AW1

Engineering living tissues with organized (micro) fiber scaffolds

Miguel Castilho

Eindhoven, Eindhoven, Netherlands

Abstract

The human body is constituted by elegant tissue structures optimally adapted through millions of years of evolution to their function in the body. While most mature tissues possess an ability to self-repair after minor damage, this regenerative capacity diminishes when faced with severe injury or disease. Recent years have witnessed remarkable strides in regenerative medicine, striving to develop functional human body parts in laboratories. Yet, the outcomes of clinical investigations often fall short of expectations. One of the reasons for this is that although man-made fabrication processes succeed on copy tissue and organs layouts, they cannot resemble the elegantly orchestrated (bottom-up) self-assembly of tissues, resulting in dysfunctional constructs. In this way, our lab research is focused on advancing living tissue manufacturing by developing bottom up self-assembly of materials and cutting-edge, high-resolution 3D(bio) printing, as well as, innovate combinations thereof. In particular, in this presentation, I will discuss our work on organized (micro) fiber-based scaffold structures for engineering functional living tissues. These structures can instruct cellular development, enabling the creating of living constructs that are able to imitate complex tissue functions. Specifically, fiber-scaffolds that guide cells in mimicking tissue functions like contractility (in cardiac and skeletal muscle), filtration and removal of waste products from the blood (in kidney) and load-bearing (in bone and articular cartilage). Additionally, I will also show how such fiber scaffolds can be used to create advanced tissue models that simulate chronic blood disorders like myelofibrosis (in bone marrow), facilitating the development of predictive drug testing models. We have found that rational design and engineering of scaffold microarchitecture, morphology and bioactive moieties binding affinity offers a promising approach to support and steer cell behavior. This enables the formation of functional extracellular matrix (ECM) that can (potentially) last a life-time and imitate complex tissue functions. During the presentation, I will also showcase examples of fiber scaffolds fabrication using various naturally-derived polymers, such as collagen and silk fibroin, by combining bottom up self-assembly of materials with 3D printing and (bio)fabrication technologies. This yield structures that approximate the hierarchical features inherent in naturally-produced fibers, a feat that has proven elusive until now and is essential for their optimal functionality.

AW2

Three decades of research on calcium phosphates for bone regeneration

Marc Bohner

RMS Foundation, Bettlach, Switzerland

Abstract

The George Winter Award lecture gives me the opportunity to reflect on my career as a researcher in the biomaterials field. After a short historical introduction, I will explain how I ended up working on calcium phosphates in the biomaterials field. I will show that it had a lot to do with chance. I will then describe four scientific interests in the past 33 years: calcium phosphate cements (CPCs), cements for bone augmentation, the link between chemistry, structure, and bone regeneration, and finally osteoinduction. Being one of the first researchers in the field of CPC and bone augmentation gave me a lot of freedom, opened many doors, and the resulting scientific work created scientific and commercial impact. Regarding the link between chemistry, structure, and bone regeneration, my co-authors and I claimed that the macroporous architecture of a β-tricalcium phosphate scaffold had no effect on bone ingrowth and that woven bone invaded interconnected pores with interconnection sizes close to 1 micrometer. These two findings disagree fundamentally with what the scientific community generally "believes": 50 micrometers and 500 micrometers are considered to be the minimum size and the optimum pore size for bone ingrowth, respectively. Regarding osteoinduction, we proposed that the uptake of calcium is essential to modulate an immune reaction towards the differentiation of stem cells into the osteogenic lineage. Generally, the release of calcium is considered to be important. How easy is it to propose fairly new findings / mechanisms in old research topics like bone ingrowth and osteoinduction? How easy is it to be critical towards the theories of well-established researchers? At the end of my presentation, I would like to briefly discuss my services to the community: as editor of Acta Biomaterialia, as councilor for the Swiss National Science Foundation, as reviewer of scientific journals and as active member of scientific societies, in particular ESB. I will conclude by thanking those who have contributed to make me the scientist I am.

AW3

Composite Polymers for Bone Tissue Engineering: Why and how to stimulate vascularization and innervation of the bone tissue?

Joëlle Amédée Vilamitjana

University of Bordeaux, Bordeaux, France

Abstract

The effective reconstruction of large bone segments remains a major unsolved problem in the clinical field, evident in cases of severe trauma, cancer treatment and reconstructive surgery. Tissue engineered approaches have mainly focused on the stimulation of osteoconductive properties of the bone substitutes, as main parameter for bone repair. These have been shown insufficient to achieve efficient bone repair, especially when the vasculature and the nerve fibres of the host bed tissue, are damaged after tumour resection and/or radiotherapy.
The importance of vascularization in bone repair has developed a robust body of literature and been considered to play a pivotal role in new bone formation. However, besides our solid knowledge on the coupling between osteogenesis and angiogenesis, and conscious of the complexity of the bone tissue, from few years, we are widening to new and innovative horizons towards other key players in bone regeneration, especially nerve fibres.
In such context, we have first developed different co-cultures models, both in 2-D (2D) and then in three (3D) dimensional structures, to dissect the biological mechanism of how sensory neurons can support osteogenesis and angiogenesis. Cell to cell communication between mesenchymal stem cells, endothelial cells and / or sensory neurons were first investigated before designing composite polymers able to favour their anchorage and respective function. In vivo analysis of the nature of the tissue after implantation in different preclinical models were used for validation of the efficiency of these scaffolds.

AW4

Biomaterials for Tissue Engineering

Antonios G. Mikos

Rice University, Houston, USA

Abstract

Advances in biology, materials science, chemical engineering, computer science, and other fields have allowed for the development of tissue engineering, an interdisciplinary convergence science. Our laboratory has focused on the development and characterization of biomaterials-based strategies for the regeneration of human tissues with the goal of improving healthcare outcomes. In a collaborative effort with physicians, surgeons, and other scientists, we have produced new material compositions and three-dimensional scaffolds, and investigated combinations of biomaterials with cell populations and bioactive agents for their ability to induce tissue formation and regeneration. We have examined the effects of material characteristics, such as mechanical properties, topographical features, and functional groups, on cell behavior and tissue guidance, and leveraged biomaterials as drug delivery vehicles to release growth factors and other signals with spatial and temporal specificity. This presentation will review recent examples of diverse biomaterials-based approaches for regenerative medicine applications and highlight emerging areas of growth.

PL1

Printing and Self-assembly of Designer Porous Materials

André R Studart

ETH Zurich, Zurich, Switzerland

Abstract

Porous materials are attractive structures for drug delivery and regenerative medicine due to their high surface area and ability to support tissue growth. In biological materials, such as bone, marine sponges and wood, Nature exploits porosity to create structures combining high mechanical efficiency, controlled permeability and tunable growth. In contrast, synthetic porous materials have yet to reach the elaborate architectural design found in their biological counterparts. To fill this gap, processing routes that enable deliberate control over the material's porous structure at multiple length scales are highly demanded. In this talk, I will show how 3D printing and self-assembly technologies can be harnessed to fabricate porous materials with unprecedented structural control and properties. The key feature of our approach is to utilize templates for the controlled generation of pores via bottom-up self-assembly and/or top-down 3D printing processes. Using oil droplets, air bubbles or dissolvable particles as templates, our strategy enables the fabrication of designer porous materials that cannot be achieved through conventional manufacturing technologies. This opens new opportunities for the development of biomaterials with predictable and programmable response.

PL2

Meta-biomaterials

Amir A. Zadpoor

ETH Zurich, Zurich, Switzerland

Abstract

Meta-biomaterials are architected biomaterials with rare or unprecedented combinations of mechanical, physical (e.g., permeability), and biological properties. The unusual properties of meta-biomaterials are due to their rationally designed micro-architectures as well as their surface functionalities. Geometry and topology of the applied micro-architectural designs play important roles in determining the effective macro-scale properties of such materials. I will present my lab's contributions to the various aspects of micro-architectural design of meta-biomaterials at different length scales, from macroscale to nanoscale, and discuss the unusual properties resulting from such designs. At the macroscale, the main concepts of patient-specific medical devices, deployable implants, and metallic clay will be discussed. At the microscale, I will present the various microarchitectural design approaches including those involving multi-material additive manufacturing, auxetic materials, and the concept of curvature and its influence on cell response. At the nanoscale, the effects of surface nanotopography on osteogenic response, immunomodulation, and bactericidal properties of meta-biomaterials will be discussed. I also demonstrate the utility of origami- and kirigami-based approaches for the integration of the benefits of all the abovementioned length scales into a single piece of meta-biomaterial. This includes the use of 4D printing techniques to create self-folding meta-biomaterials as well as reconfigurable meta-biomaterials (i.e., biomaterials with 3D-to-3D shape shifting behavior) and the incorporation of flexible electronics into shape-shifting meta-biomaterials.

PL3

Discovery is just the first step: translating scientific innovations into clinical practice

Jill A Helms

Stanford University School of Medicine, Stanford, USA

Abstract

“From bench-to-beside” captures the principal of translating basic science discoveries into therapeutic interventions but despite ground-breaking advances, an alarming number of bench successes become stranded along the road to clinic. Regulatory challenges and limited finances contribute to this bottleneck but are not the only significant factors: fundamental to an effective translation pipeline is collaboration amongst clinicians, scientists, industry experts, regulatory and healthcare decision-makers and herein lies an inherent difficulty: the motivations, knowledge base, and cultures of these professionals differ. The wider the divide between these groups, the more challenging is the translational journey.

In addition to being a member of the AO Technology Transfer Board, I am a Professor in Stanford School of Medicine’s Department of Surgery. In my laboratory, we’re working on understanding why bone and soft tissue healing declines as we age. Many such age-related changes can be traced back to sluggish stem cells, and my group has developed methods to re-activate a patient’s own stem cells for therapeutic intervention in a broad range of conditions affecting bone, cartilage, skin, and beyond. Challenges of this magnitude require “moonshot thinking” and some serious team science involving experts from various clinical disciplines and scientific training, along with regulatory consultants, industry leaders, and commercialization experts. We use this network to translate idea into action: I have raised over $30MM to fund our discovery research and >$50MM in venture funding to co-found and lead a biopharmaceutical start-up into FDA-regulated clinical trials.

Conducting clinically relevant research has always been my main objective, but it goes hand-in-hand with another goal: to diversify the entrepreneurial pipeline to include more women and people of color. I believe that education and outreach are critical to this mission, and I use every avenue available to transform the way people think about translational science and regenerative medicine to emphasize its contribution to our daily lives.

PL4

MAPing our way to functional recovery after ischemic stroke

Tatiana Segura

Duke University, Durham, USA

Abstract

Stroke is a leading cause of adult disability. Ischemic stroke is caused by a blockage in a cerebral blood vessel that deprives the brain from oxygen and nutrients. Acutely, both brain vasculature and neural cells are severely damaged in the stroke core, leading to the neurological deficit that causes disability and the influx of peripheral immune cells, including macrophages and T-cells. The massive cell death, matrix degradation, and immune cell infiltration generates a fluid filled cavity, the stroke core. This fluid filled cavity is toxic, expands and remodels overtime causing more neurological disability. My laboratory has pioneered the detoxification of the stroke core to promote functional tissue deposition to increase functional recovery. Our main technology to promote stroke core remediation is a biomaterial platform that can be injected into the stroke cavity and modulate biology through physical and biomolecular interactions. This talk will cover our latest results and approaches to utilize materials to promote functional recovery after stroke.

PL5

Advances in Suspension Bath Printing to Process Biomedical Materials

Jason A Burdick

University of Colorado Boulder, Boulder, USA

Abstract

There are a wide variety of biofabrication technologies that are being developed to process biomedical materials and cells into desired configurations for applications in tissue engineering, in vitro disease models, and drug delivery. Extrusion bioprinting is one such technology that is widely used, where an ink is deposited onto a substrate layer-by-layer to build up a 3D structure. An emergent technique is the use of suspension baths, formulated as either molecular assemblies or granular structures, that support the printing of inks anywhere into 3D space. Suspension baths are required to shear-yield to receive the ink and then self-heal to hold the ink where desired. Here, I will provide an overview of how we have designed a wide variety of suspension baths to receive biomedical materials, including hydrogels and cell aggregates (i.e., spheroids), and how we have used computational models based on ink and suspension bath rheology to model material interfaces. Applications of these baths include (i) the deposition of cell aggregates into heterogeneous cardiac tissue models, (ii) the printing of inks with shear-aligned fibers to guide cell directionality to fabricate anisotropic meniscal constructs, and (iii) the printing of cell aggregate inks where suspension baths stabilize constructs during aggregate fusion for cartilage tissue engineering. Lastly, we have combined suspension bath printing with lithography techniques (i.e., volumetric additive manufacturing) to enable multi-material printing to rapidly fabricate complex 3D constructs.

S1.1-K1

Calcium phosphate nanoparticles: versatile tools for gene and drug delivery

Matthias Epple

University of Duisburg-Essen, Essen, Germany

Abstract

Calcium phosphate constitutes the inorganic component of mammalian bone and teeth. Thus, it is inherently biocompatible and biodegradable. It will be shown how calcium phosphate nanoparticles can be prepared, loaded with drugs or biomolecules, and surface-functionalized with targeting moieties (e.g. proteins, antibodies, or peptides). Calcium phosphate nanoparticles are easily taken up by cells to deliver their cargo (e.g. nucleic acids, proteins, drugs) into the cell. Typical applications are the induction of protein formation by transfection with DNA (e.g. BMP, VEGF), the silencing of protein formation by delivery of siRNA (e.g. TNF-alpha, NFkappaB), and the delivery of antigens to induce a specific immune response (e.g. an antigen against hepatitis). A major advantage of calcium phosphate nanoparticles is their inherent biodegradability and the final clearance from the body in the form of calcium and phosphate ions after intracellular dissolution in endolysosomes. This offers many applications in biomedicine in various areas (surgery, orthopedics, immunology). Finally, imaginary risks associated with calcium phosphate nanoparticles will be discussed.

S1.1-O1

Bacterially synthesized hydroxyapatite nanoparticles from eggshell waste

Mareeswari Paramasivan[1,2], Sampath Kumar T S[1], Hemalatha Kanniyappan[1], Vignesh Muthuvijayan[1], Chandra T S[1]

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Abstract

The intensity towards the adoption of biomimetic mineralization in fabricating bone tissue scaffolds has surged owing to the numerous benefits it holds. Bio mineralization using microorganisms serves an upper hand as the approach results in the production of nano form of hydroxyapatite (HA). With the aim of integrating sustainability into production processes, incorporation of waste sources has been largely explored. Eggshells are municipal wastes discarded in hundreds of thousands of tonnes by the food industry. As sustainability scenarios advocating for sustainable food and farming predicts global egg production to reach 91 million tonnes by 2050 (Food and Agriculture Organization of United Nations). To deploy such large amounts of biowaste in biomedicine holds immense potential as eggshells comprises of calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%), organic substances (4%) and insoluble proteins.

In the present study, a novel attempt to employ sustainable raw material for biomimetic synthesis of hydroxyapatite using Serratia sp. was carried out. Eggshell waste was utilized to produce CaCl2 to aid the biomineralization process of hydroxyapatite production. Fourier transform infrared spectrometer (FTIR) results showed the presence of phosphate and carbonate groups in the ESHA crystals (Fig 1A). The morphology and particle size of the ESHA (Egg shell derived HA) nanocrystals were determined by Transmission electron microscope (TEM) and were found to be needle shaped of size 10 nm-50 nm. While, X-ray powder diffraction (XRD) analysis clearly revealed a hexagonal lattice structure and poor crystallinity. Further, the substitution of different ions inherent in eggshell were present on ESHA nanoparticles and it was confirmed by Inductively coupled plasma optical emission spectrometer (ICP-OES). ICP-OES data also estimated a higher Ca/P ratio. Such increased Ca/P ratios is ascribed to the carbonate ions substituting the phosphate, indicating the presence of B-type carbonate hydroxyapatite. Biocompatibility studies were also carried out and MTT assay revealed more than 80% cells survived, indicating non-toxicity of ESHA with fibroblast cell line, osteosarcoma, and macrophage cell lines (Fig 1B). To the best of our knowledge this is the first report to produce bacterially derived HA using sustainable precursor material from food industry waste and such sustainable synthesis of nano HA shows great promise for biomedical applications such as bone repair.

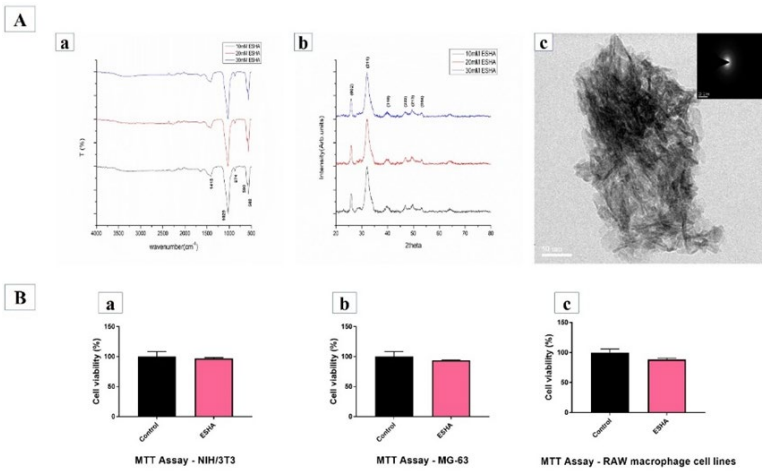

Figure 1A: a) FTIR b)XRD c)TEM analysis of ESHA nanoparticles. Figure 1B: Biocompatibility study of ESHA nanoparticles against a) 3T3 b) MG-63 c) Macrophage cell lines

S1.1-O2

Cancer chemodynamic therapy and MRI contrast using fully biodegradable iron-doped calcium phosphate nanozymes

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Abstract

Contrarily to non-specific chemotherapies, chemodynamic therapy (CDT) takes advantage of tumoral conditions, such as acidic pH and higher H2O2 concentration, to induce cellular oxidative stress, making it a cancer-specific therapy. To work, transition metal-containing nanoparticles convert endogenous H2O2 into oxygen reactive species (ROS) similarly to natural enzymes, hence the name nanozymes. In this work, fully biodegradable iron-doped calcium phosphate (FeCaP) nanozymes were developed for the CDT of triple-negative breast cancer (TNBC), by introducing 10% iron ions in different oxidation states (Fe2+, Fe3+ or a 1:1 mixture of both Fe2+/3+).

All three nanozymes were produced as amorphous needle-shaped iron-doped calcium phosphate nanoparticles with sizes below 200nm. Each iron oxidation state induced different magnetic responses, Fe3+ led to paramagnetism, while Fe2+ and Fe2+/3+ led to superparamagnetism, with the latter presenting a higher saturation (2.6 emu g-1), which can be explained through super-exchange interactions. Consequently, MRI contrast was more evident in the sample with Fe2+/3+. In terms of CDT applicability, all three nanozymes could convert H2O2 into ROS, with similar kinetics between samples, which was evaluated using the Michaelis-Menten kinetics methodology. Also, the influence of pH conditions was evaluated and the nanozymes worked better at acidic pH values, with a complete loss of activity at neutral pH, confirming their activity at tumoral conditions.

Finally, *in vitro* cultures of TNBC cells were done in physiological and tumoral pH, with groups containing H2O2 to simulate tumoral conditions. After incubation with the nanozymes, viability decreased significantly in the groups in tumoral conditions. These results were confirmed by a live-dead assay where a larger population of dead cells was detected in the same groups. To confirm oxidative stress, intracellular ROS were measured, and the groups with tumoral conditions showed a higher concentration of ROS. Finally, cell cycle analysis was conducted, and the same group's induced cell cycle arrest at the G2 phase, being indicative of apoptosis.

In conclusion, biodegradable FeCaP nanozymes are effective nanoparticles for CDT of TNBC, with great tumour specificity. Additionally, the iron oxidation state did not influence CDT activity but resulted in different magnetic responses, which translated into a better MRI contrast with Fe2+/3+.

S1.1-O3

Degradable zinc phosphate glass microspheres for applications in bone tissue engineering

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Abstract

Objective: Degradable phosphate glasses showed favourable properties for use in tissue engineering. By changing the composition of the glass, the degradation rate and ion release can be modified. Zinc oxide can work as a glass network modifier and has shown potential advantages in bone formation. Besides, phosphate glasses were able to be processed into different structural forms, like microspheres, which can be used as microcarriers. This study aims to develop zinc phosphate glass microspheres and explore the optimized size for applications in bone tissue engineering.

Materials and Methods: Zinc-titanium-calcium-sodium phosphate glasses with 0, 1, 3, 5, or 10 mol% zinc oxide were prepared and processed into microspheres. The smaller microspheres were in a size of 50-106 µm and the larger ones were in a size of 106-150 µm. The characteristics of the glasses were examined with X-ray Diffraction and Scanning Electron Microscopy. The osteoblastic cell line MC3T3-E1 was cultured on the surface of microspheres and quantified with Cell Counting Kit 8. To evaluate osteogenic differentiation, rt-qPCR and Alizarin Red S staining were performed after 14 days.

Results: We successfully made different sizes of zinc phosphate glass microspheres (Figure 1). The glass microspheres except Zn10 were able to support the adhesion and proliferation of MC3T3-E1 cell lines. The relative gene expression of Bmp2 was significantly upregulated in the smaller size of Zn3 microspheres (26-fold, $P<0.001$) and both sizes of Zn5 microspheres (smaller: 27-fold, $P<0.001$; larger: 35-fold, $P<0.001$). Meanwhile, we observed cluster formation of glasses microspheres after 14 days and the mineralization of MC3T3-E1 cell lines was enhanced as well (Figure 2).

Conclusions: Zinc phosphate glass microspheres with zinc oxide content of less than 10 mol% can be used as microcarriers for bone tissue engineering.

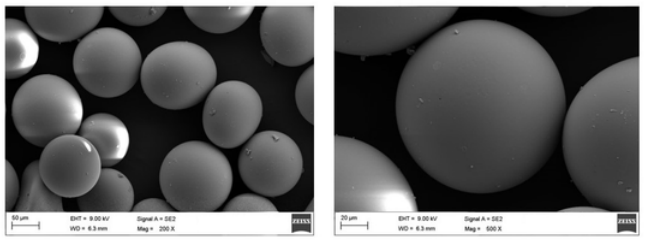

Figure 1. SEM images of Zinc-titanium-calcium-sodium phosphate glasses microspheres.

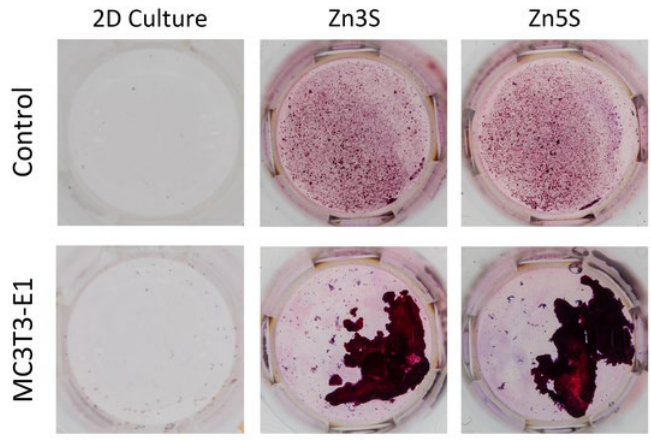

Figure 2. Alizarin Red S staining was performed to detect the in vitro mineralization of MC3T3-E1 cells. Smaller size groups of Zn3 and Zn5 were transferred in 48 well plates with cell-repellent surfaces and seeded with MC3T3-E1 cells for 14 days. The 2D Culture group were the traditional cell culture on a flat surface. The control group were the samples without the cell-seeded.

S1.1-O4

Design of novel organic-inorganic nanocomposite hydrogels for directed biomineralization and *in vivo* bone tissue formation

Babatunde O Okesola

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Abstract

INTRODUCTION. Organic-inorganic hydrogels, which combine the features of inorganic nanoscale particles with the inherent recognition motifs of organic molecules have gained prominence in various fields, including tissue engineering, nanoelectronics, catalysis, sensing, etc.[1] Self-assembly represents a powerful strategy to organize matter across multiple length scales [2,3] and offers the possibility to control spatio-temporal organisation of inorganic superstructures.[4-6] Although, controlled arrangement of specific reactive sites on a variety of biomolecules have been widely explored to control crystal growths, the possibility to harness the unique surface chemistry of nanosilicates in self-assembling hydrogels to nucleate and control the growth of biominerals within a 3D confinement will represent a new direction in biomaterials design.

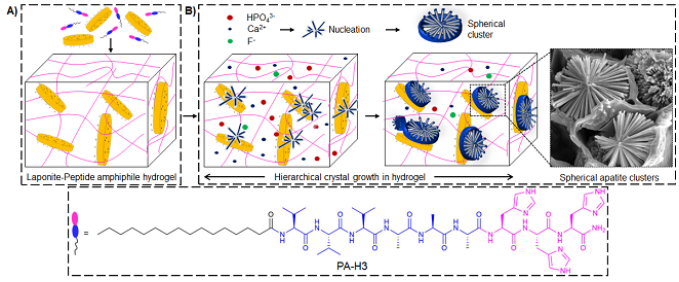


*Fig. 1. A) Supramolecular co-assembly of Laponite and histidine-rich peptide amphiphiles. B) Hierarchical biomineralization in **Lap-PAH3** nanocomposite hydrogels.*

RESULTS & DISCUSSION. Using supramolecular co-assembly, we developed organic-inorganic hydrogels integrating the unique surface chemistry and high aspect ratio of Laponite® XLG (**Lap**) nanodiscs and nanofibrillar networks of histidine-rich peptide amphiphiles (**PA-H3**) (**Fig. 1A**). The multicomponent hydrogels displayed high stiffness (~75 kPa) and self-healing property. When immersed in an aqueous solution of apatite, the hydrogels concentrated mineralising ionic species, nucleated and guided hydroxyapatite crystal growth within the pores of the 3D hydrogels (**Fig. B**).[7] Applicability of the mineralized hydrogels was assessed with *in vitro* differentiation of mesenchymal stem cells toward osteogenic phenotype and *ex vivo* neovascularization using CAM assay. Further application in a rabbit model shows that the multicomponent organic-inorganic hydrogels can promote bone and blood vessels formation with better performance than the commercial gold standard Bio-Oss®.[8]

CONCLUSION. We hope that these multicomponent **PA-Lap** hydrogels will find potential applications in other areas of nanotechnology beyond bone tissue engineering, and that this material design strategy will facilitate tailorable structure-property-function relationships in organic-inorganic nanomaterials.

ACKNOWLEDGEMENT. BOO thanks University of Nottingham for the award of Nottingham Research Fellowship.

Reference. [1] Saveleva et al, *Front. Chem*. 2019, 7, doi.org/10.3389/fchem.2019.00179. [2] Okesola et al, in Self-assembling Biomaterials, eds. H. S. Azevedo and R. M. P. da Silva, Woodhead Publishing, 2018, DOI: https://doi.org/10.1016/B978-0-08-102015-9.00019-8, pp. 371-397. [3] Okesola and Mata, *Chem. Soc. Rev.*, 2018, 47, 3721-3736. [4] Merg et al, *J. Am. Chem. Soc.* 2016, 138, 13655–13663. [5] Pigliacelli et al, *Chem. Commun.* 2020, 56, 8000-8014. [6] Kang et al, *ACS Nano* 2018, 12, 6554–6562. [7] Okesola et al, *ACS Nano* 2021, 15, 11202–11217. [8] Okesola et al, *Adv. Funct. Mater.* 2020, 30, 1906205.

S1.2-K1

Engineering an *in vitro* pathological skin model as a powerful tool to boost the validation of advanced wound dressings towards their quick market entry.

Rossella Laurano, Monica Boffito, Sara Pionato, Gianluca Ciardelli

Politecnico di Torino, Turin, Italy

Abstract

To answer to the urgent need of advanced wound care products to effectively treat and promote the regeneration of chronic skin wounds, efforts have been devoted towards the engineering of complex and multifunctional wound dressings (WDs). However, such advancements have highlighted the absence of consistent *in vitro* pre-clinical validation tools, leading to clinical trial failure. This technological gap affects, more in general, the success of applications needing a thorough comprehension of the skin physiological and pathological processes. Indeed, 2D mono- or co-culture systems are not able to mimic the skin reliably; differently, beside the unsustainable ethical approach, animal models often provide results in contrasts with *in vitro* tests. Therefore, the engineering of 3D *in vitro* skin models represents a powerful tool towards clinical translation and to boost WD validation from the bench to the market under the 3R principles. In this context, this work aimed at establishing an *in vitro* chronic wound model by combining a multi-stimuli-responsive hydrogel, bioprinting techniques and colonization with human dermal fibroblasts (hDFBs) and keratinocytes (hDKCs) (Figure_1). More in detail, methacryloyl gelatin (GelMA) with tunable functionalization degrees (55%-99%) were first synthesized through a green water-based chemistry. Then, the rheological characterization of hydrogels showed preservation of thermosensitivity and high responsiveness to Vis-light irradiation (Storage Modulus increase up to 15 kPa for 10% w/V formulations irradiated - 80000 Lux/90s). Such responsiveness was exploited to encapsulate hDFBs in the sol state and to 3D bioprint multilayer structures mimicking the morphology of chronic skin wounds. The optimal printability window was identified basing on a morphological characterization of filaments, single layers and 3D constructs. Moreover, encapsulated hDFBs showed high viability and spreading up to 7 days in normal culture conditions. Afterwards, hDKCs were seeded on the top layer achieving a liquid-air interface. The model was then validated by testing the cytocompatibility of commercial negative (i.e., ultra-high molecular weight poly(ethylene)) and positive (0.1% ZDEC polyurethane) controls giving results in accordance with 2D *in vitro* tests. Lastly, the presence of a strong and persistent ROS environment was mimicked by adding hydrogen peroxide inducing oxidative stress.

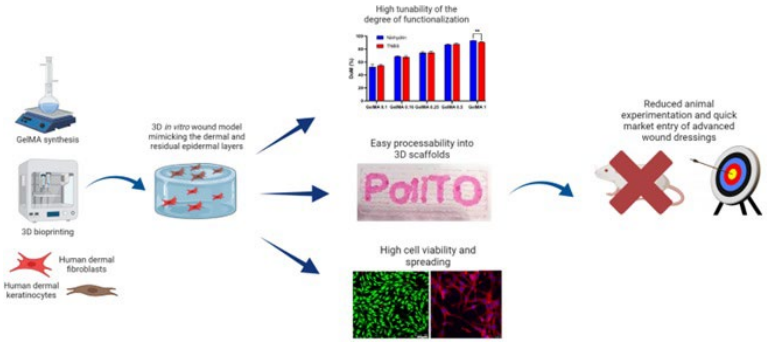


Figure 1. Schematic representation of the concept and main results.

Acknowledgment: with the support of Fondazione Compagnia di San Paolo - Trapezio Call for Proposals -Target 1 - project “Human multi-tissue platform for comprehensive evaluation of chemical toxicology on a chip”. Rossella Laurano acknowledges funding from PON “Ricerca ed Innovazione” DM 1062/2021.

S1.2-O1

Effect of an electrospun biodegradable poly(DL-lactide-co-ε-caprolactone) mesh loaded with or without antifibrotics as an alternative to commercially available polypropylene meshes for wound healing: from bench to *in vivo* approach.

Yurena Polo[1], Laura Rubio-Emazabel[1], Ana Ayerdi-Izquierdo[2], Nerea Garcia-Urkia[2], Noelia Alvarez[2], Jose-Ramon Sarasua[3], Jorge Fernandez[1], Antonio Muñoz[1]

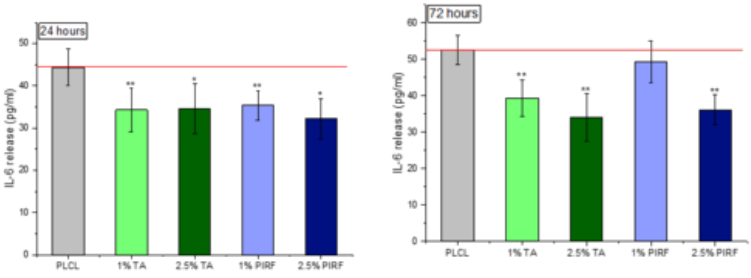
[1]Polimerbio SL, Donostia-San Sebastian, Spain. [2]TECNALIA, Basque Research and Technology Alliance (BRTA), Donostia-San Sebastian, Spain. [3]Department of Mining, Metallurgy and Materials Science, University of the Basque Country (UPV/EHU), Bilbao, Spain

Abstract

Introduction. Wound healing is a complex process in response to opening of a tissue and the absence of cellular structures. The damaged region is normally covered with a sterile bandage to promote healing and restrain infection.[1] Currently, commercially available meshes are made of physically and chemically inert, non-immunogenic, and non-toxic materials like polypropylene (PP). But, they may cause calcification, fibrosis, or inflammation, worsening the healing process.[2] To overcome this problem and provide advanced healing features, we designed a drug delivery method based on biodegradable poly(DL-lactide-co-caprolactone) (PLCL) electrospun meshes, loaded or not with antifibrotics, pirfenidone (PIRF) or triamcinolone acetonide (TA) and studied their effect both *in vitro* and *in vivo*.

Methods. PLCL was synthetized by ring opening polymerization and meshes fabricated by electrospinning loading the two antifibrotics: PIRF and TA. We performed a complete physicho-chemical characterization by hydrolytic degradation, drug release and scanning electron microscopy (SEM). *In vitro* functional analysis on RAW 264.7 macrophages investigated the effect on cytotoxicity and inflammation (interleukin 6 (IL-6) release). Finally, subcutaneous *in vivo* studies on a rabbit model following ISO 10993-6 compared the irritation, fibrosis and neovascularization indexes with commercial PP meshes.

Results. PLCL meshes were fabricated without aggregates and showed complete degradation after 120 days at 37˚C which is compatible with wound healing process. Both antifibrotics showed no cytotoxicity and an immunomodulatory effect (decreased on IL6 release) compared with the positive control (activation with lipopolysaccharides (LPS)) and the PLCL meshes without antifibrotic. *In vivo* evaluation on a subcutaneous rabbit model showed high irritation and fibrosis indexes after 2 weeks in the controls and a significant decrease only in PLCL meshes after 4 weeks. Regarding antifibrotic-loaded meshes, 2.5% TA and either 1% or 2.5% PIRF meshes showed the lowest fibrosis and irritation at both 2 and 4 weeks.



	Irritation index		Fibrosis index	
	2w	4w	2w	4w
PLCL	16,9	10,0	2,2	1,1
PP	15,0	15,8	1,6	2,3
Control	13,0	11,1	2,2	1,5
PLCL 1% TA	12,8	15,6	1,6	1,9
PLCL 2.5% TA	10,6	11,9	1,3	1,5
PLCL 1% PIRF	18,1	13,3	2,1	1,5
PLCL 2.5% PIRF	16,0	10,9	1,8	1,5

Figure 1: IL6 release on RAW264.7 after incubation with the antifibrotics. Table 1: Indexes after implantation.

Discussion & Conclusions. Compared to commercial PP meshes, PLCL decreased the irritation and fibrosis indexes after 4 weeks of implantation. This modulation was even greater than that observed on meshes containing antifibrotics, making our bare PLCL mesh a real substitute to the gold standard PP meshes.

Acknowledgments. SGIker technical services (UPV/ EHU) are acknowledged for SEM support. Also, Basque Government Hazitek ZE-2019/00012-IMABI and Bikaintek PhD grant (20-AFW2-2018-00001).
References. **(**1) Gizaw, M. et al, Bioengineering 2018, 5 (1), 1–28. (2) Saha, T. et al, OpenNano 2022, 7 (May), 100046.

S1.2-O2

Wound management: infection-resistant clay membranes for healing burns and scars

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Abstract

Every year more than 15 million people are victims of burn injuries; of them, about 4 million patients require further hospitalization due to severe burns that often develop multiple organ failure and infection, which require advanced treatments, *viz.* skin grafts. Currently, burn treatments include application of topical agents that comes with disadvantages e.g., contact dermatitis, increased antibiotic resistance, toxicity through absorption, and disruption of microbiome on the skin. The purpose of our study is to eliminate such issues by engineering a novel bandage system incorporating an easy and sustainable approach; thereafter, evaluate its capacity to reduce the proliferation of the skin wound pathogens and heal scars. We present a cost-effective, infection-resistant bandage that can be used for healing burn wounds and scars (Fig 1). One goal is to understand the interface of materials-biological interactions while developing burn wound healing bandage for casualties.

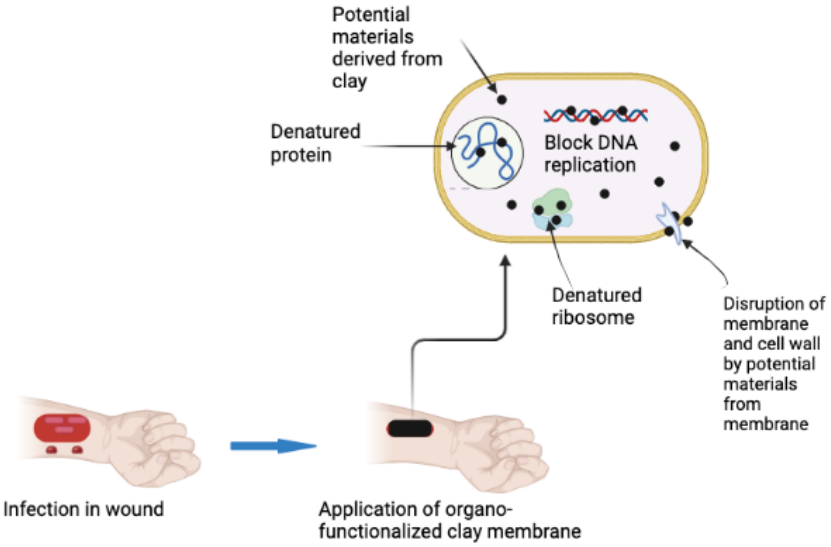

Fig 1. Development of Infection-Resistant Clay Membranes/ Films

This novel infection-resistant material has been made of hybrid clay scaffolds using optimized amounts of trimethyl glycine (TMG) precursors with Bentonite clay. These films can be functionalized with molecules or metal ions to impart antibacterial and antifungal properties. Synthesis of the hybrid clay films were carried out with metal and organic molecules e.g., silver, sulfanilamide. Samples were characterized by XRD, ATR-IR, XPS, ICP-OES, DMA, SEM to ascertain the structure, strength, compositions, and O_2-moisture transport properties of clay. XPS analyses show distinct Ag $3d_{3/2}$ and Ag $3d_{5/2}$ and N 1s peaks from the TMG present in hybrid clay films. This was validated by the presence of –CN groups using ATR-IR. DMA and XRD tests have shown mechanical durability through increasing basal

spacing due to exfoliation from TMG incorporation in the clay layers with increase in $d_{(100)}$ spacing from 1.14 nm to ordered 1.84 nm for functionalized clay (Fig 2).

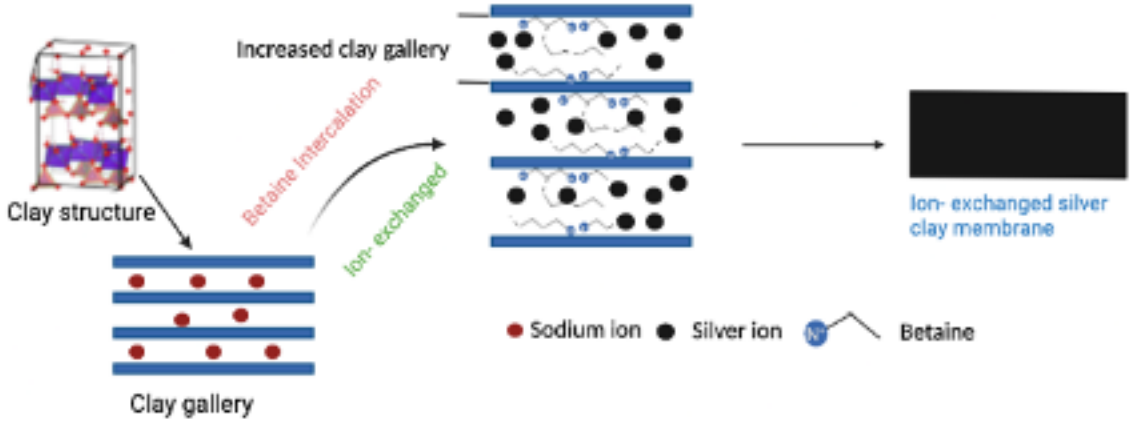

Fig 2. Strategies for Developing Hybrid Ag-Clay Films

The increase in basal spacings helps incorporate the metal ions or organic molecules that impart the antimicrobial or antifungal properties in the films. Pathogen resistance of the films was carried out using zones of inhibition for *E. coli*, *S. aureus* and *C. albicans* for 18 hours with the Kirby Bauer disk susceptibility test that showed 100% efficacy. 2nd degree burn-tests using *Silvadene* control for porcine trials showed exceptional performance of our clay dressings upon comparison with scar- and wound-healing features. These hybrid clays could be a cost-effective solution to deter infections from ESKAPE pathogens.

S1.2-O3

An Active microfluidic wound dressing for wound care

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Abstract

Severe burn injuries typically cause devastating wounds which require both early treatment strategies and consistent care to regenerate healthy tissue. Due to the lack of resources in autologous skin grafts and limitations of current wound treatments which are time-consuming and costly, burn wounds have a critical impact on the health and quality of patients' life in particular severe pain, function, and mobility loss, social stress and isolation, depression, prolonged hospitalization, financial burden, and high rates of mortality.

Current wound healing approaches such as tissue-engineered scaffolds and biomaterials are incapable of fast wound healing and having control over infections in large areas. Therefore, We have developed an active microfluidic non-adhesive wound dressing to enable long-term control of the wound environment by seeding cells and collecting exudates regularly.

We hypothesize this control will facilitate and accelerate wound closure hence wound healing and reduce rates of infection and scar tissue formation. An array of microfluidic channels enabled in situ 3D bioprinting, as therapeutic cells including fibroblasts and keratinocytes were deposited through multiple outlet ports *in vitro* and *in vivo* on a mice dermal skin surface at an early point in wound treatment to study the cell clustering and proliferation at each delivery points by DAPI, Live/Dead staining, and Masson's trichrome staining. An 'H-tree' network design was chosen to provide uniform flow and delivery to 2 and 32-port devices. The fluid flow rates and uniformity have been studied by particle tracking (PIV) measurements and numerical simulations. We hypothesize that our microfluidic device will provide dynamic control of the wound environment as well as enhance the rates of skin regeneration and reduce infection to improve therapies for burn and wound care significantly. The effect of hydrogel (e.g., Collagen and gelatin) substitutes and cell-laden hydrogels as ECM for delivering dermal cells have been studied and showed that hydrogel carriers can promote cell clustering at the outlets that can accelerate wound closure and healing process.

The scalability of the microfluidic wound dressing up to 32-port and 20 cm2 surface area will be studied further in terms of uniform cell and hydrogel delivery in time intervals. Also, this form of in situ bioprinting of cells and hydrogels will be compared to conventional 3D bioprinting in terms of cell proliferation and wound healing processing time using an *in vitro* wound model. *In vivo* experiments on big animal models will be the final step in this research.

