted Poly(beta aminoester) nanoparticles encapsulating mTOR siRNA for lung cancer therapy

Antoni Torres, Cristina Fornaguera, Coral Garcia, Salvador Borros

Institut Químic de Sarrià (IQS), Barcelona, Spain

Abstract

Lung cancer is one of the leading causes of death, mainly non-small cell lung cancer (NSCLC), due to the lack of efficient therapies. Currently, the standard of care is the administration of a chemotherapeutic drug, which, together with the low efficiency in killing tumours, produce high toxic side effect due to its systemic distribution. Nevertheless, it is well known that cancers are produced by spontaneous mutations that provoke an overexpression of tumor associated genes, such as mTOR. In this context, the use of small interfering RNA (siRNA) able to knock down these oncogenes to revert the disease may be a promising alternative. However, considering the labile character of siRNA and its poor stability in biological conditions, the use of a delivery system, such as polymeric nanoparticles, is a must. In the current work, Poly(beta aminoester) (pBAE) nanoparticles (NPs) were used for the encapsulation of mTOR siRNA and their further controlled delivery after systemic administration. in order to only transfect selectively lung cancer cells we modified the polymer adding a specific targeting moiety to the Sigma receptor, a transmembrane receptor overexpress in a diverse set of tumors like non-small cell lung cancer (NSCLC). This receptor has a high affinity to bind benzamide derivates such as anisamide. Thus, it was used for polymer functionalization. The formation of small nanoparticles (<200nm), with slight polydispersity (PDI< 0.3), cationic surface charge and high entrapment efficiency was demonstrated, with no variations due to the addition of the anisamide. These nanoparticles were capable of gene knockdown in lung cancer cell models, being non-toxic for healthy cells. Furthermore, *in vivo* studies demonstrated the potential of these nanoparticles in reducing tumor growth. Summarizing, we have designed a novel therapeutic approach for lung cancer patients based on Anisamide-targeted pBAE nanoparticles encapsulating mTOR siRNA that represents a clear first proof-of-concept studies for NSCLC therapy.

PoB.19.03

Novel, Biomimetic Poly(Glycerol Sebacate) Based Elastomers for Adipose Tissue Engineering

Reece Griffith, Biqiong Chen

Queen's University Belfast, Belfast, United Kingdom

Abstract

Adipose tissue defects, commonly caused by disease, trauma, injury or oncological surgery, can impair function and cause cosmetic irregularities. It is known that implanted materials which mimic the properties of native tissues can improve tissue regeneration when repairing tissue defects. Herein, several new biodegradable and biocompatible elastomers are reported for potential use in adipose tissue engineering. The elastomers were synthesized from a poly(glycerol sebacate) (PGS) prepolymer and a poly(ε-caprolactone) diol, which was prepared from ε-caprolactone and poly(ethylene glycol) (PEG). Molar ratios were varied to observe their effects on the properties. The results from Fourier transform infrared spectroscopy confirmed the formation of the PGS based polymers.

Contact angles between the polymer film and water were 36 – 110 ° and swelling in a phosphate buffered saline (PBS) solution ranged from 7 – 46 wt.%. The incorporation of PEG segments influenced the water contact angle and swelling degree of the polymers. Some polymers being hydrophilic indicated usefulness in tissue engineering for nutrient diffusion and cell adhesion. The new polymers had 0.20 MPa – 1.07 MPa tensile strength, 0.34 – 0.73 MPa Young's moduli and 86 – 272% elongation at break. Samples with the highest PEG content had lower moduli and strength but higher elongation at breaker.

Wet tensile tests of PBS-swollen samples were performed in a bath of PBS solution at 37 °C. The properties were 0.14 – 0.2 MPa tensile strength, 0.13 – 0.99 MPa Young's moduli and 47 – 157% elongation at break. All polymers showed highly elastomeric behaviour with no yield point seen in either the wet or the dry state. The mechanical properties of some of the polymers mimicked those seen in human adipose tissue. Cyclic tensile tests showed the polymers had practically zero hysteresis at 60% strain at 100 mm min-1 strain rate. This was maintained after 250 cycles. *In vitro* biodegradation tests found that the polymers degraded by 2.5 – 8 wt.% in a PBS and lipase solution after 120 days, indicating that they could support new tissue growth while maintaining biodegradability. Overall, some of the synthesized elastomers could be useful for adipose tissue engineering.

PoB.19.04

Playing with microfluidics: a straightforward approach to obtain chitosan nanoparticles functionalized with antimicrobial peptides

Diana R. Fonseca[1,2,3], Estrela Neto[1,2], Beatriz Custódio[1,2,4], Sofia Guimarães[1,2], Pedro M. Alves[1,2,3], Rúben F. Pereira[1,2,4], Paulo Freitas[5,6], Marco Martins[5], Cátia Teixeira[7], Paula Gomes[7], Paula Parreira[1,2], M. Cristina L. Martins[1,2,4]

[1]i3S – Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal. [2]Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [3]Faculdade de Engenharia, Universidade do Porto, Porto, Portugal. [4]ICBAS—Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal. [5]INL, International Iberian Nanotechnology Laboratory, Braga, Portugal. [6]INESC-MN, INESC Microsystems and Nanotechnologies, Lisboa, Portugal. [7]LAQV-REQUIMTE, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

Abstract

Bacterial antibiotic resistance is a public health challenge, prompting the demand to find alternatives. Among the microorganisms to which new agents are an urgent need is Helicobacter pylori (Hp). This gastric bacterium causes several gastric disorders, including gastric cancer (5th most common & 4th deadliest) and the available antibiotic-therapy failure rate is up to 40%[1-3]. Antimicrobial peptides (AMPs) have gained attention as newer options, being PexigananA(MSI-78A)reported as bactericidal against Hp. AMPs immobilization onto biomaterials overcomes the drawbacks usually associated with their performance *in vivo* (proteolytic degradation and aggregation with proteins), enhancing their bactericidal effect[4]. Here, a versatile, cost-effective, and environmentally friendly "one-pot" microfluidics system suitable for nanoparticles(NP) production and bioconjugation of any ligand containing a thiol group (e.g.,cysteine aminoacid) is proposed. MSI-78A with a terminal -SH group was directly grafted onto chitosan nanoparticles surface(AMP-NP, diameter ~113 ± 2 nm; nanoparticle tracking analysis (NTA)), using the Thiol–Norbornene "Photoclick" Chemistry. The reaction yield was ~40% (aminoacids analysis), corresponding to 96 µg/mL of AMP grafted onto NP batch (10^{11} NP/mL, NTA). The grafting success was confirmed with Fourier-transform infrared spectroscopy (FTIR), by the appearance of the characteristic absorption bands of the AMP at 1660 cm-1 (amide I) and 1530 cm-1 (amide II). AMP-NP at a concentration of 10^{11} NP/mL had a fast-bactericidal effect against Hp 26695 strain, reaching full eradication in 30min, while for the Hp J99 strain, the same bactericidal effect was achieved after 24h. These results demonstrated that MSI-78A maintained its activity after surface grafted onto NP and that the minimal bactericidal concentration was even lower than when using the free peptide (256 µg/mL). The high Hp–chitosan affinity further improved the killing effect. After exposure to AMP-NP, the bacterial membrane was compromised, being visible the formation of vesicles and release of cytoplasm content.
Furthermore, AMP-NP at bactericidal concentration were compatible with human gastric adenocarcinoma cell lines (AGS&MKN74-ATCC®-ISO10,993–5;12). Overall, the designed AMP-NP boosted the activity of MSI-78A and are promising for Hp eradication. A straightforward system to obtain AMP-conjugated chitosan nanoparticles was developed. Its main advantage is the possibility to produce, crosslink and immobilize different thiolated compounds in one single device. This system can be further explored with other biomaterials and for different applications.
[1] Rawla *et. al*.Prz Gastroenterol.,2019,14(1):26-38, [2] Sung *et. al*.CA Cancer J Clin.,2020,71:209–249
[3] Neshani *et. al*.Helicobacter,2019,p. 24, [4] Fonseca *et. al*.Acta Biomaterialia,2022,137:186-198

PoB.19.06

Exploring laser-patterning of never-dried bacterial nanocellulose (BNC) envisioning the development of immunomodulatory surfaces

Lígia Costa[1,2], Alexandre F. Carvalho[3], António J.S. Fernandes[3], Florinda M. Costa[3], Miguel Gama[1,2]

[1]CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal. [2]LABBELS –Associate Laboratory, Braga, Portugal. [3]I3N-Aveiro, Department of Physics, University of Aveiro, Aveiro, Portugal

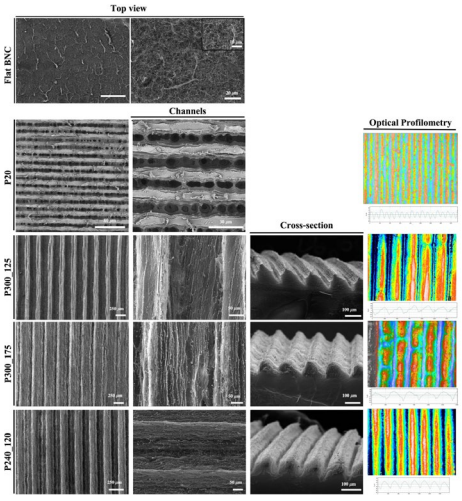
Abstract

The critical interplay between foreign body reaction (FBR) and infection is well recognized – the so called Biomaterial Associated Infections (BAI). Being central players of inflammatory responses and highly flexible cells, the selective modulation of macrophages polarization under distinct topological cues have been explored. It has been reported that elongated morphologies, induces a M2 polarization, with anti-inflammatory cytokine production [1].

BNC hydrogel is produced in pure form, possess high-aspect ratio, high fibre strength and crystallinity degree, as well as superior thermal and dimensional stability [2]. *In vitro* studies have demonstrated that BNC is non-cytotoxic and non-immunogenic. Moreover BNC presents a mild foreign body reaction *in vivo* [3].

Here, we explore laser technology for direct patterning of never-dried BNC surface with bioinspired topographies and micropatterns known for its contact-guidance efficiency. We have assessed the effect of the developed laser assisted-microtopographies on cell behavior, envisioning the further control of FBR trough modulation of macrophage-mediated immune response.

Using CO2 and UV lasers, different topographies in the range of 20-300 µm lateral feature size, were generated on BNC surface with high fidelity and reliability, in terms of lateral periodicity (Figure). The laser processing did not alter the surface chemistry of the BNC and the generated microtopographies were stable upon full hydration. The *in vitro* assays with fibroblasts have proven the efficacy of the micro-patterned BNC on oriented cell behavior, in terms of cell distribution and morphology. As expected, it has been seen that the reduction of the lateral periodicity to sizes approaching cell-size (P20) benefits contact-guidance by promoting cell alignment on the groove's direction and having a more prominent effect on cell elongation. Nevertheless, the rough periodic surface of the larger micro-structured BNC (P300_125, P300_175 and P240_120) also favors cell distribution, but in a more randomly manner, and a shift on cell morphology, when compared to flat BNC where cells maintain a rounded morphology and grow in clusters privileging cellular interconnections. This exploratory study unveiled the potential of this technology for development of textured BNC interfaces that might direct specific-cell responses and be helpful on the control of FBR.



1. Tylek T, Blum C, Hrynevich A, et al (2020) Biofabrication 12:025007.
2. Lee K, Buldum G, Mantalaris A, Bismarck A (2014) Macromol Biosci 14:10–32.
3. de Oliveira Barud HG, da Silva RR, da Silva Barud H, et al (2016) Carbohydr Polym 153:406–420.

PoB.19.07

Zwitterionic terpolymer brush with enhanced antifouling properties: a tool to prevent implant infections

Alina Pilipenco[1], Guruprakash Subbiahdoss[2], Michala Forinová[1], Monika Spasovová[1], Oleksandr Romanyuk[1], Alexandr Dejneka[1], Erik Reimhult[2], Hana Vaisocherová-Lísalová[1]

[1]Institute of Physics of the Czech Academy of Sciences, Prague, Czech Republic. [2]Department of Bionanosciences (DBNS), Institute of Biologically Inspired Materials, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

Abstract

Mitigating the ability of bacteria to adhere and form biofilms at surfaces is one of the major challenges in medical devices, implants, and bioanalytical devices operating in “real-world” conditions both in research and industry. Significant research efforts have been devoted to developing suitable bio-interface architectures, creating so-called “antifouling” barriers that resist nonspecific biomolecular adsorption and prevent subsequent bacterial adhesion and biofilm formation. Polymer brushes, composed of grafted chains with super-hydrophilic zwitterionic and non-ionic pendant groups, represent one of the most promising avenues to create robust bio-interfaces with ultra-resistant properties suitable for medical devices [1]. Here, we report a recently developed functionalizable terpolymer-brush architecture comprising *N*-(2-hydroxypropyl) methacrylamide (HPMAA), carboxybetaine methacrylamide (CBMAA) and sulfobetaine methacrylamide (SBMAA) with a tunable composition of functional groups [2]. We found an optimal ratio in the polymerization feed of 77 mol%, 20 mol%, and 3 mol%, respectively, with extraordinary resistance to bacterial adhesion. The terpolymer brush coatings demonstrated superior resistance in cell culture medium (DMEM-LG+10%FBS medium) by significantly reducing ($p \leq 0.01$) protein adsorption compared to uncoated gold surfaces. The extraordinary resistance to bacterial adsorption of the brush was first demonstrated *via* bacterial (*Staphylococcus epidermidis* ATCC 35984 and *Pseudomonas aeruginosa* PAO1 ATCC 15692) adhesion experiments under flow conditions for a time period of 2 h on a glass coverslip surface grafted with terpolymer brushes. Significant inhibition ($p \leq 0.001$) of bacterial attachment was observed on brush coatings compared to uncoated glass. These results were supported by a longer-time study of suppressed biofilm formation of *S. epidermidis* and *P. aeruginosa* in both static and flow conditions compared to uncoated glass for 20 h. *In vitro* experiments were also carried out to investigate the interactions between macrophages and bacteria, which is important in the understanding of the pathogenesis of implant-associated infections. Our results expand the research of the versatile properties of the tailored terpolymer-brush-based bio-interface toward its application as an antifouling coating.

1. Li, Q.S., et al., Chemical Reviews 122, 17073-17154, (2022).
2. Forinova, M., et al., ACS Appl Mater Interfaces 13, 60612-60624, (2021).

PoB.19.08

Rational design of a multi-compartmentalized conformable implant for brain cancer

Irene Guerriero[1,2], Cristiano Pesce[1,3], Daniele Di Mascolo[1,4], Anna Lisa Palange[1], Paolo Decuzzi[1]

[1]Laboratory of Nanotechnology for Precision Medicine, Fondazione Istituto Italiano di Tecnologia (IIT), Genoa, Italy. [2]Department of Informatics, Bioengineering, Robotics, System Engineering (DIBRIS), University of Genoa, Genoa, Italy. [3]Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy. [4]Department of Electrical and Information Engineering, Politecnico di Bari, Bari, Italy

Abstract

Glioblastoma continues to be the most aggressive among all the brain cancers. After 20 years, the clinical standard still relies on maximal safe resection of the malignant mass followed by radiochemotherapy returning a median overall survival of about 15 months. Most therapeutic approaches are impaired by the presence of the blood-brain barrier (BBB) and the biological heterogeneity fueled by the complex interconnection between malignant, immune, and normal brain cells.

Here, the authors present a mechanically conformable, compartmentalized biodegradable implant - µMESH – for the local and sustained deployment of complex combination therapies. Upon partial tumor removal, µMESH adheres to the resected cavity, circumventing the BBB and minimizing systemic toxicity. µMESH comprises a water-soluble polyvinyl alcohol (PVA) microlayer, carrying hydrophilic agents, intercalated with a poly lactic-co-glycolic acid (PLGA) micronetwork, carrying hydrophobic agents (Figure1A).

µMESH is realized *via* soft lithographic techniques, returning a PVA microlayer and a corresponding PLGA micronetwork whose geometrical and mechanical features can be finely tuned during the fabrication process. Specifically, PLGA micronetworks with openings of 5, 20 and 50 µm2 were fabricated (Figure1B-C) and compared to a continuous, flat PLGA microfilm.

First, µMESH biodegradation was evaluated under different conditions by assessing over time variations in mass, pH, NMR and FTIR spectra, and polymer molecular weight by gel permeation chromatography. Preliminary results showed that while the PVA microlayers rapidly dissolve in physiological solutions within several hours to a few days, the PLGA micronetwork biodegrades *via* a mixture of surface and bulk erosion depending on its original geometry over the course of several weeks to a few months.

Then, high-performance liquid chromatography was used to quantify the drug release from the two µMESH compartments. While PVA dissolution modulates the release of hydrophilic agents, diffusion appears to dominate the release from the PLGA micronetworks. Finally, the µMESH therapeutic efficacy was assessed *in vitro* on cancer cell monolayers and 3D-spheroids and *in vivo* on orthotopic murine models of glioblastoma. µMESH-treated mice manifested a significantly longer survival as compared to multiple controls (Figure2).

In conclusion, complex therapeutic regimens including small molecules and nanomedicines can be implemented via µMESH for the successful preclinical treatment of high-grade brain tumors.

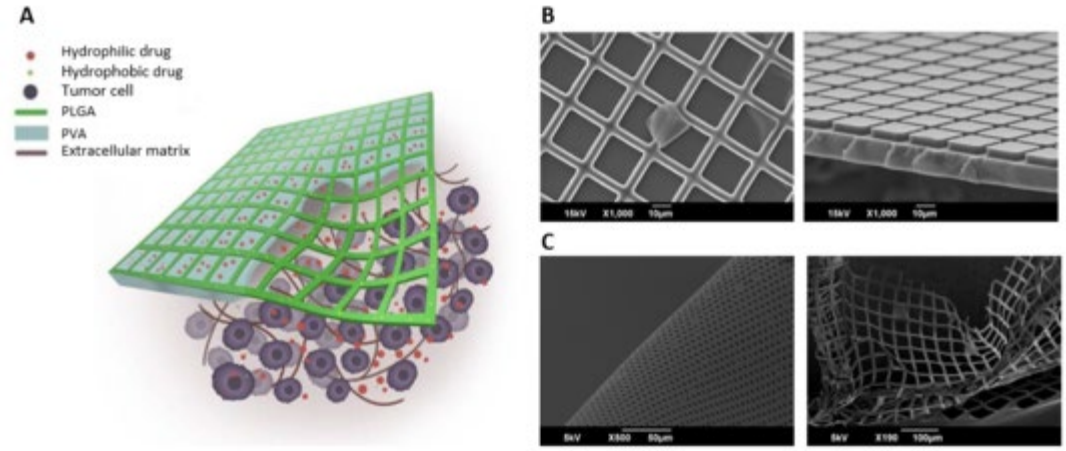


Figure1 | A.Schematic representation of µMESH; B.Scanning electron microscopy images of PLGA and PVA microlayers with openings and pillars of 20x20µm; C.PLGA openings of 5x5 and 50x50 µm.

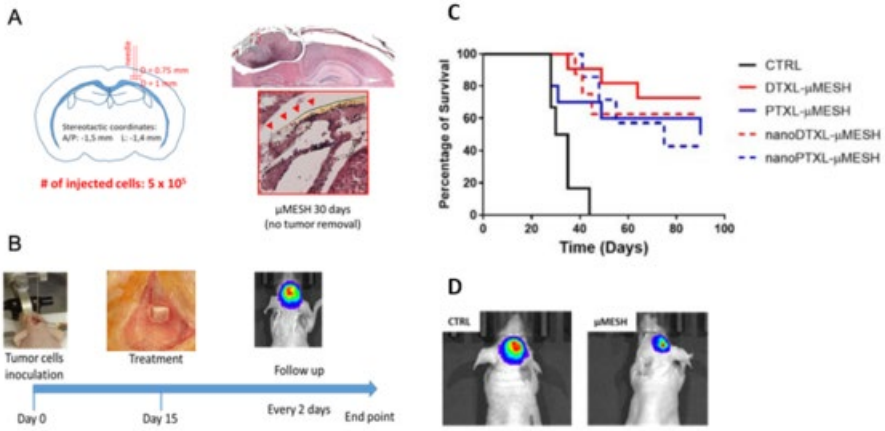


Figure2 | A.Tumor cell orthotopic implantation and histological section showing a residual portion of µMESH; B.Experimental timeline; C.Kaplan-Meier survival curves; D.Bioluminescence imaging.

PoB.19.09

Unexpected Unique Properties of Conducting Polymer

Petr Humpolíček[1], Daniela Jasenská[1], Věra Kašpárková[1], Ondřej Vašíček[2], Lukáš Münster[1]

[1]Centre of Polymer Systems and Faculty of Technology, Tomas Bata University in Zlín, Zlin, Czech Republic. [2]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic

Abstract

A large interest is currently growing in the field of combination of synthetic- and bio-polymers resulting in materials with unique properties. In the case of conducting polymers, which are especially advantageous as cell-instructive materials, the colloidal particles are important as they are the only form of conducting polymers dispersible in an aqueous environment. This characteristic makes them especially beneficial for the preparation of composite biomaterials. The polyaniline colloidal particles prepared by the chemical route are well known and frequently studied; however, we present a green, nature-friendly approach based on enzyme-based polymerization of one of the conducting polymers, polyaniline, which is synthetized via the oxidation of aniline with hydroperoxide-activated horseradish peroxidase and chitosan or poly(vinyl alcohol) as steric stabilizers. The particles prepared in this way prove, except for other properties, the interesting biological properties, mainly immunomodulatory effect. This is the first time the immunomodulatory effect of conducting polymer-based colloidal particles was observed. Moreover, the colloidal particles were able to reduce oxidative stress and inhibit the production of reactive oxidation species by neutrophils and inflammatory cytokines by macrophages. The anti-inflammatory effect observed was related to their antioxidant activity, especially in the case of neutrophils.
Acknowledgment: This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic – DKRVO (RP/CPS/2022/001).

PoB.19.10

Adhesive films based on marine-derived fucoidan and chitosan with antibacterial potential

Cátia Correia[1,2], Diana S. Costa[1,2], Ana Rita Inácio[1,2], A. Catarina Vale[1,2], Daniela Peixoto[1,2], Tiago H. Silva[1,2], Rui L. Reis[1,2], Iva Pashkuleva[1,2], Natália M. Alves[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal. [2]ICVS/3B's–PT Government Associate Laboratory, Braga, Portugal

Abstract

Developing biomaterials for soft tissue engineering and wound healing is a challenging task, as it requires the biomaterials to possess specific properties, including antibacterial properties to prevent bacterial growth and infection and adhesion abilities between the material and tissue to promote effective treatment. A promising source of antibacterial agents is marine-derived polymers, an environmentally friendly and sustainable resource for biomaterials production due to the similarities with biopolymers present in the extracellular matrix. Herein, we developed an antibacterial tissue adhesive combining two marine polysaccharides, namely fucoidan and chitosan. Fucoidan, a sulfated polysaccharide obtained from brown seaweed with antibacterial properties against gram-positive bacteria, was conjugated with dopamine (FCat) to improve its tissue adhesive properties[1]. This conjugation enhanced the antibacterial properties and FCat presented a bactericidal effect against gram-positive and gram-negative bacteria, as shown by the minimum bactericidal concentration (MBC) assay. The processability and mechanical properties of FCat were improved by blending it with chitosan and producing films by the solvent-casting method (Chit/FCat). Films from chitosan (Chit) as well as from unmodified fucoidan and chitosan (Chit/F) were also used as a control. Alcian blue staining and XPS analysis confirmed the presence of fucoidan in the films obtained from blends. The conjugation with dopamine improved the adhesion strength (21.89±1.80 kPa for Chit/FCat vs 15.82±1.65 kPa for Chit/F), presenting a close similarity to the strength of clinically used natural adhesives and higher than the strength of some synthetic adhesives in a wet environment (Fig.1a). Preliminary cytocompatibility tests were performed by culturing L929 fibroblastic cells on the surface of the films, resulting in an improved cell attachment and viability for the Chit/FCat films in comparison with Chit/F and Chit films (Fig.1b) . Overall, our results revealed that the Chit/FCat adhesive films, produced by using marine resources, had shown their potential to be applied as wound healing with antibacterial properties. Nevertheless, further studies are needed to evaluate the effectiveness of the films *in vivo*.

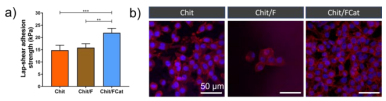


Figure 1. a) Adhesion strength and b) L929 cells attachment on the films after 7 days of culture

ACKNOWLEDGMENTS: The authors thank the financial support of FCT and European program FEDER/FEEI through PTDC/BTM-MAT/28123/2017 and PhD scholarship SFRH/BD/143209/2019, as well as the REMIX project, funded by the European Union's Horizon 2020 Research and Innovation programme under the Maria Sklodowska-Curie grant agreement no. 778078.

[1] Y. Jeong, et al., Macromol Biosci 2018, 18, e1800137.

PoB.19.11

Development of Novel Cyclodextrin-based Nanogels for hydrophobic drug delivery

Yanjing Ji

University Medical Center Groningen, Groningen, Netherlands

Abstract

Introduction. Nanogels have attracted much attention as drug carriers with excellent properties, such as high-water content, high porosity, excellent hydrophobic and hydrophilic drug binding ability, good biodegradability, and different biological activities. As a consequence, these excellent properties allow nanogels to exhibit superior performance over other nanocarriers and have promising drug delivery in disease treatment. Here in, we modified the Heptakis-(6-amino-6-deoxy)-β-Cyclodextrin heptahydrochloride (Ha-β-CD) with Acrylic acid N-hydroxysuccinimide ester (AA-NHS) to obtain the functional group of acrylamide. Afterwards, the poly(N-Isopropylmethacrylamide-*co*-Acrylamide cyclodextrin) (p(NIPMAM-*co*-AACD)) nanogels was synthesized. **Methods.** Acrylamide functionalization of Ha-β-CD was performed according to the previous report. Precipitation polymerization was used for the synthesis of p(NIPMAM-*co*-AACD)) nanogels. we characterized the modification of AACD with Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance spectroscopy (^{1}H NMR), and High-performance liquid chromatography (HPLC). The physical and chemical properties of p(NIPMAM-*co*-AACD) nanogels were studied with dynamic light scattering (DLS), transmission electron microscopy (TEM), and ^{1}H NMR. **Results and Discussion.** polymerizable acryloyl groups on Ha-β-CD molecule using AA-NHS in pH 8.5, 50 mM sodium carbonate buffer, which reacts with the free amino groups present in Ha-β-CD. Conjugation of acryloyl groups to Ha-β-CD showed changes in the FT-IR spectra. The peak of 3369 cm^{-1}, 1609 cm^{-1}, and 700-1000 cm^{-1} The new peak at 1246cm^{-1}, 1623cm^{-1}, and 1663cm^{-1} correspond to C-N stretching, C=C stretching, and C=O stretching, which indicate crosslinking of acryloyl groups with Ha-β-CD through AA-NHS. After that, the polymer was formed by radical polymerization using three different monomers: NIPMAM, AACD, and N, N'-methylene(bisacrylamide). (BIS) as cross-linker, in molar ratio 93.5:1:5.5, respectively. The polymerization was initiated by adding a solution of ammonium persulfate (APS), yielding the p(NIPMAM-co-AACD) nanogels after 4 h at 70℃ under N_2. We also synthesized p(NIPMAM) nanogels without AACD according to the above composition ratio. **Conclusions.** In this study, we introduced polymerizable acryloyl groups on Ha-β-CD molecule using AA-NHS and successfully copolymerized AACD with NIPMAM. Further study needs to focus on the degrees of acryloyl group modification, the morphology and temperature-dependent measurements of nanogel. contributed to the N-H stretching of primary amine, N-H bending of primary amine, and the glucopyranose ring, respectively.