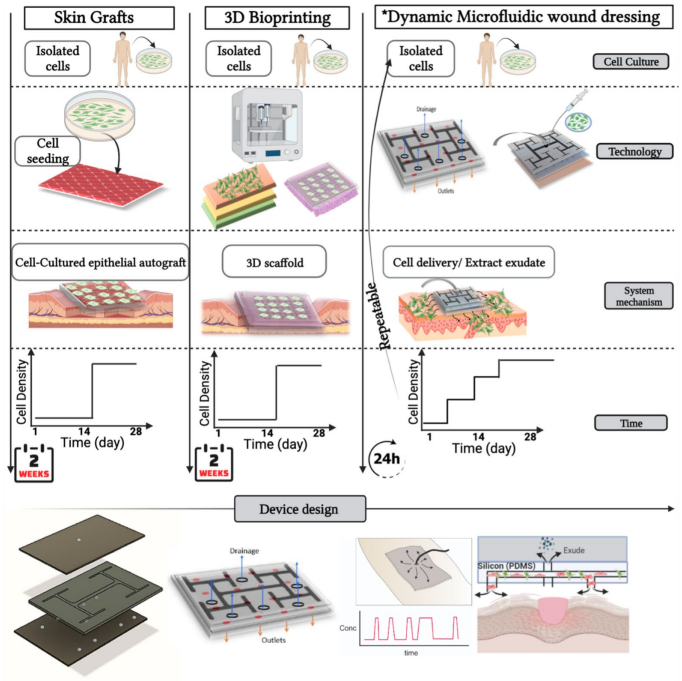

Figure 1. Conventional approaches vs dynamic microfluidic wound dressing device for wound healing application

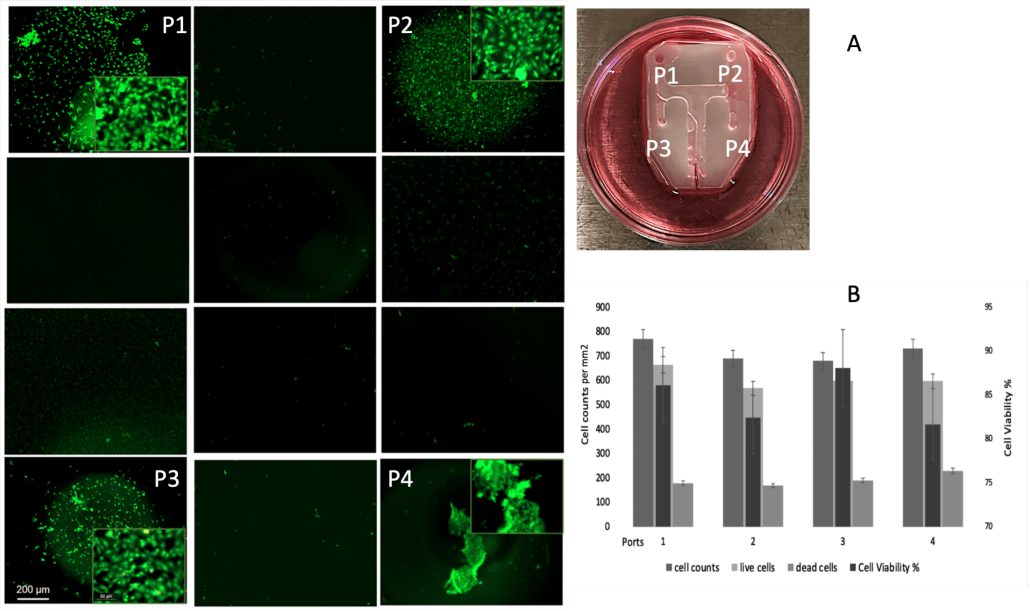

Figure 2. A) Live/Dead staining images B) cell counts and viability % of HDFa seeded on 5% wt. gelatin hydrogel through a 4-port device and incubated for 3 days.

S1.2-O4

Delivery of miRNA-loaded Peptide Nanoparticles through 3D printed PEG-Chitosan-PCL Patches for Wound Healing

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Abstract

Introduction. Wound healing is a significant global healthcare worldwide. We have used the cationic cell penetrating peptide RALA to condense miR-31 and miR-132 into nanoparticles (NPS) capable of intracellular delivery. NPs were lyophilised and loaded into biodegradable, polymer-based filaments during hot melt extrusion. Polycaprolactone (PCL) and polyethylene glycol (PEG) were used as carrier polymers to maintain stability of NPs. Chitosan was added to confer antimicrobial qualities and aid the absorption of wound exudate.

Materials and Methods. RALA was complexed with plasmid miR-31/miR-132 at a range of N: P ratios. PCL, PEG and chitosan were extruded to fabricate 2 mm diameter filaments. Filaments were then 3D printed using an ARBURG Plastic Freeformer into 1 cm^2 patches (Fig.1A). Release profiles in ultrapure water were measured via Picogreen assay. NCTC-929 fibroblast cells were transfected with NPs released from filaments and analysed by RT-PCR and AlamarBlue assay. For the *in vivo* study, wounds were created on the skin of c57bl/6 mice (N=5 per group) and treated with patches for 1 week with regular imaging of closure.

Results/Discussion. NP-loaded patches were capable of releasing around 50% of loaded miRNA (Fig.1B). NPs released from the patches were functional, producing a significant increase in miR-31 or miR-132 when incubated with NCTC-929 cells (Fig2A) with negligible cytotocitity (Fig.2B). Treatment with miR patches led to more rapid wound closure in the *in vivo* model compared to untreated and commercial control patches (Fig.2C).

Conclusion. RALA/miRNA NPs were successfully formulated, coextruded into polymer-based filaments and 3D-printed into patches. The patches successfully released functional cargo which was capable of transfecting cells, leading to miRNA upregulation with negligible cytotoxicity. This technology also outperformed commercial controls in an *in vivo* murine wound healing model.

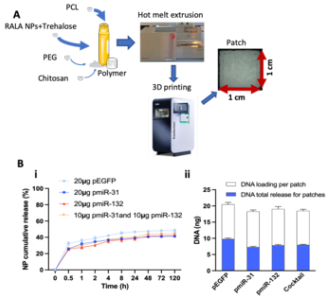

Figure 1:Schematic showing manufacturing process for RALA/miR-loaded 3D-printed patches. (B)(i) Cumulative release profile of 3D-printed patches loaded with pEGFP, pmiR-31, pmiR-132 or pmiR-31/132 NPs over 120 h. (ii) Quantification of DNA released from patches in (i) showing total loading and total released after 5 days.

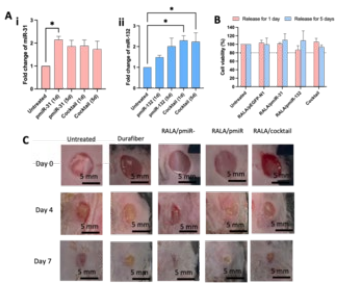

Figure 2:(A) Fold change in (i)miR-31 and (ii)miR-132 expression in NCTC-929 cells following incubation with NPs released from patches after 1 and 5 days. (B) Cell viability of NCTC-929 cells measured by AlamarBlue assay 24 h after incubation with release products of patches after 1 and 5 days. (C) Representative images of wound closure in the in vivo mouse wound healing model used to test patches over 7 days.

S1.3-K1

Design of an innovative and sustainable silk-based technological platform for bone tissue engineering

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Abstract

Nowadays there is an increasing demand of new strategies for skeletal tissues healing in large defects due to the aging population and the incidence of traumatic tissue injury, with no current therapeutic solutions for all patients. In this context, biomaterials-based products and scaffold-free strategies are having a special attention in the TERM field.

Natural polymers, for instance proteins and polysaccharides, are intrinsically multifunctional, dynamic and environment responsive, hierarchical structure. These materials offer several advantages such as excellent biodegradable and biocompatible, and high flexibility, which possess the capability of changing shape and size to promote the growth of engineered tissue within the surrounding tissues.

Among them, silk polymers are finding broad impact in biomaterial systems for a range of cell and tissue studies. Silk-based scaffold properties can be tuned through processing, i.e., dissolution and fabrication methods, so producing materials with specific physic-chemical cues, which have been demonstrated ability to activate specific pathways inducing tissue regeneration. Adding or conjugating selected biological moieties can be specifically improved bioactivity so obtaining materials for bone tissues regeneration even in pathological conditions such as osteoporosis. Additionally, three-dimensional (3D) additive manufacturing, microfabrication, and nanoscale lithography have begun to address the complexities required for manufacturing precision biomaterial-based devices across many length scales1.

Silk biopolymers and related fabrication methods has been extensively studied and applied in the last years to the design scaffolds for innovative therapeutics strategies, considering patient diversity, clinical needs, and future products commercialization. A technological platform was built up, trying to move from advanced research to bone product development, considering environmentally friendly and green technologies, recently introduced in several industrial fields including biomedical. In this context, it was re-defined protocols and fabrication procedures, introduced specific interactions with bone-related systems, improved reproducibility and scaling up to facilitate the translation into devices ready for clinical studies and subsequent commercialization2. During the seminar it will be used the silk-platform design as a model, starting from controlled silk production to standardized protocols for processing, bioactivity bone-specific and pre-clinical evaluations.

References. [1] F. Agostinacchio, et al. (2021). Trends in Biotechnologies, 39(7), 719-30; [2] A. Bucciarelli and A. Motta. (2022). Biomaterials Advances,139, 212982.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101008041

S1.3-O1

Engineering controlled drug delivery from implant coatings by electrophoretic deposition of silk fibroin in tailored structures

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Abstract

Polymer-based coatings are commonly used on medical devices to provide sustained release of therapeutic agents. Electrophoretic deposition (EPD) is an ideal method for constructing polymer coatings on medical devices due to its mild solution-based approach, short processing times, and suitability for complex geometries and porous structures. Traditionally, therapeutic drugs were mixed into the matrix polymer precursor solution before coating deposition, resulting in poor control over release kinetics. To improve drug release control, recent studies have explored incorporating other material-based drug delivery vehicles or using cross-linking agents. However, these solutions can increase complexity, cost, and biosafety concerns. As an alternative, the matrix polymer precursors can be assembled into nanostructures to regulate drug release without introducing new chemicals, which could enhance the drug release capability of EPD polymer coatings. We demonstrated this assembly strategy with silk fibroin (SF) coatings.[1, 2]

SF coatings were electrophoretically deposited using only dissolved SF molecules (SFM), only pre-made SF nanospheres (SFN), or a mixture of both at different ratios. Drug release profiles of SFN and SFM coatings were compared to investigate the mechanisms behind the superior control over drug release of SFN coatings.

The use of nanospheres as building blocks enhanced the maximum drug release amount by 1.38 times and prolonged drug release time by 21 times while retaining drug effectiveness without detectable cytotoxicity. When mixing SFM and SFN in coatings, drug release amount and duration linearly increased with nanosphere concentration and deposition time.

These findings demonstrate that the two key processing parameters (nanosphere concentration and deposition time) can be used to precisely control drug release dosage and duration. Overall, they highlight the potential of pre-defining coating architecture for controlled drug delivery.

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(2) Cheng, X.; Long, D.; Chen, L.; Jansen, J. A.; Leeuwenburgh, S. C. G.; Yang, F. Electrophoretic deposition of silk fibroin coatings with pre-defined architecture to facilitate precise control over drug delivery. Bioactive Materials 2021, 6 (11), 4243-4254. DOI: 10.1016/j.bioactmat.2021.03.046.

S1.3-O2

Development of Tissue Adhesive Microparticles for Gastrointestinal Perforation Closure and Postoperative Adhesion Prevention

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[1]University of Tsukuba, Tsukuba, Japan. [2]National Institute for Materials and Science, Tsukuba, Japan. [3]Kagoshima University, Tsukuba, Japan

Abstract

Introduction. Endoscopic submucosal dissection (ESD) has been used as a minimally-invasive gastrointestinal cancer treatment. However, ESD operation in thin tissues, such as the duodenum, often causes post-ESD perforation (hole in the gastrointestinal tract) at a high rate. Present study focused on the molecular design of tissue adhesive microparticles from decyl group-modified Alaska pollock gelatin (C10-ApGltn) for post-ESD perforation closure and wound healing [1]. Furthermore, we clarified the postoperative adhesion prevention ability of the particles [2,3].

Experiment. C10-ApGltn was synthesized through Schiff base formation between decanal (10 carbons) and amine groups of ApGltn and subsequent reductive amination by 2-picoline borane. C10-ApGltn microparticles (C10-MPs) were prepared from the obtained C10-ApGltn by coacervation, freeze-drying and thermal cross-linking. Perforation closure property of C10-MPs was evaluated referring to ASTM-F2392-04R using a duodenal tissue as an adherend. Furthermore, the postoperative adhesion prevention effect of C10-MPs colloidal gel was evaluated using a rat cecum-abdominal wall defect adhesion model.

Results and Discussion. C10-MPs and Org-MPs, which are non-hydrophobized particles, showed a spherical shape with a particle diameter of 2 to 3 µm. C10-MPs formed a colloidal gel by hydration on the duodenal perforation model and showed 2.5 times higher burst strength compared with that of Org-MPs (**Fig. 1**). Aggregation behavior of C10-MPs in water indicated that the hydrophobic interactions formed between C10-MPs led to stable colloidal gel formation and high burst strength. To evaluate underwater stability, C10-MPs colloidal gel formed on duodenal tissue was immersed in saline for 2 days. C10-MPs colloidal gel remained on the duodenal tissue for 2 days in wet environment. This result indicated that C10-MPs formed hydrophobic interactions with hydrophobic molecules (elastin, fibronectin and cell lipid bilayer, etc.) in the duodenal tissue to maintain stable adhesion in underwater environments. Additionally, C10-MPs colloidal gel degraded subcutaneously in the rat back within 2 weeks without severe inflammation, indicating the colloidal gel had biodegradability. In a rat cecum-abdominal wall defect adhesion model, the C10-MPs colloidal gel-treated group showed significantly less adhesion score than the untreated group due to the presence of a colloidal physical barrier on the wounds.

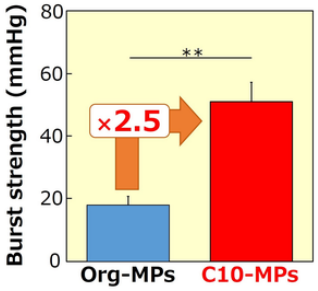

*Fig. 1 Burst strength of Org-/C10-MPs applied on the gastrointestinal perforation model. **p < 0.01*

References: [1] S. Ito, A. Nishiguchi, T. Taguchi, et al., *Mater. Sci. Eng. C*, 2021, *123*, 111993; [2] S. Ito, K. Nagasaka, A. Nishiguchi, T. Taguchi, *Acta Biomater*. 2022, *149*, 139-149; [3] S. Ito, A. Nishiguchi, T. Taguchi, *Acta Biomater*. 2023, *159*, 83-94.

S1.3-O3

Induced pluripotent stem cell-derived chondrocytes and periosteum-derived cells in a GelMA-based approach for *in vivo* osteochondral regeneration

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Abstract

Osteochondral defects are deep joint surface lesions that affect the articular cartilage and the underlying subchondral bone. In the current study, a tissue engineering approach encompassing distinct cell populations encapsulated in a biocompatible hydrogel is explored. Two different bioinks containing either human induced pluripotent stem cell (iPSC)-derived chondrocytes or human periosteum-derived progenitor cells (PDCs) encapsulated in gelatin methacryloyl (Gel-MA INX X210, BIO INX) were evaluated for their potential to regenerate articular cartilage and the subchondral mineralized tissue respectively. A GelMA hydrogel (10 wt %) precursor solution including photoinitiator in PBS was combined with 20 million cells/mL. After crosslinking the bioink in polytetrafluoroethylene moulds through exposure to UV light (365 nm, 5mW/cm²) for 10 min, constructs were cultured for up to 28 days in a serum-free chemically defined medium, containing TGF-β (10 ng/mL), GDF-5 and BMP-2 (100 ng/mL). At day 1 and day 21 of the differentiation period, cell-laden constructs were implanted subcutaneously in nude mice to evaluate ectopic tissue formation 4 weeks post-implantation. iPSC-derived chondrocyte-laden GelMA displayed increasing Safranin O positivity and collagen type II immunostaining between 14 and 28 days of *in vitro* culture, corroborated by upregulation of transcripts for SOX9, ACAN, and COL2A1. The cartilage-like matrix formed *in vitro* further matured *in vivo* as assessed by Safranin O staining, resulting in more mature hyaline cartilage-like tissue. When implanting unprimed iPSC-derived chondrocyte-laden GelMA, abundant matrix formation was observed after 4 weeks, albeit less mature than in primed constructs. In primed PDC-laden GelMA hydrogel constructs, Safranin O positivity and collagen type II immunostaining was observed *in vitro* in the outer region of the constructs at day 14, gradually expanding towards the center by day 28. Gene expression analysis of PDCs encapsulated in GelMA showed upregulation of COL10A1 to a greater extent than that of COL2A1, indicating hypertrophy. Additionally, upregulation of RUNX2 and OSX was seen. When implanting these constructs *in vivo* after 21 days of *in vitro* culture, the formation of ectopic mineralized cartilage and bone tissue was detected by micro-computed tomography (µCT). Taken together, these data suggest that iPSC-derived chondrocytes encapsulated in GelMA can generate hyaline-like cartilage tissue constructs with different levels of maturity while using periosteum-derived cells in the same construct type generates mineralized tissue and a bone ossicle *in vivo*. Therefore, the aforementioned bioinks can be an important part of a strategy to generate osteochondral tissue *in vivo*.

S1.3-O4

Dynamic Alginate-Hydroxyapatite Based Composite Containing Human Mesenchymal Stromal Cells for Critical Sized Bone Defects

Rotsiniaina Randriantsilefisoa, Gregor Miklosic, Ezgi Irem Bektas, Matteo D'Este

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Abstract

Statement of Purpose: Effective alternatives to autografts are needed to heal critical sized bone defects. We developed a dynamic bone graft substitute made of hydroxyapatite, embedding alginate beads degrading overtime serving two functions: dynamically creating porosity for cell and fluid invasion and encapsulating human mesenchymal stromal cells (hMSCs) that subsequently differentiate into osteoblasts at the defect site.

Methods: First, we fabricated oxidized alginate beads of different sizes and encapsulated hMSCs by electrospraying in a calcium chloride bath. To vary degradation rates, alginates with degree of oxidation of 2.5%, 5% and 10% were used. Second, we mixed the obtained beads with a calcium phosphate (CaP) phase (tetracalcium phosphate (TTCP) and dicalcium phosphate, anhydrous (DCPA)) and an alginate solution buffered to pH 7.4 to form a stable crosslinked alginate-hydroxyapatite matrix around the beads with a liquid-to-powder (L/P) ratio of 3/5. The obtained hMSCs-beads-CaP composite was molded into spheres and incubated in aqueous conditions until further use. To measure degradation, beads were tagged with fluorescein and the fluorescence intensity of the supernatant was followed for 12 days. The corresponding cell morphology was visualised by phalloidin/DAPI staining.

Results: Oxidized alginate beads of 200 – 500 µm were fabricated and characterized. Gradual degradation of the beads and release of the encapsulated cells was observed (Figure 1). The cells released from the beads are elongated, contrary to the retained cells, smaller and rounder.

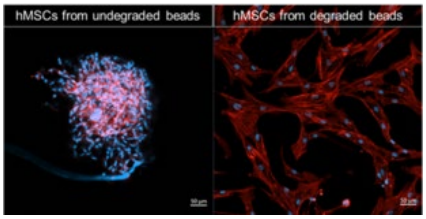

Figure 1: hMSCs morphology before (left) and after (right) bead degradation on day 9. Scale: 50 µm.

Bone graft formulations with CaPs were optimized to provide a non-decaying material in static aqueous conditions keeping a stable shape for at least 21 days (Figure 2). Upon immersion of the composite material in solution, hydroxyapatite is produced, while sodium alginate forms a water insoluble gel in the presence of calcium ions resulting in the stability of the graft. The studies also showed good molding capacity of the putties with and without beads, showing potential to fill large irregular gaps in bone.

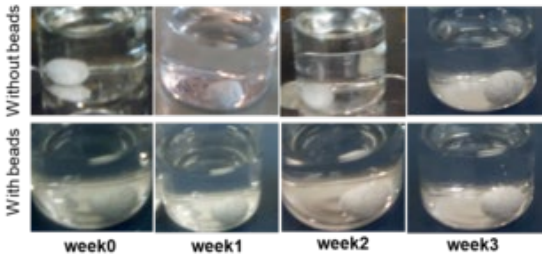

Figure 2: Putty remains stable in aqueous conditions for at least 3 weeks.

This composite brings mechanical stability and dynamic remodeling which could greatly enhance the efficacy of synthetic bone graft substitutes.

Acknowledgments: The AO Foundation and the European Union's Horizon 2020 research and innovation program under grant agreement No 857287 for financial support.

S1.4-K1

Therapeutic vascularization in regenerative medicine

Andrea Banfi

Basel University Hospital, Basel, Switzerland

Abstract

Therapeutic angiogenesis, i.e. the generation of new vessels by delivery of specific factors, is required both for rapid vascularization of tissue-engineered constructs and to treat ischemic conditions. Vascular Endothelial Growth Factor (VEGF) is the master regulator of angiogenesis. However, uncontrolled expression can lead to aberrant vascular growth, as well as non-vascular side-effects. Major challenges to fully exploit VEGF potency for therapy include the need to control *in vivo* distribution of growth factor dose and duration of expression. In fact, the therapeutic window of VEGF delivery depends on its amount in the microenvironment around each producing cell rather than on the total dose, since VEGF remains tightly bound to extracellular matrix. On the other hand, short-term expression of less than about 4 weeks leads to unstable vessels, which promptly regress following cessation of the angiogenic stimulus. Here we will briefly overview some key aspects of the biology of VEGF and angiogenesis and discuss their therapeutic implications, with a particular focus on approaches using extracellular matrix engineering with recombinant factors.

S1.4-O1

Development of a pro-angiogenic hydrogel for (bio)engineering of blood-brain barrier

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Abstract

Despite all the progress and effort that has been made in the last few decades regarding the research on neurodegenerative diseases, the complexity of the human brain and the low efficiency of a minority of suitable drugs, which are able to cross the brain-blood barrier (BBB), have halted the advance of significant breakthroughs in neurosciences. Brain-on-a-chip (BoC), an advanced microfluidic platform comprising organ models to recapitulate human physiology and homeostasis at a lower cost and higher reproducibility, has the potential to create an accurate and simple-to-use preclinical tool to screen novel neurotherapeutic drugs, by decoupling a complex organ, such as the brain, into different cellular structures, while maintaining their interconnections. A significant challenge in the development of BoC is mimicking BBB, especially the creation of an extracellular matrix (ECM) able to sustain and recreate ideal conditions for the spread, adhesion and close junction of endothelial cells for several days. In this work, a BBB in-vitro model with functional properties closest to the ones found in the human brain was developed. This BBB model consisted of a gelatin-based hydrogel incorporating heparin, as a pro-angiogenic growth factor immobilization and release molecule, and hyaluronic acid (HA), as an enhancer of the mechanical strength and regulator of the physiological processes related to BBB cells. The hydrogel 3D network was achieved by activation of carboxylic acid groups of heparin and HA with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC)/sulfo-N-hydroxysulfosuccinimide (NHS), which served as a crosslinking agent to the amino groups of gelatin (Figure 1 A, B). The physicochemical and rheological properties of hydrogels were investigated, along with in-vitro cellular studies using human endothelial cells (HUVECs), such as cell adhesion and proliferation (Figure 1C), as well as gene expression, revealing its potentiality for integration in BoCs.

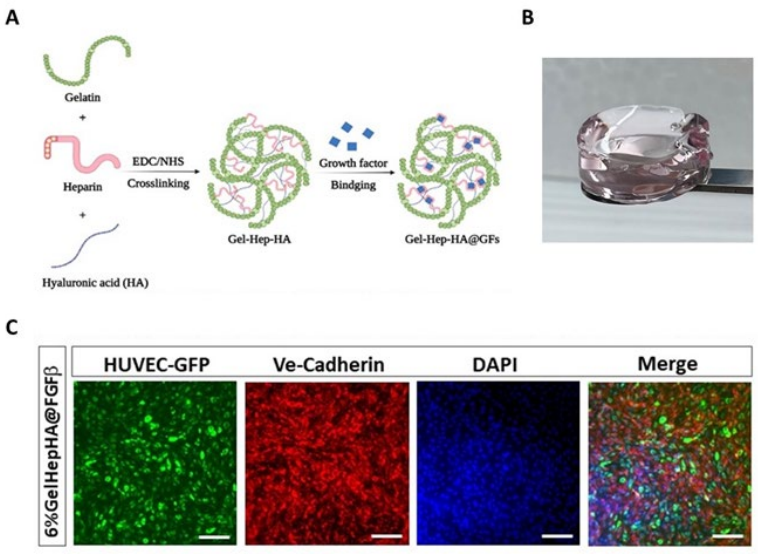

Figure 1. Active pro-angiogenic hydrogel for (bio)engineering of blood-brain barrier. A. Schematic illustration of the bio-hydrogels formed by the activation of carboxylic acid groups of heparin and hyaluronic acid (HA) with EDC/sulfo-NHS crosslinking to the amino groups of gelatin (GelHepHA). B Bio-hydrogel. C. Microscope fluorescent images and immunostaining with Ve-Cadherin (red) and DAPI (blue) of HUVEC-GPF seeded on 6%GelHepHA with FGF-2, after 7 days. Scale bars: 100 mm.

S1.4-O2

Dental Pulp Stem cells facilitate the engineering of 3D functional pre-vascularized constructs

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Abstract

The engineered reconstruction of tissues remains challenged by the lack of proper microvascularization, therefore cell-based pre-vascularized grafts may enhance the survival and integration of implanted constructs within the host. Dental pulp stem cells are a promising candidate for pre-vascularization due to their angiogenic potential and pericyte function. The present study describes the fabrication of 3D pre-vascularized tissue constructs on polylactic acid (PLA) scaffolds by employing dental pulp stem cells derived from deciduous teeth (SHED) and explores the *in vivo* kinetics of the engineered vasculature integration using multi-modal imaging.

Collagen hydrogels were held in poly-lactic acid (PLA) grids prior to subcutaneous implantation in athymic nude mice according to the following groups: 1) endothelial cells (EC) and SHED co-culture (EC-SHED), 2) EC treated with SHED conditioned medium (EC-SHED-CM), 3) SHED alone. Before implantation, cell viability was assessed inside the *in vitro* microvascular networks. Capillary parameters were measured using Skel2Graph3D Matlab® after CD31 staining. After *in vivo* implantation, early angiogenesis was investigated by positron emission tomography (PET) using a 64Cu-NODAGA-RGD tracer [4]. Mouse (IB4) and human (UEA1) fluorescent lectins were injected, and the explanted constructs were imaged with two-photon microscopy. Finally, the vasculature was analyzed by contrast-enhanced micro-CT acquisition. The PLA grids were further included and characterised by immunohistochemical analysis.

At the time of implantation, there were no significant differences in vascular volume, sprout length and tortuosity between the EC-SHED and EC-SHED-CM groups. The cell viability was 96.2% in the EC-SHED group and 94% in the EC-SHED-CM group. The *in vivo* PET at day 10 showed that the uptake of the radiotracer was significantly increased in the pre-vascularized groups compared to SHED alone, indicating higher angiogenic activity in these groups. After *in vivo* injection of species-specific lectins, multiple anastomoses of human implanted vessels with the host's vessels were detected, highlighting the perfusion of the engineered vessels. The contrast-enhanced Micro-CT acquisitions revealed a statistically significant increase in the total vascular volume in the EC-SHED PLA constructs compared to all the other groups.The presence of human adherent junctions (VE-cadherin), sensitive innervation (cGRP) and the deposition of basement membrane (collagen type IV) suggested the existence of a mature human vascular network.

This study supports the interest for pre-vascularization before implantation and demonstrates the strong potential of SHED to produce mature microvasculature, a key factor for successful tissue engineering.

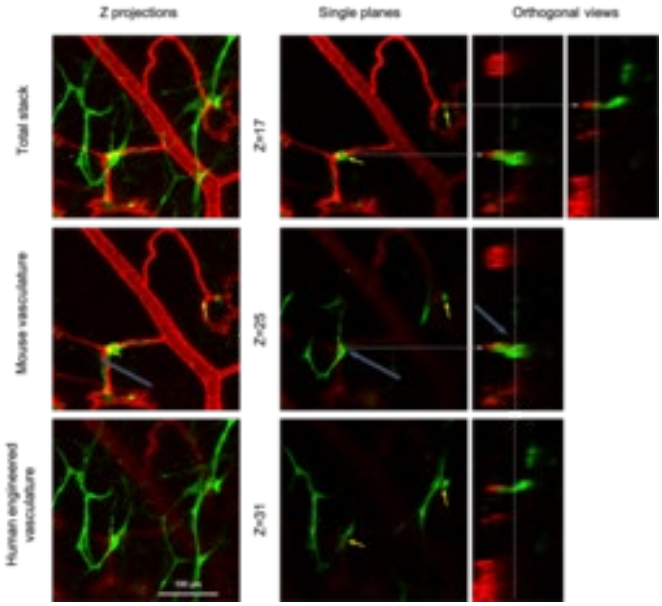

Figure. Anastomosis of the human implanted vessels (UEA1 lectin in green) with mouse vessels (IB4 lectin in red).

S1.4-O3

Heart Repair using 3D Bioprinted Patches containing Cardiac Spheroids in Alginate/Gelatin Hydrogels

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Abstract

Cardiovascular disease, including myocardial infarction (MI) and heart failure (HF), is a leading cause of death globally. Our laboratory has developed a novel approach to 3D bioprint cardiac tissues containing human cardiac spheroids (hCSs) together with alginate/gelatin (Alg/Gel) hydrogels. We have previously demonstrated that bioprinted Alg/Gel patches containing hCSs are characterised by morphological, biochemical and pathophysiological features similar to the ones of the *in vivo* human cardiac tissue. For this reason, they have been extensively used to test toxicity of drugs, as well as to model the damage that follows myocardial infarction ("heart-attack-in-a-Petri-dish"). Given their unique ability to mimic the human heart microenvironment, we hypothesised that bioprinted patches containing hCSs in Alg/Gel could protect against impaired cardiac function in an *in vivo* mouse MI model. To test this hypothesis, we compared the effects of few groups of patches in animals. Infarcted MI mice received either (i) AlgGel acellular patches, or (ii) AlgGel patches with freely suspended cardiac cells, or (iii) AlgGel patches with hCSs. Control groups included (iv) mice that underwent a sham (SHAM) procedure and (v) infarcted mice that did not receive any patch (MI). We performed cardiac function measurements *via* ultrasound imaging up to 28 days. Epicardial transplantation of patches containing hCSs was the best treatment to significantly ($p=0.010$) improve left ventricular ejection fraction (LVEF%) from 41% (MI mice) to 64%. Similarly, the infarct area was significantly reduced in mice receiving hCS patches compared to MI animals not receiving any patch. Bulk RNAseq analyses of heart tissues demonstrated similar gene expression profiles between SHAM and MI mice receiving AlgGel patches with hCSs. Altogether, our findings support the novel use of cardiac Alg/Gel patches containing hCSs to protect against MI-induced HF. Together with our clinical partners, current studies are focussing on the personalisation of bioprinted patches from both the cellular and morphological aspect, as well as to the minimally-invasive delivery of the patch to quickly translate our findings from the bench to the bedside.

S1.4-O4

Bioengineering 3D lymphatic vascular structures in synthetic matrices

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Abstract

Background. The lymphatic network serves an important role in interstitial fluid drainage and in immune cell transport. Moreover, in cancer progression, metastasising cancer cells hijack the lymphatic vessel network to spread throughout the body. Despite its importance in cancer metastasis, the regulation and regression of the lymphatic system has been largely overlooked in favour of blood vessel system. The few *in vitro* models described for lymphatics have been based on naturally derived materials. However, the usage of a synthetic polymer for *in vitro* studies, namely polyethylene glycol (PEG), allows for a more standardised and tuneable environment to study lymphangiogenesis and lymphatic vessel regression.
Methods. 3D cultures were grown by encapsulation of cells in enzymatically crosslinked TG-PEG gels. hLECs were mono-encapsulated or co-encapsulated alongside mesenchymal stromal cells (hBM-MSCs). Different cell densities and ratios of hLECs to hBM-MSCs were tested. In addition, 3D cultures were grown using a plug-and-play platform. For this, hBM-MSCs were seeded prior to hLECs and different hBM-MSC preculture times were tested. Different cell densities and ratios of hLECs and hBM-MSCs were assessed. Stimulatory effects of growth factors, namely vascular endothelial growth factors A and C (VEGFA & VEGFC) and fibroblast growth factor 2 (FGF-2) were assessed. Characterization of the hLECs was done by staining for pan-endothelial marker CD31, as well as lymphatic-specific markers Prox-1 and Lyve-1. hLEC networks were imaged with fluorescence confocal microscopy and image-based quantification of CD31-positive tube-like structure length determined the most suitable conditions.
Results. Vessel-like structures were able to form in hLEC and hBM-MSC co-culture encapsulation, when there was a cell density of at least 1 Mio/ml of each cell type. Without hBM-MSCs, no hLEC vessel-like structures formed. In the plug-and-play platform, lymphatic vessel-like structures formed when BM-MSCs were allowed to preculture for at least 3 days. Without hBM-MSCs, no hLEC vessel-like structures formed. Analysis of the CD31 signal revealed that both FGF-2 and VEGFA performed better than the lymphatic-specific growth factor VEGFC. When forming tube-like networks, the hLECs retained their lymphatic-specific marker Prox-1.
Conclusions and Outlook. A robust method for the generation of lymphatic vascular-like networks has been established. In following studies, different anti-angiogenic drugs will be applied to pre-formed networks and their regressive effectiveness will be compared to the treatment of networks of blood endothelial cells (BECs). In the future, introducing cancer cell-lines to formed hLEC networks will allow for novel studies of interactions between the lymphatic system and cancer.

S1.5-K1

Materials for mechanochemistry and mechanobiology

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Abstract

Most hydrogels used in biomedical applications display a homogenous static architecture. In contrast, natural hydrogels in tissue are highly dynamic, where internal and external forces will catalyse changes in chemistry, architecture, and mechanical properties. In this presentation I will demonstrate how materials chemistry can be used to fabricate dynamic hydrogels that mimic signaling in natural tissue. First, I will demonstrate mechanochemical linkages that release molecules in response to force. To imbue the toughness needed in mechanical applications, including durability under cyclic loading, the covalent mechanophore linked hydrogels are impregnated with a second ionically linked alginate network to facilitate toughness properties approximating natural hydrogels. Next, I will show how supramolecular chemistry can be used to develop hierarchically structured hydrogels that are self-healing and have tunable mechanical properties that approximate the characteristics of natural matrices. Finally, I will introduce a new mechanochemical approach where polymeric microcapsules are integrated within the hydrogel, and how force can trigger rupture to release bioactive molecules. Together, these mechanochemistry concepts that mimic natural processes in the extracellular matrix, provides a new approach to making force-responsive dynamic biomaterials for a broad spectrum of applications including implant adhesives and coatings, bandages, biosensors, and materials for tissue engineering.

S1.5-K2

Harnessing light activated bioinks and cell-instructive spheroids for biofabrication of functional tissues

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Abstract

Biofabrication technologies, including 3D bioprinting and bioassembly, enable generation of engineered constructs that replicate the complex 3D organization of native tissues via automated hierarchical placement of cell-laden bioinks, tissue modules, and/or bioactive factors. Photo-initiated radical polymerization combining light and photo-initiators to generate radicals for crosslinking photo-polymerizable macromers, has been widely employed in 3D bioprinting of cell-laden hydrogels [1,3]. Despite rapid advances in biofabrication technologies, no universal bioink exists, requiring optimization of bioinks for each individual biofabrication technique and specific tissue niche. Bottom-up spheroid biofabrication strategies offer flexibility to precisely arrange cellular modules to mimic the zonal structure of tissues as well as uncouple the reliance on narrow biofabrication windows and manipulate cell signaling through spatial presentation of different biomolecules.

This presentation discusses alternative strategies to provide highly tuneable bioinks that 1) promote a specific cell-instructive niche using light-activated crosslinking in high throughput modular spheroids, and 2) are printable across multiple biofabrication technologies, including extrusion-, lithography- and microfluidic-based bioprinting.

We describe the design of versatile photoinitiator system (Ru/SPS) and photo-clickable, cell-instructive gelatin-based bioinks and bioresins for biofabrication of 3D *in vitro* models. Tailoring macromolecular chemistry offered by the platform by varying photoinitiator and thiolated crosslinker (DTT, PEG-SH) concentration, we modulated the cell-instructive tissue niche for multiple cell types via: covalent incorporation of thiolated bioactives (e.g. heparinSH), nanocomposites (e.g. strontium, Laponite); di-tyrosine cross-linking of decellularized extracellular matrix (ECM) bioinks, and tailored Ru/SPS photoinitiator delivery presenting stiffness gradients in cell-laden bioinks. Examples discussed include yielding enhanced chondrogenic/osteogenic differentiation and vascular network formation [2,3,5,9].

We further discuss experience in developing hybrid tissue constructs and convergence with 3D spheroid bioassembly platforms for probing multicellular spheroid fusion, extracellular matrix (ECM) formation and stem-cell niche, offering new paradigms for high-throughput screening, “on-chip” and osteochondral tissue repair applications.

This work demonstrates advances in development of cell instructive universal bioink platforms for 3D bioprinting and regenerative medicine, and advances biofabrication and bioassembly of functional tissues towards clinical translation.

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S1.5-O1

Engineering Heart Valve Interfaces Using Melt Electrowriting: Biomimetic Design Strategies from Multimodal Imaging

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Abstract

Interfaces within biological tissues not only serve the purpose of connecting different regions but also contribute to the overall functionality of the tissue. This is especially true in the case of the aortic heart valve, which has multiple heterogeneous regions that enable its crucial function. However, in regions such as the commissure and inter-leaflet triangle, the microstructural composition and orientation remains largely unexplored.

Here, a multi-modal imaging study of the aortic heart valve interfaces was conducted using micro-computed tomography, second harmonic generation imaging and focused ion beam - scanning electron microscopy to reveal the collagen orientation, density, and recruitment in these unexplored regions. This information would then be used to inspire the biomimetic design of scaffolds for interfacial tissue engineering applications using melt electrowriting (MEW).

MEW is a high-resolution biofabrication technology capable of integrating complex microstructures into scaffold designs. Three strategies for interfacing MEW scaffolds were investigated for their morphological, tensile and flexural properties, demonstrating the superior performance of continuous interfaces.

To assist in this process, a novel software and graphical user interface was created for generating G-codes, which enabled the generation of an array of MEW scaffolds with never-before-seen complexity.

Finally, a bio-inspired MEW scaffold for the interfacial region of the aortic heart valve was designed and fabricated based on the findings of the multi-modal microscopy study. The singular scaffold incorporated continuous interfaces, gradient porosities, variable layer numbers across regions, and tailored fibre orientations.

When characterised under physiologically relevant tensile strains, the scaffold exhibited similar behaviour to porcine heart valves with respect to yield strain, hysteresis, and relaxation behaviour. This work demonstrates the ability of a bio-inspired approach to address the functional complexity of biological tissues.

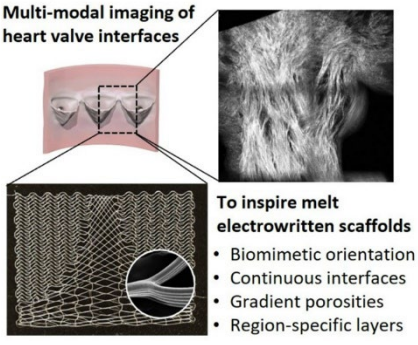

Figure 1: Overview of process used to design bioinspired heart valve interface scaffold. Showing regions of interest analysed (top left), second harmonic generation image of collagen fibres in the commissure (top right) and corresponding bioinspired scaffold (bottom left), with inset showing a continuous biomimetic interface.

S1.5-O2

Convergence of biomimetic culture methods and ECM-based biomaterials for humanisation of bioengineered *in vivo* bone models

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Abstract

The limitations of traditional rodent models in modelling complex human diseases have restricted the discovery of new markers from the human microenvironment, likely significant contributors to incurable diseases such as bone metastatic cancers. As more physiological models are needed, our previous work has leveraged calcium-phosphate coated polycaprolactone (CaP-PCL)-based melt electrowritten (MEW) scaffolds to engineer osteoblast-derived mineralised tissues *in vitro*[1]. The ectopic implantation of the resulting microtissues in immunodeficient mice formed humanised bone niches[2], and successfully served to study breast and prostate cancer mechanisms and treatment[3]. Two limitations included the long-term *in vitro* culture needed prior to implantation (6-12 weeks of *in vitro* osteogenic media (OM)) and limited humanisation of the graft. We hypothesized that gelatin methacrylamide (GelMA)-based hydrogels combined with an optimized biomimetic culture of primary human cells would address these issues. Specifically, we compared CaP-PCL MEW scaffolds and GelMA hydrogels (0.1 million primary osteoprogenitors, biomaterial volume ~60 µL) as suitable humanised bone model platforms. We supplemented the constructs with a calcium-phosphate-supersaturated medium (CaPSM) for three days[4] to boost mineralisation prior to further *in vitro* culture (4 weeks, referred to OM+), prior to implantation in NSG mice for 11 weeks. *In vitro* results showed that higher calcium uptake and ALP activity were seen after OM+ treatment. Superior mineralisation was observed in GelMA groups vs CaP-PCL. *In vivo*, micro-computed tomography (µCT) revealed earlier mineralisation in OM+ constructs. *Ex vivo* µCT showed higher bone volume fraction and superior cortical shell in GelMA compared to CaP-PCL constructs, while OM+ conditions showed higher trabecular network. Higher vascularisation and bone marrow infiltration was observed in GelMA constructs cultured after OM+ vs OM. Histology confirmed endochondral new bone formation in humanised GelMA, which is more physiologically relevant to bone formation with definitive haematopoiesis, yet CaP-PCL scaffolds showed a hybrid endochondral and intramembranous bone formation. Further analysis via SEM, EDS, and nanoindentation informed on human osteocyte network, mineral density and quality. In conclusion, GelMA following CaPSM treatment demonstrated that faster and superior humanisation could be achieved in rodents, leading the path towards advanced preclinical humanised models.

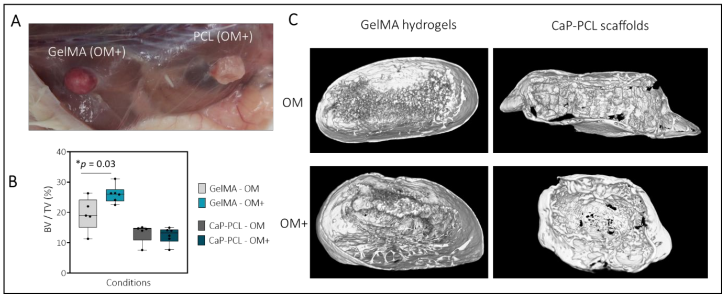

Figure. Samples after 11 weeks in vivo; A) Photos, B) Mineral Density and C) corresponding µCT images of constructs.

[1] Bock N et al. Bone Res. 7, 2019; [2] Moreno-Jiménez I, Cipitria A et al. Sci. Adv. 6, 2020; [3] McGovern JA, Bock N et al. Commun. Biol. 4, 2021; [4] Bessot A et al. Adv. Healthc. Mater. 2023. doi:10.1002/adhm.202201701

S1.6-K1

Interdisciplinary translational research on biomaterials and implants: current status and future scope

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Abstract

Biomaterials science and biomedical engineering have sustained as one among frontier and growing areas of research and innovation within the engineering science community in the world; considering the number of scientific discoveries and their societal impact. The potential economic and societal impact have driven significant research programs globally, over the last few decades. However, the outcome has not yet been too significant in India (and many developing nations), as more than 80% of the biomaterials and implants used in Indian hospitals are still imported from the North America and Europe!

This presentation will evolve around my perception that the future of science lies in breaking down barriers between fields to solve global challenges of our time and will initially focus on discussing the key challenges that the Indian translational ecosystem has been experiencing. I shall then present as how my research group has recently conducted translational research in cranioplasty surgeries using patient-specific bone flaps fabricated using 3D printed cranium models, based on the CT scan data of the patients who underwent decompressive craniectomy, in a team of neurosurgeons. Further, I shall discuss the new technologies developed in manufacturing the acetabular liners for total hip joint replacement surgeries and dental implants. This lecture will close by introducing a new concept, Biomaterialomics, which brings together Biomaterials science and Data Science.

S1.6-O1

3D-printed Airway Model for SARS-CoV-2 Infection and Drug Test

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Abstract

There is a need for a physiologically relevant respiratory model to study viral infection and evaluate potential antiviral drugs to combat the spread of SARS-CoV-2. We develop 3D-printed airway model that recapitulates the multi-layered human airway structure consisting of pulmonary endothelium, extracellular matrix, and airway epithelium through automated inkjet and microextrusion bioprinting. This 3D microarchitecture exhibits cell-cell junction and mucus secretion, which are key respiratory barriers to viral infection, and expresses ACE2 and TMPRSS2, the two main proteins involved in SARS-CoV-2 cell entry. We have used this model to investigate the response of the airway to SARS-CoV-2 infection. We found that the infection induced cytopathic effects and barrier destruction in the model over time, highlighting the relevance of the model for studying viral infection. Importantly, virus replication was effectively inhibited when the infected 3D airway model was treated with remdesivir and molnupiravir approved for the treatment of COVID-19. We were able to determine the EC50 for each drug in the model, providing valuable information on the efficacy of the drugs. The 3D-printed airway model provides a promising tool for studying viral infection and validating the efficacy of therapeutics against respiratory viruses, including SARS-CoV-2. The development of respiratory models can accelerate drug discovery and aid the development of new treatments for respiratory infections, which is crucial for global preparedness, especially during the ongoing pandemic and the emergence of new viral strains.

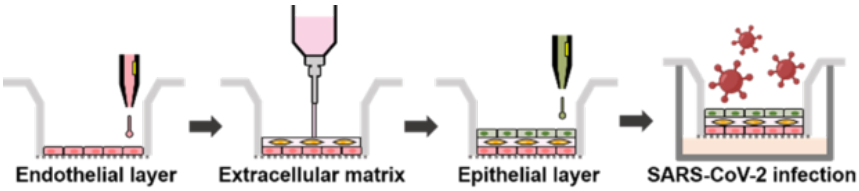

Figure 1. Fabrication schematics of 3D-printed airway model. Layer-by-layer printing of endothelial, extracellular matrix, and epithelial layer.

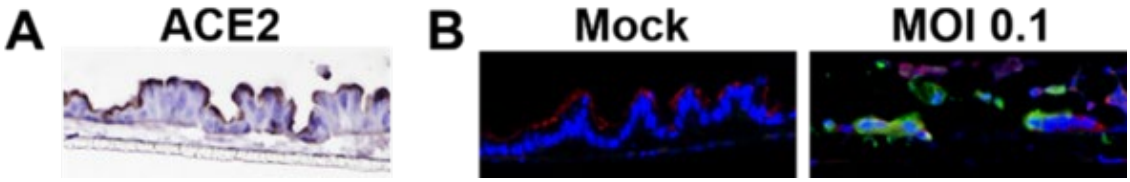

Figure 2. Cross-sectional view of three-layered airway models (A) Validation of ACE2 receptor expression (brown: ACE2, purple: nuclei) (B) 3D airway model infected with SARS-CoV-2 (10 days post-infection) (blue: nuclei, green: nucleocapsid of SARS-CoV-2, red: ACE2) (MOI: multiplicity of infection)

S1.6-O2

Piezoelectric patches preserve electrical integrity of cardiac tissue

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Abstract

Ischemic heart disease is the leading cause of death worldwide. Novel approaches have been focusing on restoring cardiac function by mainly targeting cardiac repair, however *in vivo* studies promoting both contractile and electrical functional recovery are scarce. Piezoelectric materials are capable of acquiring electric charges upon mechanical deformation. Since the heart exhibits cyclic movements, implantation of these materials on the myocardium holds great potential for designing biomaterials with self-sustained electrical stimulation, harnessing the contractile proficiency of healthy regions of the ventricle to stimulate damaged ones.

In this work, piezoelectric patches implanted on *ex vivo* rat Langerdoff-perfused working hearts and cardiac slices did not preclude tissue function and had the ability to improve its contractility, comparing with control electrically inert PCL patches. Following one month of *in vivo* implantation on the heart of a mouse model of myocardial infarction, piezoelectric patches caused an improved of cardiac electrical integrity, without precluding systolic function and attenuating adverse cardiac remodeling, with decreased left ventricle cavity dilatation and hypertrophy. Despite no alterations in histological parameters, the transcriptome of piezoelectric patch-implanted hearts was altered specifically on the more contractile/proficient portion of the left ventricle, showing a downregulation of genes associated with matrix remodeling and heart failure when compared with the PCL control, suggesting a more efficient functional recovery from the infarction by the piezoelectric group. Notably, by developing piezoelectric and PCL control patches with increased dimensions, we show these materials are applicable in the heart of an *in vivo* pig model, a relevant model that closely resembles the human heart in terms of anatomy, size and physiology. Importantly, the patches did not negatively affect the electrical integrity of healthy pig hearts, as no signs of arrhythmia were detected.

We developed, to our knowledge, the first therapeutic cell-free biomaterial-based patch with piezoelectric properties that can be directly applied to the healthy myocardium without compromising its function, not only in rodents but also in pigs, thus revealing a electrical safety profile and potential to be clinically applied in the future. Importantly, the piezoelectric patches caused a modest improvement on the overall function of the mouse infarcted heart, revealing a therapeutic potential.

Funding: FEDER through the Program COMPETE and by Portuguese fund through the FCT project PTDC/BTM-SAL/29229/2017, as well as by the European projects "RESETageing" (ref. 952266), "ERAatUC" (ref. 669088) and "REBORN" (Grant agreement ID: 101091852). LMM wishes to thank FCT for the PhD fellowship (SFRH/BD/129991/2017).

S1.6-O3

Microgel-based clickable composite bioink for 3d bioprinting of skin

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Abstract

Microgel assemblies, otherwise known as microporous annealed particles, have garnered significant interest among the palette of available bioinks for 3D bioprinting of complex 3D skin tissue architectures. These granular inks have interconnected micro-voids that can facilitate cell infiltration and tissue formation, enhance the transport of nutrients and oxygen, and facilitate long-term cell viability, creating a more permissive micro-environment compared to conventional nanoporous hydrogels. Although previous attempts have been made to replicate skin tissue by 3D bioprinting utilizing microgels, development of full-thickness skin for transplantation to promote complete skin regeneration and minimize scarring remains very challenging.

Herein, we demonstrate a novel shear thinning microgel system for skin bioprinting using human primary fibroblasts and keratinocytes. A biphasic bioink was developed comprising 150 – 300 µm porous gelatin microspheres (PGM) (Fig. 1a) encapsulated in a hyaluronan and PEG-based hydrogel supplemented with gelatin. The hyaluronan was functionalized with bicyclo [6.1.0] nonyne (BCN) and crosslinked using multi-arm PEG azide by strain-promoted alkyne-azide cycloaddition (SPAAC) click chemistry. Microgels using PGMs with and without BCN (PGM and PGM-BCN) were prepared (Fig. 1b), and the viscosity, loss tangent, and cross-linking kinetics were optimized to enable extrusion bioprinting of multilayered tissue architectures.

Human primary skin-derived fibroblasts and keratinocytes were cultured on the PGMs, yielding engineered skin tissue units (STUs), for the preparation of the microgel-based bioinks, followed by 3D bioprinting. Both PGM and PGM-BCN showed high cytocompatibility, allowing for appreciable cell densities (Fig. 1c). The cell-laden 3D printed microgels exhibited good shape fidelity, while the void fraction (Fig. 1d-e) allowed the cells to proliferate and migrate within printed structures. The possibilities to deposit the cell-laden bioinks in layered structures by 3D bioprinting allow for mimicking of dermal and epidermal compartments, which can facilitate the development of full-thickness microgel-based skin tissue constructs for the treatment of burns.

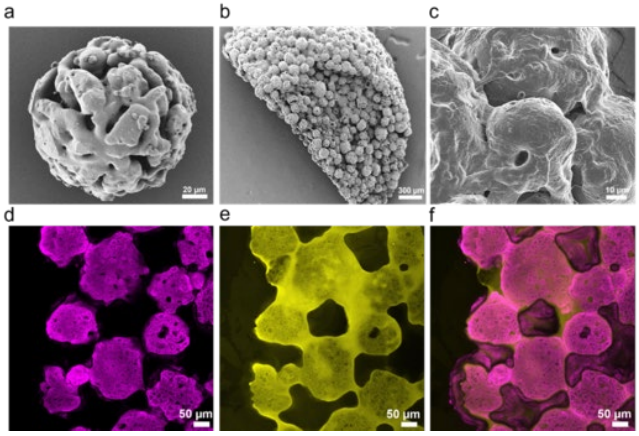

Fig 1: SEM images of porous gelatin microsphere (a), cross-section of the microgel assembly (b), fibroblasts adhesion and spreading over the microgel (c), false-colored confocal images of microgels (d), hyaluronan matrix embedding the microgels (e) and 3D stacks (f) of the microgel assembly exhibiting its intrinsic void spaces.

S1.6-O4

Immune cell surveillance of and death on silicone catheter surfaces promotes biofilm formation on the surface

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Abstract

Catheters are one of the most used medical devices. While many different materials are used to manufacture catheters, silicone is the most widely used due to its various advantages over other materials. Unfortunately, catheters, including silicone catheters, are prone to nosocomial infections. In fact, approximately 36% of bloodstream infections have been attributed to catheter use. Catheter infection leads to encrustation and biofilm formation in the catheter, rendering it useless and requiring replacement of the catheter and treatment of the infection. The question that follows is what causes biofilm formation on silicone catheter surfaces, which are otherwise quite inert. We know that circulating immune cells, specifically neutrophils and monocytes, come in contact with catheters following their *in vivo* use. These cells attempt to adhere to and degrade the catheter surface. A few reports have also shown that some of these cells may die by apoptosis on the surface. Hence, we hypothesized that neutrophil and monocyte cell death on the silicone catheter surface leads to biofilm formation. In this study, we show that prior immune cell surveillance of silicone catheter surfaces leads to a greater amount of biofilm formed on these surfaces. We also show this can be partly attributed to dead immune cell debris on the surface. Further, we demonstrate that the neutrophils and monocytes are unable to clear biofilms once they form on the surface that has been coated with dead cells. Thus, we conclude that it is necessary to prevent immune cell attachment and death on silicone catheter surfaces to prevent catheter infection and biofilm formation.

S1.7-K1

Molecular understanding and structural-based design of multifunctional biomaterials

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Abstract

Design and synthesis of highly bioinert and biocompatible antifouling materials are crucial for a broad range of biomedical and engineering applications. Among antifouling materials, zwitterionic polymers and polyacrylamides have proved so promising because of cheap raw materials, ease of synthesis and applicability, and abundant functional groups. The strong surface hydration and the high surface packing density of zwitterionic polymers and polyacrylamides are considered to be the key contributors to their antifouling property. In this talk, we review our studies on the design and synthesis of a series of zwitterionic polymers and polyacrylamides with different molecular structures. These polymers can be fabricated into different architectural forms (brushes, nanoparticles, nanogels, and hydrogels), all of which are highly resistant to the attachment of proteins, cells, and bacteria. As guided by machine-learning models, we find that small structural changes in the polymers can lead to large enhancement in surface hydration and antifouling performance, both showing a positive correlation. This reveals a general design rule for effective antifouling materials. Furthermore, zwitterionic polymers and polyacrylamides are readily functionalized with other bioactive compounds to achieve different new multifunctionalities.

S1.7-O1

Efficient protein aggregation inhibition with sulfobetaine polymers and their hydrophobic derivatives: molecular mechanisms exploration

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Abstract

Protein storage and delivery are crucial in biomedical research. However, denaturation of proteins is a major hurdle, rendering them inactive and causing undesired side-effects.[1] Small molecules, such as polyamines, vitamins, polyphenols, peptides, and non-detergent sulfobetaines, have been used to suppress misfolding and stabilize proteins.[2] Polymers, specifically sulfobetaine polymers (PSPB), have been discovered as potential agents for protein stabilization,[3] but the impact of hydrophobicity and specific interactions with proteins remains unexplored. The lack of clarity regarding the interaction between polymers and proteins has impeded progress towards developing more effective polymers.

PSBs with different molecular weights and varying amounts of hydrophobic monomers (butyl methacrylate: BuMA; hexyl methacrylate: HxMA; octyl methacrylate: OcMA) were synthesized in this study using RAFT polymerization (Table 1). The effectiveness of these polymers in preventing protein aggregation under thermal stress conditions was evaluated, and the results unequivocally demonstrated that poly-SPB is highly efficient in suppressing protein aggregation. Increasing the molecular weight and hydrophobicity of the polymer further enhanced its ability to prevent protein aggregation (Fig. 1A). The polymers were found to preserve the secondary structure of multiple proteins, including lysozyme, insulin, and lactate dehydrogenase, while protecting them from thermal stress.

Table 1 *Characteristics of Various SPB derivates (homopolymers and random copolymers) prepared via RAFT Polymerization.*

Entry		Composition				molar ratio[b]
		SPB	BuMA	HxMA	OcMA	
P1	in feed	100	0	0	0	100:0.5:2.5
	In polymer[a]	100	0	0	0	
P2	in feed	100	0	0	0	100:0.2:1
	In polymer[a]	100	0	0	0	
P3	in feed	90	10	0	0	110:0.5:2.5
	In polymer[a]	92.1	7.9	0	0	
P4	in feed	70	30	0	0	130:0.5:2.5
	In polymer[a]	64.1	35.9	0	0	
P5	in feed	70	30	0	0	130:0.2:1
	In polymer[a]	63.5	36.5	0	0	
P6	in feed	90	0	10	0	110:0.5:2.5
	In polymer[a]	91.4	0	8.6	0	
P7	in feed	90	0	0	10	110:0.5:2.5
	In polymer[a]	90.2	0	0	9.8	

[a]Determined by ^{1}H NMR; [b][monomer]:[initiator]:[RAFT agent]

Several physiochemical techniques were employed to investigate the interplay between proteins and a polymer. Isothermal titration calorimetry and surface plasmon resonance were used for quantitative analysis, revealing a weak and reversible interaction between the polymer and protein that is strengthened by increased hydrophobicity and molecular weight (Fig. 1B, C). Molecular simulations supported this finding, showing that the polymer acts as a molecular shield, preventing protein molecules from coming into close proximity (Fig. 1D). Fluorescence and CD spectroscopy provided evidence that this interaction acts as a reversible molecular shield, disrupting the aggregation pathway and preventing the formation of aggregation-prone intermediates. After removing stress, partially unfolded intermediates refold back to their native state. In conclusion, we have established that zwitterionic polymers exhibit a weak and reversible interaction with proteins, enabling them to function as molecular shields that inhibit collisions between unfolded protein chains and prevent protein aggregation. These findings provide valuable insights into protein aggregation inhibition and can aid in the development of more efficient polymeric materials.

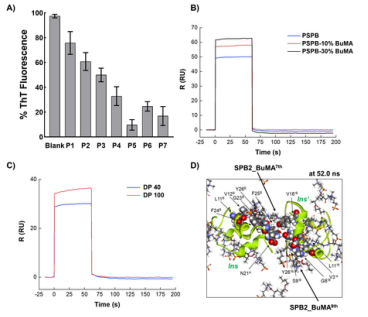

Fig. 1 *A) Insulin fluorescence in the presence of various polymers at 37°C; SPR sensorgrams of insulin binding to polymers B) with different BuMA content, C) varying degree of polymerization; and D) Molecular structure of the SPB2_BuMA system showing the polymer acting as a molecular shield.*

References: [1] Ross et al., Nature Medicine, 2004, 10, S10–S17; [2] Wang, Int. J. Pharm., 2005, 289, 1-30; [3] Rajan et al., Mater. Adv., 2021, 2, 1139-1176.

S1.7-O2

Zwitterionic polymers as next generation contrast agents for the diagnosis of osteoarthritis

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Abstract

INTRODUCTION. Osteoarthritis is a progressive joint disease which is still nearly impossible to diagnose at early stages. The low density of cartilage tissue limits radiographic detection to structural changes of bone and the reduction of the joint space width which are both features of late-stage osteoarthritis. Current (pre-)clinical contrast agents aim to detect early changes in cartilage. However, there is still further development needed as they suffer from poor specificity, slow penetration, or *in vivo* toxicity. Here, we report a zwitterionic polymer contrast agent which addresses all stated limitations (Fig. 1A).
METHODS. Our contrast agent is based on zwitterionic poly-carboxybetaine acrylamide (pCBAA) which is a highly hydrated polymer with outstanding biocompatibility and great cartilage penetration kinetics [1]. For radiographic imaging, iodinated co-monomers were synthesized to be incorporated into the polymer backbone. To investigate sensitivity to depletion of negatively charged glycosaminoglycans (GAGs) during disease [2], either anionic or cationic co-monomers were used (Fig. 1B). Additionally, a commercially available positively charged non-iodinated co-monomer was used to balance out the charges of the anionic co-monomer. Bovine cartilage explants were incubated with polymer solutions and the staining analyzed with micro-computed tomography (µCT).
RESULTS AND DISCUSSION. Cationic zwitterionic polymers give a 6-fold increase in contrast enhancement in healthy cartilage in µCT compared to anionic and a 2.5-fold increase compared to neutral polymers (Fig.1C). Healthy cartilage has a large negative charge density which leads to attractive forces facilitating penetration for cationic particles whereas anionic ones are repelled. We observed complete diffusion after 4 h of incubation. Increasing of the iodine co-monomer to CBAA monomer ratio enhances the contrast even further. Currently, we are exploring our polymers in the context of inflammatory and mechanical stimulated osteoarthritis models and test several peptide targets for the collagen network which allows specific detection of either healthy or diseased cartilage.

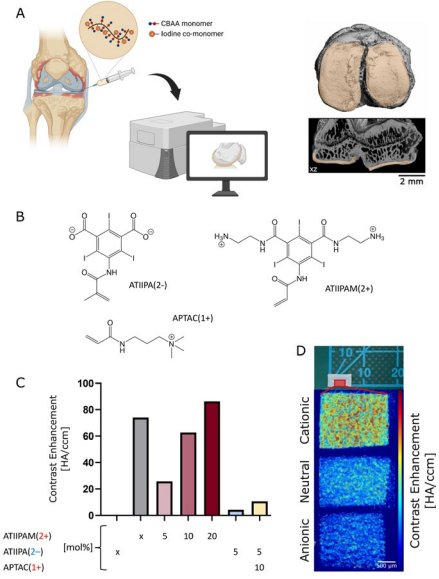

Figure 1: A) A contrast agent to visualize cartilage in micro-computed tomography for the early-stage diagnosis of osteoarthritis. B) Structure of our synthesized iodine co-monomers ATIIPA(2-) and ATIIPAM(2+) and the commercial APTAC(1+) as charge neutralizer in our polymers. C) Contrast enhancement of healthy cartilage disc induced by our polymers (0.5 mg iodine/ml). The first two bars (x) are non-polymeric co-monomers.

References. [1] Q. Li et al. Chemical Reviews (2022); [2] D. Umlauf et al. Cellular and Molecular Life Sciences (2010).
Acknowledgement. This work was supported by the Swiss National Science Foundation (Nr. 192656 to MZW).

S1.7-O3

Zwitterionic coatings on PEEK to reduce inflammatory response

Erika Roventini[1,2], Aliria Poliziani[1,2], Francesco Iacoponi[1,2], Giulia Ballardini[1,2], Leonardo Ricotti[1,2]

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Abstract

Introduction. Macrophages play a crucial role in the immune system, which initiates the inflammatory response upon encountering foreign objects in the body. However, this response can lead to foreign body reactions that cause complications in implantable devices [1]. Therefore, the investigation of coating capable to reduce the inflammatory response of macrophages is vital for various biomedical applications and could significantly improve the safety and longevity of implantable devices, enhancing their performance and reducing the risk of complications.

In this work, we combined a polydopamine coating as an adhesive primer and zwitterionic materials, used to create a hydration barrier [2]. A new protocol was proposed to functionalize a polyether-ether-ketone (PEEK) substrate, a material suitable for many biomedical applications [3].

Experimental method. A one-step strategy was used to create cross-linked zwitterionic networks on the PEEK substrate. PEEK disks were pre-wetted with ethanol and immersed in a solution containing Tris buffer solution, dopamine hydrochloride, zwitterion monomer (1:15 mass ratio), and a crosslinker (N-[3-(dimethylamino)propyl]acrylamide). Two different zwitterions, 2-methacryloyloxyethyl-phosphorylcholine (MPC) and [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium-hydroxide (SBMA), were tested. The hydrophilicity of the coated samples was evaluated by measuring the static water contact angle (WCA), while the stability was investigated with WCA measurements at different time points. Protein adsorption was detected by evaluation of BSA-FITC fluorescence. RAW264.7 cells were seeded on the coating samples and inflammatory response was evaluated by TNF-α, IL-6, and NO release after the induction of the inflammatory condition by adding LPS to the culture.

Results and discussion. The two coatings showed high hydrophilicity (WCA~10°); the MPC coating achieved higher stability over 4 months (Figure 1). In the zwitterionic coatings, protein adsorption was reduced (Figure 2A). With the MPC coating, cytokine release was lower, and NO was inhibited compared to the control (Figure 2B).

Conclusion. This work paved the way to functional coatings to mitigate inflammatory events on PEEK-based implantable devices. Future experiments will investigate the coating on different substrates to evaluate its versatility.

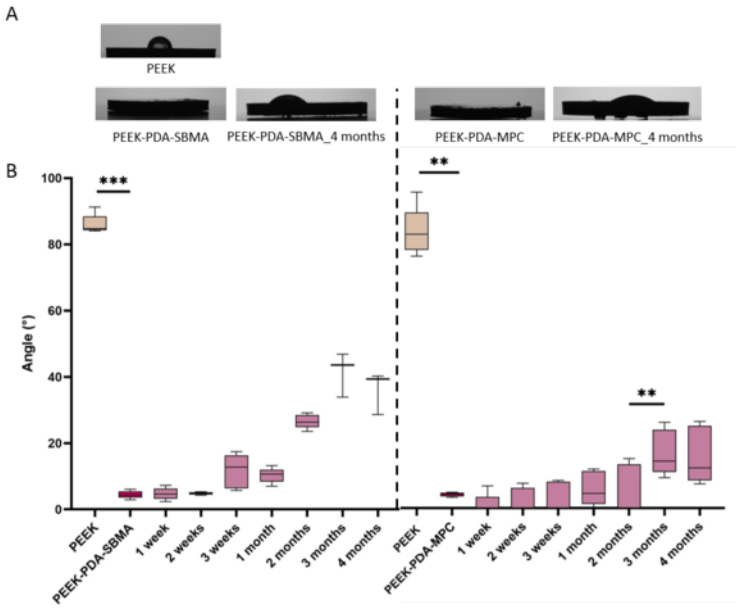

Figure 1: A) Representative photos of WCA goniometer. B) WCA evaluation.

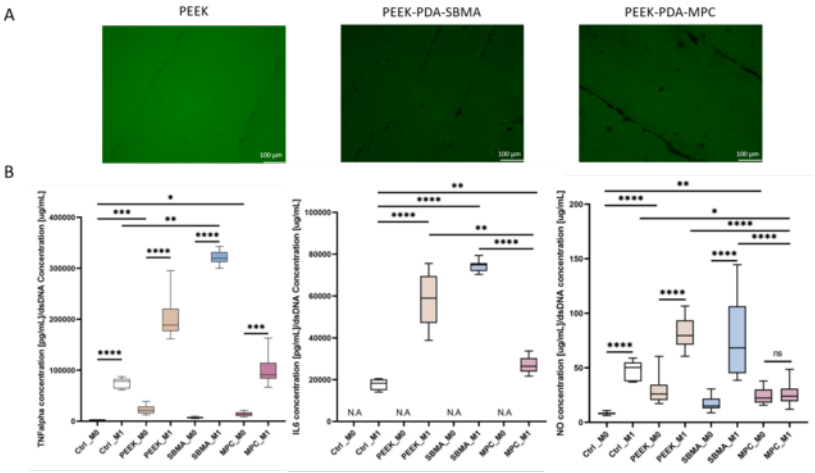

Figure 2: A) Representative images of BSA-FITC adsorption. B) TNF-α, IL-6 and NO evaluation.

Acknowledgment. This work has been funded by the European Union's Horizon-2020 research and innovation program under grant agreement No.951933, project FORGETDIABETES.

References: [1] J.M.Anderson et al., Semin.Immunol., vol.20, pp.86–100, 2008; [2] A.Chen et al., Adv.Healthc.Mater., vol.12, p.2200807, 2022; [3] I.V.Panayotov et al., J.Mater.Sci.Mater.Med., vol.27, 2016.

S1.7-O4

Zwitterionic granular hydrogels attenuate immune response both *in vitro* and *in vivo*

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Abstract

Background. Microporous granular hydrogels made of annealed microgels have attracted a lot of attention, due to their versatility and numerous advantages over bulk hydrogels [1]. While a lot of *in vitro* studies have been reported on cell-biomaterial interactions in granular hydrogels, their immunomodulatory properties and *in vivo* biocompatibility in immunocompetent animal models have not been well characterized. Moreover, while *in vivo* anti-fibrotic properties of bulk zwitterionic hydrogels have been well established [2], it is unknown whether this characteristic is also present in zwitterionic granular hydrogels.

Methods. Herein, we produced enzymatically annealed zwitterionic poly-carboxybetaine-acrylamide (CBAA) granular hydrogels using mechanical fragmentation technique, with two different microgel sizes and porosities, and studied their *in vitro* and *in vivo* immune response compared to bulk CBAA hydrogels. To study the effect of biomaterial type, we included hyaluronic-acid (HA) bulk and granular hydrogels, as a common natural biomaterial in tissue engineering. The *in vitro* immune response was studied using THP-1-derived macrophages and *in vivo* biocompatibility was studied with acellular hydrogel implantation in C57BL/6 mice for 10 weeks.

Results. *In vitro*, while bulk and granular CBAA hydrogels had no stimulatory effect on macrophages, IL6 and IL-1β genes upregulation was observed for all HA hydrogels, with granular hydrogels having more stimulatory effect compared to bulk. *In vivo*, it was shown that compared to HA bulk hydrogels, CBAA bulk and granular hydrogels and HA granular hydrogels resulted in thinner and less dense collagen capsules, as an important indicator of foreign body response. Moreover, it was shown that the number of infiltrated host cells into the hydrogels and the fraction of CD68+ inflammatory cells depend on hydrogel composition, with almost no cell infiltration into the anti-fouling CBAA hydrogels and is increased with increased porosity for HA hydrogels. Also, a higher degree of neovascularization with larger lumens was observed around all granular hydrogels and CBAA bulk hydrogels which had less dense collagen capsules. Overall, this study provides novel and useful insights into how the design of granular hydrogels can direct immune response. Moreover, it shows *in vitro* immunomodulatory and *in vivo* anti-fibrotic and anti-fouling properties of zwitterionic granular hydrogels.

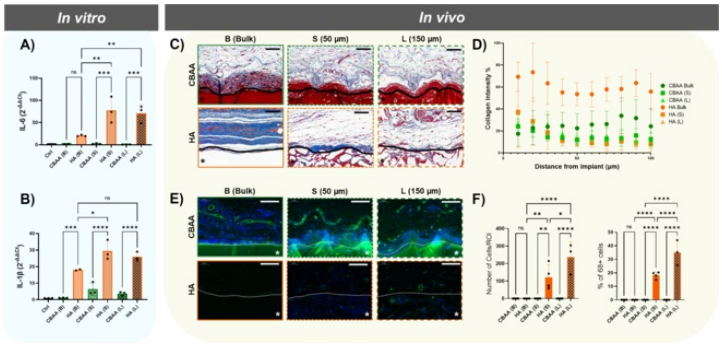

Figure A & B) In vitro qPCR data. C) Masson trichrome staining of in vivo implanted samples. D) Quantification of collagen capsule intensity E) CD31 staining for blood vessels F) Infiltrated cells quantification based on H & E and CD68 staining.

References. [1].Daly, A.C., Nature Reviews Materials, 2020; [3].Dong, D., Science advances, 2021.

S2.1-K1

Translation session co-organized by AODI

Anna Beltzung

dimpora AG, Zürich, Switzerland

Abstract

Besides staying in academia, where you stay at the forefront of the research, there are other ways where you can have a strong impact on society. As chemical/material engineers we are at the start of almost every added-value product intended to reach the market. It is then in our power to think twice about the materials that we decide to use in future products as we have the knowledge to make the right decisions. This talk will cover the journey to translating the products you've been developing since maybe your master thesis, through your PhD and maybe even your Post-Doc, into a market ready one using the case of dimpora AG, a Swiss startup revolutionizing the textile outdoor wear and sport industry.

S2.2-K1

Engineering the cell-matrix interface - understanding and guiding cell function

Claudia Loebel

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Abstract

The native extracellular microenvironment is dynamic, as cells synthesize, assemble, and remodel their surroundings during tissue development, injury, and repair. Hydrogels have evolved as valuable tools to both study mechanisms of cell-extracellular matrix (ECM) interactions (e.g., mechanobiology) and to guide desired cell behavior towards the development of new therapies (e.g., tissue repair/regeneration); however, the dynamic nature of the cell-ECM interface has been underappreciated. To address this, we are utilizing metabolic labeling techniques to visualize secreted matrix proteins to better understand how this nascent matrix influences cellular function and we are designing viscoelastic hydrogels that harness dynamic cell-hydrogel interactions. We have used these techniques to probe questions related to cellular mechanosensing in 3D, to better understand the evolution of matrix in modifying the cell-hydrogel interface in the engineering of tissues, and for the culture of organoids.

S2.2-O1

Phosphorylated focal adhesion kinase by mild reduction of cell surface proteins inhibits a RhoA/ROCK2-dependent adipogenic differentiation

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Abstract

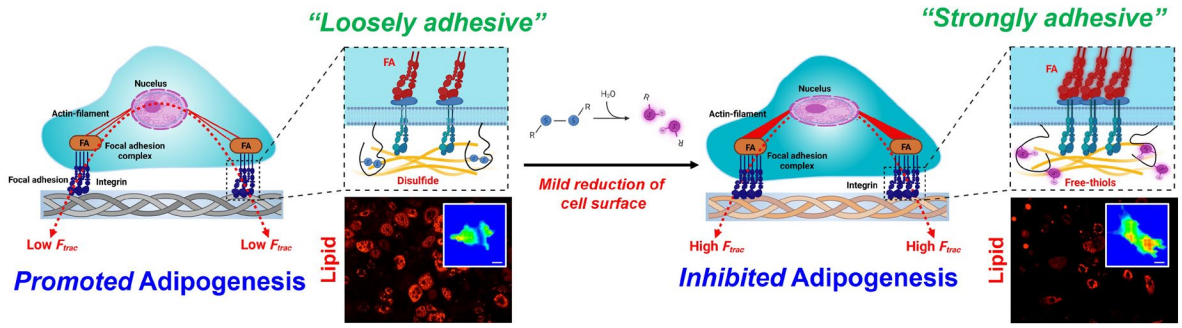

Figure: Schematic illustration of this study.

Although cell surface engineering has enormous potential to regulate various cellular functions, such as cell adhesion, morphology, migration, invasion, differentiation, and *in vivo* engraftment, less progress has been made in modulating cell fate determination through mild reduction of cell surface proteins. Thus, by harnessing the tris(2-carboxyethyl)phosphine (TCEP), a mile reducing agent with an ability to break disulfide bonds within and between proteins, we examined a molecular mechanism through which cell surface modification induces cell adhesion, spreading and fate determination with an emphasis on adipogenic potential of murine preadipocytes (3T3-L1). Our findings revealed that through mild reduction, free active thiol groups on cell surface of preadipocytes were successfully exposed and cells exhibited increased cell size, elongation, number and area of focal adhesions, leading to the inhibition of adipogenic differentiation of 3T3-L1 cells. Furthermore, by harnessing cell traction and intracellular force microscopy, we reported that cell-ECM traction force and intracellular tension were significantly increased by mild reduction of cell surface. Collectively, these results highlight the role of mild reduction of cell surface in adipogenic differentiation, which may be exploited to regulate cell fate determination.

S2.2-O2

Visible light photocrosslinkable hydrogels for breast cancer microenvironment mimicking

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Abstract

Introduction. Adipose tissue (AT) is a highly connective tissue devoted in controlling tissue homeostasis. Alterations in AT are typically associated with pathologies progression, e.g., stiffness increases due to a dysregulated collagen deposition in breast cancer [1]. Understanding mechanisms regarding pathologies development represents a challenge in the last decade. Hydrogel-scaffold based 3D *in vitro* models arose as possible solution to recapitulate tumor microenvironment (TME). Herein, to mimic pathological AT, GelMA and GelMA/HAMA hydrogels were investigated tuning their physical and mechanical features, to modulated cell-laden responses.

Experimental activities. GelMA and HAMA were synthetized [2]. GelMA (10% w/V) and GelMA/HAMA (10%:1% w/V) hydrogels were obtained by adding a visible light photoinitiator compound (ruthenium Ru, sodium persulfate SPS) and exposing polymeric mixture at 405 nm. Three different concentrations of Ru/SPS were tested. *In vitro* stability and mechanical compressive tests were performed to assess how different ratios of Ru/SPS influenced samples properties. Then, preadipocytes (3T3-L1) and human breast cancer cells (MCF7) were separately encapsulated in GelMA and GelMA/HAMA to evaluate hydrogels capability to recapitulate breast TME.

Results and Discussion. Photocrosslinking process allowed to obtain stable GelMA and GelMA/HAMA hydrogels up to 4 weeks of immersion in physiological-like environment. An increase in water uptake was identified ($p < 0.05$) by decreasing the total amount of Ru/SPS and adding hydrophilic HAMA into the polymeric mixture [1]. Mechanical properties confirmed physical features; a lower Ru/SPS amount determined softer hydrogels, and HAMA addition critically increased mechanical properties compared to GelMA hydrogels. GelMA hydrogels exhibited an elastic modulus in 2.14-7.41 kPa range (i.e., healthy AT), and GelMA/HAMA hydrogels in the 10.41 to 14.27 kPa range, reflecting pathological AT conditions (**Fig.1**) [3].

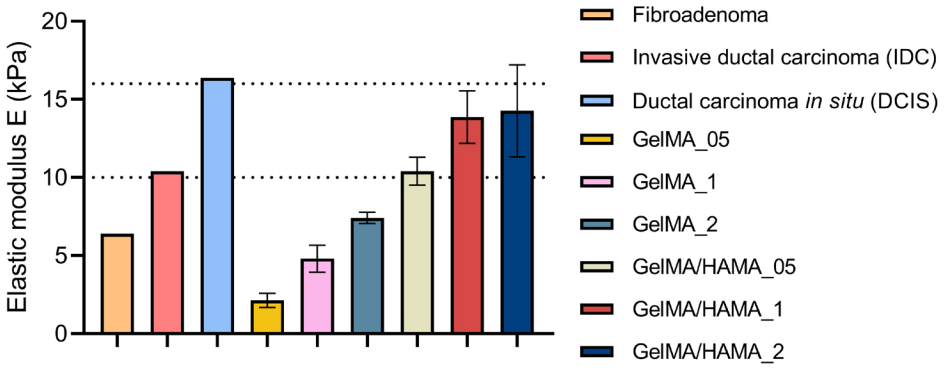

Fig. 1: Mechanical compressive tests on pure (GelMA) and hybrid (GelMA/HAMA) hydrogels. Elastic modula of obtained samples were compared with benignant breast cancer condition (i.e., fibroadenoma) and with different stages of pathological breast cancer (i.e., IDC, DCIS). Regarding developed hydrogels, GelMA_05, GelMA_1 and GelMA_2 are referred to pure GelMA hydrogels obtained with lower, intermediate and higher Ru/SPS concentration; GelMA/HAMA_05, GelMA/HAMA_1 and GelMA/HAMA_2 described hybrid hydrogels photocrosslinked with the lower, intermediate and higher amount of Ru/SPS.

3T3-L1 proliferated (AlamarBlue) and differentiated (Nile Red) better in softer GelMA/HAMA hydrogels, while MCF7 proliferated in GelMA/HAMA hydrogels and exhibited their typical tendence to aggregate in clusters (**Fig.2**) [4].

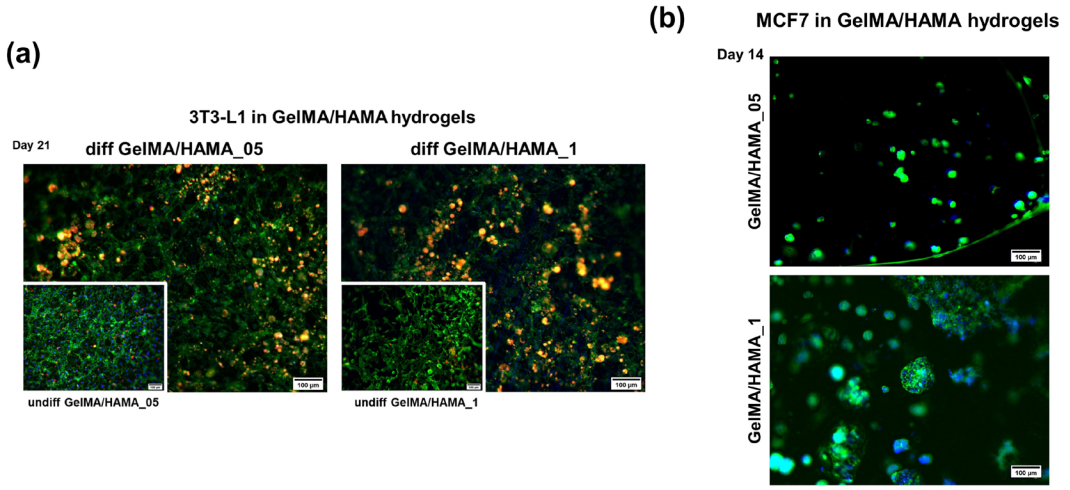

Fig. 2: Biological tests on hybrid (GelMA/HAMA) hydrogels. (a) Preadipocytes growth, proliferated and differentiated in differentiated samples. Lipidic droplets were stained via Nile Red after 21 days of cell culture; F-actin was counterstained in both differentiated and undifferentiated samples. (b) Breast cancer cells proliferated and aggregated in samples after 14 days of cell culture. Nuclea were counterstained via DAPI staining.

Conclusions. Developed GelMA and GelMA/HAMA hydrogels confirmed to be adequate in mimicking breast TME. In particular, by simply changing photoinitiator amount, we observed drastic variations in hydrogels features so to possibly mimic different steps of tumor development. Embedding a 3D printed sacrificial vasculature-like structure in Pluronic F127 allowed to obtain internal hollow channel to promote a better viability of the cells embedded in the developed hydrogels.

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S2.2-O3

Parallelized measurement of 3D single-cell mechanics using acoustofluidics

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Abstract

The mechanical properties of cells and the mechanical forces acting upon cells can play a major role in their development and function in almost all tissues of the body, ranging from musculoskeletal to immune cells. The importance of mechanobiology in health and disease is only starting to be understood, also due to a lack of appropriate tools.

The standard techniques for measuring cell mechanical properties, e.g. AFM-based nanoindentation, require a high level of expertise, provide a low throughput, and can only be employed on adherent cells in 2D. Microfluidic approaches like deformability cytometry overcome some of these shortcomings, but do not allow to track the same cell over time, or to study the cell's function simultaneously.

Here, we propose a new method that novelly allows for the simultaneous measurement of cell stiffness and cell function on a single-cell level. To track the individual changes over time on a larger cell population, a new stationary, parallelized, and compartmentalized method is employed. For this, we combine single-cell encapsulation by droplet microfluidics and fluorescent functional analysis with parallel acoustofluidic manipulation.

In acoustofluidics, a piezoelectric actuator generates a standing acoustic field inside a microfluidic chamber, which forces cells to levitate and deform in the low-pressure node. By employing this method on a cell population encapsulated individually in droplets arranged in a 2D array and placed on a fluorescence microscope, this enables the parallel measurement of cell stiffness for hundreds to thousands of single cells. Thereby the study of heterogenous cell samples and a high throughput are facilitated. Employing this method intermittently for hours to days, a time-wise tracking of single-cell mechanical properties is possible, thereby correlating dynamic functional to mechanical changes.

Since the system can be versatilely employed for any cell type, it opens up the possibility to study a variety of questions regarding the interplay between the dynamics of the mechanical properties of cells and their functionality. Thereby, this novel experimental system will facilitate valuable insights into the mechanisms behind our body's health and disease.

S2.2-O4

Hybrid pH-sensing systems for precisely probing single-cell acidification in *in vitro* tumor models

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Abstract

The tumor microenvironment is characterized by an elevated hydrogen ion concentration, which is the result of increased cellular metabolic demand and altered perfusion, e.g., oxygen availability or acidic metabolic waste products. [1] The acidity of the tumour microenvironment, which is spatially and temporally heterogeneous, [2] affects cancer initiation and progression, but also the efficacy of anti-cancer drug treatments. [3] Therefore, monitoring the local pH metabolic fluctuations is critical for understanding the basic biology of the tumour, and can also be used as a valid metabolic readout for cancer diagnosis and treatment.

Ratiometric fluorescence-based pH sensors based on micro- and nanoparticles represent reliable tools for spatio-temporal pH detection, thanks to their minimal invasive features and high reliability in terms of measurements, which are independent from probes concentration changes, instrument sensitivity and environmental conditions.[4-6].