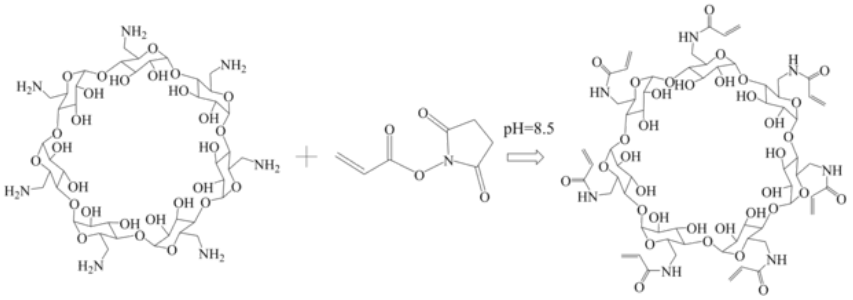


Figure 1. The synthesis scheme of acryloyl cyclodextrin (AACD).

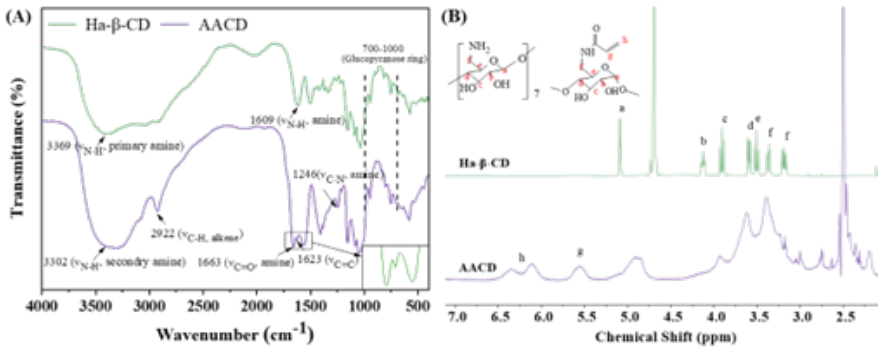


Figure 2 FTIR (A) and 1H NMR (B) of Ha-β-CD and AACD.

PoB.19.12

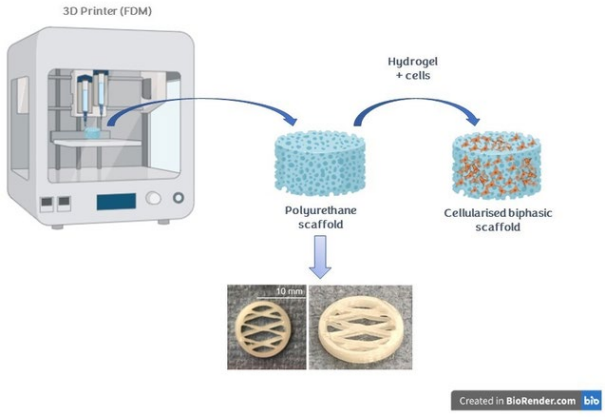
3D printed PU/GelMA biphasic scaffold as cardiac tissue model

Susanna Sartori[1], Andrea Alliaud[1], Arianna Grivet Brancot[1], Rossella Laurano[1], Monica Boffito[1], Federico Vozzi[2], Gianluca Ciardelli[1]

[1]Politecnico di Torino, Torino, Italy. [2]CNR Institute of Clinical Physiology, Pisa, Italy

Abstract

3D printing technologies have great potential in the production of scaffolds to be applied in the design of tissue models for personalised medicine, drug development and toxicological testing. The most common 3D printing technology is Fused Deposition Modelling (FDM). In FDM a thermoplastic polymer is extruded above its melting temperature to create a 3D object. Among thermoplastic polymers, poly(ester-urethane)s (PUs) represent interesting materials due to their high chemical versatility, which can be exploited to synthesize a plethora of polymers matching the requirements of different applications. For instance, degradable PUs have been extensively used in cardiac tissue engineering because of their elastomeric properties. In this study we engineered biphasic scaffolds consisting of 3D printed PU scaffolds filled with GelMA hydrogels, mimicking the young and aged cardiac tissue.



PU was synthesized using poly(ε-caprolactone) diol, 2000Da, 1,4-diisocyanatobutane and L-lysine ethyl ester. The PU was characterized by FT-IR, SEC and tensile tests. Thermal characterization was carried out by TGA, DSC and rheology, to assess polymer suitability for processing in the melt state. The PU was then microfabricated into scaffolds by melt-extrusion additive-manufacturing (fig. 1) and the extruded polymer was characterized to evaluate its stability during the printing process.

Fig.1: 3D printed PU/GelMA cellularised scaffold

The scaffolds were surface plasma-treated in the presence of acrylic acid and then grafted with fibronectin. GelMAs with two different degrees of methacryloylation were successfully synthesized as assessed by the colorimetric Ninhydrin assay and FT-IR and NMR analyses. Then, GelMA hydrogels were designed by solubilizing the polymer in a watery medium added with a catalytic amount of a water-soluble photoinitiator. Different GelMA concentrations were exploited to modulate the mechanical performances of photo-cured gels, obtained by irradiating GelMA aqueous solutions under cell-friendly conditions. Post-curing storage modulus values measured through photo-rheological time sweep tests varied between few and tens kPa. These tunable properties can mimic the cardiac aging process. Lastly, GelMA gels incubated at 25 °C under dynamic flow conditions exhibited stability up to 10 days of observation. GelMA was loaded with hiPSC-CMs, embedded in the scaffolds and photo-cured, to establish cardiac tissue models. Cells showed viability and contraction ability.

ACKNOWLEDGEMENTS: This work was supported by the European Union's Horizon 2020 research and innovation program (grant# 101037090). The content of this abstract reflects only the author's view, and the Commission is not responsible for any use that may be made of the information it contains.

PoB.19.13

TRAP-6 modified PVA materials as a potential treatment of chronic wounds

Miriam Zintl[1,2], Raffael Reichsöllner[1,3], Xiao-Hua Qin[4], Heinz Redl[1,3], Paul Slezak[1,3]

[1]Ludwig Boltzmann Institute for Traumatology, The Research Center in Cooperation with AUVA, Vienna, Austria. [2]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria. [3]AUVA Research Cluster for Tissue Regeneration, Vienna, Austria. [4]Institute for Biomechanics, ETH Zurück, Zurich, Switzerland

Abstract

Chronic wounds become an increasing clinical problem as their numbers are rising with aging population and increasing incidence of diabetes and obesity. While research on the cellular and molecular aspects of wound healing have helped to advance the understanding, diagnosis and prevention of chronic wounds, so far no suitable and effective dressing to treat all types of chronic wounds has been developed.1
Within this work, we present a novel approach to treating chronic wounds, comprising a powder [dd1] based on crosslinked poly(vinyl alcohol) modified with a thrombin receptor agonist peptide (TRAP-6) to promote wound healing. TRAP-6 activates PAR 1 (protease activated receptor), which in turn activates platelet activation and secretion. It has been shown that an upregulation of PAR1 plays a role in wound healing, and that immobilized TRAP-6 can shorten the inflammation phase in wounds and accelerate wound healing in mice.2,3
We covalently incorporated the platelet-stimulating TRAP-6 into the purely synthetic PVA carrier material by use of thiol-ene click chemistry.4 Initially, PVA was modified with norbornene groups to introduce a photochemistry-susceptible double bond, which was then covalently linked to a cystein-containing TRAP-6 peptide and used for crosslinking with a dithiol. The cohesive hydrogel material was dried and milled to obtain TRAP-6 containing particles as potential wound-treatment.
The developed material has been shown to be biocompatible via cytotoxicity tests and takes up liquid rapidly [dd2] upon contact with water. Its capacity to absorb more than 5 times its own weight is favorable in the use as a wound cover, as it ensures that wound exudate is transported away from the wound surface, adding a structural effect to the wound-healing properties induced by TRAP-6. We successfully demonstrated the biological function of the modified PVA-TRAP-6 material by proving its efficient platelet activation *in vitro* via a fluorescence-based flow cytometry method in human thrombocytes in platelet-rich plasma. Additionally, platelet aggregation was confirmed by cell counting and microscopy experiments. Initial *in vitro* cell studies with endothelial cells have shown increased activation of cells with TRAP-6 concentrations as low as 250 µM, rendering the PVA-TRAP-6 particles a promising treatment for chronic wounds.

1 Falanga, V. et al. Nat. Rev. Dis. Primer 8, 1–21 (2022)
2 Xue, M. et al. J. Invest. Dermatol. 125, 1279–1285 (2005)
3 Dugina, T. N. et al. Bull. Exp. Biol. Med. 138, 463–466 (2004)
4 Qin, X.-H. et al. Adv. Funct. Mater. 25, 6606–6617 (2015)

PoB.19.14

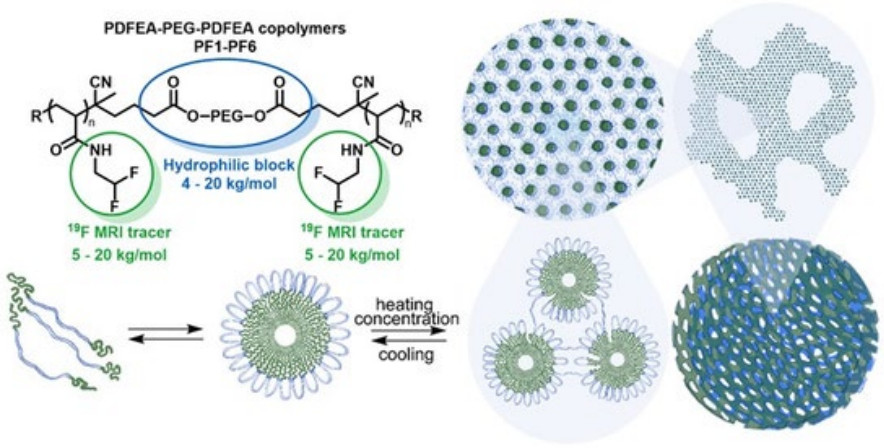
Widely applicable theranostics based on poly[*N*-(2,2-difluoroethylacrylamide)]

Kristyna Kolouchova[1], Ondrej Groborz[2], Vit Herynek[3], Laurens Parmentier[1], Anna Szabo[1], Lana Van Damme[1], Martin Hruby[4], Richard Hoogenboom[1], Sandra Van Vlierberghe[1]

[1]Department of Organic and Macromolecular Chemistry, Centre of Macromolecular Chemistry, Ghent University, Ghent, Belgium. [2]Institute of Organic Chemistry and Biochemistry, CAS, Prague, Czech Republic. [3]Center for Advanced Preclinical Imaging, First Faculty of Medicine, Charles University, Prague, Czech Republic. [4]Institute of Macromolecular Chemistry, CAS, Prague, Czech Republic

Abstract

Magnetic resonance imaging (^{1}H MRI) is a commonly used diagnostic tool that provides high-resolution morphological and anatomical information on soft tissues, with no limitations regarding sample penetration and, hence, has found an irreplaceable role in medicine.[1] Nevertheless, some anatomical structures or pathologies can be difficult to observe with standard ^{1}H MRI techniques, due to a lack of contrast with surrounding tissues. Its potential can be improved by introducing an MRI tracer. The most promising tracers are ^{19}F-based compounds, due to their high sensitivity, biocompatibility, negligible biological background along with the possibility of measuring ^{19}F in commonly used ^{1}H MRI devices. In our study, we prepared a wide spectrum of thermoresponsive triblock copolymers as 19F MRI tracers.[2] These ABA triblock copolymers are able to form nano-particles in diluted aqueous solutions and which enable transition into a physically cross-linked hydrogel upon increasing the polymer concentration. Thermoresponsive hydrogels are useful for tissue engineering applications, while the nano-particles can be used as drug delivery systems as well as for cell/tissue labelling. In general, we created therapeutic and diagnostic (theranostic) tracers enabling straightforward synthesis while serving a multitude of applications. The polymers are based on biocompatible poly[*N*-(2,2-difluoroethylacrylamide)] (PDFEA) and poly(ethyleneglycol) (PEG) polymers, of which the potential was already shown in our previous studies.[3–6]



[1]Tang, C. et al. Radiation Research (**1996**); [2]Kolouchova, K. et al. Chemistry of Materials (2022); [3]Kolouchova, K. et al. *J. Cont. Release* (**2020**); [4]Kolouchova, K. et al. *Biomacromolecules* 22, 2325–2337 (**2021**); [5]Kolouchová, K. et al. *Polym. Chem.* 12, 5077–5084 (**2021**); [6]Groborz, O.; Kolouchova, K.; et al. *Adv Health. Mater.*, 2201344 (2022)

PoB.19.15

Complex polyester-poly(glycerol methacrylate) copolymer architectures for regulating cell activation and targeted drug delivery

Ilaria Porello, Francesco Cellesi

Politecnico di Milano, Milan, Italy

Abstract

Selective cell targeting and signalling represent two major challenges in biomedical technologies and a deep understanding of the interactions between macromolecules and biological systems is essential for the development of innovative nanotherapeutics.
Complex cell behaviours are triggered by chemical ligands (proteins, peptides, polysaccharides etc.) that bind to membrane receptors and activate intracellular signal transduction. In particular, multivalent ligand–receptor recognition is often required for a variety of biological processes, including cancer metastasis, inflammation, and immune responses[1].
The current approach used to regulate nanomaterial targeting and cell activation is mainly based on conjugating ligands to the surface of the nanocarriers, although these methods present well-known limitations *in vivo*, including immune recognition, off-targeting, poor selectivity and response[2]. Recently, polymers with complex architectures (based on different ligand type, backbone flexibility and self-assembly) have been obtained through precise synthesis approaches[3]. These bioactive macromolecules can be designed to obtain multivalent binding, receptor clustering and an effective signal transduction for a therapeutic effect on stimulated cells *(Figure 1)*.
In this work, a design-by-architecture approach is proposed to identify crucial factors that affect polymer interactions with cellular response. These factors include independently adjustable architectural parameters such as main polymer chain and side chain length, number of arms/branches, monomeric unit sequences, bioactive moiety density, and position. The aim is to develop innovative polymeric materials that mimic molecular binding and signal transduction in biological systems, which could offer new opportunities for drug delivery and therapy.
Controlled-living polymerization techniques have been used to synthesize biocompatible and biodegradable polymers based on poly(glycerol/glycidyl methacrylate) (PGMA) and polyesters (PCL, PLGA etc.) with complex topology (linear, multi-arm, comb-like, brush-block) in order to achieve different functionality, self-assembly and size.
The chain length, the monomeric units, the number of arms/branches and functional groups, and the peptide sequences used for conjugation, can be varied to obtain libraries of different bioactive polymers. These biomaterials have been employed for drug encapsulation (such as doxorubicin) and they will be tested *in vitro* to induce the desired cellular responses.
Accordingly, coding the macromolecular architecture for a more efficient targeting and cell activation may pave the way for potential new therapies.

References

1. Yilmaz, G. & Becer, C. R., Macromol Chem Phys 221, (2020).
2. Pasut, G., Front Med Technol 1, (2019).
3. Celentano, W. et al., Eur Polym J 144, (2021).

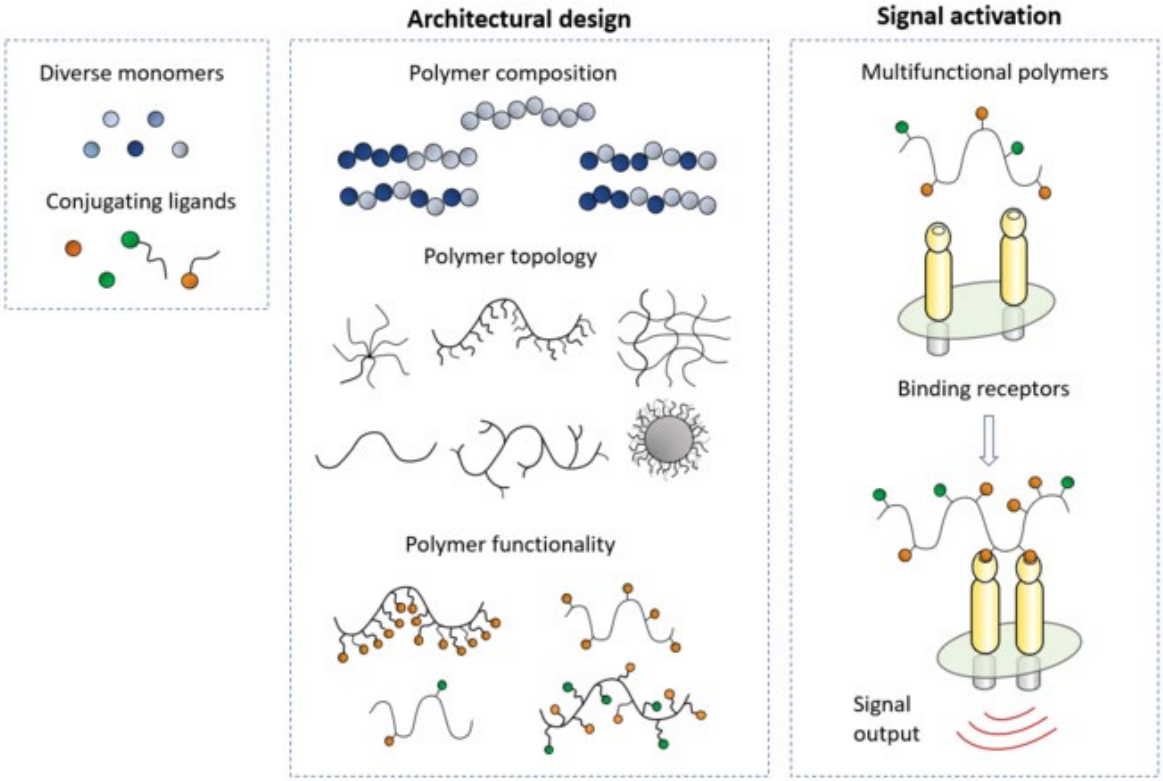


Figure 1. *Polymer architectural elements can be designed to achieve specific signal transduction in cells*

PoB.19.16

Combination of microwave-assisted polycondensation and UV-curing in polyglycerol sebacate synthesis

Mariella Rosalia[1], Davide Rubes[1], Maddalena Patrini[2], Massimo Serra[1], Aldo Boccaccini[3], Ida Genta[1], Rosella Dorati[1], Bice Conti[1]

[1]Department of Drug Sciences, Pharmaceutical Section, University of Pavia, Pavia, Italy. [2]Department of Physics, University of Pavia,, Pavia, Italy. [3]Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany

Abstract

Introduction. Polyglycerol sebacate (PGS) is a biodegradable elastomer already investigated in soft tissue engineering. Its main drawbacks are the extremely energy/time-consuming synthesis and low product reproducibility. The aim of this work is to combine and optimize microwave-assisted polycondensation and UV-crosslinking to overcome the aforementioned criticisms and obtain PGS in a sustainable manner. **Materials And Methods.** Pre-PGS was synthetised heating equimolar amounts of sebacic acid and glycerol in a closed vial at 230°C for 15 minutes, using a Biotage Initiator 2.0 (400W) microwave reactor. Purification of pre-PGS was performed by three sequential biphasic extraction in HCO32- saturated aqueous solution, HCl 1M and NaCl saturated aqueous solution. Confirmation of PGS formation was performed with FTIR analysis and monomer ratio was calculated by integration of H1-NMR peaks. Crude and purified pre-PGS acidic numbers (ANs) and Ester numbers (ENs) were determined, respectively, by titration of free acidic moieties with KOH 0.1 M and by hydrolysation of ester bonds in basic conditions at 100°C and titration of excess OH- with HCl 0.1 M. The degree of esterification was calculated as DE% = EN/(EN+AN)x100. Determination of pre-PGS molecular weight (Mw) was performed both with Gel Permeation Chromatography and Mass Spectroscopy. Incorporation of pendant cinnamates was obtained reacting cinnamoyl chloride and pre-PGS at concentration of 50% mol/mol, and adding DMAP and TEA to reactant mixture. UV-curing was achieved by irradiating PGS-cinnamate with UVB for 2 hours. **Results And Discussion.** After purification of crude pre-PGS, a yellow gel-like polymer was isolated and a purified product yield of 31.4±2.7% was obtained. Removal of unreacted sebacic acid was confirmed by a reduction of the acidic number from 26.6±0.4% to 16.6±0.4%. The formation of ester bonds was confirmed by shifts of the C=O stretching signal from 1710 cm-1 to 1740 cm-1 in the FTIR spectra, and the calculated monomer ratio was close to one. The DE% was 79.1±0.8% for purified pre-PGS. Different polymeric species were detected, from dimers to octamers, with a Mw ranging from 0.25 to 2.17 kDa and a PDI close to 1 for each species. **Conclusions.** Pre-PGS was successfully synthetized with a microwave-assisted method that extremely fastened the synthesis and avoided glycerol loss. The purification protocol was successful in removing unreacted sebacic acid. Currently, functionalization of pre-PGS with UV-crosslinkable cinnamate moieties is in progress, to actually obtain an elastomeric material that will be further characterized.

PoB.19.18

Blending PHBV and PCL with PLLA for enhanced mechanical properties

Raasti Naseem[1], Giorgia Montalbano[2], Georgia-Ioanna Kontogianni[3], Priscila Melo[1], Matthew German[1], Piergiorgio Gentile[1], Ana Marina Ferreira[1], Maria Chatzinikolaidou[4], Kenneth Dalgarno[1]

[1]Newcastle University, Newcastle, United Kingdom. [2]Politecnico di Torino, Torino, Italy. [3]University of Crete, Heraklion, Greece. [4]Foundation for Research and Technology Hellas, Heraklion, Greece

Abstract

Polymer blends offer an attractive way to combine the properties of materials in order to achieve combinations of properties which cannot be offered by a single polymer. Here we show that blending relatively small volumes of PCL and PHBV with PLLA can produce polymer blends which have improved modulus (up to 25%), strength (up to 50%) and elongation at break (up to 4000%), with *in vitro* testing showing no cytotoxicity over a two week culture period [1].

Blends were produced using twin screw melt extrusion, and FTIR and DSC were used to demonstrate that the materials were blended effectively, with DSC also used to evaluate the crystallinity of the blends. Tensile and rheological testing were used to assess modulus, strength, elongation at break, creep and viscosity.

Blending 15% PHBV with PLLA increases strength, modulus and elongation at break (Figure 1), suggesting that PHBV acts as a nucleation agent for PLLA, with more crystallinity but smaller crystals enhancing the mechanical properties across the board. Blending up to 30% PCL with PLLA reduces the crystallinity and the modulus and strength. Blending up to 10% of both PHBV and PCL results in reduced crystallinity, but improved modulus, strength and elongation at break compared to PLLA alone, indicating that the refined crystal structure arising from the PHBV still enhanced the properties overall.

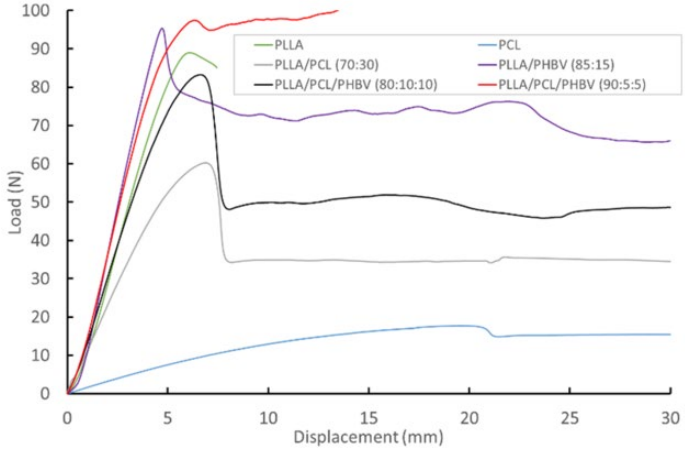


Figure 1. Tensile testing of blends of PLLA, PCL and PHBV [2].

The addition of 10% and above of PCL, or 15% of PHBV, to blends increased the creep rate and reduced the viscosity when compared to PLLA alone.

New polyester based biomaterials can be produced by blending small proportions of PCL and PHBV with PLLA through melt processing, and the produced blends have combinations of properties which cannot be provided by any of the materials individually.

References

1. Kontogianni G-I et al. Polymers. 2023, doi: 10.3390/polym15041052.
2. Naseem R et al. Molecules. 2022, doi: 10.3390/molecules27217633.

Acknowledgement: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 814410.