We applied fluorescent pH sensors to measure intracellular pH in MDA-MB-231 and MCF-7 breast cancer cells and automatically tracked and screened massive internalization events of pH sensors (**Figure 1a**). We found that the mean acidification time is comparable among the two cell lines; however, MCF-7 cells showed a much broader heterogeneity in comparison to MDA-MB-231 cells [4]. In addition, we devised a new method to precisely quantify single-cell fermentation fluxes over time by combining high-resolution pH-sensing electrospun nanofibers with constraint-based inverse modelling (**Figure 1b**). We applied our method to cell cultures with mixed populations of cancer cells and fibroblasts and found that the proton trafficking underlying bulk acidification was strongly heterogeneous, with maximal single-cell fluxes exceeding typical values by up to 3 orders of magnitude.[7] Our method addressed issues ranging from the homeostatic function of proton exchange to the metabolic coupling of cells with different energetic demands, allowing for real-time non-invasive single-cell metabolic flux analysis.

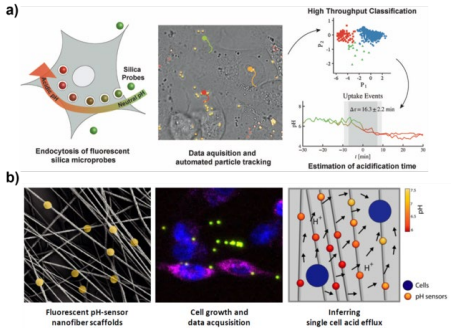

*Figure 1. Spatio-temporal detection of **a)** intacellular and **b)** extracellular acidification by means of fuorescence-based pH microsensors.*

Acknowledgments. The research leading to these results was supported from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 759959, ERC-StG "INTERCELLMED").

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S2.3-K1

Supramolecular (Bio)materials: From fundamentals to advanced solutions for pressing challenges in society

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Abstract

Supramolecular (bio)materials exhibit highly useful properties that are impossible with traditional materials but crucial for a wide variety of emerging applications in industry or biomedicine. These materials typically employ enthalpy-dominated crosslinking interactions that become more dynamic at elevated temperatures, leading to significant softening. Herein, we will discuss the development of a supramolecular hydrogel platform exploiting dynamic and multivalent interactions between biopolymers and nanoparticles that are strongly entropically driven, providing alternative temperature dependencies than typical for materials of this type. We will discuss the implications of these crosslinking thermodynamics on the observed mechanical properties, demonstrating that tuning the thermodynamics and kinetics of these crosslinking interactions enable broad modulation of the mechanical properties of these materials, including their shear-dependent viscosities, temperature responsiveness, self-healing, and cargo encapsulation and controlled release. These materials exhibit viscous flow under shear stress (shear-thinning) and rapid recovery of mechanical properties when the applied stress is relaxed (self-healing), affording facile processing though direct injection or spraying approaches, making then well served for applications in industry and biomedicine. Moreover, the hierarchical construction of these biphasic hydrogels enables innovative approaches to formulation and delivery as a diverse array of compounds over user-defined timeframes ranging from days to months. In one example, we demonstrate that these unique characteristics can be leveraged to generate vaccines exhibiting greatly enhanced magnitude, quality, and durability of immune responses. In another example, we demonstrate that these materials can be leveraged to generate new wildland fire retardant formulations enabling prophylactic treatments of high-risk landscapes for wildfire prevention. Overall, this talk will illustrate our recent efforts exploiting dynamic and multivalent interactions between polymers and nanoparticles to generate hydrogel materials exhibiting properties not previously observed in biomaterials and affording unique opportunities in industry and biomedicine.

S2.3-O1

Design of supramolecular tissue-adhesive hydrogels to prevent postoperative adhesion

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Abstract

Introduction: Postoperative adhesion is serious complication in which the surgical wounds and surrounding organs adhere with each other, causing intestinal obstruction, infertility, and abdominal pain. So far, sheet materials and two-component sprays have been used. However, sheet materials have low tissue adhesion due to their difficulty in adhering to uneven tissue surfaces and low operability under an endoscope. Moreover, two-component sprays have issues such as inefficient mixing of the two liquids caused by the spray device. Therefore, this study aims to develop supramolecular, single-syringe hotmelt tissue-adhesive hydrogels with excellent operability, tissue adhesion, and anti-adhesion properties (Figure 1) [1]. We synthesized tendon gelatin modified with ureidopyrimidinone (TGUPy). The UPy units dimerize by quadruple hydrogen bonding and amplify intermolecular hydrogen bonds, which can increase sol-gel transition temperature of gelatin-based hydrogels to achieve hotmelt properties that solubilize upon heating and form gels at body temperature. In this presentation, we will report on rheological properties of hydrogels, tissue adhesion tests, and evaluation of anti-adhesion function.
Methods: TGUPy was synthesized by the reaction of UPy units with terminal isocyanate group. TGUPy was dissolved in phosphate buffered saline at 20 wt%, and temperature dependent viscoelastic properties were evaluated by rheometer. In addition, adhesion tests were performed on porcine colon tissue in accordance with ASTM F2258-05. Rat cecum-abdominal adhesion models were used to check barrier property. Anti-adhesion performance was evaluated by scoring and histological observation.
Results and Discussion: The sol-gel transition temperature of gelatin was controlled depending on the UPy contents. The sol-gel transition temperature of TGUPy increased to about 40.0 °C, and the shear modulus at 37°C was enhanced as compared to original TG. In addition, tissue adhesion strength to the outer membrane of porcine colon tissue was evaluated. TGUPy became a low-viscous liquid upon heating, penetrated into the tissue gaps and subsequently formed hydrogels when the temperature decreased, thereby adhering to the tissues. Furthermore, anti-adhesion tests using a rat cecum-peritoneum adhesion model confirmed that TGUPy prevented postoperative adhesion. This supramolecular anti-adhesive barrier has enormous potential to prevent postoperative complications and to contribute to minimally invasive medicine as a medical device.

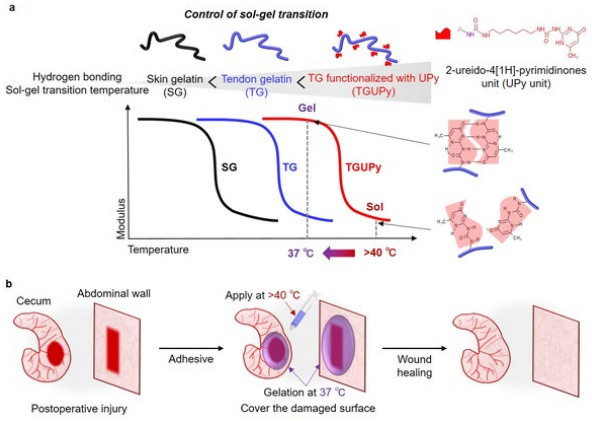

Figure 1. Schematic illustration of supramolecular, tissue-adhesive hydrogels to prevent postoperative complication.

References: [1] A. Nishiguchi, H. Ichimaru, S. Ito, K. Nagasaka, T. Taguchi, Acta Biomater. 146, 80-93 (2022).

S2.3-O2

In vivo and *in vitro* astrocyte-to-Neuron conversion by AAV encoded transcription factors embedded in bioinspired self-assembling peptide hydrogels

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Abstract

Mammalian post-developmental brain and spinal cord have largely lost neuroregeneration capability except for a few neurogenic niches. Neural injury or degeneration to this intricate organ often leads to neuron loss and impaired neurocircuits and has also been widely reported to associate with the dysregulation and activation of glial cells. The resultant active glial cells, especially astrocytes, act as a physical and biochemical barrier to neuronal regeneration that further retard the healing process of the brain after injury. Reversing this natural biological response to the central nervous system (CNS) injury has been proven to be extremely challenging using existing treatment approaches. Therefore, converting glial cells into functional neurons in situ provides an efficient way to obtain desirable endogenous neurons from a large cellular pool for ‘‘on-site’’ brain repair. As such, *in vivo* cell fate conversions have emerged as potential regeneration-based therapeutics for injury and disease.

In this study, we examined the ability of adeno-associated viral vector (AAV) encoded with genes relevant for reprogramming astrocytes (directly and indirectly) into functional neurons. Our *in vitro* results demonstrated the significant transduction and conversion efficiency resulting in successful astrocyte-to-neuron conversion through direct and indirect reprogramming (Figure 1. A, B). Moreover, the functionality of the reprogrammed neurons was confirmed by electrophysiology. The *in vivo* generation of induced neurons represents the next generation of potential treatment options for patients with brain injuries or neurodegenerative diseases. However, challenges remain with their uncontrolled dissemination, off-target toxicity, and neutralization.

To address this, we utilize a bioinspired self-assembling peptide (SAP) hydrogel to provide an extracellular matrix (ECM)-like structure to preserve damaged cells and facilitate their survival, proliferation, and differentiation. These synthetic SAP hydrogels are peptide sequences that self-assemble to spontaneously immobilize fluid via supramolecular interactions and create a highly hydrated three-dimensional. These hydrogels’ inherent biocompatibility, tunable properties, and capacity to flow and gelate in situ allow them to effectively transport, hold and release therapeutic molecules and AAVs in a spatially and temporally controlled manner while providing physical support for the surrounding tissue. These injectable hydrogels addressed multiple difficulties with viral vector delivery by shielding viral vectors, confining them to the site of therapeutic need, and minimizing immunogenicity. Our *in vivo* results in needle stick injury mice models demonstrated a significant neural regeneration upon hydrogel+AAV implantation (Figure 1. C, D). Furthermore,biomaterial implantation neither activated resident microglia/other immune cells nor further exacerbated reactive astrogliosis and subsequent glial scar formation.

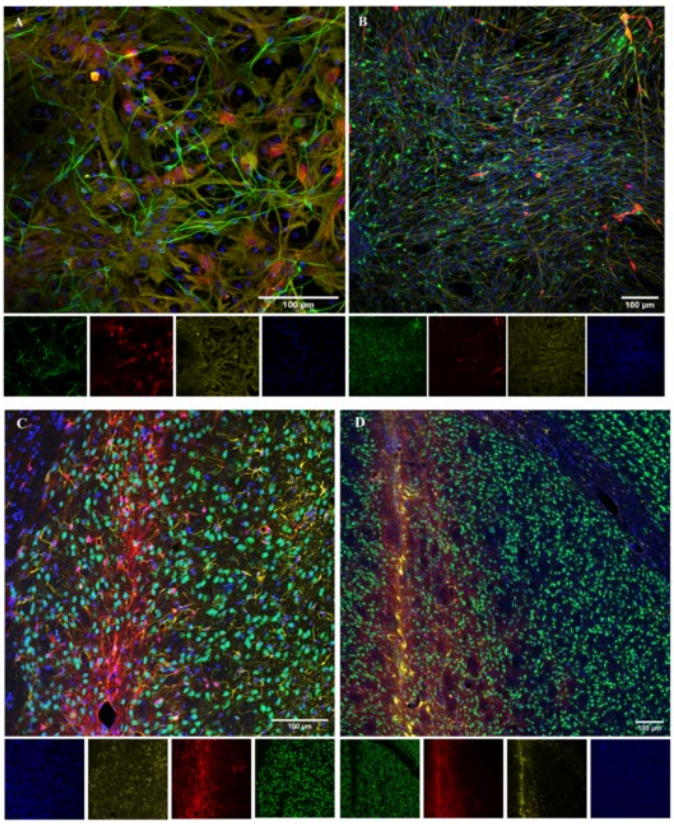

Figure 1. *Conversion of reactive astrocytes to neuron. In vitro direct (A) and indirect (B) reprogramming of astrocytes to neurons. In vivo transcription factor-AAV transduced mice (C) and empty vector transduced mice (D). Cells were immunostained with β III-Tubulin/NeuN (green), anti-mCherry (red), anti-GFAP (yellow), and DAPI (blue).*

S2.3-O3

Injectable tough double-network hydrogels with tunable physico-chemical properties, high stretchability, self-recovery and fatigue resistance

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Abstract

Injectable hydrogels have been extensively used as promising therapeutic scaffolds for a wide range of biomedical applications, such as tissue regeneration and drug delivery. However, their low fracture toughness and brittleness often limit their scope of application. Double-network (DN) hydrogel, which is composed of independently cross-linked rigid and ductile polymer networks, has been proposed as an alternative technique to compensate for the weak mechanical properties of hydrogels. Nevertheless, some challenges still remain, such as the complicated and time-consuming process for DN formation, and the difficulty in controlling the mechanical properties of DN hydrogels. In this study, we introduce a simple, rapid, and controllable method to prepare in situ cross-linkable injectable DN hydrogels composed of acrylamide (AAm) and 4-arm-PPO-PEO-tyramine (TTA) via dual Fenton- and enzyme-mediated reactions. By varying the concentration of Fenton's reagent, the DN hydrogels were rapidly formed with controllable gelation rate (5 - 60s). The rheological analysis was also processed to confirm the gelation kinetics of DN hydrogels and showed that the two networks formed sequentially. Importantly, the DN hydrogels showed a 13-fold increase in compressive strength (over 3 MPa) and a 14-fold increase in tensile strength (0.27 MPa), compared to the single network hydrogels. The mechanical properties, elasticity, and plasticity of DN hydrogels could also be modulated by simply varying the preparation conditions, including the cross-linking density and reagent concentrations. At low cross-linker concentration (< 0.05 wt.%), the DN hydrogel stretched to over 6,500 % with necking phenomena; whereas, high cross-linker concentration (≥ 0.05 wt.%) induced fully elastic hydrogels, without hysteresis. Besides, DN hydrogels were endowed with rapid self-recovery, highly enhanced adhesion, and strain-dependent conductivity, which can be applied to motion sensor. Moreover, human dermal fibroblasts treated with DN hydrogels retained viability, demonstrating the biocompatibility of the cross-linking system. Therefore, we expect that the dual Fenton/enzyme-mediated cross-linkable DN hydrogels offer great potential as advanced biomaterials applied for hard tissue regeneration and replacement, sensors, and wearable devices.

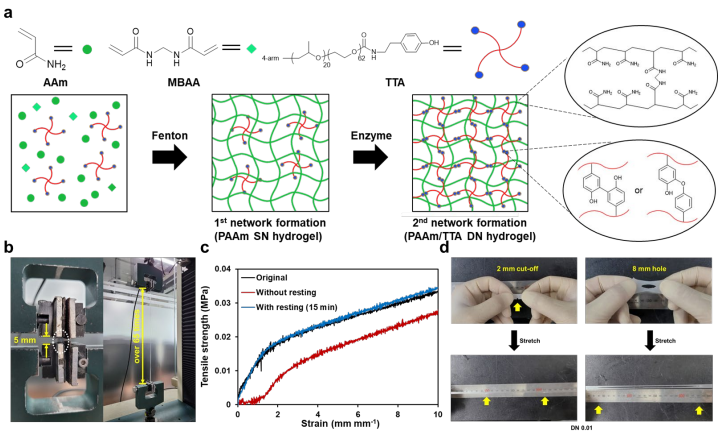

Figure. (a) Schematic representation of injectable PAAm/TTA DN hydrogel prepared by dual Fenton/enzyme-mediated cross-linking reactions (b) elongation capacity of DN hydrogel (over 6,500 %) (c) self-recovery property of DN hydrogel with 15 min of resting and (d) crack-resistance of DN hydrogels with 2 mm of cut-off, and 8 mm of hole.

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

S2.3-O4

HELP biopolymer functionalisation for production of multifunctional scaffolds with improved cytocompatibility

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Abstract

In the last two decades, the tissue engineering field has developed exponentially, and new challenges are faced especially those concerned with the production of multifunctional bioscaffolds that can promote cell adhesion, and regulate cell behavior and tissue integration with biomaterials. Genetically engineered biopolymers inspired by natural proteins such as elastin, collagen, or gelatin have high biocompatibility, a low risk of generating toxic by-products, and offer the possibility of developing scaffolds that are expected to be integrated into biological environments. Moreover, due to their proteic nature, they can be easily customised with bio-active domains to produce functional bio-based materials.

Different proteins and peptides have been produced in fusion with Elastin-like Polypeptides (ELPs), gaining the advantages that this biotechnology provides. Till now, several antimicrobial peptides (AMPs) have been fused to ELP N or C terminus. However, any of these resulting products have been used to produce hydrogels, matrices, or biomimetic components for 3D-printed scaffold fabrication.

In our lab, the Human Elastin-like Polypeptide (HELP) platform has been used to design and produce HELP-based biopolymers with cutting-edge functionality by bioactive domain fusion. A method to prepare 3D hydrogel matrices of these biopolymers has been developed. In view of realizing biomimetic components to produce biocompatible scaffolds endowed with antimicrobial properties, HELP routinely produced in our lab was successfully functionalised with the human β-defensin 1 (hBD1, NM_005218.4). Recognition sites for highly specific endoproteinases were introduced to release hBD1 from the fusion product and two main products were obtained. The incubation with the Glu-C enzyme resulted in a 47 aa peptide and a 36 aa peptide was released by the reaction with Asp-N endoproteinase. The antimicrobial activity of the biopolymer, as well as the Glu-C and Asp-N, released peptides were assayed by the radial diffusion assay revealing that the peptide released by GluC was active against E. coli. ATCC 25923. To assess the cytocompatibility of the fusion biopolymer, MG-G3 cells were seeded on different polystyrene surfaces coated with the biopolymer. Adhesion assays were performed by crystal violet staining. Finally, MG-63 cells were seeded on the 4% biopolymer 3D matrix. The results showed the improved cytocompatibility of this matrix. Therefore, we envision the possibility to integrate this fusion protein into scaffolds to realize bio-interfaces with antimicrobial activity and enhanced cell adhesion that support cell proliferation.

Acknowledgement: This work is supported by the EU-funded AIMed (grant n. 861138) and STOP (grant n. 101057961) projects.

S2.4-O1

Green nanomedicine and sustainable drug-releasing systems for regenerative medicine.

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Abstract

Environmental sustainability is a key challenge driven by an increasing consumption of natural resources. Waste materials from agriculture are promising as renewable resources due to their high availability, renewability and low-cost. Hence, more efforts are needed to the study of plant-derived materials for biomedical and pharmaceutical applications. Particularly, efficient and safe nanocarriers are demanded to exploit RNA therapies for new applications, e.g. cardiac regeneration[1]. Zein is a by-product of corn industries, representing almost 50% of corn protein. Zein nanoparticles (ZNPs) have been mostly used for the release of lipophilic drugs. Only few recent studies have proposed ZNPs for plasmid DNA release[2,3]. Pectin is a biocompatible low-cost polysaccharide extracted from citrus peel waste, promising for regenerative medicine. However, it lacks bioactivity and proper biodegradability.
Herein, we designed sustainable and efficient ZNPs for microRNA (miRNA) therapy to be embedded into injectable and printable chemically-modified pectin/gelatin hydrogels for controlled release.
Green ZNPs loaded with miRNAs were prepared by a novel method able to minimize the use of organic solvents. Physicochemical (DLS analysis, encapsulation efficiency, release kinetics) and biological (*in vitro* biocompatibility, and uptake efficacy using siRNA-Cy5 loaded ZNPs) properties of ZNPs were analyzed. Pectin was modified to introduce reactive groups and obtain crosslinkable hydrogels by blending with gelatin. Optimal pectin/gelatin compositions were selected by rheological characterizations and *in vitro* cell tests. ZNPs loaded with curcumin were also produced to provide anti-oxidant and anti-inflammatory effects.
ZNPs/miRNA showed higher encapsulation efficiency than previous ZNPs/plasmid DNA reported in the literature[2,3] (more than 90% compared to 40-65%) and high biocompatibility. Anti-oxidant activity of ZNPs/curcumin was assessed by *in vitro* cell tests. Optimised pectin/gelatin hydrogels showed injectability and printability and supported cell viability.
In conclusion, green ZNPs were optimized for the delivery of small oligonucleotides (miRNAs, siRNAs) and anti-oxidant agents (curcumin). In the future, ZNPs will be embedded into pectin/gelatin hydrogels, obtaining new green and efficient controlled drug delivery systems for advanced therapies.

Acknowledgement: This work was supported by Research and Innovation NOP 2014-2020 for Doctoral Research programmes with specific reference to Action IV.5 “PhD programmes on sustainability-based topics” and the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (BIORECAR, grant agreement No. 772168).
References: 1 Nicoletti and Paoletti et al., Nanomedicine: Nanotechnology, Biology, and Medicine, 45, 2022; 2 Regier et al., J. Nanobiotechnology, 10, 2012; 3 El Sharkawi et al., J. Drug Target., 25, 2017

S2.4-O2

Sustainable polymers of bacterial origin and their use in biomedical applications

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Abstract

Currently there is a huge need to find replacements for petrochemical-derived plastics which are not sustainable, degradable and lead to high concentrations of recalcitrant plastics. In the medical arena, currently there is a lot of use of plastics for packaging, implants, tissue engineering and drug delivery. However, there is hardly any attention paid to their sustainability and environmentally friendly aspects.
In this work we have focused on the production and use of bacteria-derived sustainable biomaterials for use in biomedical applications. Two main types of biomaterials have been focused on, including polyhydroxyalkanoates (PHAs) and bacterial cellulose (BC) (Figure 1).

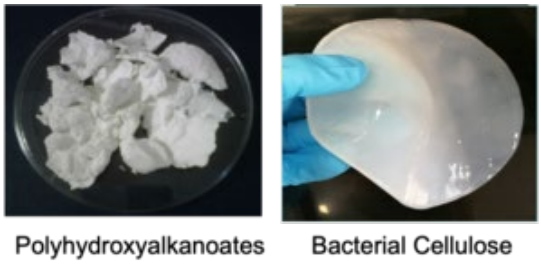

Figure 1: Polyhydroxyalkanoate and Bacterial cellulose produced using bacterial fermentation

PHAs are polyesters produced by a range of bacteria. These polymers are biodegradable in the soil and in the sea. In addition, they are also resorbable in the human body and are highly biocompatible. They are divided in to two main types, short chain length PHAs (scl-PHAs) with monomer chain length C_4-C_5 and medium chain length PHAs (mcl-PHAs) with monomer chain length C_6-C_{16}.The scl-PHAs are normally hard and brittle whereas the mcl-PHAs are soft and elastomeric in nature. Hence, we have used the scl-PHA, Poly(3- hydroxybutyrate) for bone tissue engineering, drug delivery, medical devices such as coronary artery stents, and the mcl-PHAs for cardiac, nerve, pancreas, kidney and skin regeneration. For bone tissue engineering we have used neat P(3HB) and composites of P(3HB) with Bioglass®, hydroxyapatitite and carbon nanotubes. The mcl-PHAs have been used for the development of cardiac patches, nerve guidance conduits, wound healing patches, bioartificial pancreas and bioartificial kidney. Processing techniques used include additive manufacturing, electrospinning and melt electrospinning.
BC can also be produced by a range of bacteria. It is a green polymer, is sustainable and degradable in the soil. It is also highly biocompatible and can be used in biomedical applications. Bacterial cellulose has been produced under static culture conditions. This is a highly nano-fibrillated structure and hence is a great substrate for cell attachment and growth. We have surface modified bacterial cellulose to create antibacterial bacterial cellulose. We have also used BC as a filler for P(3HB) based composites for bone tissue engineering.
In conclusion, we have successfully used bacteria-derived sustainable biobased materials for a variety of medical applications. Both PHAs and bacterial cellulose have a lot of potential in the future as sustainable materials of choice.

S2.4-O3

From agricultural waste to wound healing: sustainable development of barley extract-based bioactive dressings

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Abstract

Agriculture create huge amounts of bio-waste which can have a great negative impact on health and environment, if not properly disposed. Due to the low cost of agri-food waste and the high amount of bioactive compounds contained in it, there is a great interest in the extraction and exploitation of these molecules for the preparation of drugs and pharmaceutical products. In this context, barley has been receiving great attention. Proteins and polysaccharides are the major constituents of its grains. Among them, β-glucans are particularly interesting due to their biological properties, such as biocompatibility, biodegradability, and water adsorption capacity. In this work, mixtures of proteins and polysaccharides were extracted from barley grains, in both mild and high alkaline conditions, through a sustainable technology. Flexible and semi-translucent films, MA and HA respectively, were prepared and their mechanical properties compared with those of the skin appeared promising for skin applications [1]. In light of this, a physicochemical characterization was performed to evaluate their suitability as bioactive dressings. In particular, the swelling ability in both water and SWF, and the moisture uptake were investigated, and the results highlighting that MA exhibited a significantly higher percentage water uptake than HA (590 ± 13 % vs 270 ± 12 %), and both films are able to assure a good balance of tissue hydration, thus preventing the wound site from drying. Moreover, oxygen permeability of MA and HA (147.6 and 16.4 cm3×µm/m2×24h×Pa)×107, respectively) are comparable to other polysaccharide-based films intended as potential wound dressings. Biocompatibility was assessed through both indirect and direct tests, demonstrating that components released from the MA and HA films do not affect HDF viability and the surface properties allow a good cell attachment and proliferation. Additionally, an *in vitro* scratch test was carried out to determine the impact of released compounds on cell migration, and the results showed that HA promotes faster wound healing in fibroblasts than MA film in the first 16 hours. By lowering oxidative stress and pro-inflammatory cytokines such as IL-6 and increasing IL-10, a cytokine known for inhibiting inflammation, both HA and MA films are able of controlling the behaviour of macrophage cells in an inflammatory microenvironment. This behaviour may be ascribed to beta-glucans, which are carbohydrate components of barley extract-based films.

Reference. Razzaq et al. Carbohydrate Polymers 272 (2021) 118442

S2.4-O4

Non-woody biomass hydrogels for dermal application: Is two better than one?

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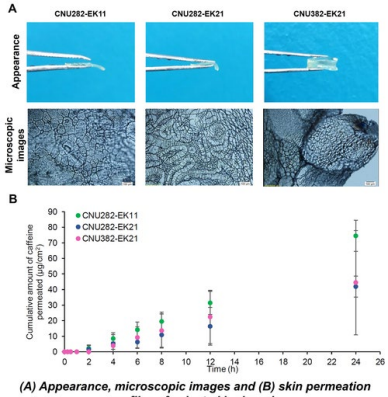
Abstract

Hydrogels are a promising platform for dermal drug delivery and biomedical applications. While native cellulose is useful for hydrogel fabrication, the environmental impact of deforestation makes non-woody biomass a more appealing alternative. Our recent report has shown the potential of using oil palm empty fruit bunch (OPEFB) in designing hydrogels for dermal drug delivery. Nevertheless, these hydrogels were too fragile to handle. In another study, we used kapok fibres to create hydrogels that were mechanically strong but less swellable. To address these issues, we combined OPEFB cellulose with kapok cellulose to create highly swollen and strong hydrogels suitable for dermal application.

Cellulose solutions (C: 2, 3, 4%w/v) were prepared by dissolving OPEFB (E) and kapok cellulose (K) (ratio of 1:1, 1:2, 2:1) in alkali solvents with different combinations of NaOH (N: 6, 7, 8%w/v) and urea concentrations (U: 2, 4, 6%w/v) before crosslinking. Both cellulose solutions and hydrogels were characterised and hydrogels were loaded with caffeine solution for skin permeation studies.

Cellulose solubility was positively influenced by OPEFB cellulose, NaOH and urea concentrations but not kapok cellulose. However, higher NaOH content can resolve the kapok solubilisation issue. After crosslinking, the swelling ratio of hydrogels prepared from 2%w/v cellulose (up to 4000%) was higher than that designed from higher cellulose content (up to 1500%). High kapok cellulose content reduced the swelling behaviour which is related to its limited solubility but NaOH content strongly improved the swelling ratio. Morphology investigations showed homogeneous porous structure at higher OPEFB and NaOH contents. Reduced porosity and thicker crosslinked network wall (Fig A) were observed at higher cellulose content (especially kapok) due to aggregation of cellulose chains. In most cases, urea content has a minor role on the hydrogel properties. The gel strength of hydrogels (>800% swelling ratio) increased with the cellulose content (4%w/v: ~47 N; 3%w/v: ~8 – 17 N; 2%w/v: ~6 – 8 N). Subsequent tests with selected hydrogels (CNU282-EK11, CNU282-EK21, CNU382-EK21) showed that a thicker hydrogel reswollen in caffeine solution achieved higher drug content despite sharing the same drug content by hydrogel weight (~27 – 28 mg/g). Nevertheless, similar skin permeation profile was obtained up to 12 h (Fig B) but CNU282-EK11 hydrogel achieved the highest drug permeated (74.5 ± 10.0 μg/cm^2) at 24 h due to a thinner structure. The hydrogels fabricated were tested biodegradable and non-toxic *in vivo*. In conclusion, combining different cellulose feedstocks can produce biocompatible hydrogels with desired properties for dermal application.



(A) Appearance, microscopic images and (B) skin permeation profiles of selected hydrogels

S2.5-K1

Smart wearable devices for on-demand healthcare applications

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POSTECH, Pohang, Korea, Republic of

Abstract

A variety of diagnostic and therapeutic devices are routinely used in the clinic. While these devices have a familiar look as wall-plugged in hard packages or placed at patients' bedsides, there has recently been a rapid expansion of new ideas on wearable healthcare devices for greatly improved patients' compliance. Here, we developed smart contact lenses and smart wearable devices for both continuous diabetic monitoring and diabetic retinopathy therapy. Smart contact lens could measure tear glucose levels as a non-invasive alternative to the conventional blood glucose tests and deliver drugs from gold coated reservoirs for the treatment of diabetic retinopathy. On the basis of these results, we also developed a smart wearable device for highly sensitive glucose monitoring in sweat for clinically feasible diabetic diagnosis. A blue-tooth system could send data wirelessly allowing patients to check their diabetic diagnosis results on the mobile phones. Furthermore, we developed cell-integrated poly(ethylene glycol) hydrogels for *in vivo* optogenetic sensing and therapy. The real-time optical readout of encapsulated heat-shock-protein-coupled fluorescent reporter cells made it possible to measure the nanotoxicity of cadmium-based quantum dots *in vivo*. Using optogenetic cells producing glucagon-like peptide-1, we performed light-controlled therapy and obtained improved glucose homeostasis in diabetic model mice. Taken together, we are currently working for the development of smart wearable devices for optogenetic cellular engineering for diagnostic and therapeutic applications. This presentation will provide the current state-of-the-art smart wearable devices for further clinical applications.

S2.5-O1

Real-time monitoring of the transepithelial/endothelial electrical resistance of a 3D-bioprinted lung model

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Abstract

With the outbreak of new respiratory viruses and high mortality rates of pulmonary diseases, physiologically relevant models of the human respiratory system are urgently needed to study disease pathogenesis, drug efficacy, and pharmaceutics. This talk introduces a bioelectronic platform based on organic electrochemical transistors (OECTs) for the real-time monitoring of barrier characteristics of 3D-bioprinted lung tissues. A three-layered lung model with an unprecedented thickness of about 10 um was fabricated by high-resolution drop-on-demand inkjet bioprinting. The 3D structured model better recapitulates the structure, morphologies, and functions of the lung tissue, compared not only to a conventional 2D cell culture model, as expected, but also to a 3D non-structured model of a homogeneous mixture of the alveolar cells and collagen. An OECT was integrated into the tissue culture platform to measure the transepithelial/endothelial electrical resistance of 3D-bioprinted lung models. We show that our platform can distinguish tissue growth in different culture conditions and disruptions in tissue microenvironments under H1N1 influenza virus infection.

S2.5-O2

Target-catalyzed Self-assembly of DNA Nanogel for Enzyme-free miRNA Assay

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Abstract

For the past decades, target-catalyzed toehold-mediated strand displacement (TMSD) cascade has been employed as an enzyme-free and isothermal reaction to amplify detection signals from DNA analytes. However, in contrast to DNA, the detection of RNA targets is very challenging due to the slow strand displacement kinetics. The rate constant of DNA-RNA strand displacement (8.62 ´ 104 M-1s-1) is ~ 35 times lower than that of DNA-DNA strand displacement (3 ´ 106 M-1s-1). Now, the key question is how to enhance the kinetics of TMSD for RNA targets to achieve pM-level sensitivity in a short detection time. To solve this problem, we propose a simple method based on the incorporation of base-pair mismatch into the stem of hairpin DNA to facilitate the formation of three-dimensional (3D) DNA nanogel for highly sensitive and specific miRNA assay. In the DNA nanogel-based FRET assay, target miRNA catalyzes the opening cycles of DNA hairpin loops tethered with a FRET acceptor into Y-shaped DNA structures, which are self-assembled with FRET donor-conjugated DNA complex into a nanogel. We placed a mismatch near the toehold to lower the hairpin's local stability and facilitate its opening by miRNA. As a result, our approach dramatically reduces the limit of miRNA detection from the nanomolar level to the picomolar level without increasing non-specific reactions. This method is particularly attractive for point-of-care (POC) miRNA assay because it does not require specialized equipment, expensive materials (e.g., enzymes), and unstable agents for long-term storage and delivery.

S2.5-O3

High-performance implantable bioelectrodes with immunocompatible topography and bioactivity for modulation of macrophage responses

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Abstract

Implantable bioelectrodes enable precise recording or stimulation of electrical signals with living tissues in close contact. However, their performance is frequently compromised owing to inflammatory tissue reactions, which macrophages either induce or resolve by polarizing to a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype, respectively. Thus, we aimed to fabricate biocompatible and functional implantable conductive polymer bioelectrodes by engineering their topographies or immobilizing anti-inflammatory cytokines (i.e., interleukin-4 [IL-4]) for the modulation of macrophage responses. Specifically, we found that these electrodes with optimal surface roughness significantly reduced macrophage recruitment, induced anti-inflammatory polarization, mitigated scarring around the implanted electrodes, and importantly allowing for recording electrocardiographic signals without substantial decreases in sensitivity. These simple and effective surface modification strategies for developing immune-compatible bioelectrodes will facilitate the development of various electronic medical devices that require high sensitivities and long-term stabilities.

S2.5-O4

Engineering dynamic materials with DNA towards adaptive biosensors

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Abstract

DNA, widely known as genetic material, is a powerful tool for programmably assembling different inorganic and organic materials into various types of nanostructures or biosensors. However, the majority of these applications do not quite fully utilize the dynamic controllability and structural adaptability of DNA strands. In this talk, I will introduce stimuli-responsive aspects of DNA-based materials, then suggest how we should utilize the techniques and materials developed towards biomedical sensing applications.

S2.6-K1

Structure and composition analyses of hierarchically organised biominerals studied using advanced multiscale *ex situ* and *in situ* microscopy and spectroscopy techniques

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Abstract

Understanding the properties of hierarchical biogenic composites and their formation is at the heart of the quest to harvest the principles behind biomineralisation for a wide range of applications in the field of medicine, environment or sustainable materials syntheses. The hierarchical nature of the biomineral and the organic matrix it forms in plays a vital role in providing the required properties, e.g. mechanical and chemical resilience as well as hardness and elasticity.

To address the topic of the hierarchical organisation key investigation tools such as Raman spectroscopy, scanning and transmission electron microscopy and X-ray based spectroscopy (small and wide angle X-ray scattering and nano-X-ray fluorescence) can be used in a multimodal fashion to analyse e.g. phosphate and carbonate biominerals such as bone, teeth, corals and sea shells across the length scale. Further, it allows for the contextualisation of high-resolution analyses of the microstructure in the wider frame of the composite hierarchy.

To study the dynamics of the biomineral formation process these methods need to be extended by *in situ* capabilities providing conditions similar to those in the biological context. Specially designed heatable flow cells can be used to enable the reproduction of the physico-chemical mineralisation conditions.

This presentation discusses, amongst other examples, the application of these techniques to elucidate details of phase transformations and structure formation of calcium phosphate in collagen, which is central to the process of bone formation. For this purpose, a polymer induced liquid precursor (PILP) based approach was chosen to obtain mineralisation characteristics similar to those found in the biological context providing intriguing insights into the mineralisation dynamics across the length scales.

S2.6-O1

Merging biomineralization and Engineered Living Materials to promote osteogenic differentiation

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Abstract

The great demand for bone implants, exacerbated by an ageing population has prompted the development of innovative materials able to foster the regeneration of damaged tissues. The emerging field of engineered living materials (ELMs) has given rise to unconventional strategies combining synthetic materials with engineered microorganisms which exhibit a unique combination of self-healing or regenerative properties, adaptation, longevity and sustainability.[1,2] In fact, the integration of a genetically modified *Lactococcus lactis* into synthetic materials has been pointed out as a controlled and efficient manner to express bone morphogenetic protein 2 (BMP2) inducing osteogenic differentiation in mesenchymal stem cells (MSCs).[2] However, engineering microenvironments that recapitulate the complexity of the bone extracellular matrix remains an ambitious challenge. In this line, biomineralization could endow ELMs with extraordinary properties. In bone, the integration and specific orientation of apatite nanocrystals into the type-I collagen fibres provide the biomineral with unique mechanical properties.[3] Biomimetic mineralization of several natural polymers is a widespread strategy in regenerative medicine due to their analogies with bone extracellular matrix. Among them, alginate stands out for its high biocompatibility, non-toxicity, low cost and easy gelification.[4]

Herein, we developed a protocol inspired by bone mineralization in which alginate (Al) gelification and apatite (HA) mineralization occur simultaneously, producing 3D mineralized gels with entrapped MSCs and genetically modified bacteria. We hypothesize that the introduction of the engineered *Lactococcus lactis* in the Al/HA system results in synergistic effects in terms of osteogenics differentiation of MSCs. Chemical composition, structure and mechanical properties were evaluated to study the effect of HA in the Al hydrogels. MSC viability and osteogenic differentiation in the absence and presence of the engineered bacteria were evaluated by a fluorescence viability staining, quantitative real-time polymerase chain reaction and immunofluorescence.

To sum up, we have developed a biocompatible 3D engineered living material to enhance the osteogenic differentiation of MSCs based on a synergistic effect between HA and an engineered *L. lactis* strain expressing BMP-2 in a controlled manner (fig.1).

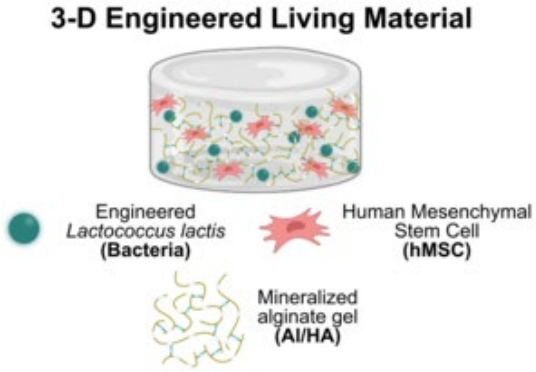

Fig.1: Graphical sketch of the proposed 3-D ELM consisting on Al/HA scaffolds entrapping the engineered Lactococcus lactis *and MSCs.*

References: [1] O'Brien, F. J., Mater. Today 14, 88–95 (2011); [2] Rodrigo-Navarro A. et al., Nat. Rev. Mater. 6, 1175–1190 (2021); [3] Delgado-López, J. M. et al., Acta Biomater. 49, 555–562 (2017); [4] Ramírez Rodríguez, G.B. et al., J. Natural polymers for bone repair. Bone Repair Biomaterials (Elsevier Ltd, 2019).

S2.6-O2

The triple point for bone graft substitutes: finding the optimal internal design of 3D printed PCL-$CaCO_3$ composites for mechanical stability, cell seeding and degradation

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Abstract

Healing of larger bone defects can be supported with a bone graft substitute (BGS) by providing a structure for bone cell proliferation and differentiation. The ideal BGS provides similar properties as the human bone, such as young's modulus, to build a stem cell niche for osteogenesis. Therefore, we investigated 3D printed polycaprolactone (PCL) scaffolds, incorporated and coated with calcium carbonate ($CaCO_3$), for the seeding with bone marrow-derived human mesenchymal stromal cells (BM-hMSC).

Scaffold designs were varied in terms of pore structure and infill density. The most suitable scaffold design was found by compression test on 3D printed structures as well as their capacity for cell ingrowth by MTT test over 7 days. To increase the mechanical stability of the scaffolds, precipitation of calcium carbonate as calcite was performed in 7 cycles (Savelyeva et al., 2017). Coated PCL-CaCO3-composites were subject to degradation tests in citric acid at pH 3, in PBS with lipase and 0.9% NaCl over 28 days, to investigate the influence of pore structure, surface degradation and calcium release, respectively. Scaffolds were analyzed by µCT, mass loss, Ca-ion release, pH and SEM. Single strands of each modification were degraded to analyze the impact on mechanical properties during degradation by 3-point tension testing.

Young's modulus of PCL could be increased by incorporation of $CaCO_3$ particles, even doubled depending on the size of the particles (Figure 1). In terms of scaffold design, an infill of 50% and triangular pores were found to be the most suitable for seeding with hMSCs in terms of attachment and proliferation rate. Differences in pore structure regarding the degradation velocity were investigated by incubation in citric acid. This degradation setup is the most comparable to *in vitro* degradation (Bartnikowski et al., 2019). Since literature is mainly focusing on degradation of PCL in PBS and lipase, these conditions were tested as well for comparability of results. While the PCL was almost completely degraded, a mineral layer was formed on uncoated samples, building a hollow shell after degradation of PCL. During degradation in 0.9% NaCl, the $CaCO_3$ coating acts as a barrier for Ca-ion release from incorporated $CaCO_3$ particles (Figure 2). Our results show a suitable internal design for PCL-$CaCO_3$ scaffolds which combine mechanical stability and successful cell seeding with accelerated degradation.

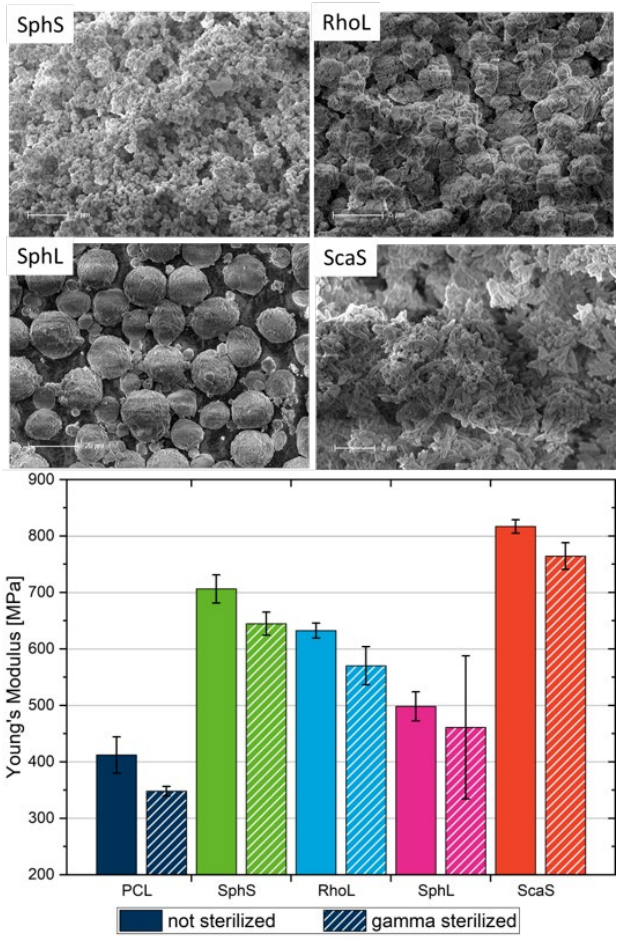

Figure 1: Improvement of young's modulus by incorporated $CaCO_3$ particles

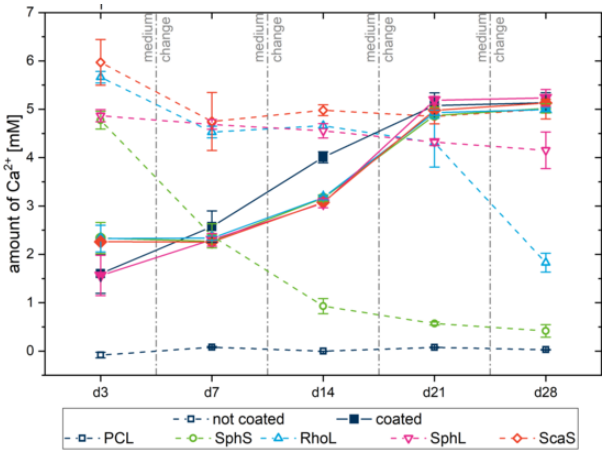

Figure 2: Ca-release during not accelerated degradation in 0.9% NaCl

S2.6-O3

Microcalcifications can trigger or suppress breast precancer malignancy potential as a function of mineral type in a 3D tumor model

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Abstract

Most early breast precancer lesions termed ductal carcinoma in situ (DCIS) contain microcalcifications (MCs), which are calcium-containing pathological minerals. The most common type of MCs is calcium phosphate crystals, mainly carbonated apatite, associated with either benign or malignant lesions. A less common type of MCs is calcium oxalate dihydrate (COD), which is almost always found in benign lesions. *In vitro* studies show that the crystal properties of apatite MCs can affect breast cancer progression. We developed a 3D tumor model of multicellular spheroids of human precancer cells containing synthetic MC analogs to link the crystal phase and properties of MCs with the progression of breast precancer to invasive cancer. Using light and scanning electron microscopy, vibrational spectroscopy, microCT imaging, and histopathology, we show that apatite crystals induce precancer cell proliferation and Her2 overexpression. This tumor-triggering effect is reduced with increased carbonate content in the MCs. COD crystals, in contrast, reduce Her2 expression compared to control spheroids with no added MC analogs. This finding suggests that COD is not randomly located only in benign lesions but may be actively contributing to suppressing precancer progression in its surroundings. Our model provides an easy-to-manipulate platform for a better understanding of the interactions between breast precancer cells and MCs, potentially providing new directions for precancer prognosis and treatment.

S2.6-O4

Bioinspired growth of oriented calcium phosphate nanocrystals arrays, towards the development of bactericidal nanostructured surfaces

Lorenzo Degli Esposti[1], Camilla Fusacchia[1], Alessio Adamiano[1], Davide Altamura[2], Cinzia Giannini[2], Riccardo Torelli[3], Maurizio Sanguinetti[3,4], Francesca Bugli[3,4], Michele Iafisco[1]

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Abstract

Crystal growth science has seen a surge of interest in the development of oriented nanocrystals arrays grown onto surfaces. These ordered nano-topographies lead to unique emergent properties, such as the antibacterial activity. For example, the surface of cicada wings is covered by tightly arranged hexagonal nano-pillars which are able to kill both Gram-positive and Gram-negative bacteria. This is caused by the excessive mechanical stretching of the bacterial membrane exerted by the nano-structured surface, which lead to membrane rupture and cell lysis. This antibacterial effect is particularly promising to counteract antibiotic-resistant bacteria, as it is a contact-based physical effect and thus bypasses the mechanisms of antibiotic resistance development. In the last few years, several artificial nano-topographies were obtained mostly by chemical vapor deposition or other top-down approaches to grow metallic or polymeric nanocrystals. However, these methods in some cases are slow, inefficient, and are limited to small surfaces; furthermore, the materials are not intrinsically biocompatible and are usually inert. On the other hand, calcium phosphate (CaP) minerals have excellent biocompatibility, and is possible to obtain nano-topographies with simple and cost-effective processes mimicking the biomineralization processes of dental enamel nanostructures. In this work I will present the developed of several CaP nano-topographies with a biomineralization-inspired bottom-up approach which allows to tailor size, shape, aspect ratio, spacing and orientation of the precipitated CaP nanocrystals (Figure 1).

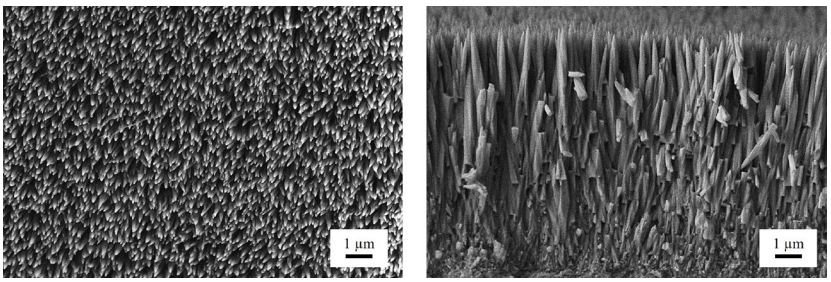

Figure 1. Example of Scanning Electron Microscopy (SEM) micrographs of ordered arrays of calcium phosphate nanocrystals.

We have studied in detail the crystal growth by advanced X-ray scattering techniques in order to understand and control the array formation and ordering. Furthermore, we have enhanced the antibacterial effect of the material through doping with antibacterial ions in order to superimpose the physical antibacterial mechanism with a biochemical one. Finally, we have assessed the biocompatibility and antibacterial activity of the nano-topographies against Gram-positive and Gram-negative bacteria co-cultured with eukaryotic cells lines.

Acknowledgement: We acknowledge the Italian Ministry of University and Research (MUR) (Prin 2020, Research Grant 202085RFNY "Antimicrobial peptides loaded inhalable calcium phosphates nanoparticles for the counteraction of antibiotic resistance: towards a new therapy for respiratory infections (AppliCare)".

S2.7-K1

Tissue replica generation: the multifaceted contributions of cells and biomaterials

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Abstract

The success of tissue engineering in restoring or replacing damaged tissues or organs relies on the ability to integrate biomaterials and living cells to create functional tissue constructs. Different aspects must be taken into account to provide a functional and effective tissue substitutive.
The design of tissue-engineered constructs requires the careful selection of biomaterials that are biocompatible, biodegradable, and capable of supporting cell growth and differentiation. Biomaterials can be functionalized with bioactive molecules, such as growth factors, cytokines, and miRNA to promote specific cellular responses.
The successful integration of living cells into biomaterials also requires a thorough understanding of cell-material interactions at the molecular level. Cells can sense and respond to physical and chemical cues from their microenvironment through a variety of mechanisms, including receptor-mediated signalling, cytoskeletal remodelling, and cell-matrix adhesion. The surface properties of biomaterials, such as charge, roughness, and hydrophilicity, can modulate cell behaviour by affecting cell adhesion, spreading, and proliferation. The mechanical properties of biomaterials, such as stiffness and elasticity, can also influence cell fate by regulating gene expression and signalling pathways.
In addition to biomaterial properties, the choice of cell type and sex is also critical for the success of tissue engineering applications. Different cell types have distinct functions and requirements for growth and differentiation. For example, stem cells have the potential to differentiate into multiple cell types and are therefore attractive for tissue engineering applications. However, the choice of stem cell source, such as embryonic, induced pluripotent, or adult stem cells, can significantly impact their differentiation potential and safety. Furthermore, the culture conditions, such as co-culture, oxygen tension, and mechanical stimuli, can also modulate cell fate and behaviour.
The spatial distribution and organization of cells within the biomaterial can also influence tissue formation and function. Therefore, advanced techniques, such as bioprinting, microfluidics, and 3D culture systems, have been developed to enable precise control over cell placement and organization within the biomaterial.
In summary, tissue engineering requires a deep understanding of the biologically relevant elements involved in cell-material interactions. The integration of multifunctional biomaterials and living cells requires the careful selection of biomaterial properties, cell type, and their organization.

S2.7-K2

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

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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 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 coculture 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.

S2.7-O1

Mucoadhesive Aurozyme for the regulation of multi-hazard signals in inflammatory bowel disease

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Abstract

A therapeutic strategy that could address colitis of multiple etiologies while restoring the dysbiosis of gut microbiota is attractive. Here, we developed a mucoadhesive gold nanoparticle (AuNP)-based nanozyme (Aurozyme) that comprehensively alleviates colitis by scavenging multi-hazard signals (reactive oxygen/reactive nitrogen species (ROS/RNS) and damage-associated molecular patterns (DAMPs). The glycol chitosan coating layer on the AuNP was of paramount importance for the catalase-like activity of nanozyme, facilitating the conversion of ROS into water and oxygen molecules. Moreover, the mucoadhesive property allowed long-term adhesion to the lesion, providing extended opportunities to relieve inflammation. In colitis-challenged mice, orally administered Aurozyme restored intestinal function and downregulated inflammation with remarkable scavenging capability against multi-hazard signals. Notably, it also augmented the abundance and diversity of probiotics that are beneficial to microbial homeostasis. Our work sheds light on nanozymes in oral formulations that comprehensively treat the complex pathogenesis of inflammatory bowel disease (IBD).

S2.7-O2

Engineered microbiota-gut-brain axis platform to address neurodegenerative disorders

Simone Perottoni[1], Lucia Boeri[1], Francesca Donnaloja[1], Lorenzo Sardelli[1], Diego Albani[2], Carmen Giordano[1]

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Abstract

Our intestinal microflora, collectively named "microbiota" is involved in a complex bidirectional communication between the gut and the brain constituting the so-called microbiota-gut-brain axis (MGBA)[1]. Increasing evidences indicate that gut microbial dysbiosis may affect brain functionality with impact even on neurodegnerative diseases. In particular, the soluble molecules secreted by the microbiota strains such as lipopolysaccharides (LPS) may increase local inflammation *in vivo* disturbing the gastrointestinal and also the blood-brain barrier (BBB) permeability thus possibly propagating inflammation to the brain where it may trigger neuronal damage. Therefore, elucidating the MGBA impairment is a key challenge that may open to therapeutic approaches in many neurological diseases.
In this perspective, the newborn organ-on-a-chip technology aims at offering comprehensive models of inter-organ crosstalk, like the MGBA, bridging the current *in vivo* models complexity with the *in vitro* tools reproducibility[2].
In the context of ERC project MINERVA[3], we have developed the first MGBA engineered multi-organ platform based on organ-on-a-chip devices hosting advanced *in vitro* cell models representing the main biological players involved in the microbiota-brain connection: the intestinal microbiota; the gut; the immune system; the BBB; the brain. Here, we present the MINERVA engineered platform development and assessment demonstrating its suitability as a tool for coupled bacterial-cellular *in vitro* model to study both physiological and pathological MGBA scenario (**Figure 1**). In particular, we assessed the *in vitro* cell performance of the represented biological systems once cultured serially connected in the platform by measuring the key biological parameters and markers both in physiological and pathological scenarios. Furthermore, we exposed the cell models interconnected in the platform to an inflammatory stimulus by allowing LPS secreted by microbiota strains cultured in a lab-scale fermenter flowing along the platform. As result, we observed that the LPS was able to elicit a cytokines mediated response in our engineered 3D Brain cell model supporting the potential role of our innovative tool to investigate MGBA triggers for neurodegenerative disorders but also to address *in vitro* drug efficacy and toxicity tests.

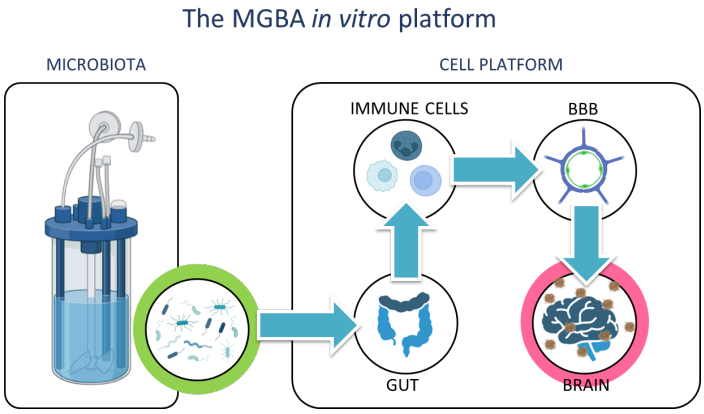

Figure 1. The multi-organ platform hosts the organ models of the main players involved in the MGBA.

References. [1] Cryan et al., Physiological Reviews, 2019, 10.1152/physrev.00018.2018; [2] Ingber, Nature Reviews Genetics, 2022, 10.1038/s41576-022-00466-9; [3] Raimondi et al., Trends in Molecular Medicine, 2019, 10.1016/j.molmed.2019.07.006.

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S3.2-K1

Macromolecular engineering of dynamic biomaterials

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Abstract

Polymer materials provide solutions to many pressing biomedical, manufacturing, and environmental challenges. However, traditional polymer materials have a limited capacity for rearrangement, presenting difficulties in their processing, use, and recycling. Engineering reversible interactions within polymer networks enables the formation of dynamic and reconfigurable materials, opening new opportunities for use and re-use of polymer networks. In this talk, we will present fundamental insight and applications of dynamic polymer networks with an emphasis on their utility in biomedical applications. We provide a framework for engineering dynamic macromolecular systems by linking molecular behavior at the reversible junctions to macroscopic properties using modeling, spectroscopy, and mechanical characterization. We then present how we use this knowledge to design biomaterials with various topologies and reversible interactions for a range of biomedical challenges, including the thermal stabilization of complex biologics and additive manufacturing of drug delivery systems.

S3.2-O1

Injectable self-assembled hydrogel platform enhances influenza vaccine efficacy and protection against potential pandemic strains

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Abstract

Due to the rapid mutability of the influenza virus, it has been historically difficult to create a broadly protective flu vaccine, leaving our seasonal influenza vaccines at less than 50% efficacy each year. Standard influenza vaccines are delivered via liquid injection, allowing vaccine components to be cleared in a matter of hours from the body. To improve immune response to vaccines, it has been shown that delivering antigen over an extended period of time can improve the neutralizing ability and breadth of the generated humoral response by mimicking the timeframe for immune interaction of a natural infection. We have developed a polymer-nanoparticle supramolecular hydrogel that can be easily mixed with flu vaccine components and adjuvants for facile injection using a standard syringe. Upon injection, the hydrogel rapidly self assembles to form a depot that will slowly release vaccine components and serve as an immune cell niche over a period of weeks.

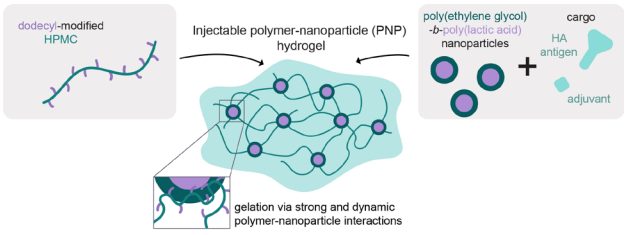

Figure 1. Schematic of PNP hydrogel mixing. A hydrophobically modified cellulose derivative is mixed with PEG-b-PLA nanoparticles and vaccine cargo to form an injectable, depot-forming hydrogel.

By tuning hydrogel material properties and adjuvant pairing, we show dramatically improved antibody titers against multivalent influenza vaccines as compared to liquid controls in a murine model. Additionally, the gel-administered vaccine maintains a greater immune response against heterologous hemagglutinin strains not included in the vaccine, including potential future pandemic strains H5 and H7. This tunable hydrogel drug delivery platform has the ability to enhance immune response across multitudes of vaccine platforms through carefully controlled drug release.

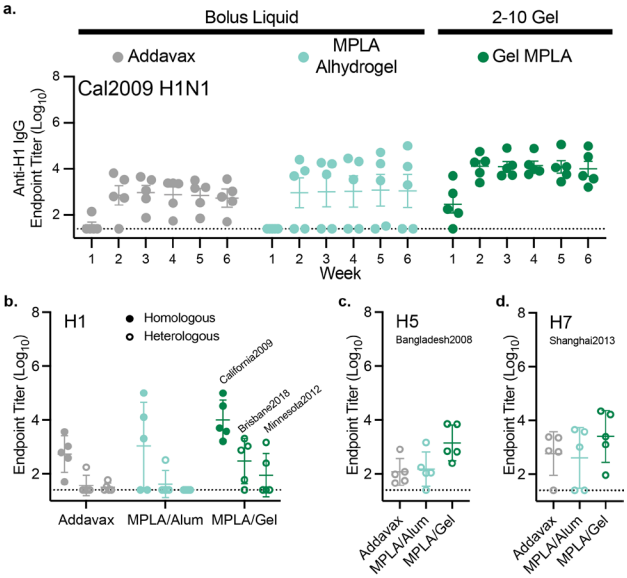

Figure 2. Anti-HA titers in mice vaccinated with a subunit trivalent influenza vaccine consisting of H1, H3, and B strains. (a) Gel group (dark green) demonstrates seroconversion against the administered HA in all animals and maintains consistently higher titers over the 6 week period as compared to two liquid controls. (b-d) Sera was tested against heterologous HA strains simulating patient exposure to mutated future influenza variants. The gel group maintains higher titers against future H1 variants (b) as well as potential future pandemic strains (c and d).

S3.2-O2

Targeted gene delivery for Huntington's disease using bioinspired self-assembling peptide hydrogels integrated with BDNF encoded AAV

Shiva Soltani Dehnavi[1,2], Richard J Williams[3], Clare L Parish[4], David R Nisbet[5,6]

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Abstract

Huntington disease (HD) is considered as a fatal, neurodegenerative disorder caused by an unstable expansion of a single polyglutamine repeat in huntingtin protein. It leads to the striatal GABAergic medium spiny projection neurons (SPNs) degeneration along with other neocortical neurons loss. Therapeutic protein delivery has been established as a beneficial strategy to treat a variety of neurodegenerative disorders. Brain-derived neurotrophic factor (BDNF), is a crucial regulator of synaptic and structural plasticity, development, morphology, higher cognitive functions, and survival of neuronal cells in the Central nervous system (CNS). As evidenced by research, moreover, the BDNF plays a crucial role in protecting of endangered GABAergic striatal neurons and primary cortical neurons from cell death. However, a number of hurdles such as short half-life, limited diffusion and cross over the blood-brain barrier, and poor pharmacokinetics has hindered the trophic factors progress towards a therapeutic intervention. As such, there is a requirement for alternative strategies to protein delivery to ameliorate these issues. Viral vector gene delivery tools are such possibilities. However, regardless of various success, some principal limitations around neutralization via the host immune system and resultant low transduction efficiencies, off-target toxicities, and virion dissemination, have prevented viral gene therapy reaching its significant potential. To enhance these systems, viral gene delivery can be integrated with engineered biomaterials to produce engineered gene delivery vehicles capable of sustained delivery, deliberate and targeted expression of proteins at the site of clinical need while decreasing off-target toxicity, and shielding viral vectors from humoral immune responses. To achieve this, I employed SAP hydrogels for the delivery of bioengineered rAAV variant (rAAV-DJ) encoded with BDNF for HD treatment. Firstly, the potential of a tissue-specific molecular hydrogel to bind and deliver rAAVDJ-BDNF-GFP was assessed. Once loaded, transduction efficiency of rAAVDJ-BDNF-GFP with and without Fmoc-SAP hydrogel on rodent neuronal cells was investigated *in vitro*. Subsequently, the optimized nanoscaffolds were implanted into the striatum of HD mice models to increase long-term survival of different neurons. Strong GFP expression proves the capability of Fmoc-SAP nanoscaffolds to deliver a functional virus. Also, *in vivo* outcomes confirmed the ability of rAAVDJ-BDNF-GFP blended with Fmoc-SAP to protect neurons and prevent tissue atrophy compared to the control (Fig. 1).

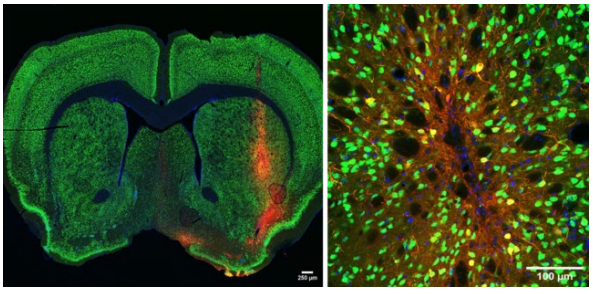

Fig.1 Injection of SAP+AAV-BDNF-GFP into the striatum of HD mice model after QA lesioning prevents the degeneration of NeuN-positive cells and tissue atrophy. Coronal sections of striatum were stained for NeuN (green), GFP (red), and DAPI (blue).

S3.2-O3

Boronate ester-based hydrogels for biomedical applications: challenges and opportunities

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Abstract

Dynamic covalent hydrogels are viscoelastic materials that can be designed to be self-healing, malleable, and injectable. These properties make them particularly interesting for a variety of biomedical applications, including drug/cell delivery, 3D cell culture, and bioprinting. Among the few dynamic covalent crosslinking reactions reported, boronate ester formation, which is the reversible reaction between a phenylboronic acid (PBA) and a diol, stands out for the stability of its precursors under physiological conditions. However, common PBAs and diols hardly react at physiological pH. Thus, designing stable and tunable boronate ester-based hydrogels is challenging, and their use remain limited.

In this context, we recently performed a combinatorial screening of PBAs and diols for efficient crosslinking under physiological conditions, using hyaluronic acid (HA) as a polymer of interest. This work led to the discovery of a new crosslinking couple composed of the ortho-aminomethyl-PBA and glucamine. We showed that, once separately immobilized on HA and mixed together, these two molecules allow for the design of hydrogels that are minimally-swelling, viscoelastic and yet stable over months, and cytocompatible. We then investigated the use of these new boronate ester-based hydrogels for two applications: cell delivery and bioprinting.

First, we developed a material-assisted cell therapy for osteoarthritis. The viscoelastic hydrogels were optimized in terms of composition and mechanical properties to enhance mesenchymal stem cell (MSC) immunomodulatory properties. We showed that the secretome of encapsulated MSCs supports macrophage polarization toward an anti-inflammatory (M2) phenotype. Interestingly, the intra-articular injection of encapsulated MSCs led to a reduction in the variability of the cell therapy efficacy in a rabbit model of osteoarthritis.

As a second application, we investigated the use of these hydrogels for the design of innovative bioinks. We successfully designed printable hydrogels that can be formed in the presence of cells before being transferred to a printing cartridge, preventing cell sedimentation. More importantly, we chemically modified the hydrogels with click and bioorthogonal chemical groups to allow a variety of post-printing modifications of the bioinks, including peptide immobilization and mechanical reinforcement. The post-printing modifications of these clickable dynamic bioinks can be controlled in space and time, paving the way for 4D bioprinting.

We will present an overview of our work, from a new molecular understanding of boronate ester-based hydrogels to their applications. We will also discuss surprising results regarding the effects of culture medium composition on the hydrogel physicochemical properties, as well as new potential avenues of research.

S3.2-O4

Engineering Dynamic Materials with DNA towards Adaptive Biosensors

Youngeun Kim

Seoul National University, Seoul, Korea, Republic of

Abstract

Programmable synthesis using nucleic acid-based materials has enabled binding and bonding of molecules or particles that sometimes do not assemble with each other via conventional methods. Whether nanoparticle-based or strictly hybridization & crossover-based, DNA strands can be used as bonding materials or fundamental building blocks to construct different types of structures including transmutable nanoparticles and DNA brick nanostructures. In this talk, I will briefly summarize recent advancements in the development of dynamic, nucleic acid-based materials, then suggest how we should utilize the techniques and materials developed towards biomedical sensing applications.

S3.3-K1

Mechanisms of Cellular Mechanosensing at the Nano- to Microscale - Impact of Interfacial Viscoelasticity and Toughness

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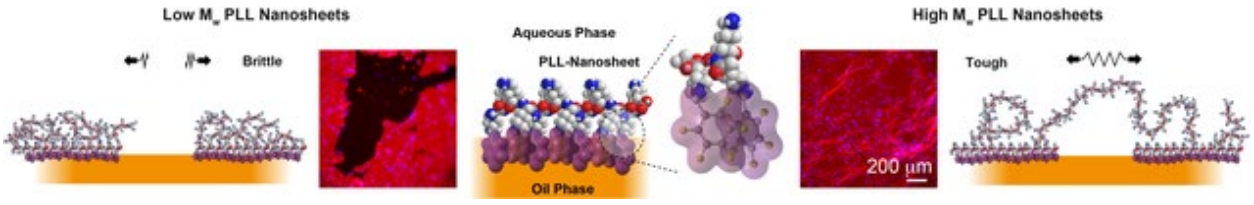
Queen Mary University of London, London, United Kingdom

Abstract

Stem cells are known to sense and respond to a broad range of physical stimuli arising from their extra-cellular environment. In particular, the role of the mechanical properties (i.e. Youngs or shear modulus, viscoelasticity) of biomaterials has extensively been shown to have a significant impact on the adhesion, spreading, expansion and differentiation of stem cells (1). In turn, cells exert forces on their environment that can lead to striking changes in shape, size and contraction of associated tissues, and may result in mechanical disruption and functional failure. Such behaviour, and the sustained remodelling of matrix, lead to the emergence of anisotropic mechanics in the cell microenvironment and associated tissues. However, studying and evidencing such processes is often challenging, as separating and disentangling different phenomena and their contribution is often not possible. In this report, we show how the design of macromolecular architecture enables the regulation of interfacial toughness and viscoelasticity and how this, in turn, controls the expansion of mesenchymal stem cells. To modulate such mechanical properties, we design polymer and protein assemblies at liquid-liquid interfaces, forming nanosheets with a broad range of mechanical profiles. To study the resulting interfaces, we use a combination of interfacial shear rheology, atomic force microscopy and magnetic tweezer-assisted interfacial micro-rheology (2, 3). We demonstrate that the nanosheets assembled display particularly high anisotropy and controlled interfacial shear moduli, viscoelasticity and toughness. In turn, cells respond strikingly to such anisotropic dynamic mechanics. Indeed, mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) are found to adhere to aqueous-oil interfaces stabilised by nanosheets. We find that their adhesion, mediated by the integrin-actomyosin machinery, is regulated by the mechanics of these interfaces and strikingly insensitive to the mechanics of the underlying liquid substrate. In turn, nanosheets are found to stabilise the formation of microdroplets and stable emulsions that support the expansion of MSCs and iPSCs, the retention of their phenotype and promotion of pro-reparative secretory profiles (4). Therefore, this works paves the way towards a new range of microcarriers, based on bioactive emulsions (or bioemulsions), for the production of stem cells and their application in regenerative medicine.



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S3.3-O1

Exploring microgel technology for high-throughput formation of osteoclasts

Johanna Husch[1,2], Nuno Araújo-Gomes[2], Niels Willemen[2], Jeroen van den Beucken[1], Jeroen Leijten[2]

[1]Radboudumc, Nijmegen, Netherlands. [2]University of Twente, Enschede, Netherlands

Abstract

Introduction: Osteoclasts are multinuclear cells of hematopoietic origin responsible for bone resorption (1). Due to their crucial role in bone homeostasis and pathologies, osteoclasts are investigated in multiple research areas relating to bone health and disease (2). However, *in vitro* research on osteoclasts remains challenging due to the intrinsic heterogeneity of the cultures (3). Consequently, there is a need for establishing pure osteoclast cultures. Here, we investigate whether encapsulation of osteoclast precursors in microfluidically generated hollow microgels composed of a tyramine-conjugated dextran shell (4) could facilitate cell aggregate formation and subsequent cell fusion thus forming a single osteoclast with predetermined number of nuclei per microgel. Due to the sacrificial nature of the microgel, generated osteoclasts can be released to obtain pure osteoclast cultures.

Methods: Primary human mononuclear cells were isolated from buffy coats and differentiated toward macrophages. Macrophages were detached and encapsulated in microgels as described previously (4). Encapsulated macrophages were differentiated toward osteoclasts for up to 29 days. During the culture period, morphology, and viability were observed using light, and fluorescence microscopy. To assess osteoclast formation with confocal microscopy, microencapsulated cells were stained for β3 integrin as an osteoclast marker.

Results: We successfully encapsulated primary human macrophages in ~80 µm diameter microgels. Cells survived the encapsulation procedure, as was demonstrated by viability of more than 80 %. Although limited aggregation of macrophages occurred, immunofluorescent staining revealed formation of a number of high quality osteoclasts within the microgels.

Discussion & conclusions: We successfully managed to differentiate encapsulated primary human macrophages toward osteoclasts. In further studies, we will investigate protocol optimization to augment cellular aggregation rates and increase production throughput to realize scalable production of high quality osteoclasts.

Acknowledgements: This study received financial support from the Twente University RadBoudumc Opportunities (TURBO) program.

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S3.3-O2

Microgels and hydrogels microparticles for biosensing

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Abstract

Microgels and hydrogels microparticles represent a versatile sub-class of hydrogels enabling new functions in biomolecule detection thanks to the highly tuneable chemistry. From the synthesis to the final read-out microgels prove advantageous and unique in overcoming issues in detection and quantification directly in biofluids. The chemical flexibility of hydrogel microparticles and microgels allows to embed several building blocks into their structure during the synthesis. Anchoring groups on particles are used to immobilize probes of diverse natures such as antibodies, enzymes, or oligonucleotide strands. In such a way particles active for the detection of a target or more than one biomarker can be obtained. Moreover, the high biocompatibility and antifouling properties of hydrogels make possible to use microgels in biosensing directly in biofluids overcoming washing and separation steps. As for the read-out steps, microgels can be easily manipulated in flow with simple microfluidics making the detection more efficient and sensitive in multiplex context. Here we are presenting a PEG-microgel platform for the detection of circulating oligonucleotides for cancer diagnosis and of circulating proteins for immunoassay.

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S3.3-O3

Liquefied core-shell capsules with multifunctional properties based on alginate interfacial gelation

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Abstract

Liquefied Core-shell structure can serve as a “semi-scaffold-free” approach that allows cells to generate 3D spheroids with *in vivo*-like cell-to-cell contacts. Here, we proposed a novel approach for fabricating of liquid core-shell capsules through inverse gelation of alginate and layer-by-layer (LBL) coating. We hypothesized that the unique properties of polyethylene imine (PEI) could be utilized to overcome the low structural stability and the limited cell recognition motifs of alginate. Furthermore, PEI can enhance the mechanical properties by forming a strong polyelectrolyte complex with the anionic groups of alginate at the interface while providing antibacterial properties and cell attachment with its hydrophilic cationic character. In the next step, alginate dialdehyde (ADA) is used to react with free amine groups of PEI to reduce the cytotoxicity of PEI (Figure 1).

The core was extruded into different concentrations of alginate bath solutions to form an interfacial membrane as the outer shell. Then, the collected beads were immersed into PEI (0.5 and 1 mg/mL) and ADA (2.5 %w/v) solutions, respectively, with washing steps in between to remove the remaining unabsorbed polymer. The morphology and structural analysis evaluated by light microscopy imaging and SEM confirmed the core formation and various shell layers (Figure 2A). SEM showed that the ALG/PEI/ADA capsules have spherical shape morphology within a size range of (2.1-5.4 mm). In addition, PEI-coated samples showed antibacterial properties to both *S. aureus* and *E. coli*, as the zone of inhibition could be detected obviously (Figure 2B). The biocompatibility test results showed a dose-dependent manner (1 to 2 %w/v of PEI). Interestingly, with lower PEI concentration, higher viability (132%) was obtained compared to non-coated (50%) and higher PEI-coated (85%) capsules, which shows the effect of charge-associated cytotoxicity at higher concentrations (Figure 2C). The present bioencapsulation protocol can be adapted to fulfill the requirements of various applications. For example, as a dynamic bioreactor system for MSCs cultivation, production of cell-based therapeutics like growth factors, *in vitro* platforms for drug screening and disease modeling, or simply as small building blocks for bioprinting using bottom-up tissue engineering approaches.

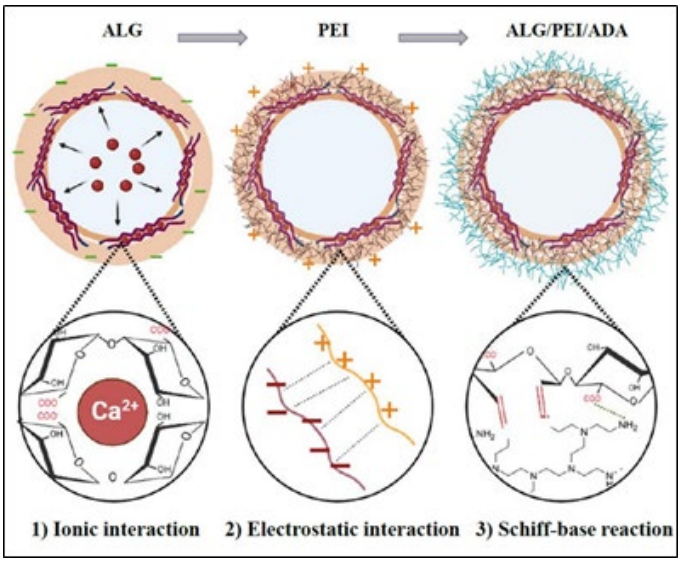

Figure 1. Schematic of possible chemical and physical bonds between ALG, Ca2+, PEI and ADA, to show crosslinking mechanism in capsule formation process.

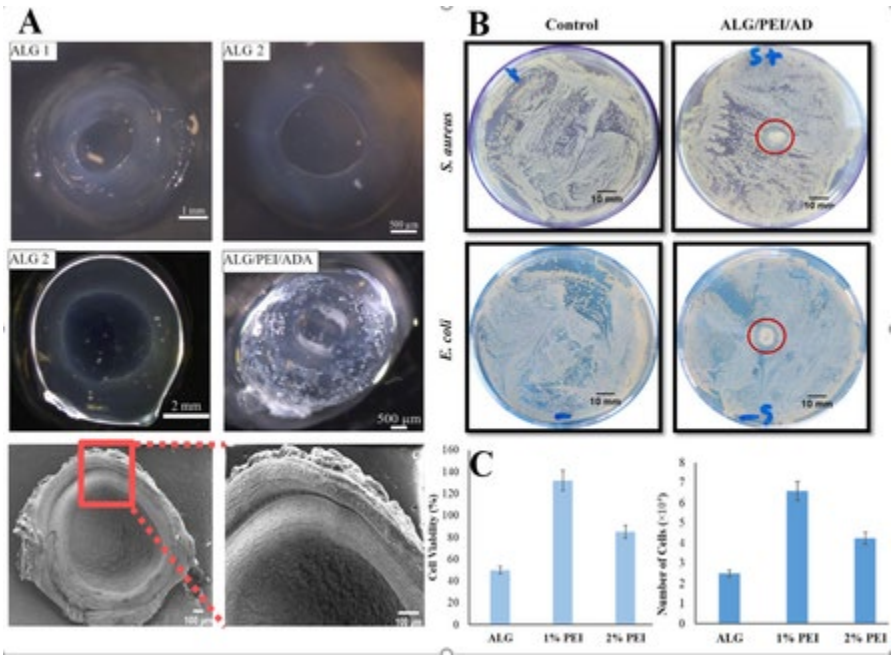

Figure 2. The results of light microscopy, SEM images (A), antibacterial activity (B), and cytotoxicity of ALG/PEI/ADA capsules (C)

S3.3-O4

Self-assembly induced-charge electrophoretic nanosensors for high throughput detection of ovarian cancer biomarker HE4 and CA125

Yi-Zhen Wang[1], Nan-Si Li[2], Hao-Han Pang[2], Ying-Pei Hsu[3], Cheng-Hsin Chuang[1], Hung-Wei Yang[2]

[1]Institute of Medical Science and Technology, National Sun Yat-sen University, Kaohsiung, Taiwan. [2]Department of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan. [3]Department of Materials and Optoelectronic Science, Center for Functional Polymers and Supramolecular Materials, National Sun Yat-sen University, Kaohsiung, Taiwan

Abstract

Among female cancers, one of the most dangerous is ovarian cancer. Clinical reports show a poor five-year survival rate of only 25% for women diagnosed at a late stage of the disease, versus those diagnosed in stages I or II, which is over 90%. This data clearly demonstrates that improved early diagnosis of ovarian cancer is urgently needed to significantly improve survival for women with this disease. Nowadays, clinical diagnosis of ovarian cancer is based on pelvic examination, abdominal sonography, computer tomography and tumor biomarkers. Carcinoma antigen 125 (CA 125) has been recognized as a clinical glycoprotein/biomarker for the early-stage detection of ovarian cancer. However, the CA125 assay alone is not ideal for the detection for ovarian cancer, but studies combining CA125 assay with other biomarkers has shown a positive increase in detection ability, such as HE4, transferrin, and mesothelin. Therefore, we develop a self-assembly induced-charge electrophoretic nanosensor (ICEN) based on Fc-tagged virus-like particle (Fc-VLPs) and two types of Au nanoclusters for high throughput detection of ovarian cancer biomarker CA125 and HE4. Our data demonstrate that the specific capture of CA125 and HE4 leads to direct signal transduction in the form of ICEN migration amount suppression above 10 pg/mL CA125 and HE4. Additionally, after particle-tracking analysis with optical microscopy, the ICEN migration amount decreases in a manner that is dependent on the concentration of CA125 and HE4 to show a significant intensity decrease for both biomarkers compared with control. This work lays the foundation for a new paradigm for rapid, simple, high throughput, and label-free active nanoparticle-based biomarker detection.

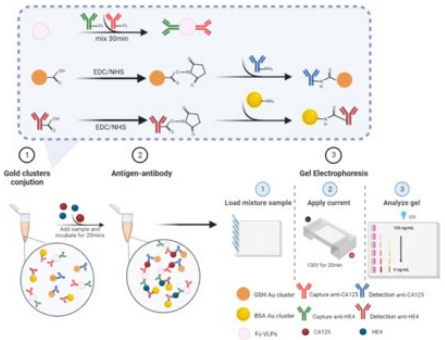

Fig.1 *Schematic of the self-assembly induced-charge electrophoretic nanosensor*

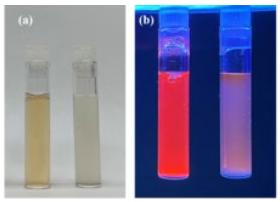

Fig. 2 *BSA Au-NCs (left) and GSH Au-NCs (Right) under visible light(a) and UV- visible light(b)*

S3.4-K1

Advanced micromaterials and modular bio-inks for multiscale tissue engineering

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Abstract

The modular design of tissues is of indispensable importance for proper organ function. Incorporating such a modular design into living matter is expected to allow engineered tissues to more accurately emulate the behavior of native tissues. Unfortunately, the toolbox to engineer hierarchically designed tissues has remained limited. To overcome this technological challenge, I have developed microfluidic droplet generation platforms for the production of enzymatically crosslinked cell-laden microgels that. Using this platform, I engineered various 3D stem cell microniches with on-demand tunable biophysical and biochemical properties to controllably program stem cell differentiation along chosen lineages to create tissue structures such as chondrons or cardiospheres in ultra-high throughput. Moreover, we used our microgel technologies to create a variety of advanced bioinks for the engineer tissues with innovative properties, which includes microporous tissues containing high density capillary networks, self-oxygenating tissues, and hierarchically organized living tissues with multi-scale designs in a scalable manner. This materials toolbox thus allowed for an unprecedented control over the design and behavior of engineered living matter. In short, I here present several microfluidic microgel-based concepts that are focused on advancing the engineering of multiscale hierarchical living tissues.

S3.4-O1

Reactive jet impingement bioprinting with porous substrates for in situ seeding into implants

Marcin Kotlarz, Charalampos Tzivelekis, Priscila Melo, Ali Babaie, Kavin Hettiarachchilage, Piergiorgio Gentile, Ana Marina Ferreira, Kenneth Dalgarno

Newcastle University, Newcastle, United Kingdom

Abstract

Reactive jet impingement (ReJI) is a bioprinting process which creates gel droplet streams through in air reaction of polymer and crosslinker solutions, as shown in Figure 1 [1]. Suspending cells in one or both of the solutions allows cell-filled-gel droplets to be created. By jetting the gel pre-cursor solutions and then producing the gel by a downstream reaction, high shear stresses are avoided and this allows high cell density gels (up to 40 million cells/mL of gel) to be created. The process is drop-on-demand, and so is able to print gel droplets onto any substrates, including rough and delicate substrates. This presentation will focus on ReJI bioprinting onto porous substrates as a method for in situ cell seeding.

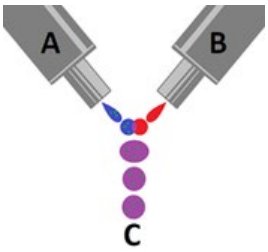

Figure 1. ReJI Bioprinting Process. Microvalves at A and B produce droplet streams of polymer solution and crosslinking solution. When droplets collide they react to form gel droplets which are deposited onto the substrate at C.

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).

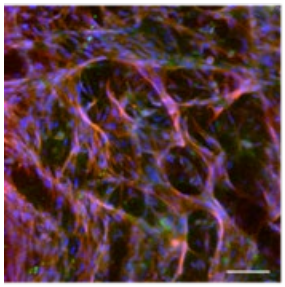

Figure 2. Confocal image of Neo-NHDF cells in a hydrogel printed onto a Calcium-alginate fibre mesh at day 3 of culture and a 30 M cells/ml print density. Blue staining indicates cell nuclei, red F-actin and green vinculin. Darker areas are the fibres. Scale bar: 100 µm

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.

References: 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.

S3.4-O2

Engineering aqueous two-phase system inks to drive cellular differentiation, alignment and functionality in 3D bioprinted constructs

Martina Marcotulli[1,2], Arianna Iacomino[1], Federico Serpe[1,3], Andrea Barbetta[4], Giancarlo Ruocco[1], Chiara Scognamiglio[1], Gianluca Cidonio[1]

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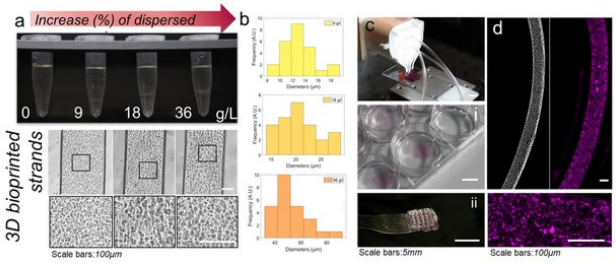
Abstract

The unparalleled ability of aqueous two-phase systems (ATPS) to reproduce microscale density gradients and interfaces is ideal for tissue engineering and regenerative medicine (TERM) purposes.
Herein we present a new class of ATPS biomaterial inks, demonstrating the possibility of 3D printing novel water-in-water (w-w) emulsions to pattern multiple cell types and drive tissue regeneration both *in vitro* and *ex vivo*. Gelatin methacryloyl (GelMA) was compartmentalised within a continuous phase made of alginic acid. The content of GelMA and the dimension of the dispersed phase were tuned to influence the ability of a library of cell types to spread and proliferate following 3D bioprinting.
ATPS was prepared from sodium chloride (NaCl) which was included at increasing concentrations (9, 18 and 36 g×l^{-1}) coupled with HEPES solution (Figure 1a) to aid cell survival upon encapsulation. Dispersed material was found to form drop-like emulsion within the continuous phase ranging from 10 and 100 µm size, depending on the concentration of NaCl-HEPES solution (Figure 1b). The ability to tune the degradation of either GelMA or alginate was demonstrated by harnessing alginate lyase or collagenase, respectively. Selective degradability was confirmed via optical coherence tomography (OCT) imaging (850nm probe).
A 3D microfluidic bioprinting approach was employed to produce and deposit ATPS inks. Interestingly, the internal micro-structure of 3D printed ATPS fibres was tuned according to printing parameters (Figure 1c,d). Dispersed phase drop-like features were modulated in size, arrangement and anisotropy depending on (i) printing speed, (ii) surface and (iii) temperature.