PoB.19.19

Surface modification of a 3D-printed polyurethane stent-graft for anti-thrombotic properties

Kim Vanden Broeck[1,2], Michel Daher[1,3], Mickaël Maton[1], Nicolas Tabary[2], Maria-José Garcia-Fernandez[1], Thomas Menard[1,4], Jonathan Sobocinski[1,4], Feng Chai[1], Bernard Martel[2], Nicolas Blanchemain[1]

[1]Univ. Lille, INSERM, CHU Lille, U1008- Advanced Drug Delivery Systems (ADDS), Lille, France. [2]Univ. Lille, CNRS, INRAE, Centrale Lille, UMR 8207 – Unité Matériaux et Transformations (UMET), Lille, France. [3]CHU de Reims, Reims, France. [4]Institut Coeur Poumon, Hospital Center University of Lille (CHU Lille), Lille, France

Abstract

Introduction. In the context of patient-specific treatment of complex abdominal aortic aneurysms, a custom-made stent-graft (SG) was fabricated by 3D printing with medical-grade thermoplastic polyurethane (TPU) [1]. A common complication after SG implantation is thrombosis in 10% of cases [2]. The strategy for preventing blood coagulation and thrombosis was to immobilize heparin on the TPU SG. A preliminary treatment with polydopamine (PDA), a biocompatible and bio-inspired adhesive polymer, and polyethyleneimine (PEI) was used as a versatile platform for the immobilization of biomolecules like heparin. PDA will allow the coating's adherence, thanks to catechol functions, and PEI will provide surface amine groups in order to graft the heparin [3]. **Experimental Methods.** TPU was immersed in a dopamine solution (2g/L, Tris Buffer 2g/L, pH=8.2, 400rpm, 5 hours) to form a polydopamine coating (TPU-PDA). Then, an immersion in polyethyleneimine (50g/L, Tris Buffer 2g/L, 400rpm, 24 hours) was performed to form covalent bonds (TPU-PDA-PEI). Finally, an immersion in heparin solution was done (1 g/L, PBS 1X, 400rpm, 24 hours) to create an electrostatic bond between heparin and TPU-PDA-PEI (TPU-Hep) (Fig. 1). The functionalization process was evaluated at each step by colorimetric dosage, Fourier-Transform InfraRed spectroscopy (FTIR, Perkin Elmer) and Scanning Electron Microscope (SEM, Hitachi). Surface properties and water contact angle (KRÜSS) were also controlled. The thermal properties were analysed by differential scanning calorimetry (DSC, TA Instruments). Cytocompatibility studies were assessed by direct contact with Human Pulmonary Microvascular Endothelial Cells (ISO10993-5). **Results And Discussion.** Colorimetric assay confirmed the increase of catechol groups (from 2.10±0.10 nmol/cm² for the TPU to 4.67±0.84 nmol/cm² for the TPU-PDA sample), of amine groups (with respectively 19.17±1.00 and 53.27±0.95 nmol/cm² for the TPU-PDA and TPU-PDA-PEI sample) and of carboxylic acid groups (with respectively 23.16±2.19 and 50.29±11.31 nmol/cm² for the TPU-PDA-PEI and TPU-Hep sample). SEM also confirmed the presence of a homogeneous coating on the TPU's surface with a small quantity of dopamine nanoparticles at each step of the process. Surface analysis revealed stretching vibration of alcohol at 3400-3500cm-1, proving the presence of functionalization. **Conclusion.** This study confirmed the

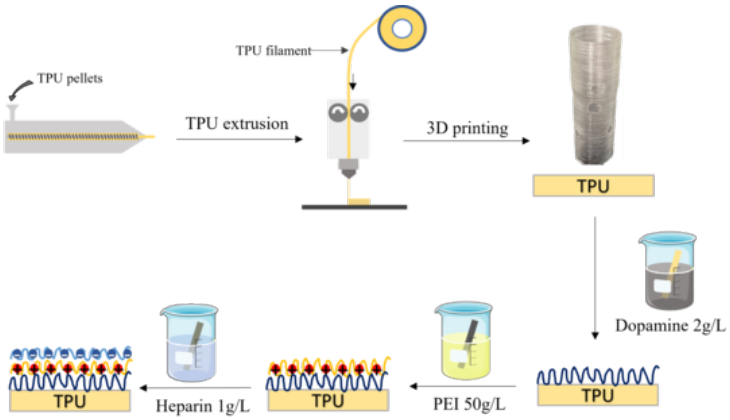


Fig. 1: Functionalization process of a 3D-printed stent-graft

functionalization of the 3D-printed TPU prototype with heparin. Therefore, this prototype is ready for anticoagulant tests and biological evaluation to confirm its anti-thrombotic properties and cytocompatibility.

REFERENCES

[1] M'Bengue et al., Pharmaceutics, 15: 456, 2023

[2] Guoquan W. and al., Exp. Therapeutic Med., 14: 1763-1768, 2017

[3] Bricout et al., Materials science and Engineering, 113: 110967, 2020

Acknowledgements
This research was funded by the Métropole Européenne de Lille (MEL).

PoB.19.20

Biodegradable polyesters blends in urological applications

Anna Sobczyk-Guzenda, Karolina Rosinska, Dorota Bociaga

Institute of Materials Science and Engineering, Lodz University of Technology, Lodz, Poland

Abstract

The aim of the work was to produce and characterize the physicochemical and mechanical properties of a mixture of two thermoplastic, biodegradable polymers with a strictly defined chemical structure at various stages of their degradation. The described material is intended to be used as a scaffold to rebuild fragments of the urethra. The blends of the tested polymers were produced by the solvent-thermal method. The base was a high molecular weight rigid polymer with a short degradation time. A second polymer was added to it, which acted as a plasticizer. In this work, polymers with three different molecular weights up to 80 kDa were tested. The obtained samples were tested for surface wettability changes, thermal properties tests by the Differential Scanning Calorimetry method and a static tensile test. On all obtained samples, degradation tests were carried out in physiological saline solution at the temperature of 37oC. In addition to the above-mentioned tests, the change in water absorption and weight loss of the obtained mixtures were analyzed. The obtained results prove that both the molecular weight of the polymer used as a plasticizer and its concentration have a significant impact on the mechanical properties of the mixtures obtained. Young's modulus varied within a wide range from 820 to 250 MPa. It took slightly lower values for the samples with the addition of polymer with higher values of molecular weights. The free surface energy increased with increasing content of plasticizing polymer from 27 to 42 mJ/m2. The greatest increase in its value was observed for low molecular weights. A significant decrease in the melting point values of the obtained samples was also observed. The degradation studies proved that during the month of storing the samples in the physiological saline, no weight losses of the samples or changes in their water absorption were observed. After this period, Young's modulus only slightly increased. Changes in these two parameters were noticed only after 2 months. Both the degradation time and the mechanical properties of the samples obtained were closely correlated with the concentration and molecular weight of the plasticizing polymer. The degradation time for such systems was in the range of 4 to 10 months.

b
“The authors would like to thank the National Centre for Research and Development (Grant no: TECHMATSTRATEG/407770/2/NCBR/2020) for providing financial support to this project”.

PoB.19.21

Modifying physical, mechanical and microstructural properties of lactide-based (co)polymers

Pieter van Delft, Caitlin Pieneman, Erik Kroeze

Corbion Biomaterials, Gorinchem, Netherlands

Abstract

Lactide-based polymers present a well-known class of materials that are applied in a large variety of resorbable products in numerous applications. They comprise a group of polymers with tunable properties, such as degradation time and mechanical strength. They have proven track records of highly positive clinical outcomes in a wide range of medical and pharmaceutical applications demonstrating excellent *in vivo* biocompatibility and biological safety. These polymers degrade by hydrolysis and are eliminated by the human body through natural pathways via the Krebs cycle.

These polymers fulfill a temporary function, allowing tissue to heal and resume its original function before the implant loses its mechanical integrity. The resorption time of devices based on these polymers can be tuned, from a polymer perspective, by tailoring the molecular weight, crystallinity and hydrophilicity of the polymer. The desired performance of the implant can be achieved through device design and manufacturing methods, as well as by tailoring the properties of the polymer itself. These properties make lactide-based polymers a very attractive class of resorbable polymers for use in medical device applications.

Corbion manufactures a wide variety of lactide-based polymers, including copolymers of caprolactone and glycolide. Our PURASORB® portfolio offers solutions for the most demanding medical and pharmaceutical applications. Our poster describes how Corbion uses chemistry to control our resorbable copolymers' microstructure, as well as related physical and mechanical properties.

PoB.20.02

New Insights into the Thixotropic Behaviour of Insoluble Collagen Type I Suspensions for Scaffold Production

Emma Z Gough, Serena M Best, Ruth E Cameron

University of Cambridge, Cambridge, United Kingdom

Abstract

Introduction. The production of freeze-dried, porous collagen scaffolds involves hydrating insoluble collagen in dilute acid then homogenising to prepare a suspension. This is normally stored at fridge temperature until it is pipetted or poured into a mold and freeze-dried to form a scaffold[1]. However, even when using collagen from a single source, these suspensions can have viscosities ranging over an order of magnitude, sometimes being unworkably viscous. This work aims to explore why such inconsistent viscosities are observed. We propose that the collagen suspensions have thixotropic behaviour which is likely to be affected by factors such as pH, impurity concentration and alkaline pre-treatment during processing[2][3]. **Methods.** Bovine dermal collagen (D-dermal) and limed bovine dermal collagen (CS-dermal) type I were supplied by Devro UK and Collagen Solutions UK, respectively. Samples were prepared by hydrating 0.5 g powder in 100 mL 0.05 M acetic acid and then homogenising in a commercial blender. Suspensions were stored at 4°C before characterisation by phase contrast microscopy and ambient temperature rheometry. Measurements were repeated after 6 months of storage and immediately after mechanical agitation. **Results and Discussion.** Both suspensions had characteristic, fibrous morphologies (Figure 1). Under standard preparation conditions, CS-dermal was more viscous than D-dermal (Figure 2). After six months of storage both had changed, notably D-dermal became 10.9 times more viscous. Furthermore, mechanical agitation for 10 or 20 seconds reduced the viscosity of CS-dermal suspension from 3.8 to 2.9 and 2.2 Pa.s, respectively. Correspondingly, flowability increased. These results are likely to be due to aggregate formation and destruction over time via reversible hydrogen-bonding interactions between collagen particles. **Conclusions.** We have demonstrated that inconsistent viscosities using a single collagen product result from time-dependent behaviour, rather than differences in homogenisation time or temperature. Collagen products behave differently, not attributable to particle morphology, hence likely affected by factors such as impurity concentration and processing conditions. These will be considered in this presentation. The results have significant implications for collagen suspension handling and processing in the production of biomedical scaffolds.

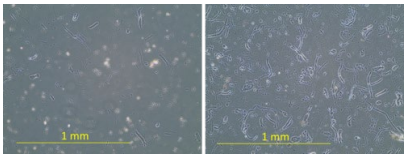


Figure 1 Phase contrast microscopy images of CS-dermal (left) and D-dermal (right) suspensions, highlighting similar collagen fibre morphologies in both types of sample

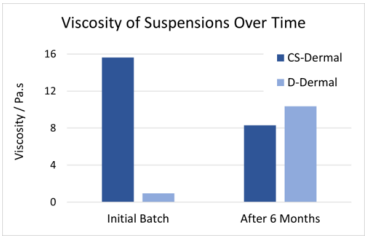


Figure 2 The viscosities of collagen suspensions (at 1 Hz shear rate), following initial production and after storage for 6 months at 4°C

References

[1] Davidenko et al., doi: 10.1016/j.actbio.2015.07.034
[2] Duan et al., doi: 10.1007/s13367-013-0014-9
[3] Hattori et al., https://doi.org/10.1093/oxfordjournals.jbchem.a022336

PoB.20.03

Mechanically compliant 3-Dimensional spongy scaffold for bone tissue engineering

Anupama Devi V. K.[1,2], Amit Kumar Jaiswal[1]

[1]Center for Biomaterials, Cellular & Molecular Theranostics (CBCMT), Vellore Institute of Technology (VIT), Vellore, India. [2]School of Bio Sciences & Technology (SBST), Vellore Institute of Technology (VIT), Vellore, India

Abstract

The possibility of using the scaffold in bone tissue engineering primarily depends on the biomaterials chosen, the techniques followed, and the qualities acquired through these features. For this application, the scaffold must be stable for at least six months prior to the formation of new bone and have mechanical strength comparable to that of bone. The use of freeze-thaw cycles for physical crosslinking is one way for enhancing the scaffold's mechanical qualities. Polyvinyl alcohol (PVA), one of the excellent thermoplastic biomaterials for enhanced elasticity, undergoes physical crosslinking through freeze-thaw cycles. While hydroxyapatite (HA) provides the natural microenvironment of the bone, the addition of polyethylene glycol (PEG) to this material has improved the material's mechanical properties through increased crystallization and structural stability through hydrogen bonding. The optimized concentration of these components was used for the fabrication of the composite scaffold through physical crosslinking by freeze-thaw cycles. The scaffolds thus fabricated were characterized for determining their physicochemical and mechanical properties such as in-vitro degradation assay and reswelling studies. It was discovered that the scaffold maintained its stable state such that, even after 160 days, only 50% of it deteriorated with the passive biodegradation process (in phosphate buffer saline (PBS)), and the degradation rate was higher during enzymatic degradation, still meeting the primary criteria required for the scaffolds. On the other hand, the mechanical property of this scaffold remained outstanding in both dry and wet states in addition to which it behaved as a reversible scaffold in the wet state that could elongate on stretching and regain back to its original state when left free. These characteristics made this scaffold peculiar and ensured that it satisfied the prime requirements for a scaffold intended for bone tissue engineering. Scanning electron microscopy (SEM) imaging enabled the visualization of the morphology of the scaffold’s cross-microporous structure. The ability of the scaffolds to support mesenchymal stem cells' cellular proliferation and their differentiation into osteogenic lineage has been investigated in accordance with the intended application. Thus, the combined outcomes of the physical, chemical and biological characterizations demonstrated the unique properties of the fabricated scaffold that includes remarkable mechanical strength and stable degradation rate thus making it ideal for its application in bone tissue engineering.

PoB.20.04

Tailorable mechanical and biochemical properties of mineralized collagen scaffolds for controlled local BMP-2 release

Leonie Schlicht[1], Anne Bernhardt[1], Johannes Jähnichen[1], Richard Richter[1], Gauri Tendulkar[2], Anja Lode[1], Michael Gelinsky[1]

[1]TU Dresden, Dresden, Germany. [2]RESORBA Medical GmbH, Nürnberg, Germany

Abstract

The high osteostimulatory nature of BMP-2 (bone morphogenetic protein 2) has been known for many years. It is clinically applied in cases of degenerative disc diseases or tibia fractures. A major problem is the inefficient delivery of BMP-2 at the fracture site. BMP-2 is only loosely bound to a collagen sponge on which it is administered and easily washed away, which results in high doses of BMP-2 to be applied. On the other hand, ectopic bone formation can be provoked by high concentrations of BMP-2. Biomimetically mineralized collagen type I (MColl), containing nanocrystals of hydroxyapatite, is known to bind and stabilize several growth factors. We could show in earlier *in vitro* and *in vivo* studies that a sustained release of the cell-attractive and pro-angiogenic factor VEGF (vascular endothelial growth factor) can be achieved with mineralized collagen as carrier. In the present study, scaffolds made of equine mineralized collagen were investigated concerning their mechanical properties and their BMP-2 release behavior. Scaffolds with varying mineral content were prepared to a) create mechanically stable scaffolds with adjustable compressive strength and to b) tailor the BMP-2 release by adjusting the available binding sites for the protein.

Starting from a mineral-to-collagen ratio similar to human bone (70:30 wt/wt %) arising from concurrent fibrillation and mineralization of equine collagen, the mineral content was reduced by blending MColl with mineral-free collagen (MFColl) to different ratios (100 % MColl, 75 % MColl, 50 % MColl, 25 % MColl) which could be verified by decreasing mineral contents measured by loss-on-ignition experiments. All tested blends showed a homogeneous distribution of the mineral phase within the scaffold. The interconnecting pore structure known from 100 % MColl was also found in the mixed variants (Figure 1). Compressive strength analyses of freeze-dried scaffolds showed adjustable mechanical properties of the scaffolds depending on their mineral content. *In vitro* ELISA studies revealed that the amount of BMP-2 released into medium could also be steered by the mineral content in the scaffold. The open pore structure of all scaffold variants supported the ingrowth and proliferation of human pre-osteoblasts analyzed by MTT staining.

In conclusion, a biomimetic material system was established that allows controlled and steady growth factor release and adjustable mechanical properties as well as cell ingrowth and proliferation.

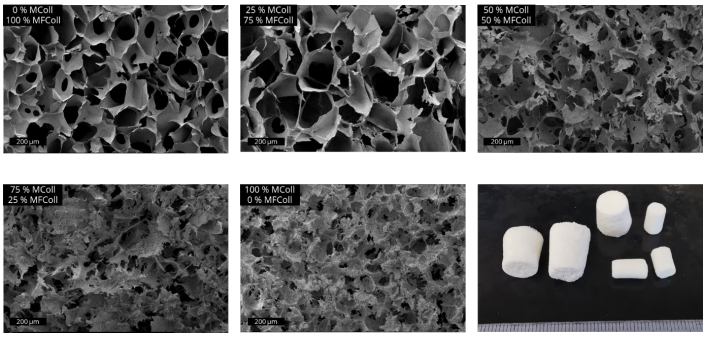


Figure 1: *SEM images of scaffolds made from blends of MColl and MFColl in different weight ratios. Macroscopic appearance of scaffolds made of mineralized collagen (right lower corner).*

PoB.20.08

Optimal Crosslinking of Layer-by-Layer Assembly Coated Porous Bone Scaffolds for Stable Mechanical Performance under Hydrated Conditions

MohammadAli Sahebalzamani[1,2], Tanya Levingstone[1,2], Helen O. McCarthy[3], Nicholas Dunne[1,2,3]

[1]Centre for Medical Engineering Research, School of Mechanical and Manufacturing Engineering, Dublin City University, Dublin, Ireland. [2]Biodesign Europe, Dublin City University, Dublin, Ireland. [3]School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom

Abstract

Introduction. Previously, we have optimised the material system and the process parameters for fabricating layer-by-layer (LbL) assembly coatings for bone tissue-engineered scaffolds[1,2]. These LbL-coated scaffolds demonstrated a 260-fold increase in mechanical performance (i.e., elastic modulus) compared to pristine polyurethane (PU) under ambient conditions. However, when tested under hydrated conditions, these LbL-coated scaffolds showed a significant reduction in mechanical performance due to water's plasticising effect. Therefore, this study aims to investigate the ability of various crosslinking processes to maintain the mechanical properties of the LbL-coated porous scaffolds under hydrated conditions, enabling them to function under *in vivo* application. **Experimental Methods**. The nanocomposite LbL-coated scaffolds were fabricated by depositing 10 quadlayers (QL) of 1 wt.% solutions of poly-l-lysine (PLL+), polyglutamic acid (PGA-), polydiallydimethylammonium (PDDA+), and 0.5 wt.% montmorillonite (MTM-) nanoclay onto a highly porous polymer-based scaffold under the optimised conditions as previously described[2] (Figure 1). LbL-coated scaffolds were chemically crosslinked using either 1-ethyl3-(3-dimethylaminopropyl)carbodiimide-N-hydroxysuccinimide(EDAC-NHS), glutaraldehyde (GA) or tannic acid (TA). Each LbL-coated scaffold was immersed in a 1 wt.% aqueous solution of EDAC-NHS, GA or TA for 12 h at 22ºC, with the pH maintained at 6. The physicochemical properties were determined using SEM and FTIR spectroscopy, and the mechanical performance under hydrated conditions (i.e., in phosphate-buffered saline (PBS) at 37ºC) was quantified as a function of elastic modulus and compressive strength. **Results and Discussion.** The coating thickness, mass and porosity of the 10 QL LbL-coated scaffold were not significantly (p-value ≥0.05) affected by the crosslinking method. However, TA crosslinking was the most effective at maintaining the elastic modulus of the 10 QL LbL-coated scaffold under hydrated conditions (Figure 2a), while also resulting in a more uniform QL coating (Figure 2b). Furthermore, FTIR spectroscopy showed a higher density of hydroxyl groups (-OH) and free amine groups of –NH2 in LbL-coating following TA crosslinking which was indicative of a greater degree of crosslinking. **Conclusion.** This study has demonstrated that crosslinking using TA can maintain the mechanical properties of the LbL-coated porous scaffolds in hydrated conditions. Furthermore, TA is considered a "green" crosslinking method, which offers improved biological properties, such as biocompatibility and antibacterial characteristics, compared to other crosslinking methods. Thus, this approach delivers LbL-coated bone tissue-engineered scaffolds capable of functioning under *in vivo* application.

References

[1] Sahebalzamani M.A (et al.), Biomater. Sci, 10 (11): 2734-58, 2022.
[2] Sahebalzamani M.A (et al.), Proceedings of the 32nd ESB Conference, Bordeaux, France, 2022.

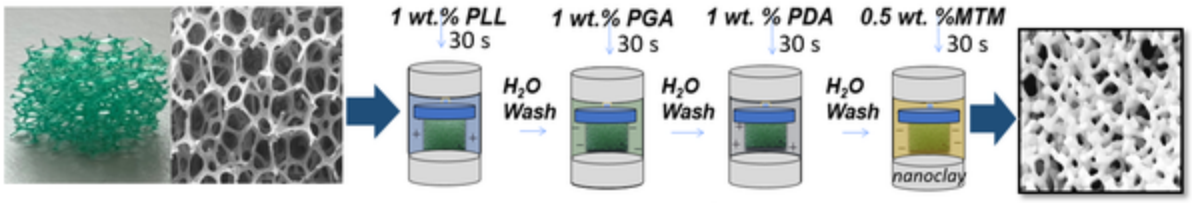


Fig 1a Fig 1b Fig 1c

Figure 1: a) Pristine open-cell porous polyurethane (PU) template (97% porosity), b) Schematic of LbL assembly nanocomposite-coating process using compression deformation (strain = 5%) and controlled flow perfusion (12 mL/min), c)Effect of multilayer LbL-assembled coating on porous structure.

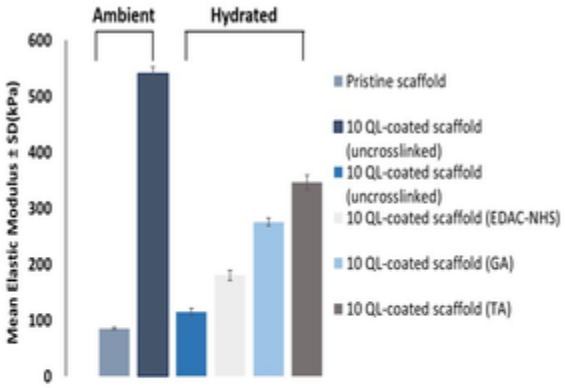


Fig 2a

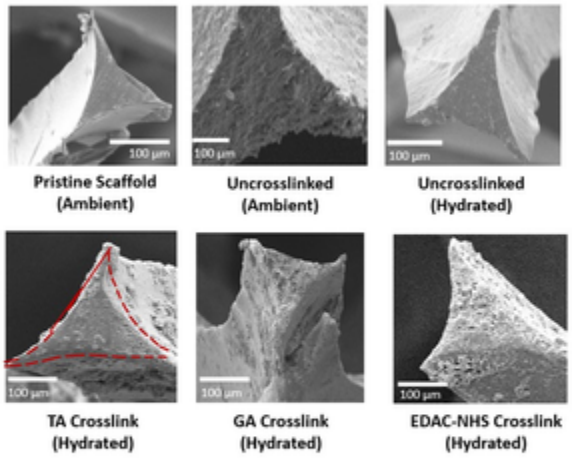


Fig 2b

Figure 2: a) Mean Elastic modulus (±SD) of 10 QL-coated porous scaffolds (n=5) as a function of pre- and post-crosslinking when tested in hydrated conditions (PBS at 37°C) for 24 h.

Figure 2: b) SEM images showing the physical change in LbL coating as a function of crosslinking method used. The dashed line indicates a more uniform LbL coating on the porous scaffold when crosslinked using TA.

PoB.20.09

Regulated porous structure by SLM technique effects on release of Sr ions incorporated into Ti surface by chemical and heat treatment

Seiji Yamaguhi, Tomiharu Matsushita

Chubu University, Kasugai, Japan

Abstract

Introduction. Selective laser melting (SLM) is a promising technology that can accurately produce micro structures with the optimal pore size and porosity for osteoconduction. In our previous studies, rolled Ti plates modified with Sr-containing calcium-deficient calcium titanate by chemical and heat treatment released about 1 ppm of Sr ions to accelerate new bone formation and exhibited high critical detaching strength. The greater effect is expected if greater amount of Sr ions is controllably released from the implant surface.In the present study, porous Ti with regulated porous structure was produced by SLM, and the effect of porous structure on release of Sr ions was investigated. **Materials and method:** Cylindrical porous Ti bodies with compact cap on both ends were made by SLM with the pore size of 300, 600, 900 µm and diameter of 5, 10, 15, and 20 mm as shown in Figure 1. All of them have the same porosity of 65 % and designed surface area of about 1,200 mm^2, except 20 mm diameter samples that have double. They were immersed in 5M NaOH, 50 mM $CaCl_2$ and 50 mM $SrCl_2$ mixed solution, and heated at 600 ºC for 1 h, and then immersed in 1M $SrCl_2$ solution. The structure, surface area, ion release and apatite formation of processed samples were anlyzed by SEM,EDX, BET, ICP and SBF soaking. **Results and discussion:** All samples have a specific micron-scale morphology due to the material-derived particles that should increase surface area. Further, nano-structured surface layers having 2.2 % Ca and 1.5 % Sr were uniformly formed due to the chemical treatment. BET revealed that the actual surface area after the chemical treatment was 162 to 355 times higher than the designed one. When the treated samples were soaked in SBF, all of them formed apatite fully on their surfaces.When examining the release of Sr ions, it was found that the porous samples released 5 to 12 ppm of Sr ions, equivalent to 5 to 12 times higher than that of the rolled Ti plate. Larger the sample diameter, slower the release of Sr ions as shown in Figure 2. **Conclusion:** Controllable release of Sr ions was observed on SLM-made porous Ti with regulated porous structure, where the release rate strongly depended on both the porous body diameter and pore size. Early and stable osteoconduction is expected with this type of material due to the controlled release of Sr ions and apatite formation.

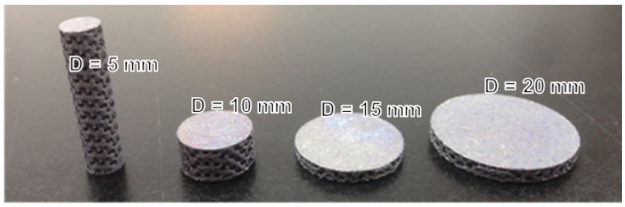


Figure 1 SLM-made porous Ti subjected to chemical and heat treatment. D: diameter of porous Ti.

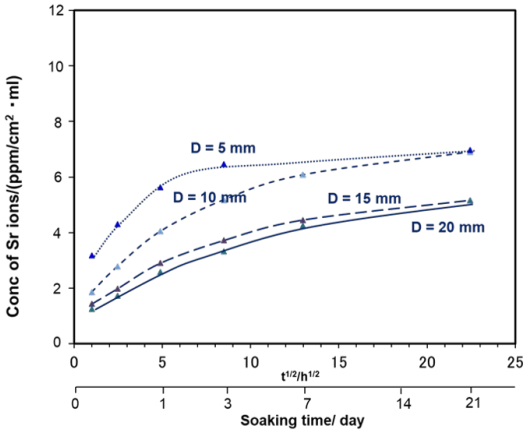


Figure 2 Release of Sr ions from SLM-made porous Ti with 300 nm in pore size that was subjected to chemical and heat treatment, depending on soaking time measured by ICP. Concentration of Sr is normalized by designed surface are and PBS volume. D: diameter of porous Ti.