Encapsulation of A549 was carried out and actin cytoskeleton arrangement was investigated over 7 days, finding a major cellular spreading and actin remodelling when 36 g×l^{-1} was used. 3D printing of C2C12 in ATPS with increasing salt concentration was found to drive cell elongation and ultimate functionality following 21 days of culture. The printing of HBMSCs in ATPS inks demonstrated the ability of higher salt content to influence mineral deposition and bone tissue formation. Chick chorioallantoic membrane (CAM) model was used to investigate the influence of micro-architectural porosity of ATPS bioprinted constructs. Implants fabricated with a greater concentration of salt were found capable of sustaining a significantly greater vessel penetration and scaffold integration.
Altogether, the novel ATPS inks here reported holds tremendous potential for the engineering of hierarchical new tissue substitutes with complex architecture, offering new opportunities to build tissue of interest with microscale precision.

S3.4-O3

Regeneration of auricular cartilage for the treatment of microtia

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Abstract

A key challenge in cartilage tissue engineering is the formation of fibrocartilage. Fibrocartilage is mechanically inferior to elastic and hyaline cartilage and its formation significantly influences the durability of cartilaginous grafts. To provide patients with a tissue engineered treatment, it is therefore vital to control cartilage tissue maturation. Here we show that the maturation of auricular grafts into elastic cartilage (Figure 1a) can be achieved by tuning the material properties and maintaining the chondrogenic capacity of human auricular chondrocytes (hAUR). Matured grafts show the deposition of elastin, collagen II and glycosaminoglycans, the absence of collagen I and achieved biomechanical properties matching those of native human auricular cartilage.

Material properties were tuned through the addition of alginate to a previously developed hyaluronan transglutaminase (HATG) bioink (Figure 1b) [1]. Morphologically, hAUR maintained a round morphology in HATG-Alg grafts compared to the spread morphology observed in HATG grafts (Figure 1d). After maturation for 9 weeks, HATG-Alg grafts achieved a 2-fold higher compressive modulus compared to HATG grafts. Importantly, HATG-Alg grafts showed the absence of collagen I and the onset of elastin deposition. The chondrogenic capacity of human auricular chondrocytes was maintained through the addition of growth factors such as FGF-2 and TGF-β3 during cell expansion (Figure 1c). The addition of these growth factors stimulated proliferation, upregulated cartilage specific genes and had similar effects on graft maturation as the addition of alginate.

Combing both approaches resulted in HATG-Alg grafts maturing into auricular cartilage matching the histological appearance and mechanical properties of native tissue. Histologically, the presence of glycosaminoglycans, collagen II and elastin and absence of collagen I were confirmed (Figure 1e). Biomechanically, grafts matched the compressive (1.6±0.1 MPa) and Hertz modulus (1.2±0.3 MPa) of native human auricular cartilage, a 10- to 20-fold increase over fibrocartilaginous grafts (Figure 1f,g).

This study presents one of the first reports on the *in vitro* maturation of human auricular grafts matching native human auricular cartilage. By overcoming the formation of fibrocartilage, these results could lead to a novel treatment for microtia patients, preventing the harvest of costal cartilage and thereby drastically improving treatment.

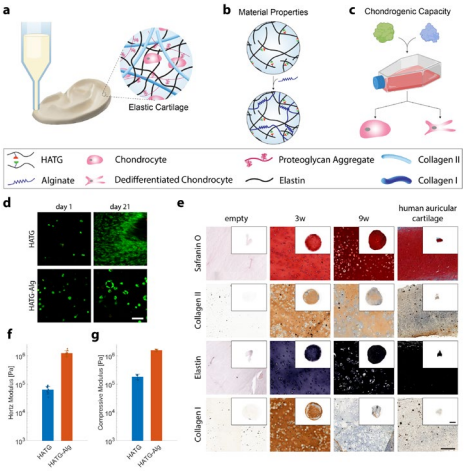

Figure 1. (a-c) Approach to mature auricular cartilage (d) Viability of hAUR embedded in the bioinks. (e) Histology of hAUR embedded in the HATG-Alg bioink. (f, g) Mechanical properties of hAUR embedded in the HATG and HATG-Alg bioink and matured for up to 9 weeks.

Reference: [1] Fisch et al. Adv Funct Mater (2021).

S3.4-O4

Generation of high cell density bioinks for bioprinting of functional cartilage grafts

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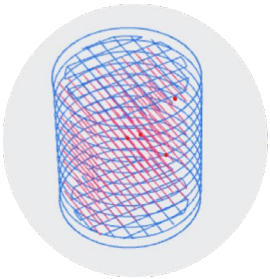
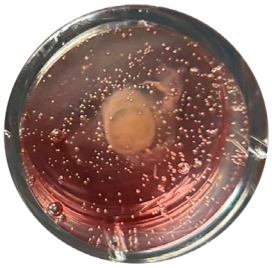
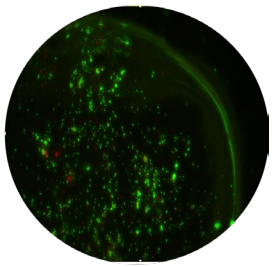
Abstract



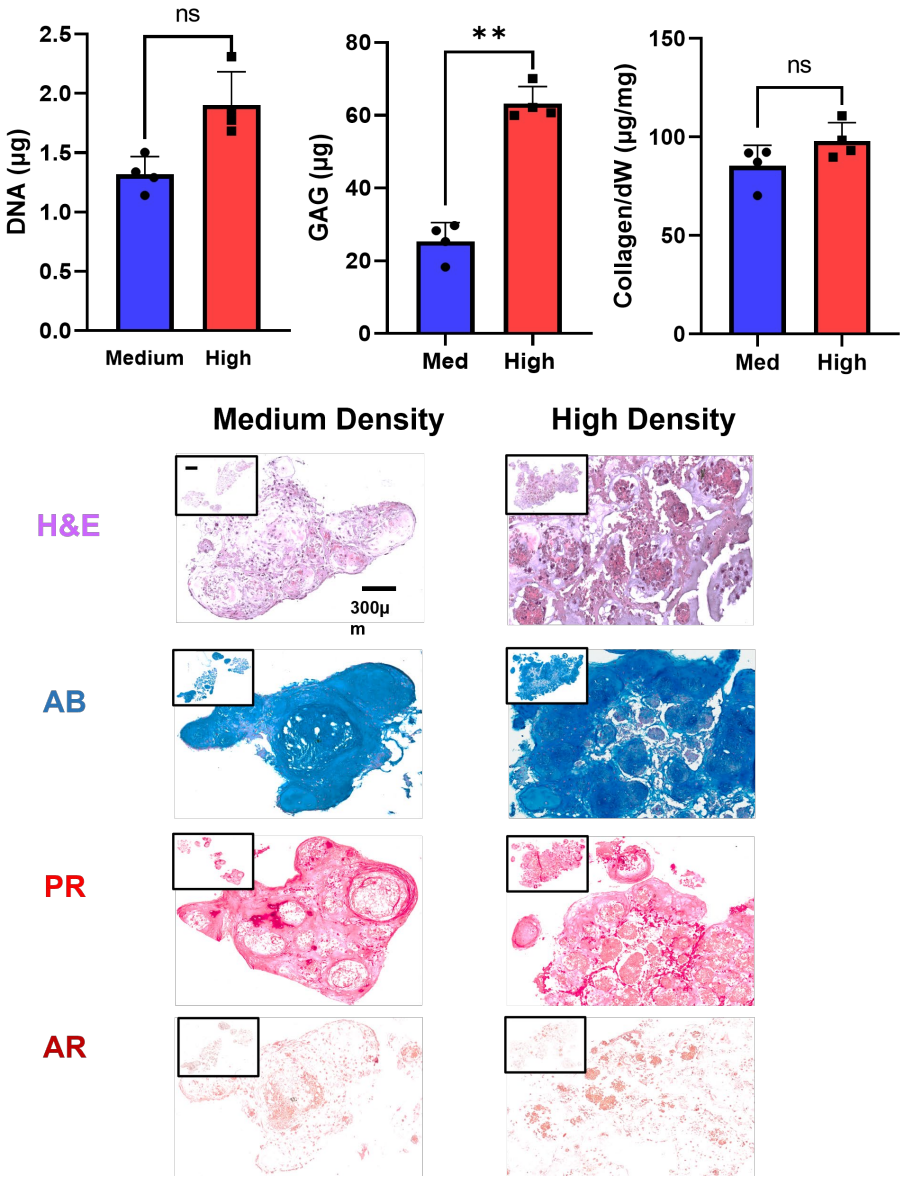
Introduction: Tissue engineering (TE) strategies aim to regenerate tissues which have lost significant functionality due to physical damage or disease. Current approaches fail to recapitulate the highly organised structure and extracellular matrix (ECM) composition of complex tissues such as articular cartilage, motivating the exploration of novel TE strategies. 3D Bioprinting enables the precise deposition of bioinks in a spatially organised, three-dimensional manner, potentially enabling the engineering of complex tissues and organs. The objective of this study is twofold. The first objective is to explore the impact of bioink cell seeding density on the engineering of articular cartilage grafts. The second objective is to explore the potential of the freeform embedded suspended hydrogel (FRESH) technique for the bioprinting of low viscosity bioinks containing high concentrations of bone marrow derived mesenchymal stem/stromal cells (bmMSCs).

Methods: MSCs were encapsulated in a rapidly degrading oxidised alginate (OA) based bioink at specific cellular densities (40 or 60 x10^6 cells/ml). These cell laden bioinks were deposited into agarose moulds and cultured in chondrogenic medium for 6 weeks. Constructs were then assessed for viability, biochemically, and histologically. A degradation study was undertaken to assess how differently oxidised alginate inks degrade over time. For the embedded printing process, material characterisation and optimization of various bioink compositions and an adapted FRESH support bath matrix were undertaken.

Results: Both the medium (40x106 cells/ml) and high (60x10^6 cells/ml) cell density bioinks supported robust chondrogenesis, with high levels of DNA and collagen, and statistically significantly higher levels of sulphated glycosaminoglycans (sGAGs) in the high density group. Histological evaluation further confirmed that both medium and high-cell density bioinks supported robust chondrogenesis. No differences in cellular viability were observed. The 4% oxidised alginate inks degraded completely over a 4-week period. Following optimization, it was possible to generate a FRESH support bath with an average, uniform microparticle size of 20µM. It was possible to accurately deposit alginate based bioinks into such a support bath, with relatively high resolution (<100µM filament diameter) and fidelity in single material, acellular printing trials.

Conclusion and future steps Initial work has been undertaken to begin the development of a novel bioprinting platform, including material, bioink, print process optimisation and characterisation. Future

studies will explore multi-material, 3D embedded bioprinting of physiologically representative constructs with spatial-temporal defined oxidised alginate boundaries, with a view to engineering cartilaginous tissues with user-defined collagen network organisation.



S3.5-K1

Targeted and sustained delivery of anti-Inflammatory drugs for treatment of injured eyes

Nasim Annabi, Yavuz Oz, Yimin Gu, Yuting Zhang

University of California, Los Angeles, Los Angeles, USA

Abstract

Introduction. Damage to the ocular surface commonly induces inflammations due to the disrupted epithelial layer of the eye. Topical administration of an eye drop solution is the most common and convenient method of delivering anti-inflammatory drugs to the eye. However, tear dilution and turnover after eye drop administration negatively affects therapeutics delivery onto targeted sites on the ocular surface.[1] This necessitates frequent administration of eye drop to ensure optimum concentration for effective treatment, which may increase the possible adverse effects.[2] To address challenges associated with conventional eye drop solutions, current strategies focus on packaging therapeutic drugs inside nanoparticles (NPs) and delivering them in a targeted manner inside the eye. In this study, we engineered novel anti-inflammatory drug loaded targeting NPs that improve corneal retention by targeting the mucin layer on the ocular surface. The sustained release of drugs from these NPs efficiently combats eye inflammation arising from ocular injuries.

Methods. NPs were fabricated by self-assembly of a phenylboronic acid (PBA) functionalized amphiphilic copolymer in the presence of loteprednol etabonate (LE) through solvent evaporation method. High-performance liquid chromatography monitored the drug loading capacity and sustained *in vitro* drug release behavior of the NPs. *In vitro* mucoadhesion of PBA-bearing NPs was studied using fluorescence spectroscopy by measuring relative fluorescence intensities of NPs with different amounts of sialic acid solutions.

Results and discussion. PBA-bearing NPs showed enhanced *in vitro* mucoadhesion behavior utilizing covalent interactions between PBA and sialic acid that is abundant in mucus. Hydrophobic anti-inflammatory drug LE was successfully encapsulated in the core of NPs by self-assembling PBA functional amphiphilic copolymer. Drug-loaded NPs were dispersed in a matrix solution and sustained release of drug in an artificial tear solution was demonstrated.

Conclusions. Targeted delivery of therapeutics with PBA-modified NPs enhanced the retention time of NPs on the ocular surface, and sustained release from these NPs reduced frequent drug administration avoiding possible adverse effects. Targeting mucus membrane also facilitated higher drug transportation into the protective epithelial layer resulting in effective treatment against ocular inflammation.

Acknowledgments. This work is supported by funding from Department of Defense Vision Research Program Technology/Investigator-Initiated Research Award (W81XWH-21-1-0869).

References. (1) Gaudana, R.; Jwala, J.; Boddu, S. H. S.; Mitra, A. K. Pharm. Res. 2009, 26, 1197–1216. (2) Shoji, M. K.; Markatia, Z.; Ameli, K.; Bayaraa, M.; Clauss, K. D.; Ugradar, S.; Lee, W. W. J. Plast. Reconstr. Aesthetic Surg. 2023.

S3.5-O1

Towards corneal limbus *in vitro* model: regulation of hPSC-LSCs phenotype by matrix stiffness and topography during cell differentiation process

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Abstract

Limbus provides a unique niche for limbal epithelial stem cells (LSC) and is a vital element in the constant renewal of the corneal epithelium. Despite of identification of its unique topography and mechanical properties, little is known about their role in LSC regulation which creates a significant gap in the development of LSC based therapies. Recent studies have highlighted the importance of the corneal mechanical environment in cell regulation *in vivo*, and in primary cell regulation *in vitro*, but we are lacking this information from different subpopulations of LSCs representing different developmental stages with possibly different regenerative capacities. Thus, there is a high demand for modeling native niche mimicking conditions *in vitro*.

Here, we investigated the effect mechanical properties of the matrix and limbal niche mimicking topography on the human pluripotent derived LSCs (hPSC-LSC) regulation *in vitro*. We selected three cell populations from hPSC-LSC differentiation trajectory and cultured them on PA gels with controlled stiffnesses (soft, medium, and stiff) and on glass control, all functionalized with 4-dihydroxy-l-phenylalanine and extracellular matrix proteins. We analyzed mechanical properties of the PA gels with atomic force microscopy and rheology and characterized hPSC-LSC viability (PrestoBlue™), morphology and protein expression via immunofluorescence staining's on the gels. In addition, we created limbal mimicking topography in polydimethylsiloxane with a customized mold and repeated same cellular analysis as with PA gels. Furthermore, we report key protein expression (including YAP, ABCG2, p40, p27) of the cells in their native environment through immunofluorescent whole mount staining of human donor corneas and imaged them with a confocal microscope with high spatial resolution.

The results show YAP-mediated response to substrate stiffness in hPSC-LSCs and correlation with ABCG2 expression. Soft PA gel promoted cytoplasmic YAP, stem cell like phenotype and ABCG2 expression in a LSC population from early phase of LSC differentiation. In addition, same subpopulation of LSC cultured on a limbal topography showed high expression of quiescent marker p27. On contrast, stiff matrix promoted nuclear YAP, high viability, and proliferative phenotype of LSCs from a later phase of LSC differentiation.

The results highlight the importance of the mechanical response and substrate preference for the cells being highly dependent on their developmental stage and mechanical memory from previous culture matrix during their differentiation. This study accentuates mechanical environment being an important design factor in cell culture and provides a significant step forward in construction of native tissue mimicking niche for hPSC-LSCs *in vitro*.

S3.5-O2

3D bioprinted stromal substitute with human keratocytes

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Abstract

The cornea is the outermost, transparent layer of the eye and is responsible of refracting the majority of the incoming light. It also serves as a protection layer against the UV exposure. The thickest component of the cornea is the stroma and constitutes 90% of the corneal thickness. Being the outermost element of the eye, cornea is also most prone to damages by external physical and chemical agents, which may cause immediate blindness due to the effect they have on the epithelial and the stromal layers. Tissue engineering is heavily involved in substituting the natural corneal layers with cell carrying or cell-free artificial layers and a certain level of success has already been achieved.

In this study, the stromal substitute was prepared from GelMA, a gelatin methacrylated with methacrylic anyhydride. Methacrylation degree was found to be 88 % using NMR. 3D printing of the polymer was carried out at 19oC using an EnvisionTEC printer in the form of 10x10 mm2 consisting of 8 layers with a thickness of ca. 500 um. The square hydrogels were crosslinked using Irgacure 2959 which was activated with UV 365 nm and then the hydrogel mesh was punched as a disc of 6 mm diameter. The hydrogel had a compressive mechanical strength of 3.12±0.53 kPa. The hydrogels were also bioprinted together with human keratocytes, which were shown to express ALDH1A1 and lumican assays proving the cells being keratocytes. Cell viability in the 3D printed stromal substitutes were followed for 21 days to show >80% viability. The light transparency of both the cell carrying and cell-free hydrogels were higher than 80% above 400 nm. The hydrogels in 3D printed (Figure 1A) and in slab form were tested for insertion into a porcine eye *ex vivo* (Figure 1B and 1C) and were found to be fully transparent and handled very well. These promising results will be followed by an *in vivo* rabbit test.

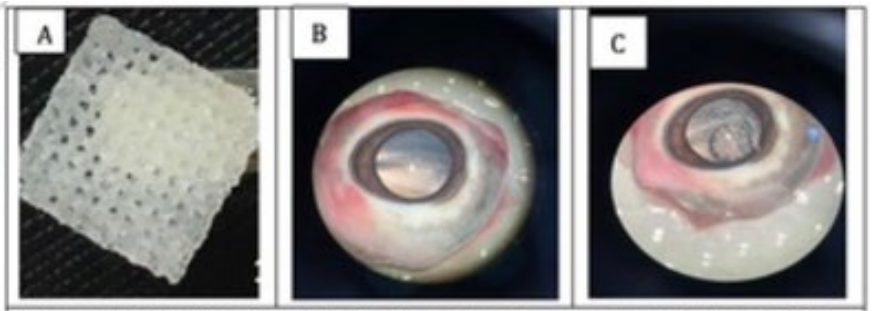

Figure 1: 3D printed GelMA hydrogel based cornea substitute and its introduction into a porcine eye. A) 3D printed hydrogel. In the porcine eye: B) hydrogel slab and C) 3D printed hydrogel

S3.5-O3

Development of a 3D structured hydrogel membrane for cellular therapy of the outer blood-retina barrier

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Abstract

The outer blood-retina barrier (oBRB), composed of a retinal pigmented epithelial (RPE) cell monolayer, on the Bruch's membrane (BM) and overlying the vascularized choroid, is disrupted in several retinal dystrophies. The main current strategies to repair the oBRB are to implant a RPE monolayer on a material mimicking the BM. However, they are not including the choroid, which will alter the graft integration and survival upon implantation. Here, we aim to develop a 3D structured membrane, for cellular therapy, mimicking the entire oBRB and co-cultured with RPE and endothelial cells (EC) derived from human induced pluripotent stem cells (hiPSC).

Membrane design. A 200 µm thick polysaccharide hydrogel was synthesized from a pullulan-dextran solution and freeze-dried (FD) to tailor its porosity. After optimization, we obtained membranes with a porous side connected to the inner porosity for the development of the pre-vascular network and, on the other side, a non-porous surface intended for the RPE monolayer.

EC seeding. To enhance EC adhesion, the FD membrane was hydrated in a collagen I solution and further FD, resulting in a coating on the surfaces and in the pores (Fig.A). Human retinal microvascular EC (HRMVEC) and EC derived from hiPSC were seeded on the porous side of the membrane and cultured for up to two weeks. Results showed that the EC entered the inner structure, where they adhered to the side of the pores and proliferated (Fig.B).

RPE seeding. To improve RPE adhesion, an additional laminin coating was added on the non-porous side where hiPSC-derived RPE were seeded and cultured for up to two weeks. As shown in fig.C, the cells were able to adhere and proliferate on the membrane, to form a monolayer, without entering in the porous network.

To conclude, we designed a membrane mimicking the oBRB structure, with a porous network suitable for the culture of ECs and a non-porous surface allowing the RPE monolayer formation. Current experiments are now focusing on co-culture experiments to study the interactions between the two cell types and to model the entire tissue.

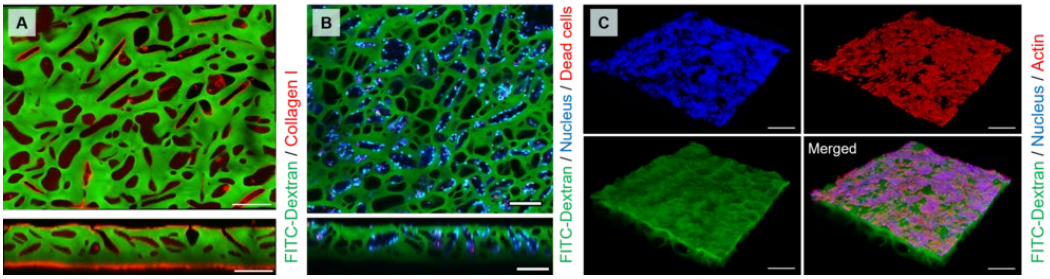

Figure: (A) Immunostaining of the collagen coating of the oBRB membrane. (B) Live and dead staining at day 7 of the HRMVEC cultured in the porous network. (C) RPE monolayer formed on the smooth side after 7 days in culture. All images were acquired with a confocal microscope, with the rough side on top in the cross-section. Scale bars are 200 µm.

S3.5-O4

3D bioprinting of corneal cell-laden inks as bioengineered corneal substitutes

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Abstract

Less than 2% of patients with corneal blindness worldwide have access to the human donor tissue needed for its conventional treatment, necessitating bioengineered strategies. While corneal-derived mesenchymal stromal cells (MSCs) have demonstrated potential as a therapeutic strategy for corneal wound healing, their inclusion within hydrogel-based corneal tissue substitutes is limited by the contractility of MSCs causing severe deformation of the shape and size of the hydrogel. This hydrogel deformation results in loss of optical transparency and alteration of the interface with the endogenous tissue. To address this limitation, we hypothesized that a hydrogel stabilized by covalent crosslinks could resist the contraction induced by corneal MSCs while maintaining cell phenotype for improved regenerative potential. Specifically, we developed a 3D printable collagen hydrogel covalently crosslinked by strain-promoted azide-alkyne cycloaddition (SPAAC), a bioorthogonal click chemistry that exhibits no cross-reactivity with cells and proteins. Type I collagen was functionalized with azides, while a 4-arm polyethylene glycol (PEG) molecule was functionalized with bicyclononyes (BCN). The bioink (collagen-azide laden with human corneal MSCs) was printed into a gel-phase support bath loaded with PEG-BCN crosslinkers. The crosslinkers diffuse into the printed construct to induce gelation of the collagen, after which the support bath can be melted away, and the self-supporting, printed structure can be recovered. Using a 3D microextrusion bioprinter, we fabricated tissue-like constructs of customizable sizes and curvatures for corneal replacement. We demonstrated the improved transparency of the SPAAC-crosslinked collagen across the visible light range compared to non-chemically crosslinked collagen (98% *vs*. 52% transmittance at 500 nm, respectively). The SPAAC-crosslinked collagen resisted deformation from encapsulated human corneal MSCs over 72 h with no detectable contraction, while the non-chemically crosslinked collagen hydrogels contracted to 20% of their initial diameters. In addition, the corneal MSCs in the SPAAC-crosslinked collagen maintained their characteristic phenotype, with high viability, expression of the keratocyte differentiation marker aldehyde dehydrogenase 3A1, and secretion of pro-regenerative cytokines similar to corneal MSCs within non-chemically crosslinked collagen. Finally, we used anterior lamellar keratoplasty rabbit models to validate that SPAAC-crosslinked collagen hydrogels remain transparent, restore the curvature of the cornea, and promote re-epithelialization for wound healing *in vivo*. Taken together, our results demonstrate a material strategy to 3D bioprint with SPAAC-crosslinked collagen in a cell-friendly manner. These corneal MSC-laden, biofabricated constructs offer potential as customizable corneal tissue replacements as an alternative to transplantation of cadaveric human corneas, which are severely limited in supply.

S3.6-K1

Nanocarbons: one family, different fits in composite hydrogel biomaterials

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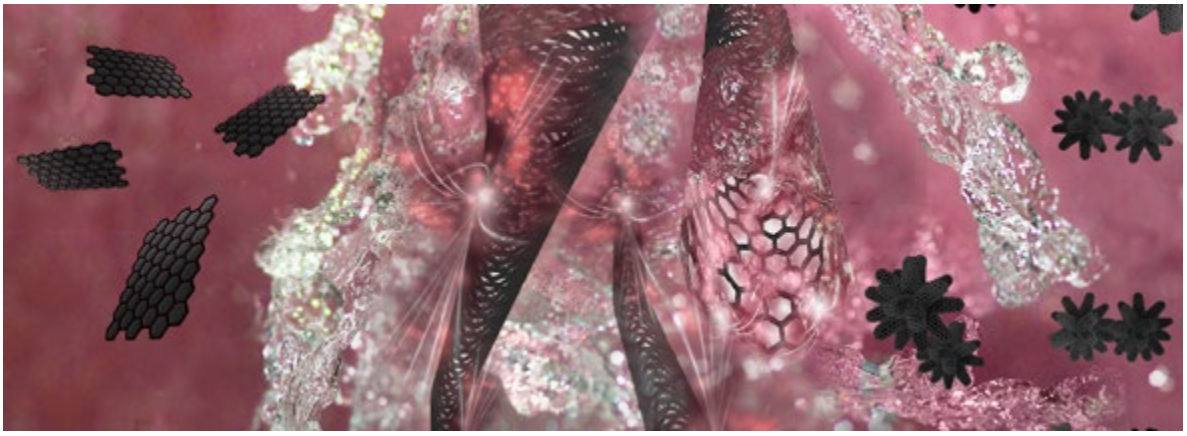
Abstract

The family of nanocarbons is diverse in size, morphology, and reactivity. They have attracted great interest in nanocomposite biomaterials to introduce new properties. However, which member of the family is the best fit for function is a question that finds different answers depending on applications. For instance, CNTs' elongated morphology is ideal to boost the activity of conductive cells, as in the nerve[1] and heart[2] tissues. They also offer an ideal template for the coating with self-assembling peptides towards hydrogel biomaterials that self-heal[3] for cell culture. Such peptides are well-suited to mimic the extracellular matrix and display bioactive motifs to guide cell fate[4,5] and to form supramolecular channels[6-8] for therapy.[9] As we learn about morphological control at the nanoscale towards functional nanocomposites, we can embark in the navigation at the exciting interface between nanotechnology and supramolecular chemistry for the emergence of new properties in smart materials and hydrogels[10] to address ananswered questions in the challenging area of tissue engineering.



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S3.6-O1

Protein-stabilized nanoparticles as the next generation of targeted MRI contrast agents

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Abstract

Magnetic resonance imaging (MRI) is one of the most used imaging and diagnostic techniques. However, it often requires the use of contrast agents, which are usually based on Gadolinium (Gd). The release of Gd and consequent toxicity have raised safety concerns, leading to significant efforts to develop safer contrast agents. A novel and mostly unexplored approach is the development of protein-stabilized nanomaterials, bringing together the magnetic properties of metal atoms complexes and nanoparticles (such as Gd complexes or iron oxide nanoparticles) with the biocompatibility and stability of proteins.[1]
Consensus tetratricopeptide repeat (CTPR) proteins are designed proteins that are particularly notable for their robustness and mutational permissibility, allowing the introduction of metal coordination sites without a substantial impact on the protein structure.[2] The use of CTPR designed proteins enables better control of the nanomaterials' properties, and offers a fully customizable platform for the introduction of specific functional motifs.[3-4]
Herein, cysteine- and histidine-based metal-binding sites were introduced into CTPR proteins for the coordination of Gd and Fe. These modified proteins were then used to generate protein-stabilized nanoparticles (Prot-NPs) with tailored relaxivity properties (Figure 1), which enable their exploration as MRI contrast agents. Moreover, two different strategies were explored to target the Prot-NPs to specific pathologies. In a first approach, Prot-GdNPs were stabilized on a cysteine-based scaffold that also contained an Hsp90 binding module. This binding module allowed us to target Hsp90, which is overexpressed in several pathologies, e.g., fibrosis and certain cancers. Likewise, protein-stabilized iron oxide nanoparticles (Prot-IONPs) were developed as contrast agents. The Prot-IONPs were grown on a histidine-based scaffold that was subsequently modified with alendronate to target calcifications in atherosclerotic plaques. This study serves as a proof-of-concept for the versatility and potential of the Prot-NPs in biomedical applications through the development of MRI contrast agents with targeting ability. Moreover, we showcase designed proteins as a platform that allows the scaffolding of diverse nanomaterials that can be finely tuned to target and treat different conditions either by simply changing the protein sequence or by straightforward post-synthetic modifications.

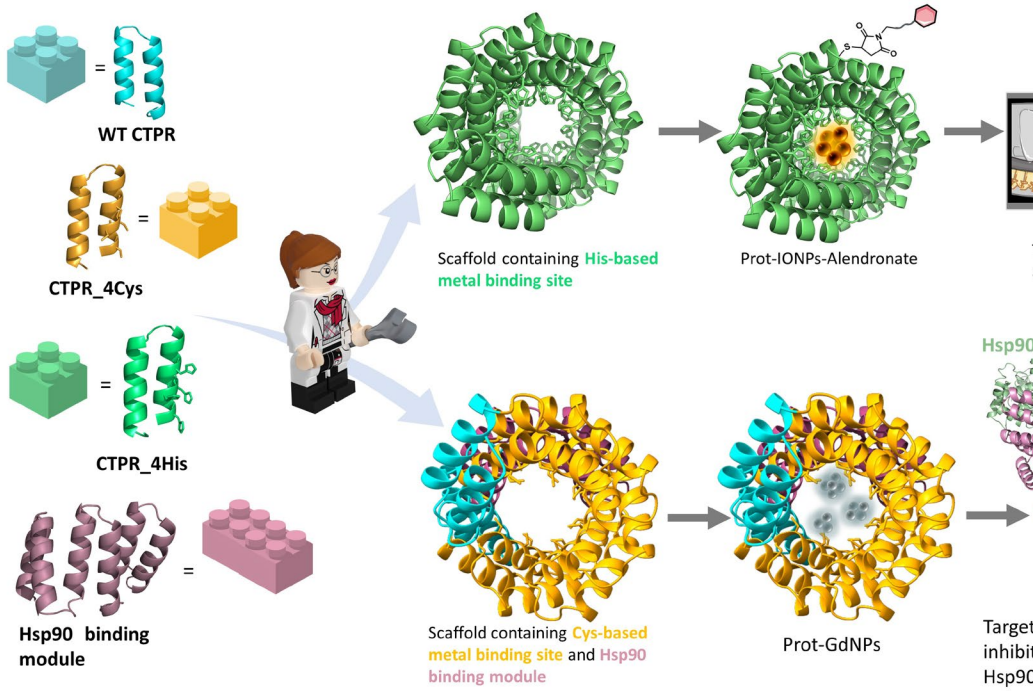

Figure 1. Designed CTPR proteins were used to generate Prot-NPs with tunable properties for use as targeted MRI contrast.

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S3.6-O2

Simultaneous bone cancer therapy and regeneration through an organic-inorganic hybrid nanocomposite

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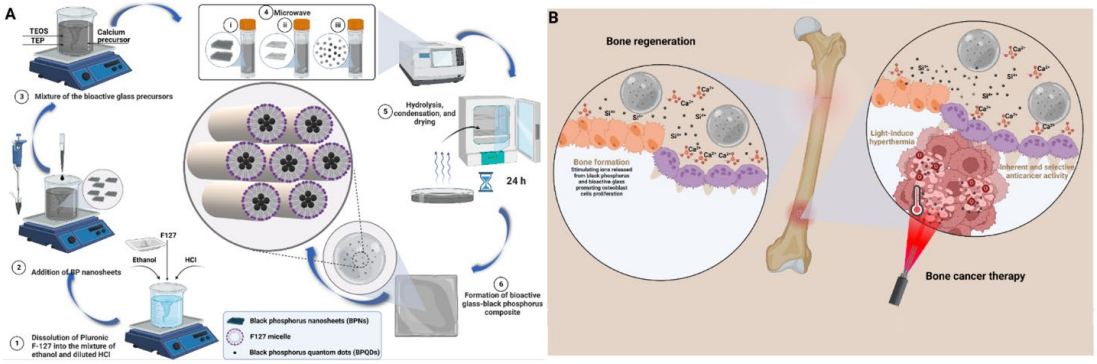
Abstract

There is an up-and-coming term recently known as theragenerative referring to biomaterials capable of inducing therapy followed by repairing/regenerating. The present study introduced an innovative synthesis strategy to fabricate a theragenerative nanocomposite composed of Pluronic-F127, bioactive glass (BG), and black phosphorus quantum dots (BPQDs) (Scheme 1). Sol-gel technique was adopted to synthesize BG-BP nanosheets composite in an organic solution followed by applying microwave in the second step. The liquid crystals formed by F127 in the medium led to the protection and encapsulation of the BP in the interior side micelles from oxidation. The aims of this study were as follows: (i) In situ synthesis of BG encapsulating BP, (ii) evaluation of the F127 and microwave irradiation effects on the physicochemical and biological properties of bare BG and BP-incorporated hybrid composites *in vitro*.



Scheme 1. (A) The two-step synthesis strategy yielding F127-BG-BPQDs. (B) Theragenerative potential of the composite.

The F127 was found to distribute the BG ions all over the composite homogenously and the microwave process has made the hybrid structure chemically more stable. Moreover, the microwave process turned the BP nanosheets into 5 ± 2 nm QDs in the structure. The *in vitro* bioactivity was found to improve in the presence of BP and formation of newly generated calcium phosphate in SBF was stimulated. The hybrid at a concentration of 150 µg mL-1 prevented osteosarcoma cells (SAOS-2) proliferation thanks to BP whereas the BG hybrid without BP did not prevent the growth rate. The apoptosis pathways were assessed through Caspase-3 expression and Annexin V staining. The ROS content and ATP levels were measured and found to be tripled and doubled for BG-BPQDs samples in the exposure of cancer cells compared to control, respectively. To reinforce the anticancer activity, the

BG-BPQDs hybrid was exposed to NIR irradiation and a stronger anticancer effect against bone cancer cells was achieved due to hyperthermia. The regenerative potential of the hybrid was determined against healthy osteoblasts and human mesenchymal stem cells; the proliferation of osteoblast cells was promoted and the osteogenic differentiation of stem cells was occurred by the BG-BPQDs nanocomposite.

A theragenerative platform composed of BG-BPQDs was synthesized through an innovative strategy. The hybrid could selectively inhibit cancer cells proliferation while stimulating the healthy cells growth. This platform has promising potential for further studies in bone cancer therapy and regeneration.

Acknowledgments. The authors would thank PRIN 2017, ACTION PROT. N. 2017SZ5WZB for financial support.

S3.6-O3

Incorporation of Graphene Oxide and Gelatin methacyrol bioinks for articular cartilage regeneration

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Abstract

The currently available treatments for repairing articular cartilage defects are unable to facilitate long-term repair, often leading to osteoarthritis and culminating in a life of pain, disability and isolation for millions worldwide. Recently, attention has shifted to the use of tissue-engineered scaffolds to repair articular cartilage defects by combining cells, biomaterials and growth factors. Graphene oxide (GO) is a nanomaterial comprised of a single layer of carbon atoms. Interestingly, the use of GO in scaffolds for articular cartilage regeneration has been shown to increase the expression of key cartilage genes and matrix components[1] [2]. However, the mechanism underpinning the chondroinductive behaviour of GO is not fully understood.

TGFβ is a key chondrogenic growth factor which plays essential roles in cartilage formation and maintenance. Here we investigated if GO can activate the TGFβ/SMAD signalling pathway as a possible mechanism underpinning the chondroinductive behaviour of GO.

Within the human chondrocyte cell line TC28a2, we have shown GO alone activates TGFβ/SMAD signalling. Induction of TGFβ signalling pathways was demonstrated through increased expression of a SMAD binding element (SBE) luciferase reporter [3], pSMAD2 immunoblotting and analysis of TGFβ response genes via qRT-PCR. By combining fluorescent lifetime imaging (FLIM) with a plasma membrane tension probe and using a mechanosensitive luciferase reporter, we have demonstrated the ability of GO to influence the mechanical properties of the matrix/plasma membrane and to activate mechanosensitive signalling pathways, which are known regulators of TGFβ/SMAD signalling.

Collectively our findings in human chondrocytes identify GO as a chondroinductive material through its ability to activate TGFβ signalling and modify mechanotransduction. Importantly, GO is able to activate TGFβ/SMAD signalling in the absence of exogenous TGFβ growth factors, thus highlighting a promising strategy for articular cartilage generation and repair.

Furthermore, we have shown GO enhances chondrogenic gene expression in pluripotent stem cell (PSC) derived chondroprogenitors without affecting cell viability. Our current work describes the development and characterization of biocompatible GO/ Gelatin Methacyol (GELMA) hydrogels[4], optimised for encapsulation and printing of PSC derived chondroprogenitors.

References: [1] F. Olate-Moya, L. et al. ACS Appl. Mater. Interfaces, 12, 4343–4357, (2020); [2] H. Shen et al., J. Mater. Chem. B, 6. 908–917, (2018); [3] S. Woods et al. Cells, 10, 726, (2021); [4] A. Xavier Mendes et al, ACS Biomaterials Science and Engineering, 7, pp. 2279–2295 (2021)

S3.6-O4

Chemical modification of carbon nanostructures enables preparation of materials for tissue engineering and regenerative medicine

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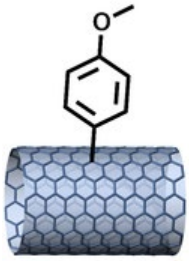
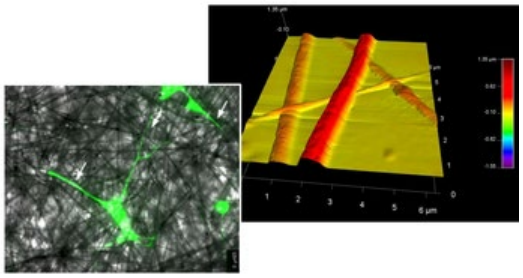
Abstract

Carbon nanostructures (CNSs), including carbon nanotubes (CNTs) and graphene-based materials, are employed as fillers in polymer phases, to confer properties such as improved electrical/thermal conductivities and mechanical reinforcement. Composites of CNSs and biocompatible polymers combine the properties of the two constituents and act as functional materials for applications including medical devices and implants, tissue engineering and biosensing.

Chemically functionalizing the surface of CNSs with suitable organic groups is an effective strategy to increase the affinity between the two phases, maximizing the desired effects while minimizing the loading and preventing cytotoxicity of the filler.





We synthetized CNT derivatives bearing organic moieties that can be efficiently dispersed in poly(l-lactic acid) (PLLA) and the resulting composites have been successfully processed in the form of free-standing homogeneous films and electrospun nanofibers. Our scaffolds were able to promote neuronal growth and differentiation starting from either SH-SY5Y human neuroblastoma cells or human circulating multipotent stem cells from peripheral blood, in the latter case even in the absence of exogenous neurotrophins.

Extending the approach to other CNSs, such as reduced graphene oxide (RGO) and carbon nanohorns (CNHs), showed that different CNS dimensionalities give rise to different properties within the composite. Such observation opens the possibility to design scaffolds for the regenerative medicine of different tissue types. For example, while nanocomposites based on highly conductive CNTs boost neuronal differentiation, less conductive CNH and RGO fillers enhance the expression of myogenic markers.

To investigate the role of electrical properties in the interactions with cells, the effect of surface potential induced by CNTs in electrospun PLLA fibers onto primary human fibroblasts was studied.

Scaffolds with improved biomimetic properties have been prepared, based on water soluble CNT derivatives dispersed in hydrogel matrices.

On the basis of these results, CNS nanocomposites based on biocompatible polymers have been prepared and used as an innovative surgical implant designed for nerve regeneration. *In vivo* tests have shown promising results in terms of biocompatibility and effectiveness in restoring.

Acknowledgements. “Centro Levi Cases” (project PRINTERS) and Department of Chemical Sciences (project P-DISC#05BIRD2021-UNIPD) of the University of Padova are gratefully acknowledged for financial support.

S3.7-K1

Current advances in Vat-Photopolymerisation of bioceramics and future directions

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Abstract

Additive manufacturing of bioceramics, commonly known as 3D printing, emerged to address the limitation of conventional techniques, to enable the fabrication of bioceramics scaffolds with intricate internal geometries resembling trabecular bone. Vat-photopolymerisation, is the most potent 3D printing technology for producing bioceramic constructs with high dimensional accuracy and resolution. In this presentation we describe a vat-photopolymerisation technique using a high-resolution liquid crystal display (LCD), as a dynamic mask-generator, to manufacture strut and sheet-based lattices constructs with unprecedented precision obtaining bioceramics with porosities above 90%, pore sizes below 200 µm, and a minimum wall thickness of 38 µm. We introduce LCD technique as the simplest and most affordable vat-photopolymerisation technique which uses only a liquid crystal display, to irradiate the resin layer with UV and display itself acts as the mask generator. The ceramic-resin mixture in LCD technique is in direct contact with the display which further facilitates the printed pixels to be matched to the shape of the beam profile (Figure 1).

Figure 1- Schematic diagram of the LCD printing of bioceramics featuring a simple build including one mobile part (build platform) and the high-resolution display, under the vat, that generates direct masking and selectively irradiates the surface of the ceramic resin layer by layer.

Nevertheless, common vat-photopolymerisation printing techniques such as LCD, digital light processing (DLP) and stereolithography (SLA) have limitations in achieving nanoscale precision and accuracy. Nanoscale printing represents the future of fabrication of bioceramics, however research on nanoscale printing of bioceramics is limited to alumina and zirconia compositions with small constructs. The light-scattering effect caused by bioceramic particles blended with photosensitive resin at high concentrations is a significant constraint in broadening the scope of this approach. Precise control over the assembly and integration of bioceramic building blocks with nanoscale accuracy and temporary stabilization is critical for successful printing at the nanoscale. Successful fabrication of bioceramic structures at the nanoscale would allow for precise control of the implant's porosity, surface roughness, and topography, leading to improved tissue integration and enhanced mechanical properties. Here we present our preliminary data on nanoscale printing of calcium phosphates using two-photon laser and demonstrate their potential for biomedical applications, specifically in the field of bone tissue engineering.

S3.7-O1

Cellular ceramics made from zirconia-toughened alumina and yttria-stabilized zirconia show good biocompatibility and a high osseointegrative potential: *in vitro* results and *in vivo* evidence from a pilot study in sheep

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Abstract

Bioceramics have shown to induce only minimal triggers to evoke inappropriate biological responses, proving their excellent biocompatibility. In orthopedics, ceramic composites, like zirconia-toughened alumina (ZTA), have long-been used for bearing applications in hip arthroplasty. In dentistry, yttria-stabilized zirconia (TZP) was introduced in the 2000's for metal-free and aesthetic dental restorations and has successfully been applied as dental implant and abutment since.

Both ZTA and TZP are applied in a novel advanced technology for direct foaming of ceramic materials, enabling manufacturing of three-dimensionally structured ceramic components with a porosity of 60 – 80%, and with pore sizes mimicking trabecular bone. The porous surface enables direct ceramic-to-bone contact and may improve implant and scaffold integration into bone tissue.

In vitro results revealed no cytotoxic behavior of cellular ZTA and TZP, with MG-63 osteoblast-like cells showing high viability (107% and 106%, compared to positive-control) after 48h-incubation with medium pre-incubated with cellular ZTA, or direct cultivation on cellular TZP discs. Scanning electron microscopy revealed good cell adhesion on struts and inside pores of both materials.

An ethically approved long-term *in vivo* pilot study in sheep revealed very good to excellent osseointegration of cellular ZTA and TZP implants both in cortical and trabecular bone. The design of the 12- and 24-week GLP-compliant study included 10 animals with two cylindric and two disc-shaped implants placed into the femoral trabecular bone and into a critical size calvarial defect, respectively. Data showed host tissue penetration to the core of the implants and full implant integration into cortical and trabecular bone. Moreover, new bone formation in direct contact to ceramic surfaces was observed. Histopathological scoring (range: 0 – 4) revealed ossification centers in almost all pores already after 12w (TZP:4.0, ZTA:3.5) with a high percentage of vascularization (TZP:3.3, ZTA:3.5). A high percentage of ossification centers showed mineralized bone after 24w (TZP:2.7, ZTA:2.8). Of note, in the pores of the trabecular implants, bone marrow was already formed after 12w.

Radiological and histopathological analyses revealed no signs of adverse reactions to the implants or implant degradation. Importantly, local host response was absent or minimal, with limited numbers of macrophages and lymphocytes present at the implant margin and host reaction scores ranging between 5.3 and 16.3 (score range: 0 – 56). Thus, results indicate very low irritant potential and good tolerability of all cellular ceramic implants tested.

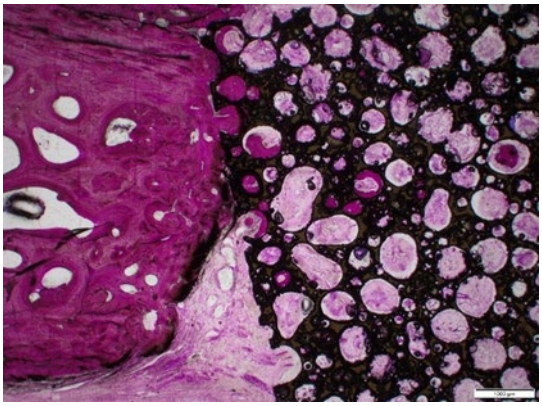

Fig 1: Cellular TZP implant; critical size calvarial defect (24w)

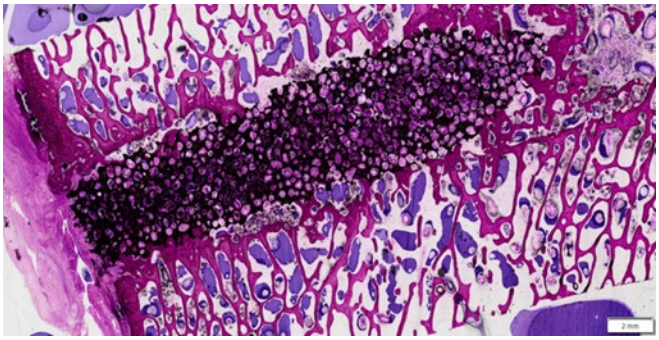

Fig 2: Cellular ZTA implant; femoral trabecular bone (12w)

S3.7-O2

In-vitro and in-vivo evaluation of a silicate (1393) and borosilicate (1393B20) robocasted bioactive glass scaffolds

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Abstract

Ion release, from the dissolution of bioactive glasses (BAGs), trigger osteogenesis and support new bone growth. However, biomedical applications of phosphosilicate BAGs are limited by their slow and incomplete conversion into HA (1393) or by their tendency to crystallize (S53P4, 45S5) thus inhibiting their sintering into porous scaffolds.

Borosilicate BAGs have been proposed as an alternative. They tend to convert faster and more completely to hydroxyapatite. Furthermore, their degradation is more controllable than in the silicate glasses. They also demonstrate suppressed crystallization tendency during sintering allowing manufacturing of porous scaffolds. Presence of boron is also hypothesized to have a beneficial impact on cells by stimulating osteogenesis.

Here, 3D borosilicate 1393B20 BAGs scaffolds were prepared by robocasting. By introducing boron and magnesium into 1393 bioactive glass, 1393B20 composition: 43.68 SiO2-10.92 B2O3-22.10 CaO-7.9 K2O-6 Na2O-1.7 P2O5-7.7 MgO (in mol%) was developed. Generally, the replacement of CaO with MgO, 1) suppresses the crystallization tendencies during sintering, 2) promotes bone repair and remodeling. Boron, increases the dissolution rate and may promote osteogenesis/angiogenesis. As such, 1393B20 scaffolds were investigated, in comparison to the traditional 1393 for its potential to support new bone growth and mineralization in-vitro and in-vivo.

Scaffolds cytocompatibility was assessed in direct culture with human adipose stem cells (hADSCs) for 1 week. Initial results showed that 1393 and 1393B20 support hADSCs viability and proliferation (Figure 1b). Scaffolds supported cells attachment and spreading while maintaining their characteristic spindle morphology (Figure 1b). Scaffold's ability to induce osteogenic differentiation of hADSCs is under investigation.

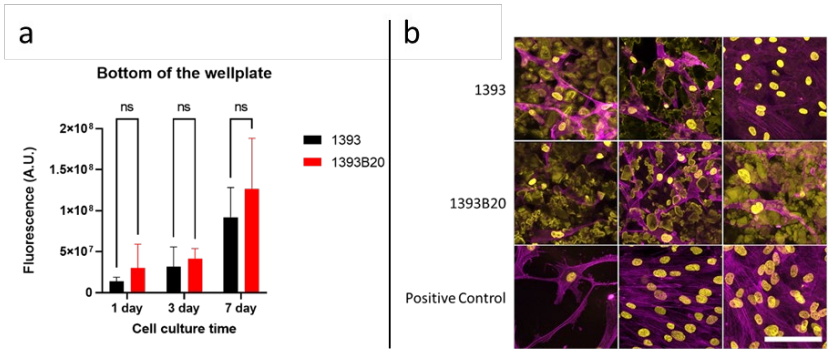

Figure 1. a) Proliferation and b) morphology with hADSCs after 7 days in direct culture with 1393 and 1393B20 scaffolds. Scale bar 100µm.

Following the in-vitro testing, an in-vivo study, using a rat calvaria defect model, was pursued. The healing of rats calvaria critical-sized defect after implantation with scaffolds was investigated (Figure 2a). At each timepoint (1, 2 and 3 months) microCT (X-ray micro-computed tomography) imaging was used to quantify bone mineralization (Figure 2b). Moreover, SEM-EDX was used to visualize the regeneration

in the defect and asses the resulting topography. Finally, histological examination is ongoing to observe the mineralized tissue.

Preliminary in-vivo results indicate that 1393B20 and 1393 scaffolds support new bone growth and show potential in hard tissue regeneration. The impact of boron on bone regeneration is in progress.

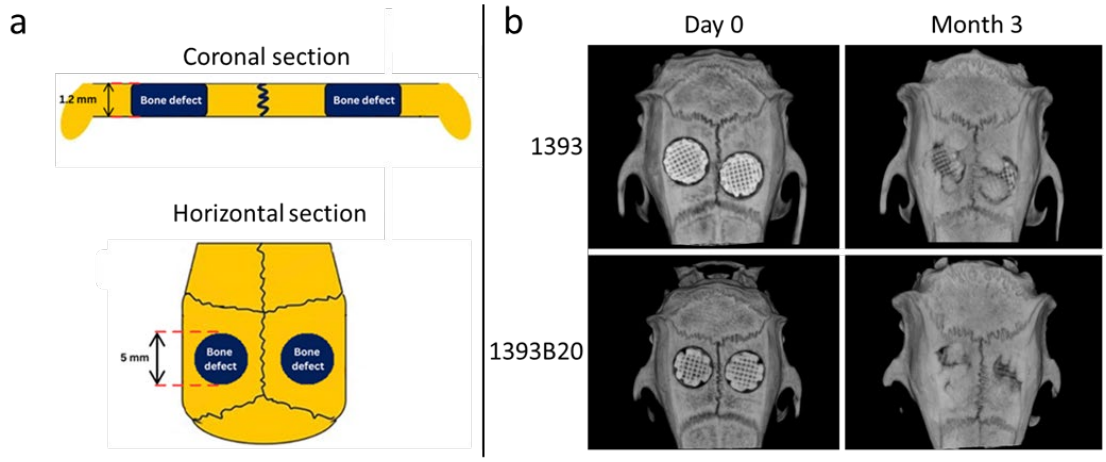

Figure 2. a) Rat calvarial critical-sized defect, b) microCT images of implanted scaffolds at day 0 and month 3.

S3.7-O3

Effect of pore-directing agent to silicon source ratio for the development of mesoporous cerium and calcium-doped silica nanoparticles for sustained doxycycline release

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Abstract

Introduction. Due to their ordered pore structure and high drug loading and release capacity, Mesoporous Silica Nanoparticles (MSNs) are regarded as promising drug carriers. The physicochemical properties of MSNs are influenced by the synthesis process or composition[1]. The dissolution of important elements of bone biology and metabolism including Si and Ca from MSNs can cause stem cells to differentiate into osteogenic lineage and produce mineralized extracellular matrix. Ce ions are beneficial for tissue regeneration due to their antioxidant, anti-inflammatory, and antibacterial properties[1,2]. The use of Doxycycline (DOX) for local drug delivery is effective in the management of various clinical infections including periodontitis or peri-implantitis. The aim of this study was the development of MSNs with enhanced physicochemical and biological properties towards effective local DOX-release.

Experimental methods. Using the CTAB-assisted Sol-Gel method, Ca/Ce-doped MSNs MCM-41 type in the composition of $60SiO_2$-35CaO-5CeO (%mol) were synthesized[1]. Different ratios of Tetraethyl orthosilicate (TEOS) (silicon source) and Cetyltrimethylammonium bromide (CTAB) (pore-directing agent) were used in an alkaline environment (Table 1). The physicochemical properties, the DOX-loading and release profiles, the hemolytic activity in human erythrocytes, and the apatite-forming ability were investigated.

Results and discussion. Different CTAB/TEOS ratios affected the textural properties of MSNs as well as DOX-encapsulation (Table 1). An increase in CTAB/TEOS ratio led to a slight decrease in particle size, pore volume, and ζ-potential, and an increase in surface area and Ce^{3+}/Ce^{4+} ratio. The precipitation of cerium-oxide in the case of MS3 according to XRD, led to exclusion of this sample. MS1 and MS2 presented improved textural characteristics and DOX-loading capacity. All MSNs appeared to be hemocompatible after 24h of incubation at all tested concentrations (0.125–1mg/ml).

Sample	CTAB (g)	TEOS (ml)	pH	CTAB/ TEOS (g/ml)	Surface Area (m^2/g)	Pore Diameter (nm)	Total pore volume (cc/g)	Particle size (nm)	Z-potential (eV)	Ce^{3+}/Ce^{4+} (%)
MS1	1	7.5	12	0.13	584	2.7 & 3.7	1.9	59	-32.3	29.7
MS2	1	5.0	12	0.20	649	2.7	1.6	50	-29.4	34.3
MS3	1	2.5	12	0.40	770	3 & 11.5	1.3	No futher Evaluated		

Table 1: Reactants' amount, textural properties, and DOX-loading percentage of the MSNs.

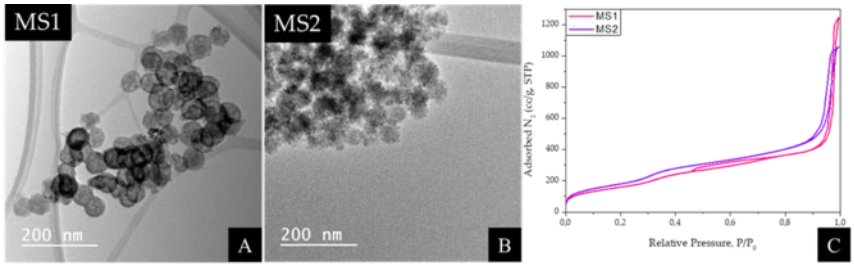

Figure 1: TEM microphotographs (A and B) and N_2 adsorption-desorption isotherms (B) of MS1 and MS2.

Conclusion. DOX-loading differences were recorded. This could be explained by the different textural characteristics of the materials. MS1 and MS2 presented adequate DOX-loading capacity and optimum textural properties and Ce^{3+}/Ce^{4+} ratio. Those MSNs may therefore be a viable option to increase DOX's bioavailability.

References. *1. Pouroutzidou G.K. et al., Int J Mol Sci. 22(2):577,2021; 2. Tsamesidis I. et al., Nanomaterials. 11(9):2189,2021*
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.*

S3.7-O4

Fabrication and characterization of formable, in-situ setting ceramic composite bone tissue scaffolds

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Abstract

Bioactive glasses (BG) exhibit the unique ability of bone bonding and have been shown to be safe and effective for bone regeneration. However, processing them into 3D porous bone scaffolds remains a challenge. This study aims to develop a new processing route to create porous BG composites. The design approach builds on our recent proof-of-concept work aimed at creating a formable composite scaffold by mixing 45S5 BG powder and a sodium silicate binder solution, which self-sets in the air. The objective of this study is to optimize the setting time and mechanical properties of the composite bone scaffolds to a range that is practical for clinical applications.

Three different methods were investigated to decrease the binder setting time through acid catalysis: CO2 gas, boric acid, and phosphoric acid. The setting time obtained by each acid catalyst was measured by tube-inversion method and the data was analyzed to create a predictive model. The results have shown that the binder setting time could be precisely controlled by adjusting the binder's pH and concentration. Real-time Raman spectroscopy was employed to gain insight into the molecular reactions that occur during binder setting using various acid catalysts. The findings indicated significant variations in the chemical structure of the solidified binder, depending on the pH level, as well as the specific acid catalyst used. The setting reaction by-products were investigated using X-ray diffraction (XRD). The mechanical properties of the solidified binders and composites set with different acid catalysts were studied in compression mode. The results demonstrated that the compression strength of the solidified binders and composites depend on the pH, binder's concentration and the type of acid catalyst. The microstructure of the solidified binders and composites were analyzed with Scanning electron microscope (SEM). Finally, porosity, phase distribution, and scaffolds' structure were verified using micro-CT, generating a 3D model of each investigated composition. Further development will involve *in vitro* and *in vivo* studies to develop a prototype kit for use in operating room.

S4.1-K1

Engineering of complex *in vitro* models

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Abstract

The selection of a proper material to be used as a scaffold, as a proper matrix, or as a bioink in 3D bioprinting approaches to support or encapsulate cells is both a critical and a difficult choice that will determine the success of failure of any tissue engineering and regenerative medicine (TERM) strategy.
In our research group we have been mainly using natural origin polymers, including a wide range of marine origin materials, for many different approaches that allow for the regeneration of different tissues. Several innovative bioinks with quite specific properties were developed and proposed for several specific uses. We have also been optimizing the respective formulations for using these novel materials in distinct biomanufacturing strategies.
Furthermore, an adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation methodologies should be developed and optimized. We have been using different human cell sources namely: mesenchymal stem cells from bone marrow, mesenchymal stem cells from human adipose tissue, human cells from amniotic fluids and membranes and cells obtained from human umbilical cords.
The potential of each biomaterials/cells combination and respective concentrations, as related to different manufacturing technologies, with details when appropriated focusing on bioprinting, to be used to develop novel useful regeneration therapies will be discussed. Several examples of TERM strategies to regenerate different types of tissues will be presented. The use of different cells and new ways to assess their interactions with different natural origin degradable scaffolds and bioinks will be described. A unique high-throughput platform to better understand material/cells interactions and optimise their performance and biological performance will be discussed. This rather innovative platform is based on the use of unique microfluidics-based approaches.

S4.1-O1

Advantages and challenges in development of perfusion-based 3D cell cultures

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Abstract

Three-dimensional (3D) cell culture systems are being increasingly used for reliable cell and tissue studies often relying on the use of biomaterials as cell supports. In this case, static conditions frequently lead to mass transport limitations with concentration gradients in the culture medium and within the scaffolds. This problem can be solved by introduction of fluid perfusion directly through the scaffold. However, fluid flow also induces hydrodynamic shear stresses to which both the scaffold surfaces and cells may be directly exposed. Thus, when designing a 3D perfusion-based culture the following critical issues should be optimized: scaffold properties, cell immobilization method, and the perfusion flowrate providing efficient mass transport at adequate shear stress levels. Here we showcase 2 model systems based on alginate hydrogels as cell supports and perfusion bioreactor, stressing on operational challenges, on one side, and valuable outcomes, on the other. Alginate microfibers were utilized to imitate extracellular matrices of soft tissues and protect the incorporated cells from the direct fluid flow exposure, while macroporous composite Ca-alginate (2 wt%) scaffolds containing hydroxyapatite particles (2 wt%) were used to imitate the bone tissue structure and immobilize cells within the pores. Both seeded scaffold types were then cultured in perfusion bioreactors (3D Perfuse, Innovation Center FTM, Serbia). Microfibers with different cancer cells (*e.g*. rat glioma C6, human U87 glioblastoma, human NCI-H460 lung cancer cell lines) were produced by simple extrusion into a gelling bath containing Ca ions (180 mM). However, each cell line required separate optimization regarding alginate concentration (1.5–3 wt%), extrusion parameters and culture conditions. For example, Ca-alginate microfibers degraded under the flow of RPMI-1640 culture medium so that barium (45 mM) was selected for alginate gelation for lung carcinoma cell immobilization. Also, it was shown that fluid in the perfusion bioreactor partially flows through the hydrogel (~10 nm/s interstitial velocity) adversely affecting these cells. Still, cancer cells in alginate microfibers have shown decreased sensitivity to anticancer drugs as compared to monolayer cultures. In the second series, murine K7M2-wt osteosarcoma and MC3T3-E1 preosteoblast cell lines seeded in porous composite Ca-alginate scaffolds formed cell aggregates within the pores, which became denser with higher amounts of reticular fibers under the medium flow (~60 µm/s interstitial velocity, ~5-15 mPa shear stress) as compared to static cultures. Overall, the performed studies demonstrate the additional effort required in setting 3D culture systems but also increased possibilities for exploiting *in vitro* physiological-like environments.

S4.1-O2

A new PNS-CNS-on-chip to explore the therapeutic potential of neuro-targeted trimethyl chitosan-based nanoparticles as a treatment for spinal cord injury

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Abstract

With 500k new cases worldwide per year, spinal cord injury (SCI) carries substantial individual and societal costs. So far, there are no treatments available to promote neuronal survival and/or regeneration. Recently, gene therapy has emerged as a powerful tool to promote neuronal repair and regeneration. However, the effectiveness of this approach relies on the development of delivery vectors that are efficiently targeted and suitable for clinical use. Previously, we proposed the use of nanoparticles based on thiolated trimethyl chitosan (TMCSH) to mediate targeted gene delivery to peripheral neurons upon an intramuscular administration. Here we are exploring the capacity of these nanoparticles to effectively carry siRNA to promote neuroprotection/regeneration in the central nervous system (CNS) neurons after a peripheral administration. To attain neural targeting, we functionalized the TMCSH-siRNA polyplexes with 50kDa non-toxic carboxylic fragment of the tetanus toxin, known to be neurotropic and retrogradely transported along axons to reach CNS, via a PEG spacer. To test our hypothesis, we have developed a new microfluidic model mimicking the interface between the peripheral and central nervous system (PNS-CNS) neural cells. TMCSH showed good ability to complex siRNA (68-75%, depending on the TMCSH:siRNA ratio) and the resulting polyplexes showed nanosizes (114-203nm), low polydispersity index (≤0,25), spherical morphologies and positive zeta potential values (9-25mV), making them suitable for cellular uptake without causing toxic effects in the neuronal cells (neuronal cell lines, primary cultures and *ex vivo* models). The targeting ability of our polyplexes was confirmed through flow cytometry, atomic force microscopy and confocal microscopy image analysis using our microfluidic devices under flow conditions. The targeted polyplexes' uptake increased in neuronal cells and decreased significantly in non-neuronal, indicating neurospecificity. In addition, under flow conditions, the targeting moiety allowed an increased polyplexes' binding. The retrograde transport of our nanoparticles along the axon to the cell body of neurons was also characterized through STED super-resolution microscopy and spinning disk confocal microscopy. Our PNS-CNS microfluidic device allowed to mimic the bio-interaction between the polyplexes and neurons and monitor the intracellular trafficking. Furthermore, since our devices have integrated microelectrode arrays, polyplexes' biocompatibility as well as the biological effect of a therapeutic siRNA against PTEN are being evaluated. Our study shows the potential of TMC-based vectors to be used as non-viral gene carriers for delivering therapeutic nucleic acids to PNS and CNS and thus it can be a promising therapeutic approach for SCI and other neurological conditions.

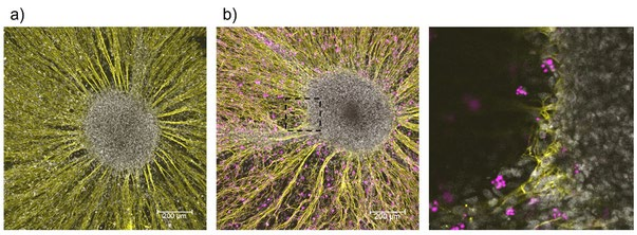

Fig.1. Image of an embryonic rat dorsal root ganglion (DRG) explant incubated, for 24 h, with TMCSH-Cy5-siRNA polyplexes N/P4. a) Non-treated DRG. b) Cy5-siRNAmi-polyplexes-treated (magenta). Neurons: βIII-tubulin (yellow); nuclei: Hoechst (grey).

S4.1-O3

Development of 3D cell-printed neural network to simulate brain microenvironment and its application to neurodegeneration study

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Abstract

The brain has complicated physiology, including a brain-specific extracellular matrix, axonal network, and compartmentalized structure (i.e., white and grey matter). The brain may lose its own functions including cognitive function, motor control, and homeostasis once the physiological structure disrupted. One of the severe neuropathies is neurodegeneration induced by progressive neuron cell death and neural network disruption. There are numerous factors to induce neurodegeneration such as environmental factors (e.g., alcohol consumption, diesel exhaust particles) and pathophysiological factors (e.g., neuroinflammation and neurovascular disruption). However, the neurodegeneration from various factors is not sufficiently elucidated yet due to the lack of reliable cerebral model that simulates humanized neural networks and microenvironments. Especially, direct effects of alcohol consumption dose and period on cerebral neurons for alcoholic neurodegeneration are not yet elucidated despite the neurotoxicity of alcohol in the epidemiological investigation. Therefore, a cerebral research platform is required that recapitulates compartmentalized cerebral structures based on brain-specific neural networks for elucidating neurodegeneration, including alcoholic atrophy. Here, we suggested a 3D cell-printed neural network model to study alcoholic neurodegeneration by incorporating brain-specific material (porcine brain decellularized extracellular matrix, P-BdECM) and external stimulation for the rapid formation of mature neuronal networks. First, we showed that our developed P-BdECM has a human nervous system favorable protein composition by proteomic analysis. Furthermore, the P-BdECM can promote neuron maturation and stabilize neuroglia cells. Based on this neurologically favorable material, we tried to build a compartmentalized axonal neural network model *in vitro* with 3D cell printing. Briefly, we constructed a cell culture system with neural progenitor cell-laden bioink (Matrigel and BdECM hybrid) and built an acellular region on the system for axonal elongation to recapitulate axon-soma compartmentalized structure. We optimized the width of the acellular region (~200 um) to induce axonal outgrowth only without cell migration into the axon-elongated region. In addition, to induce rapid cytoskeletal rearrangement and activation of the axonal outgrowth cone, we treated neurotrophic factor (BDNF) and AC electric field to the printed neural models. Optimized BDNF enrichment (~10ng/ml) and native-like electrical stimulation synergistically enhance axonal elongation in the acellular region for a compartmentalized neural structure without cellular apoptosis. Further, we optimized ethanol treatment concentration by confirming the reduction of mitochondrial activity of matured neurons for alcoholic neuropathy modeling. Through the study, we expected that the robust axonal neural network model under recapitulation of the brain microenvironment can be applied to neurodegenerative disease research.

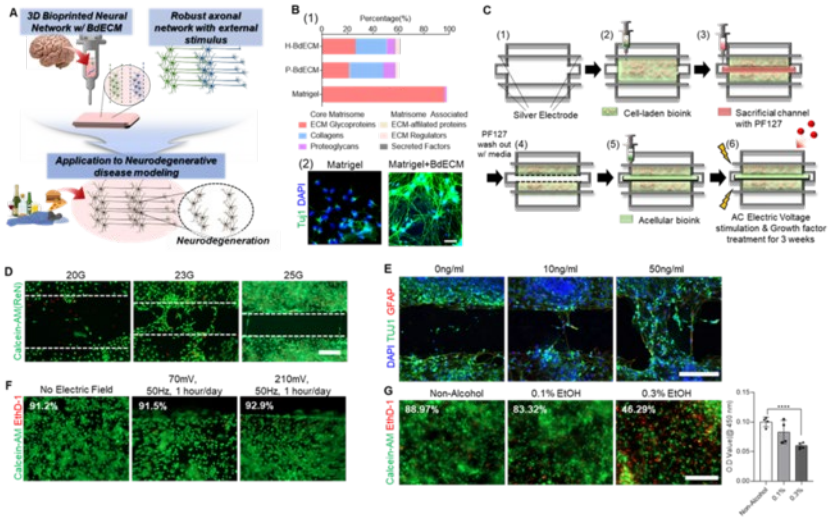

Figure 1. *(A) Schematic of 3D Bioprinted Neural Network for Neurodegenerative disease study (B) Matrisome Comparison for human-BdECM, porcine-BdECM, and Matrigel; Enhanced neural differentiation with BdECM (C) Fabrication process for 3D bioprinted neural network and enhanced axonal elongation strategy (D) Axon-soma compartmentalization by controlling acellular region width (E) Promoting axonal elongation with neurotrophic factor treatment (F) Cell viability under electrical stimulus (G) Alcohol-derived neural apoptosis; All scale bars in figure represent 200 um*

S4.1-O4

Engineering spherical membranes for inhalation tests in the presence of synthetic mucus

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Abstract

Inhalation tests are fundamental for assessing drug and substance absorption. Standard in-vitro models are based on flat 2D semipermeable membranes at the air-liquid interface. Only recently some studies attempted to replicate the spherical alveolar geometry [1, 2] or the presence of a mucus layer [3]. However, they still lack lung properties such as stretchability. To this end, we developed transparent spherical and streacheable membranes which replicate the alveolar architecture in a more accurate manner.

2D and 3D Agarose-Gelatin (AgGel) membranes were fabricated by cast-drying 1%-5% w/v agarose–gelatin solutions in custom moulds. Mechanical tensile tests were performed at a constant strain rate (0.2 s^{-1}). A549 cells were seeded (100.000cell/cm^2) on the membranes and in PET Transwells as control. Transcellular and paracellular transport was investigated with FITC-labelled dextran (0.5 mg/mL) and rhodamine (10 µM). Transepithelial electric resistance (TEER) and Alkaline Phosphatase (ALP) release was also evaluated.

The membranes resulted highly transparent and elastic in the range of pathophysiological strains (5-17%) [1] with an apparent elastic modulus = 1.07±0.35 MPa and failure stress = 0.13±0.03 MPa. Figure 1 shows that cells adhered forming an uniform monolayer on AgGel membranes. Moreover, AgGel3D presented lower TEER, FITC and ALP values with respect to AgGel2D and PET controls, suggesting that curve substrates provide physiological culture conditions for lung epithelial cells. Indeed, in standard culture conditions, A549 typically presents non physiological high TEER values and ALP is known to be overexpressed in cancer [3, 4].

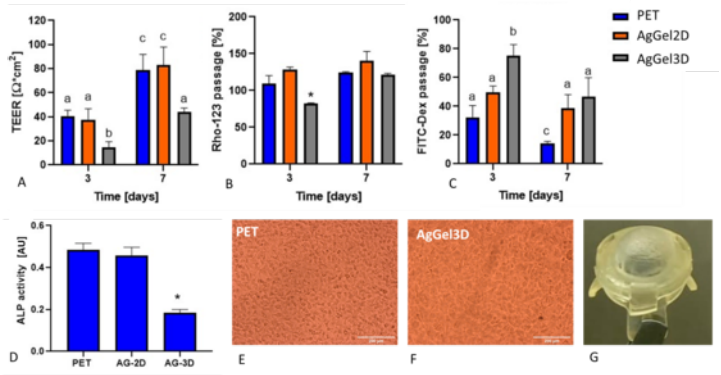

*Fig.1: A) TEER measurements, B-C) FITC and P-gp passage, D) ALP release, E-F) Brightfield images of A549 cells on PET and AgGel membranes, G) spherical membrane on cell-crown inserts. Different letters and * indicate statistical differences (p<0.05)*

The spherical membranes were also interfaced with fluidic compartments (Fig.2) fabricated by stereolitographic printing, replicating blood and air flow as well as breathing.

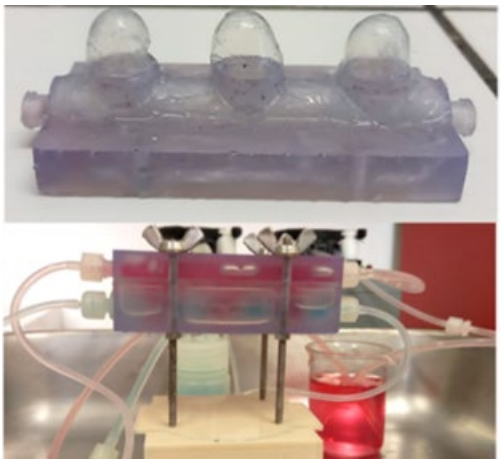

Fig.2 Sperichal membraned interfaced with fluidic compartments

Further tests are on-going to investigate cell behavirour in dynamic conditions and to develop synthetic mucus gels able to replicate healthy and diseased mucus rheology and hence investigate substance adsorption in the presence of mucus and inflammatory cells. These results represent a step forward toward the definition of advanced in-vitro inhlatiation systems as human-relevant preclinical testing tools alternative to animal tests.

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S4.2-K1

The mechanics of tendon biology

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Abstract

The field of tendon biology, long understudied, is rapidly gaining ground. On one hand, the tendon field is profiting from ever increasing mechanistic clarity within the broader disciplines of immunology, biophysics, matrix biology and mechanobiology. On the other hand, powerful technologies in molecular biology, biotechnology, bioimaging and data science are opening unprecedented experimental possibilities for labs everywhere.

Our research group, like others, has harnessed some of these advances to identify novel mechanisms of cellular mechano-transduction (mechanical stimuli and their sensors). We now find ourselves confronting challenges in unwinding the daunting complexity that lies both upstream and downstream of these sensors. This talk aims to introduce the cell and tissue engineering approaches we have devised to disentangle cell-system cross-talk in tissue homeostasis, damage repair, ageing, and disease.

More broadly, this talk aims to map our current knowledge on tendon structure, function, physiology, damage, and repair. In this map, we consider the tendon as a complex physiological system with tightly coordinated interplay between a mechanically regulated core and an “extrinsic tendon compartment” that consists of synovium-like tissues interfacing the immune, vascular, and nervous systems. This conceptual framework interconnects diverse aspects of tendon physiology and pathophysiology and provides a unifying picture that may help in understanding human disease and how to better treat it.

S4.2-O1

Two-pole electrospun PCL/collagen scaffold and physically differentiated ADSCs, a secure and effective combination for tendon regeneration

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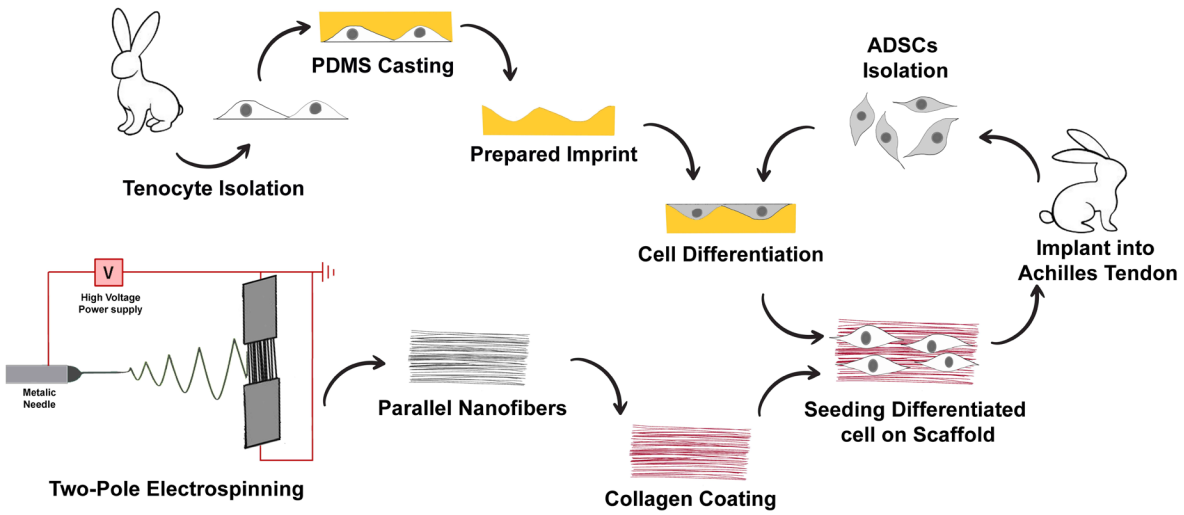
Abstract

Tendon injuries through trauma and sports are among the most frequent in humans and horses, which unfortunately recover slowly and insufficiently. New strategies in tissue engineering seek to accelerate this difficult regeneration process and increase the similarity of the regenerated tissue to the normal tendon. The regeneration of the tissue may be greatly aided by the transplantation of differentiated stem cells and a scaffold that closely resembles the structure of the target tissue. Using a tenocyte-imprinted substrate, the current work evaluated the regeneration ability of ADSCs induced to adopt a tenogenic phenotype. To create a suitable framework for regeneration, highly paralleled Polycaprolactone (PCL) nanofibers were created using two-pole electrospinning and then coated with collagen to resemble the size and the content of tendon fibers. This experiment's main goal was to evaluate the regeneration capability of the scaffold seeded with the differentiated ADSCs through the physical method (imprinting) in the rabbit model. Results from *in vivo* therapy point to differentiated ADSCs placed on the PCL scaffold for tendon restoration having astonishing therapeutic potential.



S4.2-O2

mRNA activated matrices: a tridimensional system for articular cartilage repair.

Héctor Rilo-Alvarez[1], Adriana M. Ledo[2], Mónica Lopez-Peña[3], Fernando M. Muñoz[3], Anxo Vidal[4], Marcos Garcia-Fuentes[5]

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Abstract

Articular cartilage has a critical role in the normal function of musculoskeletal system, being crucial for allow proper joint mobility. Friction forces and several disorders can generate structural damage over the patient lifetime, and this tissue lacks the ability to self-regenerate. Actual treatments for damaged cartilage do not trigger a complete regeneration process and tends to form fibrotic cartilage with worst mechanical and functional properties [1]. As an alternative therapy, we developed and tridimensional matrix system, called mRMATRIX, involving the administration of pro-chondrogenic factors in form of mRNA nanocomplexes. The objective is to enhance the therapeutic potential of pro-chondrogenic factors by releasing them in a sustained way and combining those with the physical support of a polymer matrix [2].

The stimulatory signals introduced in the mRMATRIX system were the transcription factor SOX9 and the growth factor TGF-β3. The bioactivity of the mRNA codifying for both therapeutic factors was enhanced by enhancing its stability with a specifically designed poly(A) tail of the mRNA and by the introduction of chemically modified nucleotides in the synthesis process. These modifications generate a more stable and less immunogenic mRNA.

Pro-chondrogenic potential of mRMATRIX activated with SOX9 and a combination of SOX9 and TGF-β3 was evaluated in cell cultures of mesenchymal stem cells quantifying the expression of chondrogenic factors and the production of extracellular matrix components like glycosaminoglycans. The promising results obtained in these studies [Fig 1] led us to evaluate the therapeutic potential of mRMATRIX in knee-defect rabbit models.

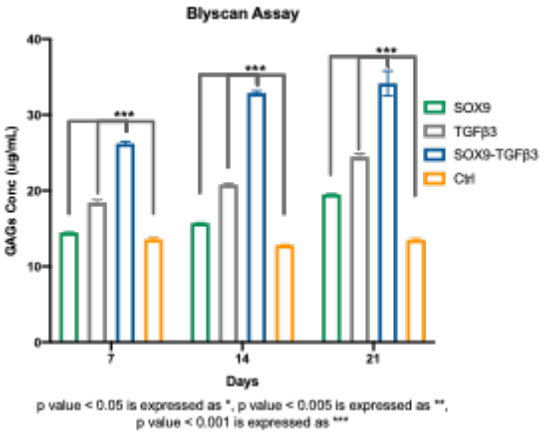

Fig 1. Determination of Glycosaminoglycans in the extracellular matrix of new formed tissues in vitro.

Results reflected the therapeutic potential mRMATRIXs activated with SOX9, which generate significant overexpression of chondrogenic markers both *in vitro* and *in vivo*. Additionally, the system activated with both stimulatory signals achieved a better recovery in cell cultures and animal assays [Fig 2].

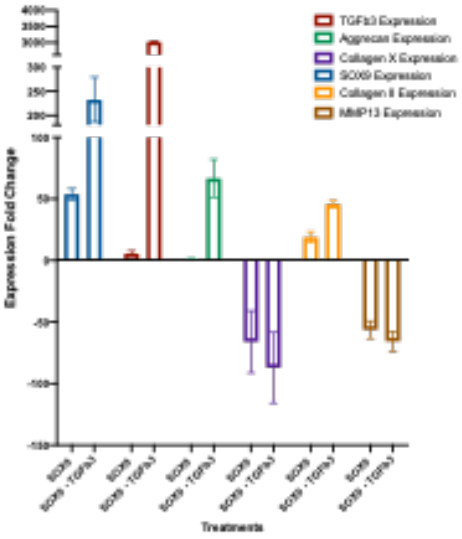

Fig 2. Expression fold change of pro-chondrogenic markers in vivo after the treatment with mRMATRIX activated with both therapeutic factors.

In conclusion, the mRMATRIX system shows great potential as an innovative therapy for articular cartilage regeneration. Particularly the system activated with SOX9 and TGF-β3 mRNA is a promising treatment option for cartilage damage repair that merits more advanced *in vivo* evaluation.

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S4.2-O3

Role of compression and shear in latent TGF-β1 mechanical activation

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Abstract

Transforming growth factor beta-1 (TGF-β1) is among the most studied growth factors in chondrogenic differentiation of human Mesenchymal Stromal Cells (hMSCs). TGF-β1 is secreted by hMSCs in an inactive latent form and can be activated by multiple mechanisms, including mechanically. Previous studies reported the mechanical activation of latent TGF-β1 under loading (Li et al. 2010; Gardner et al. 2017). Nonetheless, the role of compression and shear forces has not yet been investigated in an in-vitro model. In this study, we investigate the impact of compression and shear forces separately, on latent TGF-β1 mechanical activation using a custom-made bioreactor.

Thermoplastic polyurethane was printed at 15% (TPU15) and 16,88% (TPU16.88) infill densities with gyroid infill (Ultimaker). Scaffolds (Ø8mm, 4mm height) were then punched out. Polyurethane foam (PU) scaffolds were manufactured as described elsewhere (Gorna and Gogolewski 2006). All samples were impregnated with fibrin, prepared using fibrinogen and thrombin from human plasma (Sigma-Aldrich) to a final concentration of 25 mg/ml and 2 U/ml, respectively. Compression (10%, 15%, 20% at 1Hz) or shear (0.01 Hz, 0.1 Hz, 1 Hz) alone was applied for 6h in cell culture medium containing 50 ng/mL latent TGF-β1 (R&D Systems). All the groups were compared with free swelling (FS) controls. Active TGF-β1 was detected in the conditioned medium (ELISA DuoSet DY240, R&D Systems). The scaffolds were washed with RIPA buffer to extract bound TGF-β1 and ELISA was performed on the washed-out liquid.

Active TGF-β1 levels were higher in the media from shear protocols with respect to compression (Figure 1). Moreover, the concentration of active protein increases with increased frequency of shear stress. After RIPA washings, the concentration of total TGF-β1 was higher for scaffolds treated with compression protocols compared to shear protocols (Figure 2). Higher compression percentages showed higher total TGF-β1 concentration.

The results show that shear forces alone at the interface of the scaffolds are activating latent TGF-β1 more than compression forces. Moreover, activation seems more likely to occur at higher frequencies. Additionally, compression pushes the protein inside the scaffolds, as proved by the higher levels of total TGF-β1 found inside compressed scaffolds. These findings suggest that latent TGF-β1 is activated by shear stresses at the interface of force application and is then pushed inside the scaffold by compression stresses. Future studies will be carried out to deeper investigate the interactions between shear forces and protein activation.