PoB.20.10

Additive manufactured porous titanium with graded structure balancing bone ingrowth and antibacterial activity: Mechanical property and ion-release capacity

Seiji Yamguchi[1], Mahmoud Elroubi[1], Phuc Thi Minh Le[1], Seine A Shintani[1], Hiroaki Takadama[1], Tomiharu Matsushita[1], Hisashi Kitagaki[2], Shintaro Honda[3], Yaichiro Okuzu[3], Shunsuke Fujibayashi[3]

[1]Chubu University, Kasugai, Japan. [2]Osaka Yakin Kogyo, Osaka, Japan. [3]Kyoto University, Kyoto, Japan

Abstract

Introduction. Porous titanium (Ti) can induce bone ingrowth and used as orthopedic and dental devices. We reported that porous Ti with 300-900 µm in pore sizes showed moderate bone ingrowth in which larger pores effectively promoted bone ingrowth. We also demonstrated that Ti plate formed with iodine-containing calcium titanate by NaOH-$CaCl_2$-heat-ICl_3 (iodine treatment) exhibited both antibacterial activity and bone formation. An iodine-treated porous Ti with smaller pores is expected to slowly release iodine ions and useful for preventing surgical site infection that leads to adverse patient outcomes. In this study, a special porous Ti with graded structure in which the pores ranges from 900, 600 and 300 µm from outer to inner was fabricated by selective laser melting (SLM), and its mechanical properties were evaluated. Apatite formation and ion release of the porous Ti subjected to the iodine treatment were examined. **Materials and method.** Porous A, B and C having 54% porosity and pore sizes of 900, 600 and 300 µm, respectively and porous D with the graded 900-600-300 µm pores were designed. Rectangular (12×22×9mm^3) and cylinder (ϕ6×9mm^3) of which side surfaces were dense walls 0.2 mm in thickness were made by SLM (Figure 1). Some of them were subjected to iodine treatment. Mechanical properties of the porous samples were evaluated by FEM simulation, static compression and dynamic compression fatigue test (10 kN × 10^6 cycles, according to ASTM 2077). The structure, ion release and apatite formation of samples were analyzed by SEM, ICP and SBF soaking. **Results and discussion**. The FEM revealed uniform stress distribution for all samples. All the rectangular samples showed elastic deformation under 10 kN and their elastic modulus were about 1.6 GPa. The porous D ran out at the fatigue test without plastic deformation. When the cylinder samples were subjected to iodine treatment and soaked in SBF for 3 days, apatite particles almost fully covered porous samples except C in which it

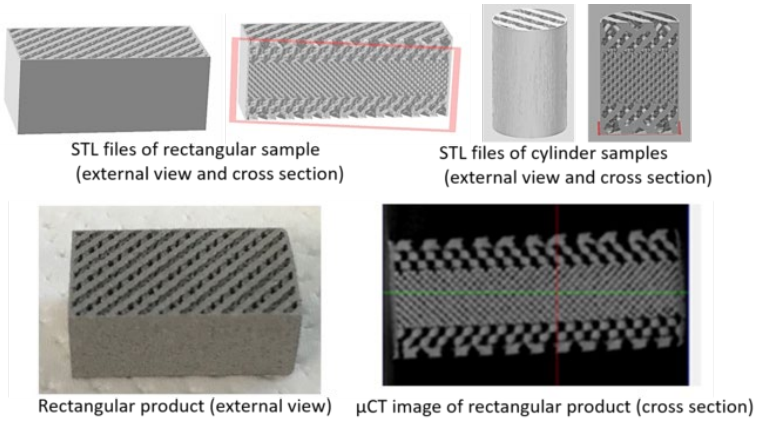


Figure 1: Designed (upper) and product (lower) of porous D (12 × 22 × 9mm^3 and ϕ6 × 9mm^3) with graded structure.900µm pores suitable for bone ingrowth was arranged outer side, and this gradually changes to smaller ones (300µm) toward inside that is suitable for sustained ion release.

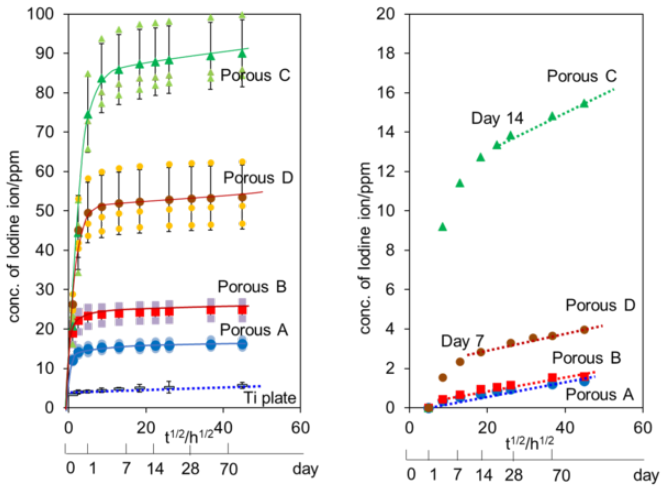


Figure 2. Iodine ions released from porous A-D subjected to iodine treatment in PBS (by ICP measurement). Left: Total concentrations. Right: Accumulated concentrations after 1 day.

was about 50 % coverage. A slowest lease of iodine ions was observed for porous C followed by porous D>B≒A; total ion concentrations were 90, 53, 25 and 16 ppm, respectively (Figure 2). These results indicate that porous D showed balanced properties in apatite formation and ion-release capacity. **Conclusion.** The porous D should be useful for orthopedic and dental devices since it has sufficiently high mechanical strength and exhibited apatite formation and sustained release of iodine ions after iodine treatment.

PoB.20.11

Ice-templated collagen scaffolds for culturing alveolar organoids

Vivien Alves Passing[1], Daniel V. Bax[1], David J. Barrett[1], Kyungtae Lim[2,3], Emma L. Rawlins[2,3], Ruth E. Cameron[1], Serena M. Best[1]

[1]Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, United Kingdom. [2]Wellcome Trust, CRUK Gurdon Institute, University of Cambridge, Cambridge, United Kingdom. [3]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom

Abstract

Introduction. There has been an increasing interest in using 3D organoid structures to investigate lung cell development. The lung consists of many cell types, including alveolar epithelial type 1 and type 2 (AT1/AT2) cells. Organoids are a useful research tool, but current culturing methods lack the spatial, biomechanical and biochemical cues of the native extracellular matrix (ECM) required to promote and maintain cellular phenotype[1]. Ice-templated collagen scaffolds are a suitable platform to understand how the ECM directs the phenotype and self-organisation of alveolar-derived cells, as their architecture and mechanical properties can be tightly controlled[2]. Here, we simplify the lung's complexity by using AT2 organoids to understand the effect of crosslinking scaffolds on these cells. **Experimental Methods.** 1 wt.% dermal collagen in 0.05 M acetic acid was freeze-dried at -25ºC to produce scaffolds, which were analysed using X-ray microtomography and SEM. The scaffolds were 0, 30 or 100% crosslinked with EDC/NHS, where 100% corresponds to a molar ratio of 5:2:1 of EDC:NHS:collagen. AT2 organoids[3] (mainly AT2-like cells mixed with proliferating cells and very few immature AT1-like cells) were dissociated into single cells and 130,000 cells were seeded on top of each scaffold. Samples were imaged after 10 days in culture. **Results and Discussion.** Freeze-drying produced a scaffold architecture similar to the lung ECM[4] (Fig.1a) with an average pore size of 142 µm (Fig.1b). The scaffolds had a high interconnectivity (Fig.1c) and a percolation diameter of 152 µm.0% crosslinking led to unstable scaffolds, causing them to visibly shrink in culture. Fig.2 shows that the AT2-like cells populated the pores of the scaffolds and were guided by the pore architecture. Cells agglomerated into clusters under all crosslinking conditions; at 0% more, smaller clusters appeared to form. The cells maintained their AT2 lineage as shown by the expression of the Surfactant Protein C-GFP (*SFTPC-GFP*) reporter gene. **Conclusion.** Collagen scaffolds with an architecture similar to the native lung ECM were produced. AT2-like cells colonised the scaffolds and maintained their AT2 lineage under all crosslinking conditions, but there were more, smaller clusters at 0% crosslinking. This work offers novel insights into the crosslinking conditions needed to culture AT2-like cells on collagen scaffolds.

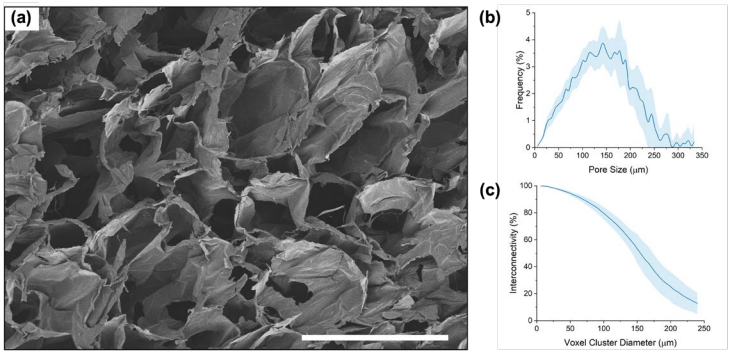


Figure 1: (a) SEM revealed that the collagen scaffold had a highly interconnected, porous structure, similar to the lung. Scale bar = 500 µm. (b) The pore size distribution of the scaffold was skewed towards larger pores and (c) it maintained a high interconnectivity even at large voxel clusters, making the scaffolds suitable for cell infiltration.

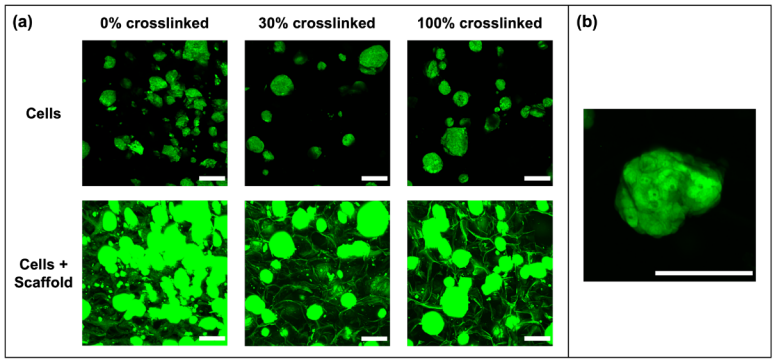


Figure 2: (a) AT2-like cells expressed the SFTPC-GFP reporter gene, a type 2 alveolar cell marker, which makes them fluoresce in green. As collagen and GFP fluoresce at similar wavelengths, the cells had to be oversaturated to visualise the scaffold. The images were thresholded to either show just the cells or both the cells and scaffold. (b) Representative image of an AT2 cluster on a 30% crosslinked scaffold. Single cells including nuclei can be seen. Scale bars = 100 µm.

Acknowledgements

VAP acknowledges EPSRC, Cambridge Trust and Royce Institute (EP/P024947/1;EP/R00661X/1).

References

1. Y.-W.Chen *et al.*,Nat.Cell Biol.**19** (2017)
2. K.M.Pawelec *et al.*,J.R.Soc.Interface **11** (2014)
3. K.Lim *et al.*,Cell Stem Cell **30** (2023)
4. Y.Luo *et al.*,Sci.Rep.**8** (2018)

PoB.20.12

Ways to enhance mechanical and biological properties of biomimetic 3D-printed calcium phosphate bone grafts: PLGA-based strategies.

Linh Johansson[1,2,3,4,5], Yago Raymond[5], Cédric Labay[1,2,3,4], Miguel Mateu Sanz[1,2,3,4], Maria-Pau Ginebra[1,2,3,4,6]

[1]Biomaterials, Biomechanics and Tissue Engineering Group (BBT), Department of Materials Science and Engineering (CEM), Universitat Politècnica de Catalunya · BarcelonaTech (UPC), Barcelona, Spain. [2]Barcelona Research Centre in Multiscale Science and Engineering, UPC, EEBE, Barcelona, Spain. [3]Biomedical Engineering Research Center (CREB), UPC, Barcelona, Spain. [4]Institut de Recerca Sant Joan de Déu (IRSJD), Esplugues del Llobregat, Spain. [5]Mimetis Biomaterials S.L., Barcelona, Spain. [6]Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology, Barcelona, Spain

Abstract

More than 2 million bone graft procedures are performed annually worldwide. A market in which calcium phosphates (CaPs)-based synthetic biomaterials are progressively substituting the allografts and xenografts options to face the need for bone grafting solutions - typically associated with bone damage caused by trauma, osteoporotic fractures, infections, or tumor removal - while preventing ethical consideration, immune diseases, and volume issues. Modern digitalized processes developed in tissue engineering during the last decade have made possible the emergence of patient-specific solutions by using 3D printing to create personalized synthetic bone grafts [1]. This technology not only opens the doors for the customization of the external shape of the bone grafts for reconstruction of defects with complex geometries but also allows to control the internal pore-architecture. However, despite their excellent biological properties, the brittleness of the current porous CaP bioceramics is a serious stumbling block that limits their clinical applications.

The objective of this study was to assess the relevance of adding PLGA in the manufacturing process to enhance the mechanical properties and biological behavior of 3D-printed low-temperature set CaP scaffolds. Two options were evaluated: i) adding PLGA directly in the CaP ink (PLGA-ink) and ii) incorporating PLGA as a coating of 3D-printed CaP scaffolds (PLGA-coating). The influence of the added PLGA on the physico-chemical properties, mechanical performance and *in vitro* biological properties was assessed using pristine biomimetic CaP scaffolds as control (CTRL).

Among the improved mechanical parameters, a remarkable enhancement by 3.5-fold of the compressive strength was observed for the PLGA-ink composite, reaching the upper limit of the human cancellous bone range (2-14 MPa), while the PLGA-coating revealed a 1.4-fold increase compared to CTRL (Fig. 1a). Moreover, the biological properties assessed on hMSC and MG63 cell lines revealed excellent biocompatibility for both PLGA-composites, with the PLGA-ink presenting the best performance in terms of osteoinduction, with an upregulation of osteogenic markers ALP and RUNX2.

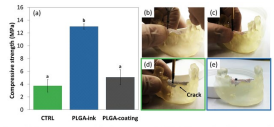


Fig. 1: (a) Compressive strength for biomimetic CaP (CTRL), PLGA-ink and PLGA-coated scaffolds; (b-e) Screwability of CTRL and PLGA-ink scaffolds: (b) perforation with a ø 1.2 mm drill, (c) fixation with a ø 1.5 mm screw, (d) fractured CTRL scaffold during fixation, (e) intact fixed PLGA-ink scaffold. In (a) different letters indicate statistically significant differences between conditions.

Finally, the study aimed to evaluate the applicability of these scaffolds in real indications in the maxillofacial sector, that require appropriate handling properties, among which the fixation of the implant in the host bone is a critical aspect. A 3D-printed prototype of the PLGA-ink composite was successfully perforated and fixed with a dental screw to a biomodel in a challenging knife-edge ridge indication in the jaw as a proof of concept for their screwability performance (Fig. 1b-e).

[1] Y. Raymond et al., MRS Bull., 47 (2022) 1–11.

PoB.20.13

Ice-templated Hierarchically Porous 3D Silica Nanoparticle Assemblies as Implants for Controlling Anticancer Drug Delivery

Sandeep Kumar Palvai, George Newham, Stephen D Evans, Zhan Yuin Ong

University of Leeds, Leeds, United Kingdom

Abstract

Tumor regrowth in surgical margins after cancer surgery remains a major challenge for the successful treatment of solid tumours[1,2]. Implantable drug delivery systems such as Gliadel® that are inserted at tumour resection cavities to eradicate residual tumour cells play a major role in combating tumor recurrence after surgery. However, poor tissue adherence, short drug half-lives and lack of control over the drug release kinetics from drug-eluting depots has been a major challenge in achieving prolonged therapeutic effects. Porous materials have become increasingly popular for controlled drug delivery systems owing to their ability to control the release of various therapeutic drugs, vaccines, and genes. However, most of the current approaches used to design the porous materials for drug delivery applications involve usage of organic solvents and post-synthetic modifications, which may not be compatible with wide range of drug molecules.

Herein, we report the first use of unidirectional freezing as a 'green' method to develop hierarchically porous three-dimensional assemblies of silica nanoparticles with tunable porosity for controlling drug release. Firstly, we synthesized monodisperse porous silica nanoparticles (PSiNPs) of various sizes ranging from 25 nm to 150 nm using polyelectrolyte complexes as a template[3]. Directional freezing of as-synthesized PSiNPs at -22 °C resulted in fibrous 3D structures, characterized by FE-SEM. BET analysis of the fibres revealed a tunable and stepwise increase in interparticle pore sizes from 17 to 74 nm as the size of the PSiNPs used for fibre assembly increased from 25 to 150 nm.

Further, anticancer drug (doxorubicin, DOX) loading was achieved by co-mixing DOX with PSiNPs of various sizes before directional freezing. A faster drug release from fibres with larger interparticle pores was observed in PBS (pH = 7.4) indicating the pore size-controlled, tunable drug-releasing abilities of these fibres. Moreover, a prolonged release of up to three weeks from the fibres containing DOX in both the interparticle and intraparticle pores of the fibres was demonstrated.

Finally, the drug-loaded fibres were formulated into a pluronic F127 hydrogel to facilitate the administration of therapeutics into the surgical cavity. This study demonstrates a novel and 'green' approach of designing implantable drug delivery systems with tunable drug release properties for the post-surgical treatment of cancer.

References:

1. Adv. Funct. Mater.2022, 32, 2205038.
2. Int. J. of Nanomedicine, 2022, 17, 2203–2224.
3. Newham G et al., J. Colloid Interface Sci., 584:669–683, 2021

PoB.20.15

Collagen from marine sponge *Chondrosia reniformis* on the production of porous scaffolds for tissue engineering

Miguel S Rocha, Catarina F Marques, Ana C Carvalho, Eva Martins, Rui R Reis, Tiago H Silva

University of Minho, Guimarães, Portugal

Abstract

Collagen is extremely similar in vertebrates and invertebrates, being the most abundant structural protein in both. Currently mammalian by-products are still the main collagen sources, but risks of zoonosis and religious/social constraints have encouraged the quest for alternative sources, namely for biomedical applications. *Chondrosia reniformis*, a marine sponge considered a potentially sustainable collagen source, renders a more glycosylated protein than other collagens in Metazoa (1, 2). Cell attachment and proliferation are favoured by the high abundance of glycosaminoglycans, motivating the study of this alternative collagen on tissue engineering scaffolding, with the hypothesis of improved regenerative potential (3).
Earlier studies have hinted at the potential use of *C. reniformis* collagen for cosmetical and biomedical applications (4, 5), therefore our work's aim was to use it on the production of porous structures by freeze-drying and evaluate their performance as templates for cell culture in the perspective of engineering different tissues. A range of different crosslinkers (EDC, genipin and glutaraldehyde) and concentrations were employed and their effect on the physicochemical and mechanical features of the developed scaffolds were evaluated by SEM, microCT, rheometer and regarding their degradation and swelling rates. It was determined that the natural crosslinker genipin was the most promising, due to its low cytotoxicity and the scaffolds' suitable pore size and interconnectivity for cell culture. The biological response was studied by *in vitro* cell culture experiments, assessing cytocompatibility to ATDC5 (chondrogenic cell line), BJ (fibroblast), EA.hy926 (umbilical vein cell line), and ASC (adipose stem cells).
It was determined that the genipin-crosslinked scaffolds are suitable for tissue engineering approaches. Thus, this study further demonstrates that *C. reniformis* collagen is beneficial for biomedical applications, being a promising and versatile alternative towards human regenerative therapies.

Acknowledgements: Funding from Portuguese Foundation for Science and Technology under Do*Mar PhD scholarship of MSR (PD/BD/143091/2018), from the European Regional Development Fund (ERDF) through North Portugal Regional Operational Program (NORTE2020), part of PORTUGAL2020 Partnership Agreement, under the structured program of R&D&I ATLANTIDA - Platform for monitoring the North Atlantic Ocean and tools for the sustainable exploitation of marine resources (NORTE-01-0145-FEDER-000040).

References

1. M. Gökalp et al., Aquaculture. 548 (2022).
2. R. Garrone et al., J. Ultrasructure Res. 52, 261–275 (1975).
3. H. Sodhi, A. Panitch, Biomolecules. 11, 29 (2020).
4. D. Swatschek et al., Eur. J. Pharm. Biopharm. 53, 107–113 (2002).
5. M. Pozzolini et al., Mar. Drugs. 16, 111–130 (2018).

PoB.20.16

Design and evaluation of a crosslinked chitosan-based scaffold containing hyaluronic acid for the articular cartilage tissue engineering

Salim Hamidi[1], Henry Chijcheapaza-Flores[1], Frédéric Cazaux[2], Bernard Martel[2], Nicolas Blanchemain[1]

[1]Univ. Lille, INSERM, U1008 – Advanced Drug Delivery Systems and Biomaterials (ADDS), Lille, France. [2]Univ. Lille, CNRS, UMR 8207 – Unité Matériaux et Transformations (UMET), Lille, France

Abstract

Articular cartilage is a connective tissue covering the articulating surfaces. (1) It plays an important role in the movement of the joint. However, once damaged, cartilage is unable to regenerate spontaneously. Consequently, this leads to articular degenerative diseases like knee osteoarthritis which affect 560 million people worldwide. (2,3)

Polymeric scaffolds are frequently used in different therapeutic approaches to generate tissue-engineered cartilage because of their ability to mimic the extracellular matrix of cartilage. Among those polymers, chitosan and hyaluronic acid defined as natural polysaccharides share a similar structure to glycosaminoglycan of the articular cartilage. (1,2)

This study aims to produce a cytocompatible scaffold-based chitosan chemically crosslinked to modify its stiffness, this was proved to impact chondrocytes behavior by increasing the rigidity of the scaffold and serves as an additional mechanical stimulus to promote chondrocytes growth and proliferation. (1)

According to our patented method (4) we prepared hydrogels composed of 5% w/v chitosan (CHT) and 1% w/v of hyaluronic acid (HA), water, and 1 % v/v of lactic acid, oxidized maltodextrin (MDo: 0.4%) was used as a crosslinking agent of CHT. In the first study, the crosslinking reaction between CHT and MDo was tested without HA. The porous scaffolds were obtained by freeze-drying hydrogels (P= 0.06 mbar, T°= -53°C, 48h).

The water uptake capacity of the scaffolds was determined by swelling test in PBS (pH= 7.4, 37°C) for 24h and completed by Dynamic Vapor Sorption technic (DVS). The degradation study was performed in PBS without or with lysozyme (0.5 mg/ ml).

The swelling ratio of the porous scaffold was significantly reduced in the groups CHT + 0.4% MDo (423 % ± 47 %) compared to the control groups (1647 % ± 250 %), and the DVS showed also a decrease of 8.5% of moisture content for the crosslinked groups at 95% of relative humidity, this behavior is explained by the formation of a covalent network that limits the mobility of the CHT polymer chains and scaffold swelling, in addition, these results were confirmed by the degradation study where it was observed a decreasing tendency in both conditions (PBS, PBS + Lys) with average weight loss respectively (16%, 22%) for CHT+0.4% MDo and (35%- 96%) for uncrosslinked CHT.

In conclusion, the crosslinking reaction has been well demonstrated between CHT and MDo and improves significantly the properties of CHT, as a prospect, it's interesting to study the crosslinking/biological effect compromise including HA.

References:

1. Subramanian A, Lin HY. Crosslinked chitosan: Its physical properties and the effects of matrix stiffness on chondrocyte cell morphology and proliferation. J Biomed Mater Res A. 1 déc 2005;75A (3):742-53.
2. Correia CR, Moreira-Teixeira LS, Moroni L, Reis RL, van Blitterswijk CA, Karperien M, et al. Chitosan Scaffolds Containing Hyaluronic Acid for Cartilage Tissue Engineering. Tissue Eng Part C Methods. juill 2011;17(7):717-30.
3. Pereira TV, Jüni P, Saadat P, Xing D, Yao L, Bobos P, et al. Viscosupplementation for knee osteoarthritis: systematic review and meta-analysis. BMJ. 6 juill 2022 ;378 : e069722.
4. Blanchemain N, Martel B, FLORES C, CAZAUX F, CHAI F, TABARY N, et al. Procédé de fabrication d'hydrogel à base de chitosan et de polyélectrolytes chargés négativement et matériau poreux alvéolaire issu dudit hydrogel [Internet]. WO2017001808A1, 2017 [cité 13 mars 2023].

PoB.20.18

Combination of hydrogel with PolyHIPE materials for 3D cell culture

Sweeta Akbari, Mart Kroon, Minna Kellomäki, Jari Hyttinen

Tampere University, Tampere, Finland

Abstract

Polymerised high internal phase emulsions (PolyHIPEs) are macroporous emulsion-templated polymers produced by the polymerization of the internal phase. PolyHIPE scaffolds are promising solutions for different tissue engineering and regenerative medicine applications. A biocompatible 3D scaffold with high porosity and interconnectivity has the potential to be used for tissue regeneration as substrate for better cell attachment, nutrition flow, infiltration and cell migration within the host tissue and *in vitro* cell culture models. In the past two decades, tissue engineering field has witnessed a transition from two-dimensional (2D) monolayer cell culture to three-dimensional (3D) approaches. This method allows cells to grow and interact with surrounding extracellular matrix better mimicking the natural 3D environment. In this work, four types of polyHIPE-Gellan Gum (P-GG 0% to P-GG 1%) materials based on the concentration of Gellan Gum (GG) were fabricated. Results indicated that higher concentration of GG increases the droplet sizes in HIPEs as shown in Fig.1. Scanning electron microscopy (SEM) and Microcomputed tomography (MicroCT) allowed us to find the effects of hydrogel concentration on the microstructure and porosity of polyHIPE materials. Highly interconnected networks with pore size ranging from 200 µm to 2 mm was obtained and the results support the application of these 3D scaffolds for cell culture. In addition, different methods of testing and characterizations such as droplet size distribution, zeta potential, FTIR, TGA, rheological studies, mechanical testing, degradation studies and a human fibroblast cell culture were included in the study.

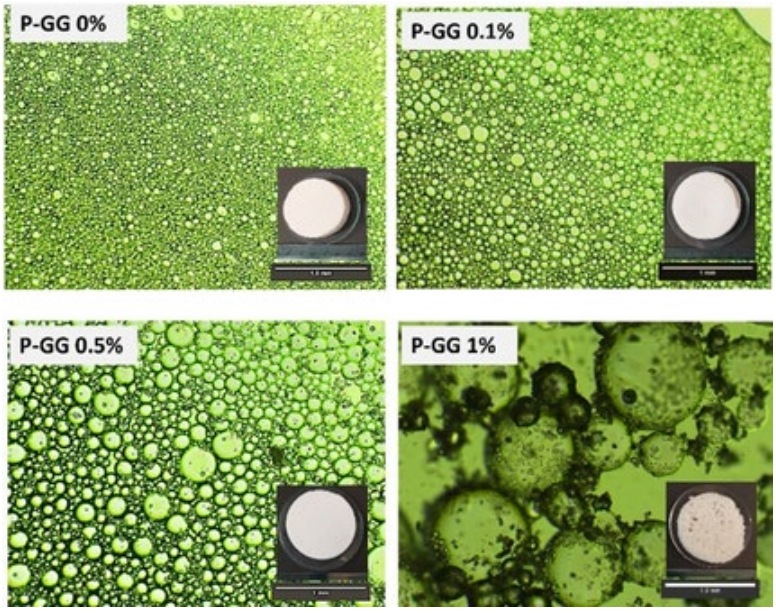


Fig.1 Optical microscopy images of HIPEs at different concentrations of gellan gum, scale bar is 1mm.

Keywords: PolyHIPE, hydrogel, 3D scaffold, emulsion, cell culture

PoB.20.19

Poly(L-lactide-*co*-glycolide)/poly(isosorbide sebacate) electrospun fibers as a biodegradable platform for vascular regeneration

Monika Śmiga-Matuszowicz[1], Jakub Włodarczyk[2], Małgorzata Skorupa[1,3], Dominika Czerwińska-Główka[1], Kaja Fołta[1], Małgorzata Pastusiak[2], Małgorzata Adamiec-Organiściok[4,5], Magdalena Skonieczna[4,5], Roman Turczyn[1,6], Michał Sobota[2], Katarzyna Krukiewicz[1,6]

[1]Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology, Gliwice, Poland. [2]Centre of Polymer and Carbon Materials, Polish Academy of Science, Zabrze, Poland. [3]Joint Doctoral School, Silesian University of Technology, Gliwice, Poland. [4]Biotechnology Centre, Silesian University of Technology, Gliwice, Poland. [5]Department of Systems Biology and Engineering, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, Gliwice, Poland. [6]Centre for Organic and Nanohybrid Electronics, Silesian University of Technology, Gliwice, Poland

Abstract

Vascular regeneration is a complex process limited by minimal regeneration of blood vessels. Current strategies for improving the outgrowth of endothelial cells include the use of synthetic scaffolds. Materials like polytetrafluoroethylene or poly(ethylene terephthalate) are inert and stable but exhibit poor elasticity and are non-degradable. Other strategies include the use of biodegradable polymers, such as poly(glycolic acid) (PGA) and poly(lactic acid) (PLA), and especially their more successful copolymer, poly(L-lactide-*co*-glycolide) (PLGA), which improves their mechanical properties, stability, and limits the release of toxic residues.

In this study, we propose a scaffold material based on electrospun fibers constructed of poly(L-lactide-*co*-glycolide) and poly(isosorbide sebacate) (PLGA/PISEB). Electrospinning of the PLGA/PISEB blend resulted in a formation of two populations of fibers with average diameters of 1.35 µm and 4.66 µm. Viability assay of human umbilical vein endothelial cells (HUVECs) cultured on the scaffold proved its biocompatibility. Additional analysis of gene expression of HUVECs showed improved pro-angiogenic profile and anti-inflammatory effect induced by the PLGA/PISEB. PLGA/PISEB fibers were observed to swell and disintegrate over the course of a 12-week hydrolytic degradation and form highly developed structures. It is expected that the fibrous structure will allow the cells to penetrate and integrate within the scaffold after implantation, whereas with the progression of degradation the structure will stabilize the newly formed endothelium.

Reference:
Śmiga-Matuszowicz et al. Biodegradable Scaffolds for Vascular Regeneration Based on Electrospun Poly(L-Lactide-*co*-Glycolide)/Poly(Isosorbide Sebacate) Fibers, Int. J. Mol. Sci. 2023, 24(2), 1190.

Acknowledgements:
This research was funded by the Silesian University of Technology, grant number 04/040/SDU/10-22-03 and 04/040/SDU/10-21-01, and by the National Science Centre, Poland (OPUS 2019/35/B/ST5/00995).

PoB.20.20

Porous chitosan/bioglass composites, dedicated to filling bone defects, enriched with biologically active peptide fibrils - production and properties

Monika Biernat[1], Anna Woźniak[1], Lidia Ciołek[1], Justyna Sawicka[2], Milena Chraniuk[3], Mirosława Panasiuk[3], Piotr Bolin[3], Beata Gromadzka[3], Sylwia Rodziewicz-Motowidło[2], Zbigniew Jaegermann[1]

[1]Łukasiewicz Research Network - Institute of Ceramics and Building Materials, Cracow, Poland. [2]University of Gdansk, Faculty of Chemistry, Department of Biomedical Chemistry, Gdansk, Poland. [3]Institute of Biotechnology and Molecular Medicine, Department of *In vitro* Studies, Gdansk, Poland

Abstract

Biocomposites for filling bone defects and rebuilding bone tissue should be biocompatible, non-toxic, and non-carcinogenic materials [1]. They should support the reconstruction of damaged tissues and have the appropriate microstructure to act osteoconductively. In addition, the arrangement and size of the pores should allow good cell migration and facilitate the delivery of nutrients within the implant. Bioactivity is also significant in creating a connection between the implant and the bone tissue and preventing bacterial infections after implant placement. Therefore, the acting of such biomaterials should be multifunctional and comprehensive and should overcome the problems arising during their application.
One of the natural polymers used to create scaffolds is chitosan. It is characterized by high biocompatibility and has antibacterial and hemostatic properties [2]. In addition, osteoconductive properties make it useful in hard tissue engineering, where bioglasses often enhance their mechanical properties and biological activity. Bioactive glasses are a component of composites that can induce bioactivity, osseointegration, and bactericidal effect of composites [3]. Another option for introducing additional composite functions is using additives, e.g., biologically active peptides [3]. The introduction of peptides in the form of fibrils seems particularly attractive regarding the prolonged action of peptides.
The work aimed to obtain new multifunctional porous composites and to determine the properties of them. The composites were obtained by thermally induced phase separation using chitosan, bioglass doped with an antibacterial element, and biologically active peptide fibrils. It was indicated, that the obtained porous materials are bioactive and non-cytotoxic concerning human osteoblast ,and they support cell proliferation. They have also antibacterial properties against Staphylococcus aureus and Pseudomonas aeruginosa. Based on the test results, the relationships between the composition of the composites and their properties were determined. It was indicated also that components of the composite work synergically what is of crucial importance in the context of the final properties of the composites dedicated to bone implantation.

References:
[1] Haugen H., et al. Bone grafts: which is the ideal biomaterial?, Journal of Clinical Periodontology (2019) 46(S21) 92-102.
[2] Di Martino A., et al. Chitosan: A versatile biopolymer for orthopaedic tissue-engineering, Biomaterials (2005), 10.1016/j.biomaterials.2005.03.016
[3] Kaya S., et al. Mesoporous silica-based bioactive glasses for antibiotic-free antibacterial applications, Materials Science and Engineering C (2018), 10.1016/j.msec.2017.11.003
Funding: This research was funded by The National Centre for Research and Development, Poland, grant No. TECHMATSTRATEG2/406384/7/NCBR/2019

PoB.20.21

The effect of calcium phosphate particles on degradation process of porous polymer scaffolds for bone regeneration

Monika Biernat[1], Anna Woźniak[1], Agnieszka Szabłowska[1], Agata Kurzyk[1], Paulina Tymowicz-Grzyb[1], Adrian Najmrodzki[1], Agnieszka Antosik[1], Joanna Pagacz[1], Piotr Szterner[1], Andrzej Plichta[2], Piotr Wieciński[2], Mirosław Kasprzak[1]

[1]Łukasiewicz Research Network - Institute of Ceramics and Building Materials, Cracow, Poland. [2]Warsaw University of Technology, Faculty of Chemistry, Warsaw, Poland

Abstract

The process of designing composites based on polymers with ceramic fillers should take into account material degradation. This process may adversely affect the functions of the body [1]. The course of degradation of biodegradable materials is a critical factor affecting the healing of a bone fracture, as it may affect its physicochemical properties. It is known that some incorporation of ceramic fillers into the polymer matrix, e.g. PLA, can accelerate degradation at the polymer matrix/particle interface [2]. On the other hand, there are reports that PLA composites without the addition of fillers showed a greater weight loss during degradation compared to composites with the addition of HA or tricalcium phosphate as a filler [3,4].

In our research, composites based on polylactide (PLA) and four types of hydroxyapatite (HA) in the form of whiskers or hexagonal rods were obtained by thermally induced phase separation. Materials were incubated in phosphate buffered saline for 12 weeks at 37°C. The effect of the type of filler on changes in physicochemical properties during hydrolytic degradation was compared. This study presents test results for compressive strength, pore size distribution, weight loss and molecular properties.

The results showed a significant reduction of PLA Mw in the composites. In addition to reducing the Mw, our study showed that the addition of HA led to a reduction in the weight loss of the composites during incubation. This indicates that the introduction of HA in the form of whiskers or hexagonal rods into the polymer matrix can prevent its degradation. The pore size distribution effectively increased after the degradation process. The compressive strength of the tested composites sometimes increased after degradation in relation to the starting materials, although it did not change significantly.

References:

[1] Q.Q. Qiu, W.Q. Sun, J. Connor, Sterilization of biomaterials of synthetic and biological origin, in: Compr. Biomater., 2011.

[2] C. Delabarde, C.J.G. Plummer, P.E. Bourban, J.A.E. Månson, Accelerated ageing and degradation in poly-L-lactide/hydroxyapatite nanocomposites, Polym. Degrad. Stab. 96 (2011).

[3] T. Niemelä, Effect of β-tricalcium phosphate addition on the *in vitro* degradation of self-reinforced poly-L,D-lactide, Polym. Degrad. Stab. 89 (2005).

[4] N.C. Bleach, K.E. Tanner, M. Kellomäki, P. Törmälä, Effect of filler type on the mechanical properties of self-reinforced polylactide-calcium phosphate composites, in: J. Mater. Sci. Mater. Med., 2001.

Funding: The Foundation for Polish Sciences granted the project “Multifunctional composites biologically active for applications in regenerative medicine of bone system” (POIR.04.04.00-00-16D7/18) that was carried out within the TEAM–NET programme financed by the European Union under the European Regional Development Fund.

PoB.20.22

3D printing as a tool for cartilage engineering: novel bioinks based on methacrylated gelatin, poly(aspartic) acid, hyaluronic acid and biofermentative chondroitin.

Celeste Di Meo[1], Lauren De Grave[2,3], Nicola Alessio[4], Sandra Van Vlierberghe[2], Annalisa La Gatta[4], Chiara Schiraldi[4]

[1]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy. [2]Polymer Chemistry and Biomaterials Group (PBM), Centre of Macromolecular Chemistry, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium. [3]Aachen-Maastricht Institute for Biobased Materials (AMIBM), Brightlands Chemelot Campus, Maastricht University, Geleen, Netherlands. [4]University of Campania "Luigi Vanvitelli", Naples, Italy

Abstract

Injuries to the articular cartilage and menisci can lead to degeneration which may ultimately result in arthritis. Solutions for slowing down the progression to arthritis would have high clinical and socio-economic impact. Current surgical approaches produce only short-term relief of symptoms (Kwon et al., 2019). Tissue engineering is now widely recognized as the most promising approach for prompting regeneration of damaged cartilage. Even if studies targeting cartilage engineering have strongly intensified, a “gold standard” biomaterial is still lacking. The main limitation is represented by the difficulty in providing the biochemical cues needed for the maintenance of the chondrocytic phenotype for proper extra-cellular-matrix (ECM) formation. Methacrylated gelatin (gel-MA) is largely used since it is biocompatible, biomimetic, and 3D-printable, with this latter feature allowing for taking advantage of the current most advanced technology in scaffold development. Notwithstanding the encouraging results collected up to now, improvements in terms of mechanical and stability features as well as of chondrocytes response are needed for successful cartilage regeneration.

On these grounds, we propose to combine gel-MA with poly(aspartic) acid (PASP) and glycosaminoglycans (GAGs) expecting improvements in both biophysical properties and biochemical signaling. PASP is biocompatible, biodegradable and easily printable (De Grave et al., 2023). Hyaluronan (HA) is exploited for its ability to interact with chondrocyte surface receptors positively affecting cell proliferation, ECM secretion and phenotype regulation (La Gatta et al., 2017). Unsulfated biofermentative chondroitin (BC) is gaining more attention, showing interesting anti-inflammatory and chondrocyte protection effects (Vassallo et al., 2021).

In this research, porous scaffolds based on gel-MA/PASP combined with HA or BC, at diverse concentrations, were developed by indirect 3D-printing. The resulting hydrogels were characterized *in vitro* in terms of gel fraction, hydration, mechanical properties, GAGs release profile and primary human chondrocytes response. Results indicated that the presence of GAGs did not significantly affect the gel fraction which was around 80-90%. Hydrogels swelling was in the range 1200-1400% and 480-580% in water and PBS, respectively and was comparable in the presence/absence of GAGs. Young Modulus of the scaffolds always resulted slightly less than 1kPa. The hydrogels were seeded with primary cells derived from human tissue (i.e. mesenchymal-stem-cells hMSC). Cell-laden scaffolds were investigated to assess their ability to favor the differentiation and the maintenance of a specific phenotype. The differentiation *in vitro* of MSC towards chondrocytes was monitored by evaluating specific biomarkers expression (i.e. Col-II, Aggregan and Sox-9) at gene and protein level.

PoB.20.25

hBMSC-Seeded 3D Chitosan-Gelatin-Genipin Scaffolds Enhance Extracellular Matrix Mineralization When Cultured Under Perfusion Flow

Gabriele Boretti[1,2], Emanuele Giordano[2,3,4], Mariana Ionita[5,6,7], George Mihail Vlasceanu[5,6], Ólafur Eysteinn Sigurjónsson[1,8], Paolo Gargiulo[1,9], Joseph Lovecchio[1,9]

[1]School of Science and Engineering, Reykjavík University, Reykjavík, Iceland. [2]Laboratory of Cellular and Molecular Engineering "Silvio Cavalcanti" - Department of Electrical, Electronic and Information Engineering "Guglielmo Marconi" (DEI) , University of Bologna, Cesena, Italy. [3]BioEngLab, Health Science and Technology, Interdepartmental Center for Industrial Research (HST-CIRI), Alma Mater Studiorum - University of Bologna, Ozzano Emilia, Italy. [4]Advanced Research Center on Electronic Systems (ARCES), University of Bologna, Bologna, Italy. [5]Faculty of Medical Engineering, University Politehnica of Bucharest, Bucharest, Romania. [6]Advanced Polymer Materials Group, University Politehnica of Bucharest, Bucharest, Romania. [7]eBio-Hub Research Centre, University Politehnica of Bucharest-Campus, Bucharest, Romania. [8]The Blood Bank, Landspitali - The National University Hospital of Iceland, Reykjavík, Iceland. [9]Institute of Biomedical and Neural Engineering, Reykjavik University, Reykjavík, Iceland

Abstract

Tissue-engineered bone tissue grafts are considered a promising alternative to traditional natural donor bone grafts, as they offer the potential for standardized protocols. However, the successful implementation of these grafts in clinical practice still presents challenges. One of the key aspects that needs to be addressed is the selection of an appropriate biomaterial or scaffold to support cell differentiation within a three-dimensional (3D) environment. Various natural and synthetic biomaterials have been explored for this purpose.

In our recent research, we introduced chitosan/gelatin/genipin hybrid scaffolds reinforced with graphene oxide (GO), which exhibited promising osteogenic potential. Nevertheless, there are concerns about the use of graphene and graphene-related materials (GRMs) in medical applications. Further testing and a comprehensive understanding of their behavior in biological systems are necessary.

To enhance the osteogenic potential of the chitosan-gelatin-genipin scaffolds without GO, we propose incorporating a perfusion flow into the differentiation protocol. It is well established that a dynamic environment can improve diffusion gradients, particularly within 3D tissue constructs, thereby enhancing their functional behavior. Bioreactor systems have been employed in several studies to achieve this goal and promote cell phenotype commitment.

Therefore, in this study, we cultured human bone marrow-derived stem cell (hBMSC)-seeded 3D chitosan-gelatin-genipin scaffolds under perfusion flow conditions. We investigated the early osteogenic processes and assessed the extent of extracellular matrix (ECM) mineralization. The results of this investigation provide a promising perspective for potential clinical applications.

PoB.22.01

Neural interfacing biomaterials coated with the firmly tethered neuro-specific lipid bilayer

Md Lemon Hasan[1,2], Ga Eul Kim[3], Mahmoud A. Elnaggar[3], Chun Ho Kim[4], Yoon Ki Joung[3]

[1]Korea Institute of Science and Technology, Seoul, Korea, Republic of. [2]Korea University of Science and Technology (UST), Seoul, Korea, Republic of. [3]Korea Institute of Science and Technology (KIST), Seoul, Korea, Republic of. [4]Korea Institute of Radiological and Medical Sciences, Seoul, Korea, Republic of

Abstract

The limitation of short-term usage of neural interfacing devices encouraged the development of neuro-specific biomaterials. Our hypothesis is that a biomaterial covered with the neuronal cell-derived membrane possessing neural cell adhesion molecule (L1CAM) can promote neuronal adhesion and activation and minimize immune responses at the condition of neural implantation. To demonstrate the hypothesis, we prepared the titanium surface modified with a PC12 cell membrane-derived lipid bilayer, covalently tethered on the surface (PM-TLB). Anti-fouling studies informed us that PM-TLB was sufficiently resistant to the fouling of plasma proteins as well as the adhesion of blood components and bacteria. Cell studies demonstrated that PM-TLB is specific to neuronal cells and non-specific to astrocytes and macrophages, clearly shown in a normal condition and an inflammatory condition. The neuronal activation study supported that PM-TLB improves the outgrowth of neurites and activation stages more than the poly(L-lysine) polymer, which is the most used substrate for neuronal cells. These results conclude that PM-TLB is an efficient surface modification showing selective modulation against neurons and the immune system, promoting neuronal interaction and suppressing neuroinflammatory responses for applications to neuro-implantable devices.

PoB.22.02

Anchor peptides: a robust and versatile technology for the functionalization of medical and healthcare materials

Niklas Herrmann[1], Manuela Garay-Sarmiento[1], Alexander Boes[1], Elisabeth Heine[1], Felix Jakob[1], César Rodriguez-Emmenegger[2,1], Ulrich Schwaneberg[3,1]

[1]DWI-Leibniz Institute for Interactive Materials, Aachen, Germany. [2]Institut de Bioenginyeria de Catalunya, Barcelona, Spain. [3]Institute of Biotechnology, RWTH Aachen University, Aachen, Germany

Abstract

Textiles in medicine and healthcare regularly act as fomites as microorganisms can accumulate and proliferate on the surface, which allows the pathogens to spread through direct or indirect contact or aerosolization. These pathogens may then infect patients, particularly those who are immunocompromised or have direct portals of entry. Many surgical-site infections (SSIs) can be traced back to airborne particles carrying microorganisms which settle on the surgeon's hands, instruments and implants. In Europe, an average of 2 – 5% of surgeries are followed by SSIs that not only impair the patient's well-being but also cause additional costs of up to 19 billion € per year for their treatment.

Here we present peptide-based adhesion promoters (anchor peptides) that can be fused or conjugated with functional moieties. Anchor peptides are short amphiphilic peptides with sizes ranging from 20 to 100 amino acids that bind from aqueous solutions to natural[1] and synthetic[2] surfaces including metals. Tailor-made anchor peptides are thus applicable in many fields, including biotechnology, catalysis, nanotechnologies, medicine, and agriculture. To generate an antimicrobial and antifouling surface, we combined the anchor peptide LCI with an antimicrobial enzyme (endolysin) as well as with antifouling polymer brushes. We call this coating Kill&Repel[3]. By exhibiting repellent and bactericidal properties, it limits the initial stages of bacterial adhesion onto the surfaces, thus reducing bacterial load. If, however, bacteria manage to surpass the repellent barrier, the endolysin is capable of killing them upon contact and the released cell debris is subsequently repelled. We applied the Kill&Repel coating onto a PCL wound dressing, thereby preventing its colonization by Streptococcus and demonstrated that sessile colonies of Streptococcus were killed by the immobilized endolysins in an agar contact assay. In addition, the coating showed outstanding antifouling properties towards blood plasma and fibroblasts as well as a >90% reduction of planktonic bacteria within one hour.

The toolbox character of the anchor peptide technology opens a revolutionary approach for the functionalization of biomaterials in medicine and healthcare.

[1] Meurer, R., Kemper, S., Knopp, S., Eichert, T., Jakob, F., Goldbach, H., Schwaneberg, U., Pich, A. (2017). Angew. Chem., Int. Ed., 56, 7380-7386

[2] Rübsam, K., Weber, L., Jakob, F., Schwaneberg, U. (2018). Biotechnol. Bioeng., 115, 321-330

[3] Garay-Sarmiento, M., Witzdam, L., Vorobii, M., Simons, C., Herrmann, N., Santos Pereira, A., Heine, E., El-Awaad, I., Lütticken, R., Jakob, F., Schwaneberg, U., Rodriguez-Emmenegger, C. (2022). Adv. Funct. Mater., 32, 2106656

PoB.22.03

A study of surface parameters causing a change in bacterial activity over magnesium alloys for future implant materials

Vivek Singh Yadav, Lalit Mohan Pandey

Indian Institute of Technology, Guwahati, Guwahati, Assam, India

Abstract

Abstract: RF sputtering is one of the physical deposition approaches to develop a desired surface modification for attaining successful implant surface properties. This technique provides a roughness range from 0.2 mm to 1mm with a uniform and smooth surface modification. It also shows a significant influence on the bacterial and cell adhesion behavior over the implant surface. In the present work, silver nanoparticles were deposited on magnesium alloy to enhance the bacterial activity and to simultaneously control the degradation rate. Various surface properties were quantified, such as surface wettability, hardness, coating adhesiveness, protein adsorption and cell adhesion over the surface. Surface characterization and biomineralization of deposited surfaces were performed through an X-ray diffraction pattern, FESEM imaging, Fourier transformation Infrared ray, and Raman spectroscopy. The XRD analysis showed strong silver 2 Θ peaks at 32.26°, 73°, which confirmed silver deposition and was further compared with JCPDS # 01-072- 2108. AFM (2 × 2 µm) and 3D profilometer (1000 × 1000 µm) images showed the roughness (Ra) change and speculated its influence over the in-vitro performance. The correlation between the two techniques was developed to create an optimal surface roughness, confirming the maximum antibacterial repulsive nature over the surface. In 2 × 2 µm with deposition time, the roughness factor (Ra) had decreased and resulted in a smooth nano finish on the surface of the AZ31 substrate. However, for 1000 × 1000 µm images roughness factor (Ra), after the addition of silver nanoparticles, showed higher peaks and troughs, providing surface with a high roughness parameter. At the same time, while observing the nature of the surface through contact angle measurement, bare AZ31 substrate showed hydrophilic behaviour at a contact angle of 72°. Notably, with an increase in silver content over the surface, the hydrophilic behaviour of substrates decreased, shifting towards hydrophobic nature in a linear trend, with an increase in deposition time of 7, 9, and 11 hours, elevating contact angles (of silver nanoparticle deposited AZ31) as 40.1°, 55 ° and 71°, respectively.

Keywords: Surface parameter, Surface roughness, Magnesium alloys, Anti-bacterial activity, Cell adhesion,

PoB.22.04

Coating of neurovascular stents to improve their efficiency

Dyhia Kersani[1], Marie Lacaze[1], Eric Largen[1], Benoit Ho-tin-noe[2]

[1]Balt Group, Montmorency, France. [2]Univ Paris Cite, INSERM UMR-S 1144, Paris, France

Abstract

Around the world, there are 12.2 million new strokes per year, one every 3 seconds. 101 million people worldwide are living with stroke aftermath, this number has almost doubled over the last 30 years[1]. Strokes are divided in two categories: ischemic Stroke that occurs when a blood vessel supplying blood to the brain is obstructed due to the formation of blood clot (thrombus). Hemorrhagic Stroke occurs when there is a loss of elasticity of the vessel wall leading to the formation of aneurysm. The aneurysm rupture causes a cerebral hemorrhage. Endovascular treatments are usually used to treat these disorders by using metallic medical devices called stents.

1. The development of mechanical thrombectomy with the use of a “stent retriever” provides an attractive strategy, alternative to the conventional systemic thrombolysis which can potentially lead to lethal cerebral hemorrhage. However, the efficacy of the existing devices in capturing the thrombi is suboptimal and the surgeon must often perform several passages to pull out the thrombus. One of the objectives of this work is the chemical functionalization of stent retriever to improve the capture of the blood clot and prevent its fragmentation.
2. Flow diversion stent was a major breakthrough for the endovascular treatment of intracranial aneurysms. Nevertheless, beyond aneurysmal occlusion, metallic flow diversions can induce endovascular reaction due to the foreignness of metal devices, hampering its endothelization across the aneurysm and arterial healing of intracranial aneurysm. The second objective of this work is the functionalization of flow diverter stents by a biomolecule leading to the improvement of reendothelialization and enhancing the hemocompatibility of the stents.

As the stents (flow diverters and stents retriever) have complex geometries, their surface characterization to demonstrate the effectiveness of the grafting method is challenging. In this work, Fluorescence Microscopy technique has been used to demonstrate the presence of the biomolecules on the surface of the different stents. Also, the efficiency of the biomolecules coated on the stents has been evaluated using different characterization methods. This industrial research work aims at improving the efficiency of intracranial stents and proposing better treatments for patients.

References:

[1] World Stroke Organization (WSO): Global Stroke Fact Sheet 2022

PoB.22.05

Exploring the Properties of Zwitterionic Polymer Brushes using Surface Enhanced-Raman Spectroscopy

Monika Spasovová[1,2], Marek Procházka[1], Josef Štěpánek[1], Ivan Barvík[1], Ondřej Kylián[3], Markéta Vrabcová[2], Milan Houska[2], Alexandr Dejneka[2], Nicholas Scott Lynn[2], Hana Vaisocherová Lísalová[2]

[1]Charles University, Faculty of Mathematics and Physics, Institute of Physics, Ke Karlovu 5, 121 16, Prague, Czech Republic. [2]Institute of Physics of the Czech Academy of Sciences, 182 00, Prague, Czech Republic. [3]Charles University, Faculty of Mathematics and Physics, Department of Macromolecular Physics, V Holešovičkách 2, 180 00, Prague, Czech Republic

Abstract

Polymer brush (PB) films represent a powerful tool to control the physical and chemical properties of a surface. PB-nanocoating composed of various monomers can be used to tune the properties of a surface for a wide variety of applications: to produce antibacterial surfaces for medical applications, surfaces functionalizable with biorecognition elements for biosensing, or surfaces with thermo- or pH-responsive behaviour. We have been focusing on the development and research of zwitterionic PBs that are bio-compatible, functionalizable, and furthermore, represent the state-of-the-art in suppressing non-specific interactions with biomolecules (fouling). These grafted-from PBs are synthesized via surface-initiated atom transfer radical polymerization, where different thicknesses can be achieved via altering the time of polymerization.

Properties such as thickness, ability to absorb water (swelling), surface density, and homogeneity are crucial for a PB's ability to suppress fouling. However, these properties are difficult to characterize, where a single technique cannot yield all the pertinent information. The combination of ellipsometry and infrared spectroscopy – both widely used, non-destructive, rapid, and relatively undemanding – can be used for fast sample characterization that yields information on the thickness and chemical purity of the samples. However, these techniques do not provide any direct information about homogeneity and polymer chain density.

To address this issue, we have been developing a novel technique for determination of homogeneity and permeability of PBs using surface enhanced Raman scattering (SERS) microscopy. Based on the used probing SERS agents, we can determine the permeability for molecules of different sizes and charge, where furthermore, the extent of SERS hotspots gives information about the density of the brush. Moreover, this technique allows to determine the permeability of proteins into the brush which could lead to: (i) a better understanding of fouling principles from complex biological samples such as human plasma, (ii) an elucidation of how far into the PB the biorecognition elements penetrate upon functionalization of the PB. The former will help with design of PBs with stronger anti-fouling properties and the latter with design of PBs for functionalization in label-free biosensing, providing surfaces that yield lower limits of detection.

PoB.22.06

Supramolecular biomaterials encompassing peptide amphiphiles, biopolymers and graphene oxide for tissue engineering

Maria Lopes[1], Joana Sousa[2], Paula A. A. P. Marques[2], João Borges[1], João F. Mano[1]

[1]CICECO, Department of Chemistry - University of Aveiro, Aveiro, Portugal. [2]TEMA, Department of Mechanical Engineering - University of Aveiro, Aveiro, Portugal

Abstract

The extracellular matrix (ECM) is an intricate supramolecular network of glycosaminoglycans and structural and functional proteins assembled across different length scales that provides a wide range of signaling cues to support cell functions. The similarity between biocompatible and biodegradable natural-origin polymers and native ECM has been leveraged to design tissue engineering (TE) strategies for repairing damaged tissues, however their weak structural stability and mechanical properties limit their use.[1] Moreover, biophysical cues of biomaterials have been highlighted as critical components in the modulation of stem cells' behavior and fate.[2] As such, due to the enhanced mechanical properties assigned by graphene-derived materials as well as the ECM-mimetic fibrillar structure ensured by amphiphilic peptides (PA), these materials have been consistently used in the development of TE strategies.[3,4]

In this work, we developed supramolecular multicomponent biomaterials encompassing poly-L-lysine and hyaluronic acid biocompatible polymers, PA and GO by combining molecular self-assembly and Layer-by-Layer assembly technology to instruct cell behavior. The secondary structure and morphology of the co-assembled materials were studied by circular dichroism, ATR-FTIR, and scanning electron microscopy (SEM). The successful fabrication of supramolecular multilayered thin films was monitored in situ by quartz crystal microbalance with dissipation monitoring. Their physicochemical and morphological properties were assessed by water contact angle, atomic force microscopy and SEM. The *in vitro* biological performance of the developed multicomponent supramolecular matrices, exhibiting distinct mechanical and topographical cues, was assessed at different time points, using mesenchymal stem cells, with the thin films imparted with the synergistic effect of GO and PA revealing enhanced cell viability and cytoskeletal alignment when compared to the control substrates. These results suggest the potential use of such hybrid biopolymer/peptide/GO supramolecular multicomponent biomaterials in bone or cardiac TE.

This work was funded by the European Union's Horizon Europe research and innovation programme under the grant agreement No. 101079482 ("SUPRALIFE"). The financial support by FCT through the PhD grants (J.S. SFRH/BD/144579/2019, M.L 2020.05210.BD), and Assistant Researcher contract (2020.00758.CEECIND, J.B.) is gratefully acknowledge. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC).

References

1. M. Abbasian *et al.*, *Int. J. Biol. Macromol.*, 2019, **134**, 673 - 694.
2. A. Higuchi *et al.*, *Chem. Rev.,* 2013, 113, **5**, 3297–3328.
3. A. F. Girão *et al.*, *ACS Appl. Mater. Interfaces*, 2020, **12**, 38962−38975.
4. Z. Álvarez *et al.*, *Cell Stem Cell*, 2023, **37**, 50.

PoB.22.07

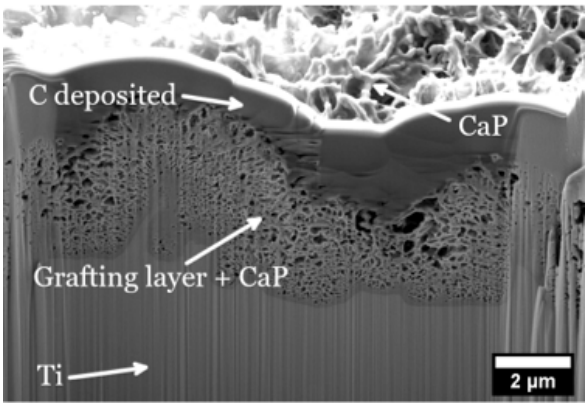
Controlled biomimetic approach for implant surface modification

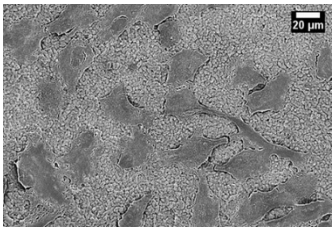
Agnese Carino[1], Simon Berner[1], Philipp Gruner[1], Federico Dalcanale[2], Michael de Wild[2], Walter Moser[3], Simon Göddeke[4], Burkard Höchst[4], Andrea Testino[5]

[1]Medicoat AG, Mägenwil, Switzerland. [2]School of Life Sciences FHNW, Muttenz, Switzerland. [3]Atesos Medical AG, Aarau, Switzerland. [4]Hager & Meisinger GmbH, Neuss, Germany. [5]Paul Scherrer Institut, Villigen, Switzerland

Abstract

Titanium and its alloys are the most frequently used biocompatible materials in medical engineering. The addition of a bioactive ceramic coating accelerates the osseointegration process. Relatively thick (tens to hundreds of µm) calcium phosphate coatings can be obtained using well-established processes, i.e., plasma spray. Only a few thin coatings methodology has been studied and is available for medical implants. We propose a biomimetic approach: a cost-effective protocol for Ti surface modification by a wet route. As a result, a calcium phosphate nanotextured layer is grown on the implant surface. The process can be applied to sandblasted and acid-etched surfaces, such as the gold standard in the dental field (SLA). The protocol consists of a multistep treatment. It generates a thin (~1µm) chemically bonded nanotextured porous layer of Ti-based ceramics on the metal surface (grafting layer, GL). A synthetic bone (calcium phosphate) grows within its porosity and on its surface under biomimetic conditions, according to an accelerated controlled method (Fig 1). The bone-like coating does not mask the underlying microroughness. The implant's surface is homogeneously nanotextured. We demonstrated the adhesion properties of the surface modification upon professional implantation into synthetic saw bone material (30 pcf) and cortical bone of calf tibia up to a load of 60 Ncm. Fatigue tests after coating were conducted according to the ISO 14801 standard to ensure mechanical integrity. Cross section (obtained by Focused Ion Beam, FIB) and surface chemical analyses (Energy Dispersive X-ray, EDX) confirmed that the surface is unaffected by mechanical stress. The bone-like coating is firmly grafted on the surface, the layer is preserved, and no delamination is detected. Biocompatibility was tested with human osteosarcoma MG63 cells. Cytotoxicity and alkaline phosphatase activity (ALP, after two weeks) for osteoblast differentiation were investigated. The same proliferation rate was observed on the control (SLA) titanium surface. The cells exhibited a healthy and spread morphology (Fig 2). Four different substrates were investigated to study osteoblastic differentiation. Our bone-like surface showed an ALP activity double that SLA reference. The process stability and the data collected on the surface properties demonstrate a high potential for the biomimetic approach as surface treatment, among others, for dental implants. The bone-like surface modification, applied as a showcase on dental screws, can be used to implement and optimise Ti-based permanent implants, such as craniomaxillofacial, spinal, and orthopaedic implants.





PoB.22.08

Surface functionalization of medical devices by plasma treatment and peptide immobilization for antimicrobial coatings

Mohadeseh Zare, Artemis Stamboulis

University of Birmingham, Birmingham, United Kingdom

Abstract

Despite recent progress in the strategies of antimicrobial coatings for orthopedic implants, developing a technique to selectively eliminate bacterial attachment without affecting the osteoblast cell adhesion and proliferation remains a research precedence. Up to now, coating strategies based on antimicrobial peptides (AMPs) can substantially tackle infectious problems where bioinspired peptides can be immobilized through covalent attachment to the implant surfaces. The covalent immobilization allows long-term activity and sustained release of AMPs to inhibit bacterial colonization and subsequent biofilm formation. However, this approach still requires higher antimicrobial activity, resistance to enzymatic degradation, long stability, and lower cost of the industrial scale.

The present study proposes a reliable technique for antimicrobial peptide grafting on the implant surfaces using plasma treatment to increase the antimicrobial activity and stability of the coating. This approach involves the surface functionalization of titanium implants with primary amine groups (–NH2) by plasma nitriding to attach AMPs (KR-12) covalently via polymeric linking arms. These linking arms directly affect the AMPs conformation and their antimicrobial activity.

Analytic methods such as X-ray photoelectron spectroscopy (XPS), Fourier-transform Infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and Confocal Raman Spectroscopy (CRS) are used to confirm the successful covalent attachment of the AMPs on the surfaces. The XPS and FTIR results prove that after plasma treatment, 15% of the atoms on the surface were attributed to NH2 groups where AMPs can be attached with the highest efficiency without showing any toxicity. The uniformity of the amine groups and immobilized AMPs on the surface were approved by CRS and AFM results. Besides, the *in vitro* antibacterial activity of the AMP-immobilized implant evaluates its efficiency against Staphylococcus aureus and Escherichia coli as implant-related infections. Moreover, Biological tests assess the cell compatibility, adhesion, and proliferation of the proposed antimicrobial coating.

This peptide-immobilized based coating, involving plasma treatment and polymeric linking arms, provides an opportunity for bacterial infection prevention for a variety of orthopedic implants with a positive impact on cell adhesion and growth.

PoB.22.09

EXTRACELLULAR MATRIX-MIMETIC PEPTIDE HYDROGELS FOR CONTROLLED CELL ADHESION AND TISSUE FORMATION

Vytautas Cėpla[1,2], Airina Mazėtytė-Godienė[1], Tadas Jelinskas[1], Romuald Eimont[1,2], Tomas Rakickas[1], Ramūnas Valiokas[1,2]

[1]Department of Nanoengineering, Center for Physical Sciences and Technology, Vilnius, Lithuania. [2]UAB Ferentis, Vilnius, Lithuania

Abstract

The design of extracellular matrix (ECM)-like scaffolds has become one of the powerful concepts toward the development of artificial tissues, both for *in vitro* and clinical applications. Due to the high cost and challenging purification processes of full-length ECM proteins, synthetic peptides offer an attractive alternative as the functional blocks of the tissue engineering scaffolds.
We have been previously involved in the development of a series of hydrogels that are based on collagen-derived synthetic peptides Cys-Gly-(Pro-Lys-Gly)$_4$ (Pro-Hyp-Gly)$_4$ (Asp-Hyp-Gly)$_4$, hereafter Cys-CLP, and optionally contain different functional peptide extensions: the fibronectin cell adhesion sequence RGDSPG (Cys-CLP-RGD)[1], collagen binding sequence DGEAG (Cys-CLP-DGEAG) or laminin adhesive motif IKVAV (Cys-CLP-IKVAV)[1]. Here we report on synthesis of ultrathin hydrogel coatings containing the above peptide blocks employing a one-pot strategy. Namely, we have performed initiator free, UV-controlled self-initiated photografting and photopolymerization reaction (SIPGP)[2] on glass-type and plastic substrates. For this purpose, we functionalized the ECM-like Cys-peptides via Michael addition reaction with photoactive methacrylate group using the 3-(acryloyloxy)-2-hydroxypropyl methacrylate reagent, thus obtaining methacrylated peptides. By combining the methacrylated peptides, 2-hydroxyethyl methacrylate, PEG methacrylate and methacrylic acid monomers in SIPGP we successfully synthesised 15-50 nm thick, mechanically stable ECM-mimetic peptide hydrogels. The hydrogel coatings were tested with different cell lines (endothelial, epithelial, fibroblast and other). We demonstrate the benefits of the ECM-like coatings for fabrication of chip substrates for controlled cell culture and tissue formation. Also, we demonstrate the benefits of our strategy in photolithographic patterning of surfaces and discuss potential bioanalytical, tissue engineering and regenerative medicine applications.

[1] Balion Z. et al., Front Bioeng Biotechnol. Sep 4;8:773, 2020.
[2] Cėpla V. et al., ACS Appl. Mater. Interfaces, 12 (29), 32233–32246, 2020.

PoB.22.10

Bioactive coatings of titanium implants containing Sr and Zn. From nano self-assembling monolayers to biohybrid sol-gel coatings

Gerardo Asensio[1], Ana M. Hernández-Arriaga[2], M. Auxiliadora Prieto[2], Agustín R González-Elipe[3], Sanjukta Deb[4], María Rosa Aguilar[1,5,6], Blanca Vázquez-Lasa[1,5,6], Luis Rojo[1,5,6]

[1]Institute of Polymers Science and Technology, Madrid, Spain. [2]Biological Research Center – Margarita Salas, Madrid, Spain. [3]Materials Science Center of Seville, Seville, Spain. [4]Division of Tissue Engineering and Biophotonics, King's College, London, Spain. [5]Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy-Spanish National Research Council, Madrid, Spain. [6]Consorcio Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, Madrid, Spain

Abstract

Introduction. The development of bioactive coatings for titanium implants that can stimulate specific host tissue responses are a major goal in biomaterials science[1]. Hence we propose the chemical functionalisation of titanium substrates (Ti-Cp) by two methodologies: i) the preparation of hybrid sol-gel coatings composed of a graft copolymer of chitosan and polyvinyl caprolactam (Ch-*g*-PVCL) and the alkoxide precursors (3-glycidyloxypropyl)trimethoxysilane (GPTMS) and methyl trimethoxysilane (MTMOS), including also the strontium-and zinc-vitamin B9 complexes (SrFO and ZnFO) with demonstrated osteogenic ability, and ii) the formation of self-assembled monolayers (SAMs) with bioactive phytic acid (PA) derivatives bearing Sr^{2+} and Zn^{2+} (SrPhy, $ZnPhy^2$). **Experimental.** Ti-Cp substrates were activated by immersion in NaOH 5M at 60°C for 24h. Hybrid sol gel-coatings were fabricated by hydrolysis, condensation and coupling reactions between GPTMS, MTMOS and Ch-*g*-PVCL, (Figure 1). SrFO and ZnFO containing samples were fabricated by previous dissolution of each metallic complex in the catalyst. The coatings were physicochemically characterized by solid ^{13}C-^{29}Si-NMR, Raman, EDX, and contact angle measurements. SAMs preparation was carried out by the direct condensation reaction with the corresponding phytate compound solution (pH 4, 1mM) promoted by heat treatment (80°C). Four experimental groups were obtained (Ti-PA, Ti-SrPhy, Ti-ZnPhy and Ti-SrPhy/ZnPhy). Their osteogenic and antibacterial performances were assessed *in vitro* in osteoblasts and *Streptococus mutans* cultures. **Results.** The optimization of the procedure followed for the sol-gel approach yielded to homogeneous coatings of ≈40µm thickness. Spectroscopic analysis by $^{13}C/^{29}Si$-NMR, Raman and EDX suggested the covalent anchoring of Ch-*g*-PVCL into the silica network, and hydrophilicity of all coated samples was significantly increased respect to Ti-Cp. Moreover, phytate-based SAMs sustained the proliferation of osteoblast cells over 14 days of culture while Ti-SrPhy, Ti-ZnPhy and Ti-SrPhy/ZnPhy overexpressed ALP activity and matrix mineralization in comparison to Ti-PA. Besides, all phytate-SAMs significantly reduced biofilm deposition of *Streptococus mutans* cultures as determined by colony forming units' viability, crystal violet staining, LIVE/DEAD and SEM imaging (**Figure 2**). **Conclusions.** The chemical composition of sol-gel coatings exhibited promising potential as bioactive systems whereas phytate SAMs shown excellent osteogenic and antibacterial properties *in vitro* for their application in oral implantology.

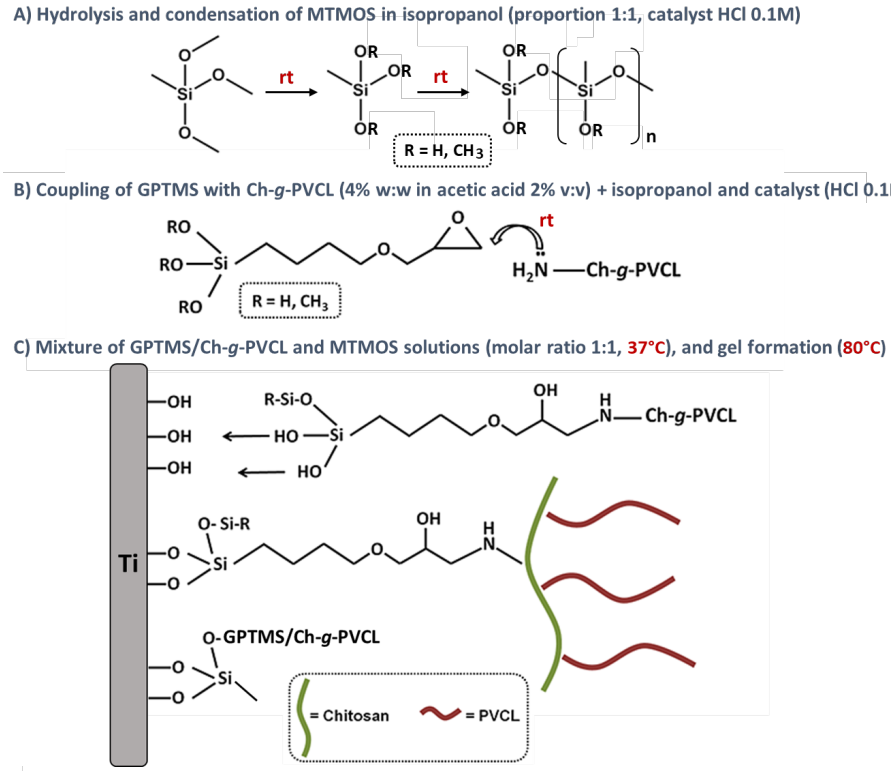


Figure 1. Procedure followed for the fabrication of sol-gel coatings.

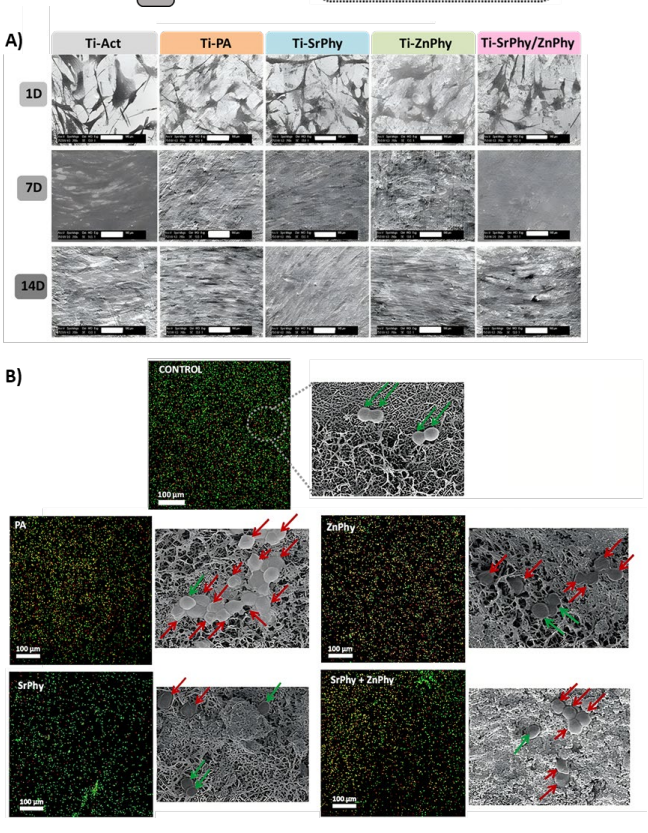


Figure 2. *In vitro* performance of phytate-SAMs. A) SEM observation of osteoblasts cells cultured over time. B) LIVE/DEAD and SEM imaging of *Streptococus mutans*.

References:

1.Asensio_G._J.Clin.Med_8:1982 _2019

2.Asensio_G._Appl.Surf.Sci_30:156818_2023

Acknowledgements:

MICINN(PID2020-114086RB-10), CAM(IND2018/BMD-9485, P2022/BMD-7406), Nanomedicine-CSIC.

PoB.22.11

Biologically-relevant interactions at the polyurethane surface: *in silico* and *in vitro* studies

Paulina Chytrosz-Wrobel[1], Monika Golda-Cepa[1], Kamil Drozdz[2], Monika Brzychczy-Wloch[2], Piotr Kubisial[1], Waldemar Kulig[3], Lukasz Cwiklik[4], Andrzej Kotarba[1]

[1]Faculty of Chemistry, Jagiellonian University, Krakow, Poland. [2]Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland. [3]Department of Physics, University of Helsinki, Helsinki, Finland. [4]J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic

Abstract

Introduction. Understanding solid–water interface interactions play an essential role in the development and application of biomaterials since they define a playground for most biochemical reactions and physiological processes. The oxygen plasma-functionalized polyurethane surfaces that can be successfully used in contact with tissue of the respiratory system were prepared and investigated. **Materials and methods.** In the experiments, the influence of oxygen plasma modification on physicochemical properties was studied with the use of AFM, ATR-IR, DTA, XPS, SIMS, water contact angle measurements and supplemented with biological tests (A549 cell line and two bacteria strains *S. aureus* and *P. aeruginosa*). **Results and Discussion.** No structural changes were found under the influence of plasma, but a strong change in surface properties influencing the biocompatibility of the tested polymer was observed (e.g. wettability). The unmodified material is hydrophobic (Θ_{H2O} = 105°), and the completely hydrophilic surface is obtained after plasma modification with the use of the following parameters: pO_2 = 0.14 mbar and t = 5 min. It was found that the most important parameter controlling the degree of modification of the polyurethane surface is the oxygen pressure in the generator chamber. It is worth underlining that the initial value of the contact angle is not recovered, even after 28 days. The oxygen plasma-modified polyurethane improves the adherence of adenocarcinoma human alveolar basal epithelial cells. The surfaces with improved biocompatibility were also tested in the context of the risk of biomaterials-centered infections and it was revealed that oxygen plasma-modified surfaces unfortunately promote bacterial adhesion. The results provide the background for the development of polymeric biomaterials with improved surface biocompatibility and point out the necessity of parallel testing of biocompatibility and risk of infection. Moreover, the experimental findings were supplemented with the molecular interpretation gained by the MD simulations employing a newly developed, fully-atomistic model of an unmodified and plasma-functionalized polyurethane material to characterize in detail the polyurethane–water interfaces at the nanoscale. The experimentally obtained polar and dispersive surface free energy components agree with the calculated ones verifying the adequacy of the developed model. It was determined that the substitution of 20% of polymeric chains ends by their oxidized versions corresponds to the experimentally obtained plasma-modified polyurethane surface and indicates the saturation of the surface with oxygen functional groups.

Acknowledgment: This study was financed by NCN Poland project DEC-2019/35/D/ST5/03107.

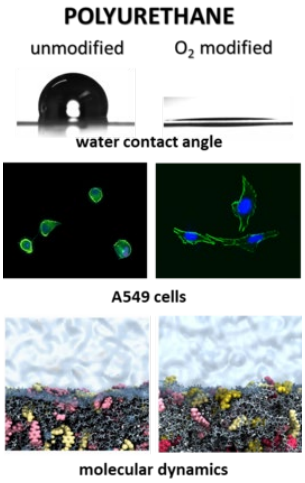


Fig. 1 The summary of performed *in vitro* and *in silico* studies for polyurethane surfaces.

PoB.22.12

Biocompatibility of plasma etched polymers – the role of crystallinity

Monika Golda-Cepa[1], Paulina Chytrosz-Wrobel[1], Paulina Gogacz[1], Kamil Drozdz[2], Monika Brzychczy-Wloch[2], Andrzej Kotarba[1]

[1]Faculty of Chemistry, Jagiellonian University, Krakow, Poland. [2]Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland

Abstract

Introduction. The most important aspect that has to be taken into consideration while designing new biomaterials is the competition for the available surface between eucaryotic cells and microorganisms, called the "race for the surface". The outcome of this contest is critical for the patient since if bacteria attach to the medical device, they colonize it and produce an antibiotic-resistant biofilm. The main factors that determine eucaryotic and procaryotic cell adsorption are chemical composition, surface free energy, and roughness. The surface properties of polymers can be modified with the use of plasma treatment. During the modification process, at first, the surface functional groups are formed. Upon prolonged time of plasma treatment the etching is observed, which begins with chain scissions of the backbone. As a result, substantial changes in surface morphology can be observed after exposure to plasma. **Experimental Methods.** Polyurethane (amorphous), parylene (semicrystalline) and polyethylene (crystalline) polymeric films were used. The XRD data were collected on a Rigaku Miniflex System and used for the crystallinity assessment of polymeric materials. Plasma treatment was performed using Femto system (Diener Electronic). Changes in surface wettability were followed by contact angle measurements (Surftens Universal, OEG) and surface topography was determined with the use of an atomic force microscope (AFM, NanoWizard 4 XP Brucker). The polymeric materials were also evaluated in terms of their biocompatibility (A529 cell line) and adhesion of bacteria cells (*S. aureus, P. aeruginosa*). **Results and Discussion.** Upon plasma treatment, the surface of initially hydrophobic polymers (Θ_w=90º) turned hydrophilic (Θ_w=20-5º). No changes in crystallinity were observed for the amorphous polyurethane, while for semicrystalline materials crystalline domains grew from 4 nm to 7 nm. Significant changes were also observed in terms of surface topography (R_{RMS}). The amorphous polyurethane after plasma etching became smoother, while for semicrystalline polymer nanotopography was formed. The premise behind the observed effect is that during exposure to plasma, amorphous regions of polymers are preferentially etched, while the more compact crystalline domains are less affected and more etch-resistant [1]. Adenocarcinoma human alveolar basal epithelial cells' attachment to the plasma-etched surfaces was significantly higher than to unmodified ones. Moreover, the plasma-etched surfaces of semicrystalline polymers were resistant to bacterial biofilm formation.

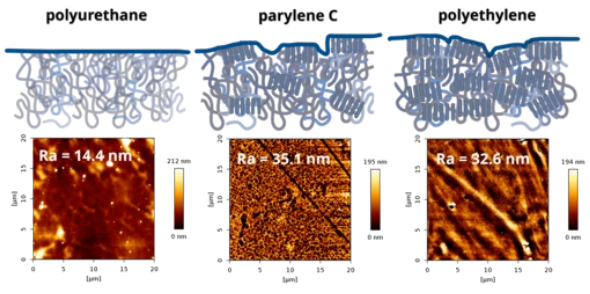


Fig. 1 The effect of oxygen plasma treatment on surface topography for polymers with different crystallinity.

References

[1] M. Golda-Cepa et al. RSC Advances 5.60 (2015): 48816-48821.

Acknowledgments. This study was financed by NCN Poland project awarded by decision number DEC-2019/35/D/ST5/03107.

PoB.22.14

Tuning responsivity of neural maturation via topographical and biochemical cues

Jagoda Litowczenko-Cybulska[1], Jacek Kamil Wychowaniec[2], Karol Załęski[1], Łukasz Marczak[3], Krzysztof Tadyszak[4], Katarzyna Fiedorowicz[1], Charlotte Jennifer, Chante Edwards-Gayle[5], Barbara Małgorzata Maciejewska[6]

[1]NanoBioMedical Centre, Adam Mickiewicz University, Poznan, Poland. [2]AO Research Institute Davos, Davos, Switzerland. [3]European Centre for Bioinformatics and Genomics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland. [4]Institute of Macromolecular Chemistry CAS, Prague, Czech Republic. [5]Diamond Light Source, Harwell Science and Innovation Campus, Didcot, United Kingdom. [6]Department of Materials, University of Oxford, Oxford, United Kingdom

Abstract

Peripheral nerve damage is a common clinical complication of traumatic injury, and leading to sensory and motor function failures. The gold standard for nerve gaps treatment is the use of autologous nerve grafts, but this approach has several disadvantages, including limited availability, loss of native function, and neuroma formation [1]. An alternative strategy to standard autografts is the use of a nerve conduit, which is a tubular structure made of synthetic/biological materials designed to bridge the sectioned nerve gap. Their main advantage is accessibility and no need of using sacrificing donors [2].

In pathological conditions, the success of neuronal regeneration requires neurite outgrowth. Improving neuron regeneration can be achieved through the use of topographic cues, suitable materials with defined stiffness/roughness and bioactive materials. Topographic cues are known to guide axon outgrowth, depending on the cue size [3]. Graphene oxide (GO) is utilized as a scaffold due to protein adsorption and desired surface roughness for cell adhesion [4]. Hyaluronic acid (HA) is a highly abundant component of the nervous system extracellular matrix and plays a signaling role in regulating cell processes. HA has potential as a bioscaffold due to its bioactivity, but its utilization is limited by its poor mechanical properties. Nonetheless, incorporation of synthetic polymers, such as polycaprolactone (PCL) can improve these properties.

Here we demonstrate that topography of groove patterned PCL and composite PCL-GO have a strong impact not only on the cell alignment and neurite outgrowth but also on the neural maturation. Our results demonstrate the specific combination of topography can lead to selective differentiation of NSCs without any exogenous supply (Fig 1.).

The second part of our studies include the fabrication and biological impact of neurotubes obtained by PCL fibers 3D printing and HA-based hydrogels augmented with bioactive agents. We compare the effect of the materials on the maturation of neural cells, by the evaluation of their morphology and detection of neural markers (Fig 2.). We believe that the presented 3D tubes hold translational potential for potential nerve graft fabrication.

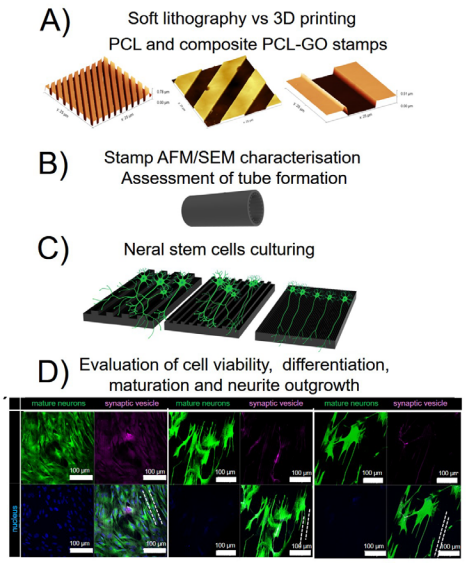


Fig. 1. A) Schematic representation of AFM morphological characterization of PCL and PCL-GO stamps representing different groove aspect ratio, obtained by soft lithography and 3D printing techniques with the 3D reconstruction of the AFM height images. B) After obtaining patterned stamps we demonstrated that both PCL and PCL-GO composite stamps could be effectively wrapped into 3D tubes holding translational potential for potential nerve graft fabrication C) Scheme of the patterned polymeric scaffold stamps with representation of neural outgrowth observed for NSCs-neurons. D) Representative confocal images of NSCs immunostained for mature neurons markers (green) and synaptic marker (violet) cultured for 21 days on patterned matrices.

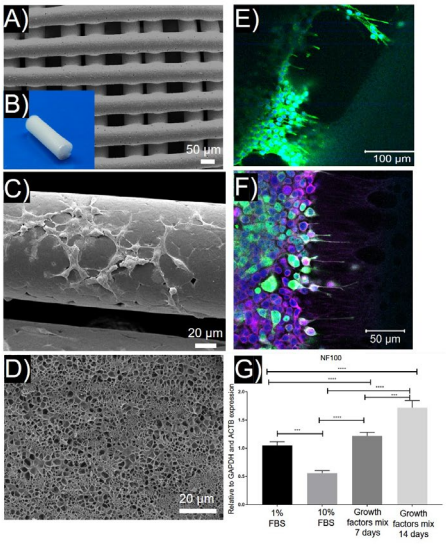


*Fig. 2. A) SEM morphological characterization of 3D printed PCL grid which we used to form B) multilayerd tube. C) SEM image of DRG neural cells cultured on PCL grid. D) Porous interconected structure of hyaluronic acid-based 3D bioprinted crosslinked hydrogels visualized by CryoSEM E) Representative confocal images of DRG neural cells immunostained for F-actin (cytoskeleton, green) and nucleus (blue) for 7 days within hydrogels. F) Confocal images of DRG neural cells with detection of late neuronal (green) and synaptic vesicles (violet) markers. G) qPCR for the mRNA expression level of neurofilament-200 neural gene expression after culturing with lower (1%) and standard (10%) FBS content and with selected active agents mix at day 7 and 14. Error bars represent SD (n = 3), determined using one-way ANOVA;*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

Acknowledgements

The authors acknowledge financial support from the Small Grant Scheme 2020 (NOR/SGS/GrooveNeuroTube/0321/2020-00), 038/04/NŚ/0044 and START 2022 Foundation for Polish Science.

References

[1] Papadimitriou L. et al. Mater Today Bio. 2020. doi:10.1016/j.mtbio.2020.100043.
[2] Litowczenko J. et al. Bioact Mater. 2021. doi:10.1016/j.bioactmat.2021.01.007
[3] Litowczenko J. et al. J Biomed Mater Res A. 2019. doi:10.1002/jbm.a.36733.
[4] Wychowaniec J.K., et al. J Mech Behav Biomed Mater. 2020. doi:10.1016/j.jmbbm.2019.103594.

PoB.23.04

XPS-Surface analyses of plasma-treated ceramics for dental applications

Thomas Eickner[1], Susanne Stählke[2], Jakob Brief[3], Barbara Nebe[2,4], Niels Grabow[1], Volkmar Senz[1]

1 Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany. 2 Department of Cell Biology, Rostock University Medical Center, Rostock, Germany. 3 VITA Zahnfabrik H. Rauter GmbH & Co. KG,, Bad Säckingen, Germany. 4 Department Science and Technology of Life, Light and Matter, University of Rostock, Rostock, Germany

Abstract

Introduction
Biomaterials for replacing teeth partially or entirely are implanted in patients for prosthetic rehabilitation. Due to the increasing use of dental implants, their further development and continuous surface quality improvement are the subject of current scientific research. Titanium is certainly one of the most frequently used materials. One of the best-studied alternatives to titanium is bioinert zirconia (ZrO2).

ZrO2 is compatible with soft and hard tissues and fulfills the mandatory functional and aesthetic requirements. The biocompatibility is also reflected in the higher vitality and proliferation of fibroblasts on yttrium-stabilized zirconia compared to titanium. In this context, the surface´s exact composition and chemical nature of the surface are of main central and the subject of this study.

Methods
Polished Yttria-stabilized zirconia discs (ø = 12 mm, h = 1.5 mm) were activated by a cold atmospheric pressure plasma jet kINPen®09 (Neoplas Tools GmbH, Greifswald, Germany) as described in [1]. X-ray photoelectron spectroscopy (XPS) was conducted on a sample following plasma treatment (ZrO2+Ar) and one reference sample (ZrO2) on a K-Alpha XPS System (Thermo Fisher Scientific GmbH, Dreieich, Germany). The Al-Kα line at 1486.6 eV was used. Survey scans and detailed spectra of characteristic peaks (C1s, O1s, Si2p, Zr3d, Y3d) have been taken in area scans (3x3 point) and finally summed up. Measurements were performed twice on the same points to observe possible influences of the XPS.

Results and Discussion
XPS revealed differences between samples in the elements observed. The areas under the specific signals for silicon (Si) and carbon (C) decreased in ZrO2+Ar compared to ZrO2 and increased for yttrium (Y), zirconium (Zr), and oxygen (O). [1]
This finding indicates the oxidation of C and Si. Complementary, spectra of Y and Zr revealed chemical states at higher binding energies for ZrO2+Ar, indicating the formation of metal carbonates or silicates. The second measurement showed changes in signals for ZrO2+Ar. Spectra developed back, finally matching the control. Thus, XPS led to surface alteration, probably due to ablation processes.
The research was funded by the PROSEC gGmbH (Progress in Science and Education with Ceramics, grant contract no. 2020-02003). Financial support by the German Research Foundation (DFG) within the “Major Research Instrumentation” funding program (Project number: 441723937) is gratefully acknowledged.

References
[1] Staehlke, S.; Brief, J.; Senz, V.; Eickner, T.; Nebe, J.B. Optimized Gingiva Cell Behavior on Dental Zirconia as a Result of Atmospheric Argon Plasma Activation. Materials 2023, 16, 4203. https://doi.org/10.3390/ma1612420**.**

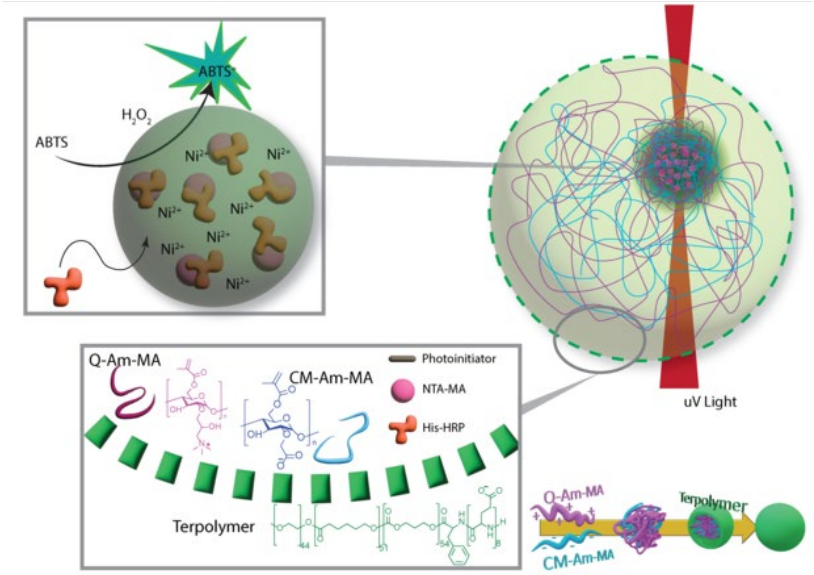
PoB.23.04

3D printing of crosslinked polymer organelles within artificial cells

Yiğitcan Sümbelli, Alexander B. Cook, Jan C.M. van Hest

Eindhoven University of Technology, Eindhoven, Netherlands

Abstract



Artificial tissue engineering is an emerging concept in the regenerative medicine field that combines synthetic cell engineering and tissue engineering, to achieve advanced degrees of spatiotemporal control over cellular behavior during the maturation period of engineered tissues. The deposition of synthetic cells along with native cells within an engineered tissue model is enhanced by the inclusion of cellular functions in the synthetic cells, such as signalling molecules and growth factor excretion. One approach to introduce functionality within synthetic cells is via hierarchical compartmentalization. Such subcellular organization can have multiple benefits including: spatial separation of incompatible components, protection of biomolecules from degradation, and local variation of concentrations. Engineering functional artificial cells containing multiple compartments within an engineered tissue along with native cells can lead to the development of hybrid tissues that offer a more reliable system for controlling cellular behavior in engineered tissues.

In this research, we develop a light-based 3D printing method to introduce artificial organelles within complex coacervate artificial cell droplets. These membrane-stabilized, liquid-liquid phase-separated droplets, are comprised of oppositely charged amylose polymers, methacrylated quaternized amine amylose (MA-Q-Am) and methacrylated carboxymethyl amylose (MA-Cm-Am), to create positively charged artificial cells containing a crowded subcellular environment. Additionally, the introduction of methacrylated-nitrilotriacetic acid (MA-NTA) to a targeted region of the artificial cell, via laser light irradiation, allows localized affinity-based binding of a model hexahistidine-tagged protein cargo. Local uptake of the cargo molecule is observed under confocal laser scanning microscopy. As a further demonstration of this model artificial organelle, functional hexahistidine-tagged horseradish peroxidase (HRP) is incorporated into these 3D printed organelles to show spatially specific conversion of ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) into its fluorescent cationic radical product.

PoB.23.06

DESIGN OF ORIENTED STRUCTURES FOR SKELETAL MUSCLE REGENERATION THROUGH NEAR-FIELD DIRECT-WRITING ELECTROSPINNING

Laura Armenio[1], Silvia Fare'[1,2], Lorenza Draghi[1,2]

[1]Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy. [2]National Interuniversity Consortium of Materials Science and Technology (INSTM), Florence, Italy

Abstract

Introduction: Thanks to the thinner filaments obtainable, near-field direct-writing electrospinning (NFES) can reach higher accuracy hence a more precise replication of ECM dimensions than other rapid prototyping techniques, but its use to process natural polymers for tissue engineering is still limited. This study aims at fabricating through NFES nature-derived polymeric scaffolds for skeletal muscle tissue engineering. **Methods:** A NFES setup (Figure 1) was assembled and validated with gelatin B (GelB) and Bovine Serum Albumin (BSA) 10:1 solution in 70% v/v acetic acid, depositing fibers in parallel arrays (20x20 mm^2) or circles (Ø=26 mm) and optimizing XY speed, electric field and, only for parallel arrays, fiber spacing. Then, multilayered parallel fiber arrays made by methacrylated gelatin (GelMA) and BSA in 70% v/v acetic acid were patterned with a fiber spacing of 330 µm onto GelMA/BSA hydrogels (Ø=6 mm, thickness=1 mm) for an easier manipulation of multilayered parallel fibers during biological evaluation, varying the total deposition iterations (300, 600, 900) and photo-crosslinked. Morphology of single fibers and multilayered samples was evaluated by SEM and mean fiber diameter and alignment degree were investigated by ImageJ.*In vitro* biological tests with C2C12 cells (cell density=20000 cells/cm^2) were conducted on patterned GelMA/BSA hydrogels. **Results:** The NFES setup allowed a high-fidelity collection of various fiber patterns with an easy XY motion programming. 22% w/v GelB/BSA concentration led to continuous fiber deposition in various environmental conditions. In both linear and curved trajectories, control over fiber deposition and straightness was reached at 8000 mm/min speed except for some coils observed in confined regions and submicrometric fibers were reproducibly obtained at an electric field of 1.5 kV/mm. In parallel arrays, spacing up to 500 µm provided submicrometric straight fibers. In patterned hydrogels (Figure 2), the highest alignment degree (~70%) was obtained at 300 iterations and 83% of fiber diameters was suitable for skeletal muscle regeneration (below 3 µm). C2C12 myoblast cells interaction with samples after 24 h demonstrated cellular viability and adhesion onto multilayered NFES fibrous patterns. **Conclusions**: A custom-assembled NFES system allowed to easily and precisely deposit oriented fibers with a reproducible morphology comparable to ECM and to design with nature-derived polymers oriented biocompatible architectures.

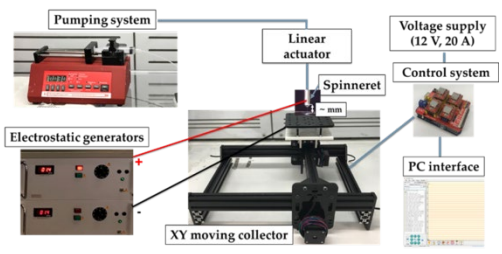


Figure 1: NFES setup scheme

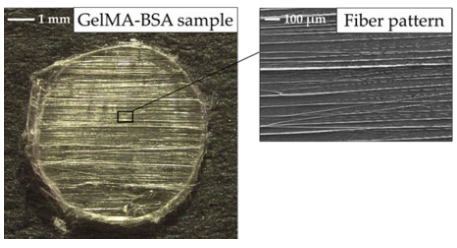


Figure 2: GelMA/BSA hydrogels patterned with multiple layers of GelMA/BSA fibers through NFES

PoB.23.07

pH Responsive Soft Actuator for Biomedical Applications

Pijush Ghosh, Amit Kumar, Nitish R Mahapatra

Indian Institute of Technology Madras, Chennai, India

Abstract

Hydrogel-based pH-responsive bilayer actuators exhibit bidirectional actuation due to the differences in the concentration gradient developed across the thickness, and the relative volume expansion due to swelling. Most of the pH-responsive bilayers with bidirectional actuation contain a particular pH value or a region across the pH spectrum at which they remain in a zero-curvature or unfolded state. We have termed this zero-curvature state as a 'non-morphing point.' Generally, pH-responsive actuators designed for targeted drug delivery show bidirectional (clockwise/anticlockwise) actuation around a single non-morphing point. The same pH-responsive system cannot be applied for drug release at another site with a different functioning pH. Thus, having a pH-responsive system with multiple non-morphing points is highly desirable. We developed a bilayer film of chitosan (CS) and carboxymethyl cellulose (CMC) crosslinked with green crosslinker citric acid (CA) with tunable non-morphing points across the pH spectrum by modulating the concentration and crosslinking density of the layers involved. The performed MTT assay on CS/CMC bilayers showed no cytotoxicity effect on the H9c2 cell line. The CS/CMC bilayer films actuate clockwise direction in acidic pH and an anticlockwise direction in basic pH. The actuation of these bilayers leads to permanently folded or irreversible structures. To attain the reversibility of these bilayers, we performed alternate acid-base treatment and ethanol treatment. We were able to attain reversibility 5 times using alternate acid-base treatment, whereas the actuated bilayer films were reversible 8 times when treated with ethanol. Drug release experiments were performed with FITC and EtBr as model drugs loaded in CS and CMC layers. Moreover, the clockwise/anticlockwise actuation of the bilayer around the non-morphing point can facilitate or inhibit the release of a drug. The clockwise actuation resulted in 55% FITC release and inhibited EtBr release to 4%; anticlockwise actuation resulted in 50% EtBr release and inhibited FITC release to 5%. We also demonstrated the possibility of mimicking nature-inspired actuation behavior from these bilayer systems by engineering different geometries over thin-film structures.

PoB.23.08

Designing the next generation of biomaterials through screening hybrid cell-microparticle spheroids

Maryam parvizifard, Francesca Giacomini, Pamela Habibović, Roman Truckenmüller, Zeinab Tahmasebi Birgani

Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands

Abstract

Introduction. In tissue engineering, biomaterial scaffolds help restore tissue functions by providing mechanical support, mimicking extracellular matrix (ECM), and instructing or maintaining cell differentiation[1]. Tailoring the physicochemical and mechanical properties of mesoscale scaffolds necessitates the time-consuming and costly exploration of numerous biomaterial formulations. Therefore, our group has recently suggested cell-microparticle spheroids as an alternative three-dimensional, miniaturized model for early-stage assessment of biomaterials[2]. We aim to demonstrate the potential of this model combined with a design-of-experiment (DoE) approach, as a smart screening tool to design tailored biomaterials. To that end, we prepared a library of microparticles from three model biomaterials often employed in bone regeneration, including gelatin (GEL), poly(lactic-co-glycolic acid) (PLGA), and hydroxyapatite (HA), with different particle sizes and concentrations, to be then screened for their osteogenic properties in the cell-microparticle spheroids. **Materials and methods.** GEL microparticles were purchased. PLGA microparticles were fabricated in an oil-in-water single emulsion and oxygen-plasma treated. Similar oil-in-water emulsion method followed by sintering at 1100 °C was used to fabricate HA microparticles. Microparticles were co-seeded with human mesenchymal stem cells (hMSCs) in non-adherent in-house-made microwells. Spheroid formation and hMSCs' metabolic activity were assessed. A DoE method was used to determine the minimum essential testing formulations made by combining three microparticles with two sizes at five concentrations to be screened. **Results and discussion.** Small and large PLGA, HA, and GEL microparticles had average diameters of 53.6 and 89.7, 57.3 and 94.2, and 42.1 and 81.0 µm, respectively. In the hybrid spheroids, the metabolic activity increased over time, with no significant difference between similar biomaterials with different sizes (Figure 1). To cover the design space, the DoE method defined a screening with 38 microparticle formulations as the next step. **Conclusion.** We prepared a set of microparticles, tested their biocompatibility in hMSC spheroids and defined their minimum essential screening formulations. We will next screen these formulations for their osteogenic potential to demonstrate the potential of the hybrid cell-microparticle spheroids as a screening tool for designing tailored biomaterials.

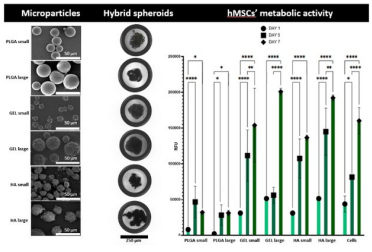


*Figure 1. Microscopy images of microparticles and hybrid hMSC-microparticle spheroids, and hMSC's metabolic activity in spheroids over time (*P<0.01,**P<0.001 and ****P<0.0001).*

Acknowledgment: This research was supported by the Materials-Driven Regeneration Gravitation Program of the Dutch Research Council and the Dutch Province of Limburg.

References:

1. Thakuri et al. Advanced Healthcare Materials 7, 2018. 2. Fois et al. Small 18, 2022.

PoB.23.09

Tailoring Mechanical Properties for 3D-printable Hydrogels by Implementing a Statistical Modelling Approach

Giulia Maria Di Gravina, Ferdinando Auricchio, Michele Conti

University of Pavia, Pavia, Italy

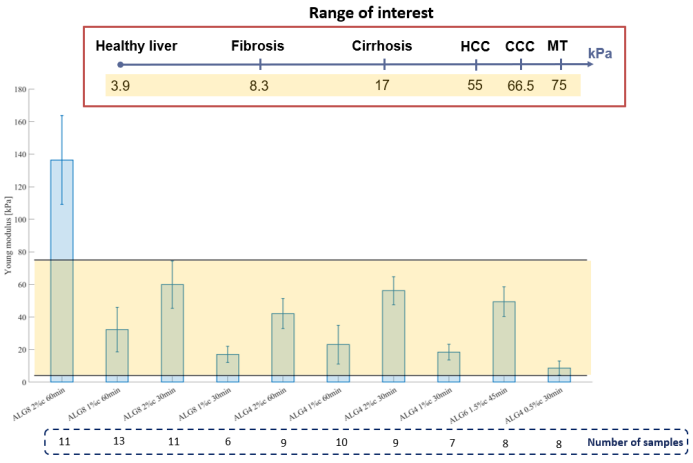
Abstract

Extracellular matrix (ECM) stiffness is recognized to have a role in influencing cell behaviour, both in physiological and pathological tissue conditions. During disease progression, the ECM is dysregulated and reorganized, with an increasing overproduction and deposition of molecules, leading to progressive tissue stiffening over time and an alteration of mechano-sensing pathways for cells. Tissue engineering offers different approaches for studying disease progression, e.g., biomaterial-based platforms. Literature offered several examples of biomaterials that can be tailored in terms of stiffness and viscoelasticity, going to mimic the spectrum of biomechanical properties shown by disease tissues at their different stages. However, in most cases, the tuning is performed through a trial-and-error method, without a full understanding and control of the variables at play.

Here we propose an empirical mathematical approach to predict and replicate precise stiffness values. As a proof-of-concept, we used an alginate-based hydrogel and we modulated its Young modulus by playing with three factors: alginate concentration (x_1), $CaCl_2$ concentration (x_2), and crosslinking time (x_3). We chose as an example of application the liver, which can be subject to a degenerative process characterized by tissue stiffness ranging from 4 kPa (physiological condition) to 17 kPa (cirrhosis condition), up to 75 kPa (metastatic tumour condition).

The workflow was organized in the following way. First, we explored the stiffness resulting from different combinations of x_1, x_2, and x_3 through uniaxial compression mechanical tests, with the goal to cover the stiffness range of interest (Figure) and to define the levels of each factor (Table), that is necessary for the second phase, based on 2^k factorial design with a central point. This allowed us to assess that all the input factors and their interactions have a significant effect on the output, and for this reason to keep all the variables for the final phase. Here, we defined a second-order model by using response surface methodology. Finally, the model was validated by reproducing the three condition stages.

The next steps will consist of biological tests, to correlate cell behaviour with hydrogel stiffness, and printing characterization, to obtain, in the end, a 3D-printed hydrogel-based *in-vitro* model with mechanical properties that can be tuned in a controlled way.



Range of interest vs. mechanical characterization results

Levels for each factor

Input factor	**Levels coded**		
	-1	**0**	**+1**
Alginate concentration (mg/mL), x_1	4	6	8
$CaCl_2$ concentration (mg/mL), x_2	0.5	1.5	2.5
Crosslinking time (min), x_3	30	45	60

PoB.23.10

Non-invasive porosity measurement of commonly used biomaterials by nuclear magnetic resonance

Sylwester Domański[1], Mateusz Urbańczyk[2], Agnieszka Zakrzewska[1], Marta Klak[1], Michał Wszoła[1]

[1]Polbionica, Warsaw, Poland. [2]Institute of Physical Chemistry, PAS, Warsaw, Poland

Abstract

Main aim of conducted research is application of non-invasive, accelerated measurement of restricted diffusion by nuclear magnetic resonance (NMR) for estimation of soft materials (GELMA, GELGMA) porosity.
Porosity is one of the main factor taken into account during the design and synthesis of a material established for biomedical and 3D bioprinting application. The most widely used methodology for pore size measurement of biopolymers is electron microscopy for instance SEM or TEM. Both of them demand long and invasive sample preparation method what do not allow conduct of porosity measurement for sample in native (non-dehydrated) form. Recently, nuclear magnetic resonance (NMR) diffusion experiments have been widely exploited in the studies of structures and fluid transport properties of porous materials. The applications of the method include rocks, glass beads, wood, ionic liquids, cells, sol–gel-made silica particles, polymers, aerogels, zeolites, metal organic frameworks, etc. In presented research this methodology was successfully applied to porosity measurement of two different type of biomaterial GELMA and GELGMA in the very first time.
Conducted studies encompassed influence of numerous factors on porosity of widely used bioinks such as: a) type of biomaterial, b) concertation of pre-polymer, c) time of sample irradiation by UV-Vis light, and d) photoinitiator concentration. Furthermore, data obtained for this non-invasive methodology was successfully corelated with data from SEM measurements and biological properties of corresponding samples. Collected data revealed high correlation between pores size and biomaterial physicochemical properties and proved measurement of restricted diffusion by nuclear magnetic resonance being a powerful tool for establishing pore size and level of pore connection in native material without destroying sample.

PoB.23.11

pH sensitive halloysite-cyclodextrin coated materials for intestinal targeting

Adi Ghebaur, Sorina Alexandra Garea

University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, Romania

Abstract

Inflammatory Bowel Diseases (IBD) like Crohn disease (CD) and Ulcerative Colitis (UC) are inflammatory disorders which affect the entire gastrointestinal tract and the colon respectively characterized by an immune reaction of the body against its own gastrointestinal tract. These diseases have a huge impact on life quality of patients due to its long term consequences, affecting people professional carrier and social life [1]. The encapsulation of mesalazine within the clay tubes (HNT) once they are functionalized with methacrylate polymerizable moieties by silanization reactions with different agents targets the drug at colon level. The attached methacrylic functional groups exhibit the ability to inhibit the release of the active substances within the media with acid or neutral pH value [2]. Taking into account the tubular structure with high porosity of HNT, the using of this clay as drug host represent an important advantage related to the capacity of drug encapsulation [3]. However the drawback related to the mesalazine encapsulation through the physical adsorption process can lead to an objectionable quick release in the colon. In order to realize the coupling of the β-CDX supramolecular structure at the surface of the HNT a chemical modification of the polysaccharide is required also. On this line chemical reaction for grafting of different methacrylate agents on the β-CDX structure will be performed. In the next step, a photopolymerization processes under VIS light is performed to react the methacrylated HNT and β-CDX respectively and the clay is coated. The modified β-CDX chemically bounded with methacrylated HNT will also be loaded with mesalazine by ticking of an immediate therapeutic effect of the drug in the affected area.

Short Biography:

1. A.R. Ciurezu, L.D. Lupu, D. Olteanu, Rev. Med. Romana, 2009, LVI
2. M.Z. Khan, Z. Prebeg, N. Kurjakovic, J Control Release, 1999, 58 (2), 215-222
3. Y. Lvov, W. Wang, L. Zhang, R. Fakhrullin, Adv Mater, 2016, 28 (6), 1227-1250

Acknowledgement

Executive Agency for Higher Education and Research Funding (UEFISCDI) and National Research Council (CNCS) are gratefully acknowledged for the financial support through the PN III research project 'New technology for pH sensitive hybrid materials based on halloysite and cyclodextrin for Inflammatory Bowel Diseases treatment' no. 604PED/2022.

PoB.23.12

Multifunctional Bioinks for a 3D printed Pulmonary Artery Model

Uxue Aizarna - Lopetegui[1], Clara García - Astrain[1,2], Luis Liz-Marzán[1,2,3], Malou Henriksen-Lacey[1,2], Dorleta Jiménez de Aberasturi[1,2,3]

[1]CIC biomaGUNE, Donostia, Spain. [2]Centro de Investigación Biomédica en Red de Bioingeniería Biomateriales, y Nanomedicina, Donostia, Spain. [3]Ikerbasque Basque Foundation for Science, Bilbao, Spain

Abstract

The combination of biomaterials with living cells and stimuli-responsive materials can be exploited to generate 3D printable bioinks for the fabrication of complex *in vitro* models which mimic the natural tissue microenvironment. With the aim of aiding our understanding of the underlying mechanisms of native tissue functions and pathophysiology, we have developed a pulmonary artery model that recreates the physical forces to which cells are exposed to during arterial pulsation. Hybrid multifunctional formulations have been developed including a stimuli-responsive polymeric ink that mimics arterial wall, and an extracellular matrix-derived bioink for the more internal artery wall layer fabrication. The hybrid biofunctional layer provides multifunctionality including responsiveness, physical contractile and expansion changes, mimicking the physical changes to which arterial cells are exposed during the pulsatile blood flow process. The living bioinks have been composed of human pulmonary artery smooth muscle cells embedded in a decellularized extracellular matrix-based formulation and human arteria-derived endothelial cells suspended in cell media, which have been proven to be biocompatible and provide a suitable environment for cell growth. All inks have been characterized in terms of chemical and mechanical properties, printability, homogeneity, and biocompatibility, after which 3D bioprinting has been used to build the vascular tissue model. The suitability of 3D printing for processing hybrid and diverse multicomponent materials for the fabrication of an arterial 3D model, resembling native tissues and microenvironments, has been verified. Moreover, we have observed that the application of cyclic stimuli to the model resulted in the upregulation of genes known to be associated with the endothelial-to-mesenchymal transition (EndoMT), proving the biological interest of the model.

PoB.23.13

NIR-fluorescence image guided surgery for ceramic fracture removal in hip revision surgery

Robert Nißler[1,2], Elena Totter[3,2], Sebastian Gottfried Walter[4], Dimitri Nowack[5], Alexander Gogogs[1,2], Inge Katrin Herrmann[6,2]

[1]Nanoparticle Systems Engineering Laboratory, ETH Zurich, Zürich, Switzerland. [2]Particles-Biology Interactions, Department of Materials Meet Life, Swiss Federal Laboratories for Materials Science and Technology (Empa), St. Gallen, Switzerland. [3]Nanoparticle Systems Engineering Laboratory,ETH Zurich, Zürich, Switzerland. [4]University Hospital Cologne, Department of Orthopedics, Traumatology and Reconstructive Surgery, Köln, Germany. [5]Deutsche Zentrum für Orthopädie, Department of Orthopedics and Trauma Surgery, Eisenberg, Germany. [6]Nanoparticle Systems Engineering Laboratory, ETH Zurich, Zurich, Switzerland

Abstract

Hip arthroplasty is a safe and highly standardized treatment of symptomatic and progressed osteoarthritis and is therefore entitled as 'operation of the 20th century'. Due to demographic changes, about 635,000 arthroplasties are estimated to be performed solely in the USA in 2030. With the numbers of primary implantations on the rise, the total number of complications and implant loosening increase alongside, resulting in increased total demand for revision surgeries. A major percentage of implants are installed with a ceramic head due to its favorable strength and low abrasion. However, up to 0.05% of all implanted ceramics will break within their lifetime, creating a major orthopedic problem requiring ceramic fragment removal. This process can be challenging as the complete removal of especially small fragments is limited due to the difficult visualization. Here, we introduce for the first time a NIR-fluorescence based image guided surgery system for ceramic implant fragment removal. We present characterization data obtained by chemical and optical analytics for the most common ceramic implant types and demonstrate that indeed, fragments can be readily visualized in a label-free manner. This technique makes detection of small fragments in the millimeter range possible, even at several millimeters of tissue depth. The presented label-free optical imaging method can be translated into clinics in a straightforward manner, using low-cost equipment thus rendering the removal of fractured ceramic implants considerably safer and more efficient.

PoB.23.14

Comparative studies of different cell types under the influence of hypothermia as a basis for optimizing biomedical applications

Jana Markhoff[1], Jacqueline Hofrichter[2], Steffen Mitzner[2], Niels Grabow[1]

[1]Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany. [2]Fraunhofer Institute for Cell Therapy and Immunology, Department of Extracorporeal Therapy Systems, Rostock, Germany

Abstract

Changed temperature conditions due to hyper- or hypothermia can cause functional effects in biological systems. A drop in core body temperature (normally approx. 36.9°C) below 35°C is defined as hypothermia (HT), categorized into endogenous, induced and accidental HT, according to the cause. Depending on the reduction in temperature, a gradation into mild, moderate or severe hypothermia is classified. A slowed metabolism and disturbance of both, oxidative processes and the acid-base balance occur as a result of reduced temperature. Increased wound infection, inhibited wound healing and altered immune modulation are documented, as well as impaired bone remodeling.
Tissue-specific cell types, human coronary artery smooth muscle cells (HCASMCs) and human cardiac microvascular endothelial cells (HCMECs) were investigated with regard to their reaction to different temperature gradations for selected biomedical applications and potentially age-related and perioperative hypothermia. In the course of this, different exposure periods (24h, 72h, 6d) were compared, as well as the influence of immediate temperature change versus gradual temperature change to 28/34/37/40°C. Against the background of visceral surgical operations, cardiopulmonary bypass grafting, as well as vascular stenting, endothelial cells were included in the investigations. Possible changes in cell viability and morphology were assessed. The extent to which reduced temperature conditions have a cytotoxic effect and have effects on oxidative stress levels and inflammatory behavior, as well as cell apoptosis and cell cycle, were also evaluated. In addition, an impedance-based measuring method allowed conclusions to be drawn about altered proliferation and adhesion behavior.
In the literature, the effects of hypothermia on cells are not homogenous and varying in culture duration, temperature levels, cell types and cell stimulation. In this work, the influence of the selected hypothermia levels on HCASMCs and HCMECs was mainly shown for metabolic cell activity resulting in increased cell activity in dependency to increasing temperatures. Thereby HCMECs seemed to be more sensitive especially to lower temperature. Cell density was reduced at lower temperatures in a cell-dependent manner without alterations in cell morphology. Only a low temperature- and time-dependent influence on the production of reactive oxygen species and cell cycle stages was revealed without clear tendencies. Furthermore, there was no evidence of increased cytotoxicity or apoptotic processes. The evaluated levels of inflammatory markers were at the lower detection limit for both cell types, with only slight synthesis of IL-6 in HCASMCs for all temperatures.

PoB.23.15

Exploring biomaterials towards battery-free medical electronics

Joana O. Gomes[1,2], Cátia R. S. Rodrigues[3], Ana C. Silva[3], João O. Ventura[3], Inês C. Gonçalves[1,2], André M Pereira[3], Andreia T Pereira[1,2]

[1]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal. [2]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. [3]IFIMUP – Instituto de Física de Materiais Avançados, Nanotecnologias e Fotónica, Departamento de Física e Astronomia, Faculdade de Ciências,, Porto, Portugal

Abstract

Internet of Medical Things (IoMT), which uses biosensors to exchange data between medical devices and users, are the next advance in the biomedical field. Also, pacemakers, left-ventricular assist devices, and brain stimulators are used to treat diseases. Batteries are required to power them and have limitations, including the need for replacement, size/weight, and the risk of toxic content leaking[1]. Thus, there is a demand for a biocompatible, endless energy source. Triboelectric nanogenerators (TENG) can convert mechanical energy into electricity by contact-separation or sliding between 2 materials, allowing energy scavenging from body movement and muscle contraction/relaxation. However, most TENGs are cytotoxic and thrombogenic. This study bypasses these barriers by proposing the use of 2 hemo/biocompatible FDA-approved materials, poly(2-hydroxyethyl methacrylate)(pHEMA)[2] and extended-polytetrafluoroethylene (e-PTFE).

In contact-separation mode, pHEMA generates 100V, 4.7µA, and 0.68W/m2, using polytetrafluoroethylene (PTFE) as a reference. Our findings reveal that graphene oxide(GO) can be used to tune pHEMA's triboelectric properties in a concentration-dependent manner. At the lowest measured concentration (0.2% GO), the generated outputs rise to 194.5V, 5.3µA, and 1.28W/m2 due to the increase in pHEMA's surface roughness. Triboelectric performance decline as GO concentration rises, plateauing at 11%, where outputs are 51V, 1.76µA, and 0.17W/m2 lower than pHEMA's. Increases in resistance from 14W-470WM, zeta-potential from -7.3 to -0.4mV, and charge decay periods from 90-120 ms within the increase in GO's concentration, point to GO's function as an electron trapping site, which decreases the triboelectric output. All materials charged a 10mF capacitor in 200s and turned on 20 LEDs. The triboelectric output was steady throughout 3-hour durability test.

A novel configuration of TENGs was established to assess the triboelectric features of ePTFE. 3 electrode types were investigated, and it was revealed that silver and carbon electrodes were stable, while PEDOT:PSS was deteached from TENG in the presence of fluids. Using a silver electrode, a 100nF capacitor was charged, resulting in a voltage of 23.6V and the lighting of 1 LED.

All the TENGs configurations, based on pHEMA and ePTFE, were cytocompatible towards fibroblasts (HFF-1).

Our findings demonstrated that TENG-based in FDA-approved biomaterials could be used to power an electrical medical device by harvesting energy from body motion.

Acknowledgements:SFRH/BD/147811/2019,2021.01807.CEECIND,2022.05030.PTDC, UID/NAN/50024/2019,Maria de Sousa award(53/2021).

References: 1-Q. Zheng, et al Nature Reviews Cardiology 18(1)(2021) 7-21. | 2-A. T. Pereira,et al H. Bergmeister; I. C. Gonçalves, Biomaterials Science 9(2021)3362-3377

PoB.23.16

Innovative Carbon Black/P(3HB) composites: Promising materials for bone tissue engineering

Mahendra P Raut, Andrea Mele, Nicholas Farr, Annabelle Fricker, David Gregory, Cornelia Rodenburg, Ipsita Roy

Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, United Kingdom

Abstract

Currently, bone disorders and associated conditions are rising rapidly worldwide. Conventional treatment methods such as allografts, autografts, and xenografts, are being employed to restore bone functions but are not always successful due to several limitations. To combat these limitations of existing treatment methods, scaffold-based bone tissue engineering strategies are now emerging as a promising alternative, aiming to generate biocompatible and biodegradable biocomposite scaffolds with osteoconductive or osteogenic properties to restore normal bone functions. Poly(3-hydroxybutyrate), P(3HB), is a polyhydroxyalkanoate (PHA) biopolymer, emerging as an outstanding biomaterial for bone tissue engineering due to its high Young's modulus, high tensile strength, and osteogenic properties.
This study aims to improve further the mechanical strength of P(3HB)/CM by combining them with various carbon materials (CM) in composite scaffolds, P(3HB) and evaluate their *in-vitro* biocompatibility with the human osteoblast cell line (MG-63), considering their potential use in bone tissue engineering. Carbon materials can add additional features to composite scaffolds such as a large surface/volume ratio, good biocompatibility, controlled biodegradability, and chemical stability.
In this study, we successfully scaled up P(3HB) production to 30L pilot-scale capacity using B. subtilis, polymer extracted, purified, and used for the preparation of the P(3HB)/CM composite scaffolds. The P(3HB)/CM composite scaffolds were characterised by tensile testing, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and surface analysis by Scanning electron microscopy (SEM). Human osteoblast (MG-63) cells were seeded on various P(3HB)/CM composite materials and assessed for cell biocompatibility using live/dead cell assay and resazurin-based cell viability assay. The overall results showed improved thermomechanical and structural properties of the composite scaffolds. Neat P(3HB) and P(3HB)/inkjet composites were observed to be the best performing composites with respect to cell growth and proliferation, indicating that Ink-jet carbon as a filler could be a promising strategy for the development of sustainable biomaterials for bone tissue engineering.

PoB.23.17

Simple fabrication of a conductive bilayer film with a cellulose nanoparticle composite suspension

Ji Un Kang, Jiho Hyun

Seoul National University, Seoul, Korea, Republic of

Abstract

The conductive bilayer film can be applicable in bioelectronics including transfer printing, energy harvesting and soft wearable devices in the future. Here, we described the fabrication of a bilayer film with carboxymethylated cellulose nanofiber (CM-CNF) and liquid metal (LM). By evaporating a composite suspension containing CM-CNF and LM, an LM@CM-CNF bilayer film could be fabricated. Interestingly, it consisted of an LM rich conductive layer and CM-CNF rich nonconductive layer, which would be a unique structure to be applicable for the soft bioelectronics. Meanwhile, the sedimentation of heavy particles could be a critical issue in the fabrication of a 3D structure with the suspension. It can be a problem in the most of fabrication processes. In contrast, we took an advantage of the sedimentation of LM droplets to fabricate a bilayered material system having a LM rich region in the LM@CM-CNF film. The thickness of an LM rich layer of the LM@CM-CNF film could be easily controlled by adjusting the sedimentation rate of LM droplets. To control the sedimentation rate of LM droplets in the CM-CNF suspension, the concentration of CM-CNF and the size of LM droplets were considered. The increase of CM-CNF concentration decreased the sedimentation rate of LM droplets resulting in the reduced thickness of an LM rich layer. But, the increase of the LM droplet size accelerated its sedimentation resulting in a thicker LM-rich layer of the LM@CM-CNF film. Furthermore, the conductive properties of a composite film were optimized by the sedimentation rate of conductive particles.

PoB.23.18

Light-induced nanoscale deformation in azobenzene thin film triggers rapid intracellular Ca^{2+} increase via mechanosensitive cation channels

Heidi Peussa, Chiara Fedele, Huy Tran, Elina Mäntylä, Arri Priimägi, Soile Nymark, Teemu O. Ihalainen

Tampere University, Tampere, Finland

Abstract

Cells in our bodies are constantly subjected to mechanical stimuli such as stretch, compression, osmotic stress, and shear. The mechanical information cells receive from their physical environment co-regulates their form and functions, thus allowing cells to adapt to their niche. The physical cues rising from the environment are often locally sensed by specific and highly dynamic protein assemblies at the cell membrane, e.g., in integrin-rich focal adhesions or by large mechanosensitive (MS) ion channel complexes.

Calcium-mediating MS ion channels, such as Piezo1 and the Transient Receptor Potential (TRP) channels, are particularly interesting for local mechanosensing due to the dual function of calcium. In addition to affecting the electrical potential difference of the cell membrane, calcium also acts as a universal second messenger, participating in numerous cellular signaling pathways, such as contraction, proliferation, secretion, vesicle trafficking, protein synthesis, and apoptosis. Furthermore, Piezo1 function has been linked to cell-cell junctions, focal adhesions and the actin cortex, making the mechanically induced calcium signaling even more complex. However, studies of these fast cellular mechanosensitive responses have proven to be difficult, partly due to the lack of proper tools to locally manipulate the cells mechanically.

Light responsive materials offer an interesting approach to study how local mechanical perturbation affects cells. Azobenzene-containing light-controllable materials have been recently proposed as smart biointerfaces, as their topography can be precisely manipulated via visible light. Photopatterning such materials with different sinusoidal microtopographies has shown to be very effective in driving cellular morphology and migration.

Herein, we exploit the dynamical properties of a light responsive azobenzene-based molecular glass (disperse red 1 molecular glass – DR1-glass) as a dynamic cell culturing substrate for the mechanically activated calcium dynamics in an epithelial monolayer. We first optimized the light-induced formation of topographical features by tuning irradiation parameters of a common confocal microscope. We show that local nanoscale material deformation and deformation dynamics cause strong calcium transients in the cells. Based on pharmaceutical experiments and a Piezo1 knockout cell line, we propose that Piezo1 channels, TRPV4-channels, the actin cytoskeleton as well as E-cadherins are involved in sensing this deformation. Finally, the flexibility of this approach helped reveal that cells respond distinctively to direct mechanical stimuli and to signals rising from neighboring cells, thus highlighting the role of cell-to-cell communication in the tissues' complex mechanoresponses.

PoB.23.19

Molecular Orientation and Optimization of Membrane Dyes Based on Conjugated Oligoelectrolytes

Ji-Yu Zhu, Guillermo Carlos Bazan

National University of Singapore, Singapore, Singapore

Abstract

Conjugated oligoelectrolytes (COEs) are amphiphilic, optically active, and fluorogenic molecules that spontaneously associate with lipid bilayer membranes. As such, COEs are gaining attention as molecular reporters, particularly in the context of exosome detection by conventional techniques, such as flow cell cytometry. Questions nonetheless remain on how to best design COEs for optimal performance and on the geometry of lipid bilayer intercalation. In response, we provide a series of oligo-phenylenevinylene based COEs with varying lengths and number of charged groups that can be used to address the uncertainties above. Examination of the organization within lipid bilayers through polarized fluorescence microscopy shows that the optical transition moments are perpendicular to the bilayer plane. Molecules thus span the bilayer with the conjugated segment flanked by the hydrophobic phospholipid tails. COEs initially form a less organized layer on the vesicle periphery, most reasonably reflecting electrostatic association prior to intercalation. Emission in the lipid bilayer, relative to the solvated state, is more pronounced with increasing conjugated segment length. Uptake experiments show that longer dimensions and increased numbers of charges allow for a higher degree of association with cells. The shorter the core length, the faster the rate needed to achieve saturation of emission. The number of charges also accelerates reaching the final state.

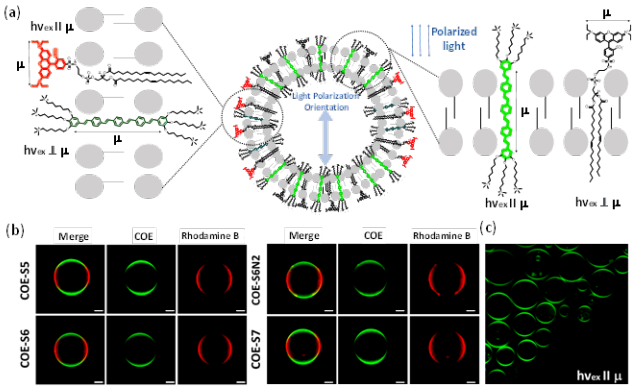


Figure 1. (a) Cartoon illustration of model giant unilamellar vesicles (GUVs) incorporating COEs and Rhodamine B-labeled lipid depicting their preferred relative molecular orientations. (b) Fluorescence polarization microscopy imaging of a single GUV stained with 1 µM of each COE and Rhodamine B (1 mol %). Scale bar: 10 µm. (c) Fluorescence polarization microscopy imaging of multiple GUVs stained with 1 µM COE-S6.

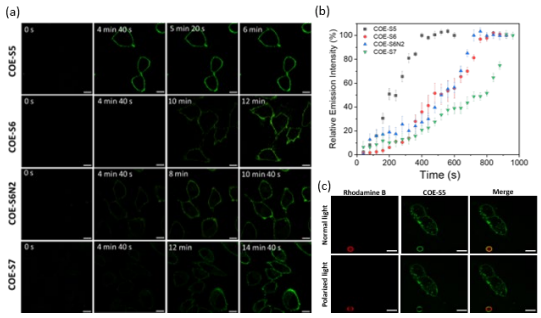
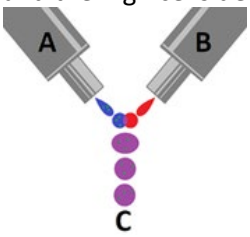
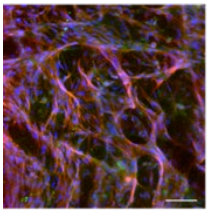


Figure 2. (a) Confocal imaging of A549 cells stained with 1 µM COE molecules for the "light up" mechanism. (b) Dependence of time on membrane intercalation of COEs. Relative emission intensities were taken from confocal images of A549 cells stained with 1 µ M COEs. (c) Normal fluorescence microscopy imaging (top) and polarized fluorescence microscopy imaging (bottom) of GUV prestained with Rhodamine B-lipid and A549 cells followed by stained by 1 µM COE-S5. Scale bar:10 µm.

In order to demonstrate the flexibility of the approach we have printed cell-filled-gels at high cell densities onto both fibre [2] and bioceramic porous substrates. The bio-ink used was a collagen-alginate-fibrin gel which was developed at Newcastle [3]. Mesenchymal stromal cells, cardiomyocytes and fibroblasts have all been printed at cell densities of up to 40 million cells per mL of gel.
In all cases the cell viability is high. Cells are initially within the CAF gel, so that the cells do not wash off, and the high cells densities encourage the cells to migrate into the porous substrates (Figure 2).



ReJI Bioprinting Process. Microvalves at A and B produce droplet streams of polymer solution and crosslinking solution. When droplets collide they react to form gel droplets which are deposited onto the substrate at C.



Confocal image of Neo-NHDF cells in a hydrogel printed onto a Calcium-alginate fibre mesh at day 3 of culture and a 30 M cells/ml print density. Blue staining indicates cell nuclei, red F-actin and green vinculin. Darker areas are the fibres. Scale bar: 100 µm

The ReJI bioprinting system offers a new approach to in situ cell seeding which offers potential for in clinic cell seeding, as the immediate handle-ability means that there is no need to wait for cells to attach, as would be the case for cells seeded in media, and the high cell densities which are achievable boost bioactivity.

1. Ribeiro, R et al. Biofabrication, 2018. doi: 10.1088/1758-5090/aaf625.
2. Kotlarz, M et al. Bio-design and Manufacture, 2022. doi: 10.1007/s42242-022-00192-5.
3. Montalbano G et al. Materials Science & Engineering: C, 2018. doi: 10.1016/j.msec.2018.04.101.