Acknowledgements: *Funded by EU-OSTASKILLS, grant No. 101034412)*

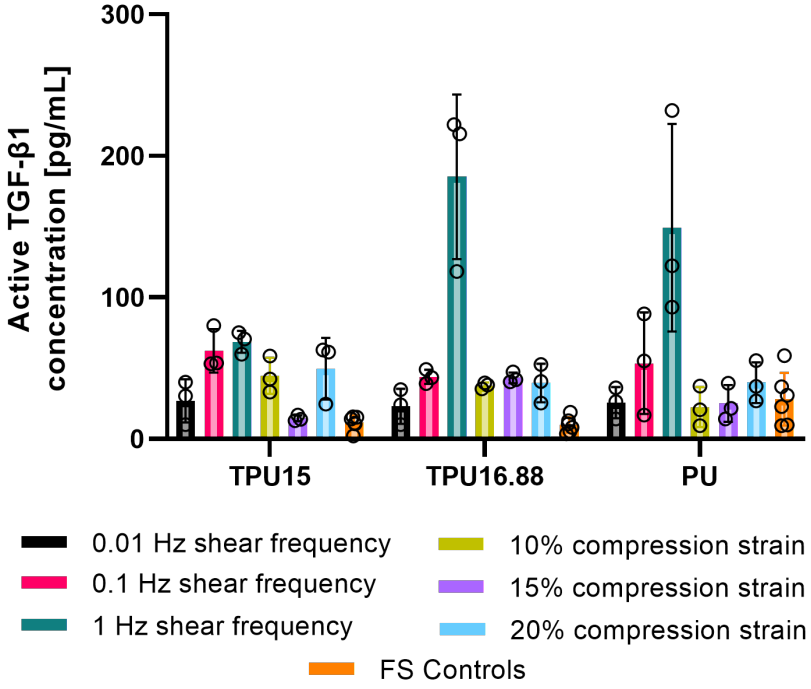

Figure 1: active TGF-β1 concentration levels for shear and compression stresses, detected in media.

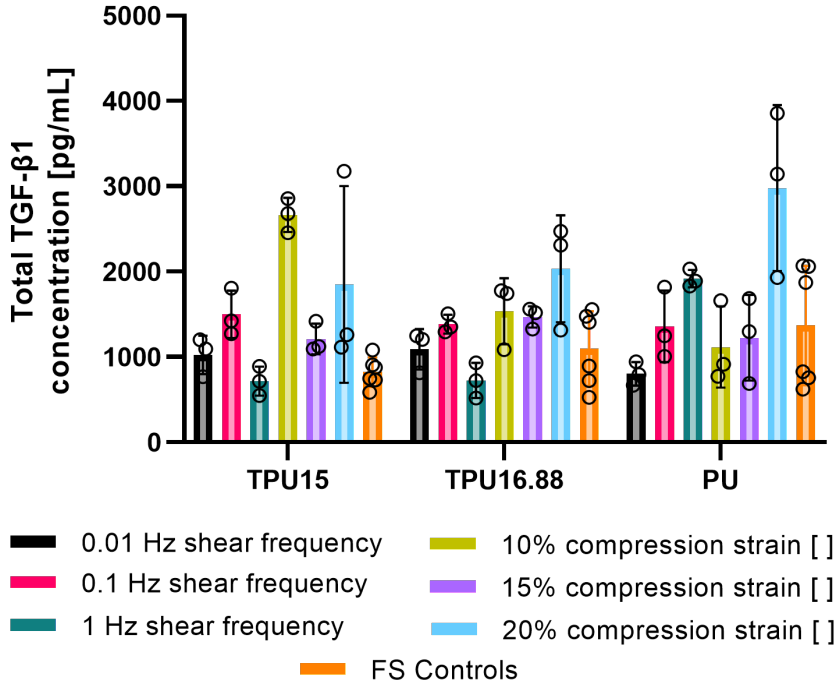

Figure 2: total TGF-β1 concentration levels for shear and compression stresses, detected in the wash-out liquid from the scaffolds.

S4.2-O4

Development of thermo-responsive hydrogels to support the response of osteoarthritic chondrocytes to mechanical loading.

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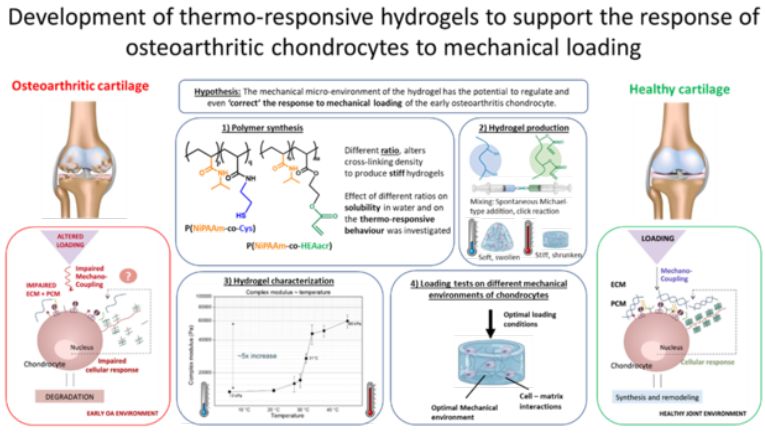
Abstract

Osteoarthritis (OA) is the most common joint disease and has a high disabling impact on patients' lives. Among adults over 60 years of age, approximately 10% of men and 13% of women suffer from OA which causes a high economic burden. To date, no effective cure to prevent or slow down OA progression is available, with the only treatment for end-stage osteoarthritis consisting of joint replacement. Our team aims to investigate how the mechanical micro-environment of the hydrogel has the potential to regulate and even 'correct' the biological response of the early OA chondrocyte to mechanical loading. To this end, a hydrogel with tuneable viscoelastic properties has been developed with the ambition to ultimately allow precise modulation of the micro-environment of the chondrocyte while leaving the cells in situ.

Two different copolymers were successfully synthesized both containing a thermoresponsive N-isopropylacrylamide (NiPAAm) co-monomer and a cross-link monomer consisting of either a thiol or an acrylate functionality. Copolymers with different percentages of cross-link monomer, ranging from 1% to 10%, were synthesized. Higher amounts of cross-link monomers will yield higher cross-linking densities producing tougher hydrogels. In contrast, the solubility of the polymers decreases with increasing amounts of cross-link monomers. Therefore an optimal incorporation percentage of cross-link monomers was determined in order to yield the toughest hydrogels possible.

Mixing both polymer solutions, solubilized together with human primary chondrocytes, induces the formation of a polymer network by Michael-type "Click" reaction yielding robust thermo-responsive hydrogels. Cell viability within the hydrogel was confirmed by caclein AM staining. By changing the temperature, the water content and the mechanical properties of the hydrogel and in this manner the micro-environment of the chondrocytes, can be controlled. At low temperatures, the hydrogels are soft and swollen, 90% of the weight of the hydrogel consisting of water. Increasing the temperature will toughen the hydrogel and water will be extruded, decreasing to only 50 w%. At low temperatures, the complex modulus remains constant around 13 kPa. A clear transition was observed at 31°C. After transition towards high temperatures, a new plateau was reached with a complex modulus of 60 kPa.

This five-fold increase in complex modulus causes drastic changes in the micro-environment of the embedded chondrocytes. Different loading regimes will be applied on chondrocytes under different mechanical micro-environmental circumstances. More insight into the role of the chondrocytes' mechanical micro-environment in the impaired mechanoresponsiveness may assist the development of future effective physical exercise programs.



S4.3-K1

Communication at biointerfaces

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Abstract

The integration between tissue and material is usually a prerequisite for the safety and successful clinical function of the device. Through the decades a complete understanding of the specific material-tissue interface has however not been available. It has not even been a prerequisite for clinical introduction. The subsequent years of clinical performance usually tell the clinicians and the manufacturer about issues where improvements are needed, resulting in new versions of the device, sometimes bi-annual in order to be able to compete and hold market shares. Some manufacturers learn by retrieval of devices and adjacent tissues whereas others do not. In the future, a thorough understanding of the material-tissue interface is likely to be more important. An increased sophistication of the devices, for example being able to report what is going on in the interface and to be time and functionally programmed, could be one reason. Another reason is the likely demand for life-long performance of the interface even in severe and compromised tissue conditions.

The recent decades have witnessed a revolution of techniques to study the fine details of the biological and material components of the interface. The methods enable a detailed analysis of the properties of the inserted material as well as the cellular and molecular components in response to the introduction and presence of the material. A systematic, interdisciplinary approach is likely to be a good strategy for an increased understanding of how the interface will evolve and behave over time. Given the wealth of different methodologies, commonly applied *in vitro* and/or *in vivo*, the choice of experimental models and selection of methods can open amazing doors for the most curious researcher.

During the lecture, examples of experimental and clinical models will be presented, ranging from small and large animal models to opportunities to obtain human samples. Focus is on the demonstration of a set of methods and analytical tools employed for the morphology of the interface as well as the routes for communication between cells in the interface. Examples are selected from the author´s previous and current projects on osseointegration of amputation prostheses, guided bone regeneration and large skeletal defects. The challenges of compromised tissues, for example after radiation treatment, and infection are discussed. Throughout the presentation emphasis is on how cells respond to and communicate after the encounter with material components in normal and compromised, local tissue environments.

S4.3-O1

Prevention of aortic anastomotic leakage using a surgical adhesive based on catechol group-modified Alaska pollock gelatin

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Abstract

Introduction. Aortic anastomotic leak is a potentially fatal complication that can occur after the treatment of aortic dissection or aneurysm. Several surgical adhesives have been used to prevent this complication, but all have problems regarding tissue adhesion or biocompatibility. On the other hand, mussels have been reported to be able to adhere to various substrates in water using the interaction of catechol groups [1]. In the present study, we developed a surgical adhesive composed of boric acid-protected catechol groups-modified Alaska pollock-derived gelatin (Cat-ApGltn) and a poly(ethylene glycol)-based crosslinker (4S-PEG). We evaluated the tissue adhesion and biocompatibility of this surgical adhesive [2].

Materials and Methods. Cat-ApGltn was synthesized by the reductive amination of 3,4-dihydroxybenzaldehyde to the amino groups of original Alaska pollock-derived gelatin (Org-ApGltn). The degree of catechol group modification was determined by the 2,4,6-trinitrobenzene sulfonic acid method. Adhesives were *in situ* prepared by mixing of Cat-ApGltn and 4S-PEG. Burst strength measurements were performed according to ASTM (F2392-04) using fresh porcine aorta. Biocompatibility was evaluated by implanting the cured adhesive under the back skin of rats.

Results and Discussion. Cat-ApGltns with various modification degree from 5 to 35mol% were successfully synthesized with high yield (90%~). 8Cat-ApGltn adhesive (modification degree: 8mol%) showed 2.3 times higher than that of the Org-ApGltn adhesive, and 3.9 times higher burst strength compared with commercial fibrin adhesive (Fig. 1). The average systolic blood pressure in healthy humans is 127 mmHg. Therefore, 8Cat-ApGltn adhesive can be applied *in vivo* to tissues subjected to high pressure, such as the aorta. The high tissue adhesion of Cat-ApGltn adhesive is presumably due to the formation of physical and chemical crosslinks of catechol groups in the bulk and at the interface with the tissue.

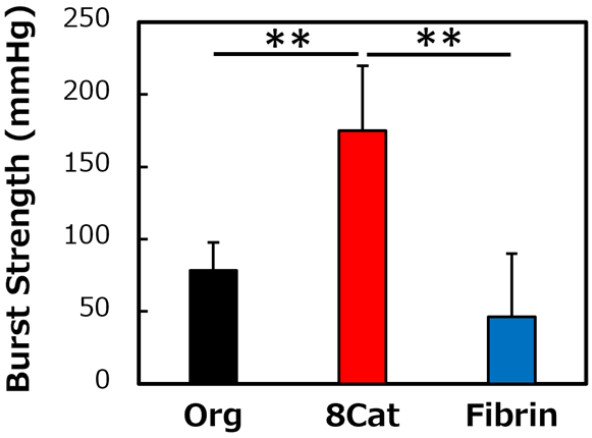

*Fig. 1 Burst strength of Org-ApGltn, Cat-ApGltn and Fibrin adhesives for sealing a porcine aorta. **P < 0.01.*

In addition,8Cat-ApGltn adhesive subcutaneously implanted in rats showed excellent biocompatibility and was completely degraded within 2 months (Fig. 2).

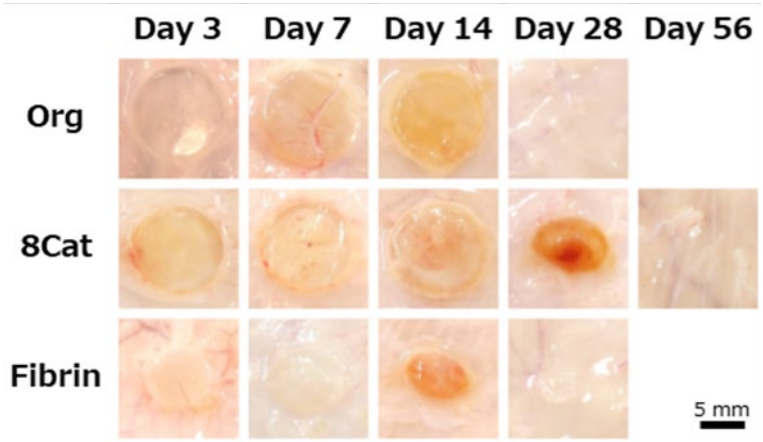

Fig. 2 Images of Org-ApGltn, Cat-ApGltn and Fibrin adhesives implanted subcutaneously in rats for various periods.

According to the above results, the 8Cat-ApGltn adhesive is biocompatible and tissue-adhesive property, and is a useful wound closure material for aortic anastomotic leakages.

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S4.3-O2

Effect of surface properties on the vascular biocompatibility of 3D-printed shape-memory alloys for the development of smart cardiovascular implants

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Abstract

Recent developments on additive manufacturing of metals have a great potential to provide a new source of functionality, in addition to more efficient fabrication processes. In particular, the biomedical industry can truly benefit from this technology for the development of personalized cardiovascular implants such as cardiac valves or intravascular stents. The properties of the blood-contacting surfaces of these cardiovascular implants are crucial for the correct integration of the implant in the patient's body and avoid restenosis, a repeated narrowing of the blood vessels mainly caused by an excessive growth of smooth muscle cells.

In this work, we used selective laser melting (SLM) to manufacture nitinol samples, a shape memory alloy with interesting properties for deployable medical devices because of the superelastic properties or shape memory effect. Different surface treatments, such as chemical etching, electropolishing or a combination of both, were applied on the 3D-printed samples, originating surfaces with different roughness, composition, corrosion resistance or surface energy properties. The adhesion, morphology and biocompatibility of these different surface-treated nitinol samples was evaluated on human endothelial and smooth muscle cells, the predominant cells forming the vascular tissue. Cell proliferation was also evaluated on non-treated or surface-treated nitinol samples, with the aim to find a suitable surface for an early endothelialisation of the implant, thus preventing restenosis. In addition, gene and protein expression analysis were performed to study vascular cells behaviour in terms of endothelialisation and inflammatory responses.

Overall, these results provide further insights into the influence of surface properties of metallic implants on vascular cells, paving the way for the use of different surface treatments to modulate vascular behaviour and improve the development of novel smart cardiovascular implants by additive manufacturing.

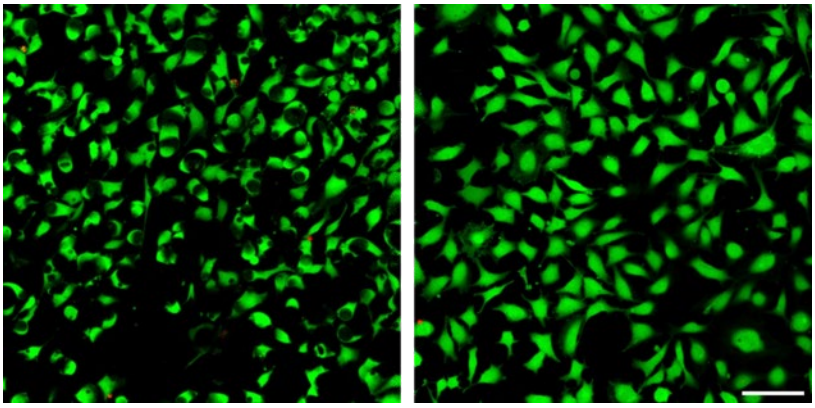

Figure: Live/dead assay of human endothelial cells cultured for 24h on non-treated (left) or treated (right) surface of a 3D printed nitinol sample. Green: alive cells, red: dead cells. Scale bar: 100µm.

S4.3-O3

Influence of training in bone healing around bio-resorbable implants imaged by scattering methods

Irene Rodriguez Fernandez[1], Thomas Bretschneider[2], Omer Suljevic[3], Tilman Grünewald[4], Nicole Sommer[3], Annelie Weinberg[3], Helga Lichtenegger[2], Andreas Menzel[1]

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Abstract

In recent years, bio-resorbable magnesium implants have gained interest as treatment options for bone fractures. Their advantages include their mechanical properties, their excellent immunological response during healing, and the reduced need for a second intervention to remove the implant. In previous studies [1], it could be shown that implant placement and degradation alter the bone structure. The preferred nanostructural orientation changes significantly from along the femur shaft to an orientation following the implant surface direction. However, these studies were carried out without a controlled regime of physical activity.

In this study, we wanted to expand these studies by including physical activity to the treatment and determine what impact (if any) physical training has in bone healing and mineralization around bone implants. For this purpose, we used synchrotron scattering techniques such as small-angle X-ray scattering (SAXS) and small-angle scattering tensor tomography (SASTT). SAXS can yield information about the changes in orientation of bone nanostructure in 2D as a response to the degradation of the Mg implant but is limited to thin slices and 2D information of the orientation. To overcome this limitation, SAXS results were combined with SAXS tensor tomography, which yields spatially resolved 3D anisotropy information. Using these two techniques, we have been able to characterize the role physical activity plays in healing and remodeling of bone around Mg implants. Our measurements suggest that that such training leads to a higher degree of orientation of hydroxyapatite (HA) in bone and found that training might lead to a quicker response of the bone metabolism. These results are highly relevant for understanding degradable implants' behavior and are expected to be of clinical significance in the treatment of bone fractures.

References: [1] M. Liebi, V. Lutz-Bueno, M. Guizar-Sicairos, B. M. Schönbauer, J. Eichler, E. Martinelli, J. F. Löffler, A. M. Weinberg, H.C. Lichtenegger, T. A. Grünewald (2021) 3D nanoscale analysis of bone healing around degrading Mg implants studied by X-ray scattering tensor tomography. Doi: 10.1101/2020.11.09.375253

S4.3-O4

Characterizing materials-tissue interface and *in vivo* bone regeneration of critical-sized bone defects loaded with osteogenic ceramic granules

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Abstract

Critical-sized bone defects occur in cases of major orthopedic fracture and bone loss or tumor resection, and they have an increasing prevalence. However, current repair products and clinical practice have their limitations. We have developed a combinatorial approach that employs ceramic bone graft granules (monetite) with a highly osteogenic coating, poly(ethyl acrylate) (PEA), which induces the unfolding of fibronectin (FN), increasing BMP-2 osteogenic growth factor binding[1].

The newly developed medical device was implanted in rat femora for 10 weeks. A segmental bone defect of 5 mm was filled either with one monetite (5x3mm, diameter-height) or two (2.5x1.5mm). The monetites were either plain or coated with PEA, FN and BMP-2 (10 µg/ml). After the sacrifice, the rat femora were embedded in poly(methyl methacrylate) (PMMA). A multiscale characterization of non-decalcified tissue was implemented using high-resolution microcomputed tomography (microCT) at 7µm voxel size, controlled-angle cutting[2], histological staining, polarized light imaging and back-scattered electron microscopy (BSE).

First, microCT images were analyzed with Dragonfly software to quantify the newly formed bone. The samples containing coated monetites showed a statistically significant bone volume increase both in the immediate materials-tissue interface and in the whole defect volume in all cases (p-values<0.01), quantitatively demonstrating the effectiveness of the coating for osteogenesis. In addition, the effect of the monetite size/amount was also studied: the use of two smaller monetites showed a significantly higher normalized bone volume than the one monetite strategy (p-value<0.0001). Second, the histological sections obtained from each sample provided information about the soft tissue surrounding the monetites. Goldner's trichrome, alcian blue and picrosirius red staining with polarized light imaging showed that the animals with coated grafts presented higher amounts of collagen than the non-coated ones. In addition, the BSE images showed seamless interfaces between monetite-new bone, with greater bone infiltration in the coated grafts, where the osteocyte lacunae could be clearly identified. These infiltrations were not observed in the uncoated grafts. Altogether, we demonstrate increased newly formed bone and improved materials-tissue interface with the proposed strategy.

In conclusion, this work proved the efficacy of the administration of ceramic bone graft granules coated with osteogenic compounds (PEA, FN and low-dose BMP-2) for bone regeneration.

Acknowledgements: H2020 HEALIKICK project (874889).

References: [1] Cheng, Z.A., Alba-Perez, A., et al., Adv.Sci. 2019, 6, 1800361. [2] Moreno-Jiménez, I., Garske, D.S., et al., MethodsX 2021, 8, 101480.

S4.4-K1

One health experiences in research: useful convenience or essential collaboration?

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Abstract

One Health is loosely defined as an interdisciplinary approach to optimising the health of people, animals and the environment by recognising and explicitly embracing their interconnectedness. The concept has been around for many decades but has grown strongly in the last fifty years, particularly in public health and especially in infectious disease control. Although widely embraced by the veterinary community, uptake in the medical disciplines has been slower, leading to calls for greater awareness and training among medical students and graduates.

But why is One Health important and what relevance has it to the material sciences, or indeed, to ESB2023? Is it a useful term, merely to entice funders of collaborative projects, or does it positively impact the quality of design and conduct in research?

We argue a One Health approach has both personal and systemic benefits that impact directly on the quality of research at all levels and stages. It recognises the heterogeneity of the world in which we live and the value of collaboration in researching that complexity. It acknowledges one's professional limitations and, thus, the need for complementary skills from other disciplines. Finally, it endorses team working and values the contribution of all members. A recent, expert opinion piece extended the concept to include equity and parity, as well as describing the role of those involved in One Health as one of stewardship (Adisasmit et al., 2022).

For a One Health approach to work, there not only needs to be a paradigm shift in personal understanding, but an institutional response in training, in corporate thinking and in research design. This has implications for funding and resource management, but will lead to greater understanding, acceptance and in the quality of outputs.

In this symposium, we seek to inform and stimulate debate – all in an atmosphere context of equity and parity!

S4.4-K2

Implementing the one health concept in academia: an opportunity to break walls and expand biomaterials concepts

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Abstract

Biomaterials science is inherently interdisciplinary. As a scientific field, biomaterials science emerged approximately 50 years ago. However, we have witnessed tremendous progress in this field. While this progress is primarily driven by medical needs, it also owes its success to the appeal of integrating biomedical sciences and engineering, which attracts scientists. Biomaterials science plays a crucial role in various areas and serves as a convergence platform with other disciplines. Bioprinting, microfluidic systems, and micro-electronic sensor/actuator systems for diagnosis are just a few examples. One area that is likely to expand is the application of biomaterials science to food safety, particularly through the use of biosensors.

The ability of biomaterials scientists to break barriers and establish connections with other fields is a fantastic asset that will be invaluable in addressing major societal challenges. Even before the COVID-19 pandemic, experts in the health field recognized the need for effective collaboration among environmental, physical, and social scientists to tackle the diverse health challenges we face today. This gave rise to the concept of One Health (OH). The OH approach acknowledges the connection between humans, animals, plants and the environment (physical, social and emotional) and aims to contribute to a multidisciplinary response, through synergy between specialists and organisations of the different areas of knowledge. Its goal is to contribute to a multidisciplinary response by fostering synergy among specialists and organizations from different areas of knowledge. In essence, OH is not just a scientific field but also an attitude and a culture.

Implementing OH, like any change in mindset, is challenging. This presentation will share lessons learned from the strategic OH program of ICBAS, a biomedical school at Porto University. ICBAS is one of the top biomedical schools in Portugal, offering undergraduate and postgraduate training in medicine, veterinary medicine, bioengineering, environment, marine sciences, and biology. Through its faculty, ICBAS is also affiliated with major research centers in these fields. Given its breadth of focus on human, animal, and environmental health, ICBAS is an ideal setting to assess the feasibility of implementing the OH concept within an academic institution.

S4.4-O1

One step closer to in-vivo implantation in sheep by customising the Young's modulus of polycaprolactone and thus nitric oxide releasing 3D-printed vascular grafts

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Abstract

Introduction: Development of nitric oxide (NO) controlled release systems in 3D-printed biodegradable small diameter vascular grafts (SDVGs) is a promising strategy to improve in-vivo graft performance by promoting tissues regeneration and long-term patency. Previously, we developed a nanocarrier-based NO controlled release system in 3D-printed SDVGs. This system demonstrated significant reduction of NO burst release and 18 days of release in the physiological range (0.5-4 × 10-10 mol.cm-2.min-1). The NO-releasing SDVGs significantly increased the migration and proliferation of endothelial cells, which is promising for in-situ graft regeneration. In order to implant the developed grafts in carotid artery of sheep, the aim of this study is to decrease the Young's modulus of polycaprolactone and thus printed SDVGs.

Materials and methods: To decrease the Young's modulus of polycaprolactone (PCL) and thus 3D-printed SDVGs, different concentrations of plasticizer have been added to PCL (Mw 80000) solution in tetrahydrofuran and stirred for 30 minutes. The resulting solutions were casted and allowed to dry under the hood for 48h. The mechanical properties of the prepared films were evaluated using a SANTAM STM-20 testing machine fitted with a load cell of 100 N. The samples (n=6, 20 mm x 10 mm x 1 mm) were tested at a constant strain speed of 50 mm/min to determine the ultimate tensile strength, elongation, and Young's modulus based on the stress-strain curves and the initial linear slope.

Results: The low flexibility of printed PCL is a limitation for suturing and implanting the graft in-vivo. One potential solution lies in reducing the interchain interaction within the PCL structure to decrease the Young's modulus, a parameter indicative for the material's flexibility. Comparing with Young modulus of PCL (282 ± 49.49 MPa), addition of 40 wt% plasticizer resulted in a significant decrease of Young's modulus (160.5 ± 13.435 MPa) which is in the optimum range for SDVGs' fabrication. Based on these promising results, implantation of the developed NO-releasing SDVGs in carotid artery of sheep seems possible in the next step.

Conclusion: Our results showed that Young's modulus of PCL is tunable by changing the concentration of plasticizer. Due to the extensive application of PCL in different fields, the customization of Young's modulus with this plasticizer-based approach represents a step forward in the development of biodegradable scaffolds with tissue regeneration capacities. The principle is applicable to tissue engineering and drug delivery systems, providing a promising avenue for the development of medical devices.

S4.4-O2

Welfare monitoring in the novel ovine thoracic limb, bilateral, single-toe ostectomy model (OBST)

Nanett Kvist Nikolaisen[1,2], Christian Nai En Tierp- Wong[3], Thomas Colding-Rasmussen[4], Michael Mørk Petersen[4,5], Daniel Hutchinson[6], Michael Malkoch[6], Casper Lindegaard[1], Stine Jacobsen[1]

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Abstract

Sheep are commonly used as model animals in orthopaedic research, due to relevant translational properties. There are only a few large animal fracture models aimed at investigating the healing of non-weight-bearing fractures. Therefore, we developed the ovine thoracic limb, bilateral, single-toe ostectomy model (OBST), with off-loading of the fractured digit achieved by applying a wooden block on the hoof of the contralateral toe. The purpose of this study was to describe and evaluate animal welfare of this novel large animal fracture model.

To comply with the 3 Rs of animal experimentation (reduction, refinement, replacement), researchers are obligated to continuously strive to improve conditions for research animals. This is only possible with valid and accurate methods for identifying manifestations of pain and stress.

Through intense and multifaceted monitoring, we aimed to assess welfare with several methods intended for the identification of the subtle pain expression of sheep in experimental settings. Four sheep underwent ostectomy in the lateral toe of each front limb. Ostectomies were repaired with 1.5 mm locking plates and 1.5 mm locking screws (DePuySynthes, Switzerland). The limb was then bandaged. Post-surgery, the animals were non-restrained single housed in pens. Medical treatment consisted of antimicrobials (penicillin, gentamycin) for 7 days, non-steroidal anti-inflammatory drugs (flunixin) until day 8 after surgery, and opioids (butorphanol tartrate) if deemed necessary.

The sheep were monitored with activity trackers (FitBark, USA), video monitoring, a pain score developed to assess orthopaedic pain in sheep, and a validated sheep pain facial expression scale for pain monitoring of sheep (EUVII framework program, AWIN project).

The study is still ongoing; preliminary findings indicate that the animals exhibit minimal pain. Within the first 48 hours after surgery, all animals scored 1 or 2 on a lameness scale ranging from 0 to 4. No animals showed severe lameness, and lameness subsided within 12 days. Moreover, an increase in activity was observed in the initial 1-2 days, with a subsequent return to normal activity on day 2-6 post-surgery.

This novel OBST model allows the evaluation of fracture healing and treatment in non-weight-bearing tubular bones. The OBST model has the benefit of excellent animal welfare compared to other ovine fracture models involving weight-bearing bones. The intense and multifaceted monitoring of the animals will provide a comprehensive evaluation of our novel model and may be adopted in other ovine fracture models, many of which are very sparsely described in terms of animal welfare and stress.

S4.5-K1

Drug delivery for tissue regeneration and ageing

Lino S. Ferreira

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Abstract

In the first part of my talk, I will present different formulations composed of light-activatable nanoparticles for gene editing. Formulations allow *in vitro* spatial control in gene editing after activation with near-infrared light. We will demonstrate the potential of the formulations *in vivo* through three different paradigms: (i) gene editing in neurogenic niches, (ii) gene editing in the ventral tegmental area to facilitate monitoring of edited cells by precise optogenetic control of reward and reinforcement, and (iii) gene editing in a localized brain region via a noninvasive administration route (i.e., intranasal). In the second part, I will speak about formulations for the release of mRNA. Clinical implementation of mRNA-based therapies warrants formulations capable of delivering them safely and effectively to target sites. I will present our recent results about the identification of polymeric nanoformulations for the efficient transfection of fibroblasts. Overall, the results presented will highlight the use of high-throughput screening strategies to rapidly identify chemical features towards the design of highly efficient mRNA delivery systems targeting fibrotic diseases.

S4.5-O1

Targeting the senescence program in the blood-brain barrier with senotherapeutic nanoparticles

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Abstract

Background**:** Aging is the leading cause of cognitive dysfunction and neurodegenerative diseases. The function and structure of the blood-brain barrier (BBB) are impaired during physiological aging and in the absence of comorbidities. BBB disruption, followed by blood-to-brain extravasation of neurotoxic molecules, is associated with cognitive loss and increased risk of cerebrovascular diseases [1, 2]. A potential trigger of BBB aging and disruption is the accumulation of senescent cells, which accumulate due to an inefficient apoptosis. In the last 6-7 years, senotherapeutics have been proposed to revert the senescent burden by inducing cell apoptosis or reducing senescent cell secretory signals [3]. Although the use of these compounds for the treatment of chronic diseases is under intense scrutiny in ongoing clinical trials, their clinical translation to the brain is limited due to low BBB permeability and potential side effects in brain cells. Here, we investigated the use of nanoparticles (NPs) to release more preferentially senotherapeutics in senescent cells located at the BBB, increasing their safety and therapeutic effect. These biodegradable NPs have been designed to deliver senotherapeutics only in cells presenting high SA-B-galactosidase activity. We demonstrate the full potential of the formulation in both *in vitro* and *in vivo* tests.

Methods**:** A library of 42 enzyme-responsive NPs containing Navitoclax (senotherapeutic) was synthesized. NPs are cleaved at the glycosidic linkage when in the presence of β-galactosidase, an enzyme overexpressed in the lysosomes of senescent cells. We evaluated the anti-senescence properties of the NPs against senescence brain microvascular endothelial cells, and comparing to the soluble senotherapeutic. Moreover, we evaluated the formulation's therapeutic effect in aged rats regarding the anti-senescence program, inflammation, BBB permeability, and recovery of cognition.

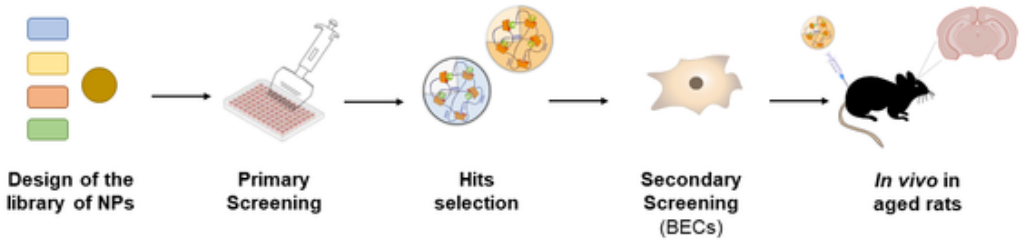

Figure 1. Schematic representation of the design of the study. *These steps include: (i) synthesis of the library of 42 nanoparticles (NPs) encapsulating a senotherapeutic; (ii) primary screening accessing cell viability in artery endothelial cells; (iii) selection of 2 hits that selectively killed senescent cells; (iv) cell targeting in senescent and healthy brain endothelial cells (BECs); (v) in vivo in aged rats to evaluate function and structure ameliorations upon administration of NPs.*

Results**:** NPs were efficiently internalized by cells after 4h of exposure and were able to selectively kill senescent endothelial cells after 48h, more efficiently than soluble Navitoclax and at a lower dose. To evaluate the *in vivo* therapeutic effect of the senotherapeutic formulation, 20 months old Wistar rats were used for the injection of one of the NPs. Preliminary results indicate that the selected hit accumulates in the brain vasculature, reduces the endothelial senescent burden, and improves spatial memory, therefore ameliorating brain function dependent on aging. Overall, we have developed a promising pharmacological strategy to eliminate senescent BBB cells.

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S4.5-O2

Engineering nanoparticles for an efficient delivery of senolytics to the aged liver

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Abstract

Background: Age-related changes in liver function have a significant impact on systemic aging and rise the prospect to have age-related diseases [1]. Recent data showed that aging mice revealed a continuous and noticeable accumulation of liver sinusoid endothelial cells (LSECs) expressing numerous senescence markers [2]. Senolytic compounds with preferential cytotoxic activity for senescent cells, such as Navitoclax, can ameliorate or even revert pathological manifestations of senescence in numerous preclinical mouse disease models [3]. However, translation of senolytic therapies to human disease is hampered by their suboptimal specificity for senescent cells and toxicity.
The goal of the current work is to develop a therapeutic platform for an efficient delivery of senolytics to the liver. We demonstrated the potential of the nanoformulation targeting senescent cells in both *in vitro* and *in vivo* tests.
Methods: A library of polymers was synthesized by the reaction of bisacrylate monomers with different amines. The nanoparticles were formed by nanoprecipitation of the polymers in aqueous solution in the presence of Navitoclax.
Results: Herein, we synthesized a library of 40 nanoparticles in which the nanoparticles were designed to be disassembled preferentially by senescent cells (Figure 1). The bioactivity of the nanoformulations were evaluated by high-throughput screening in endothelial cells to identified the best hits that deliver efficiently the senolytic drug in senescent cells, while preserving viability of nonsenescent cells. For the best two formulations, we determined the IC50 to access the senolytic index. The results show that navitoclax encapsulated nanoparticles have an improved senolytic index (~490X) over soluble navitoclax (~11X) and indicated that this effect is mainly mediated by an efficient delivery of navitoclax to senescent cells and higher degree of protection of nonsenescent cells from cytotoxic activity as well. Next, we have confirmed the advantages of one of the candidate formulations in a naturally-aged rat animal model. The results indicate that the selected hit accumulates preferentially in the liver (~65%). Efficacy of the therapy was evaluated by animal frailty and liver function analyses.
Conclusions: Taken together, we provided a potentially versatile nanoparticle strategy to efficiently deliver senolytic drugs to the aged liver with reduced toxicities.

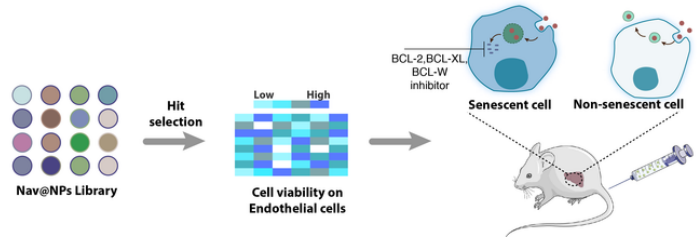

Figure 1. Scheme illustrating the design of the study.

Funding: This work was supported by RESETageing H2020-Twinn-2019 (ref:952266), "Agendas/Alianças mobilizadoras para a reindustrialização" (ref:C630926586-00465198) and through FCT projects: SenoLive – (2022.02783.PTDC) and PTDC/BTM-SAL/5174/2020. References: [1] Aging Cell 2018, 17, e12829; [2] Hepatology 2023, DOI: 10.1097/HEP.0000000000000207; [3] Nat Med 2016, 22, 78.

S4.5-O3

Injectable hydrogels loaded with hybrid nanoparticles for cell/tissue specific microRNA delivery: towards novel advanced therapies for cardiac regeneration

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Abstract

Cardiovascular diseases are the leading cause of death worldwide. Myocardial infarction (MI) causes the irreversible loss of cardiomyocytes and the formation of a scar tissue, leading to heart failure[1]. Recently, the delivery of microRNAs (miRNAs) able to induce direct cell reprogramming (DR) has emerged as a new possibility for *in situ* myocardial regeneration. In this context, we demonstrated that transient transfection of human adult cardiac fibroblasts (AHCFs) with four microRNA mimics ("miRcombo") using a commercial transfection agent triggers DR of AHCFs into induced cardiomyocytes (iCMs) *in vitro*[1,2]. In this work, we designed safe and efficient hybrid nanocarriers for *in situ* DR of AHCFs into iCMs by intramyocardial injection and injectable self-healing hydrogels for their local administration.

Hybrid nanoparticles loaded with miRNAs (H-NPs/miRNAs) were prepared and surface functionalized with a cell-specific ligand following a patent pending method. H-NPs/miRNAs were characterized physico-chemically. AHCFs were transiently transfected with H-NPs/miRNAs and their viability, miRNA uptake and transfection efficiency were assessed. Hydrogels (HYDs) based on alginate (Alg), alginate dialdehyde (ADA) and gelatin (GEL) were prepared having a double crosslinked network, and thoroughly characterized. *In vitro* release of H-NPs, loaded with model siRNA-Cy5 and embedded into HYD was analysed. The ability of free and released nanoparticles to induce miRNA-mediated DR of AHCFs into iCMs *in vitro* was analysed. Biodistribution was assessed by trials in infarcted mouse model.

H-NPs/miRNA showed higher miRNA encapsulation efficiency (99% vs 65%) and biocompatibility (80-100% vs. 50-60%), similar cell transfection efficiency and superior *in vitro* DR ability than a control commercial transfection agent. Optimized HYD showed injectability, self-healing behaviour, biomimetic stiffness and supported AHCF adhesion. H-NPs/miRNA could be delivered through the injectable HYD and interestingly the system was also printable. Mouse trials showed higher biodistribution in the cardiac tissue and reduced accumulation into the liver and the kidneys for ligand-functionalized *vs*. unfunctionalized H-NPs/miRNA.

In conclusion, ligand-functionalized H-NPs/miRNA were efficient, safe and stable and ensured cell-targeted delivery. By changing miRNA cargo and H-NP surface functionalization, other applications could be explored in the future. Enhanced local retention by nanoparticle delivery through the self-healing HYD is under evaluation.

Acknowledgement: This 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 (BIORECAR project) and from Fondazione Compagnia di San Paolo.

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PoA.7.11

Stimuli-responsive 3D printed hydrogel composite with drug-releasing short-filaments for infected wound healing

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Abstract

Developing an efficient wound dressing has gained significant attention in the biomedical field, as infected wounds can cause severe complications that negatively impact human health. Creating an optimal environment for wound healing and tissue remodeling is crucial. Hydrogel dressings have become increasingly popular for skin repair due to their oxygen permeability, ability to absorb wound exudate, and moisture retention properties[1]. Additionally, electrospun materials offer unique properties such as biodegradability and the ability to control drug release, which makes them potential candidates for treating infected wounds[2]. Electrospinning is a simple method for producing ultrafine fibers that range from nano- to micrometers in diameter. Fibers can be used as drug delivery systems, allowing for controlled and on-demand drug release with the addition of stimuli-responsive particles. The main aim of this study was to develop a multi-functional 3D-printed hydrogel composite for infected wound healing. Ketoprofen-loaded poly(lactic-co-glycolic acid) (PLGA) mat incorporated with gold nanorods (AuNRs) was structured to the short filaments (SFs) using the aminolysis method (Fig. 1A). SFs were loaded into 3d printing ink composed of gelatine-methacrylate (GelMA) and alginate sodium (AS) (Fig. 1B). Introducing photo-responsive AuNRs in SFs significantly accelerated the ketoprofen release under near-infrared (NIR) light exposure. The ketoprofen release of the activated platform by NIR light, compared to the non-irradiated system, exhibited a significant elevation of the drug release resulting from the response to the stimuli (Fig. 1C). The composite dressing also showed excellent photo-thermal performance and good mechanical properties. The stability of the print before and after NIR irradiation was also investigated. Moreover, 3D-printed hydrogel demonstrated antibacterial activity under the NIR laser due to the photo-thermal activity, leading to E. coli eradication after multiple times of exposure. Evaluated tests and achieved results paved the way toward further composite's ex vivo and *in vivo* application in the field of infected wounds.

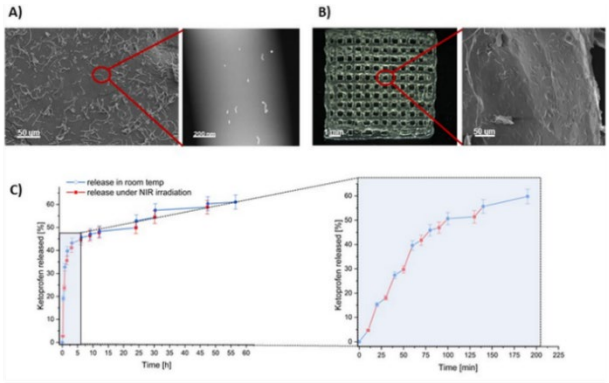

Fig. 1. Micrographs of A) PLGA SFs after aminolysis containing AuNRs and B) 3D printed composite with SFs, C) Ketoprofen release curves.

Acknowledgments: This research is supported by the National Center for Research and Development (WPC2/NanoHealer/2021)

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S4.6-K1

Multi-scale evaluation of bone adaptation around magnesium-based implants

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Abstract

Biodegradable magnesium (Mg) implants have found their way into the clinics but are still not widely used. Many open questions with respect to biological interaction remain to be answered. An important approach is to understand the impact of the degradation of Mg-based implants on the different hierarchical levels of bone. We have performed a scale bridging analysis on how degrading Mg-based materials influence the bone structure from the tissue level across the lacuna-canaliculi network (LCN) down to the ultrastructural level.

To obtain tissue level information, different biodegradable Mg-based screws (e.g. Mg-10Gd, Mg-4Y-3RE and Mg-2Ag), as well as titanium (Ti) screws were implanted into rat tibia or rabbit femur for healing times between 1 and 9 months. The bone-to-implant contact, bone volume fraction and implant degradation rate were studied using high-resolution synchrotron radiation (SR) micro computed tomography and histology. In addition, the elemental traces were characterized using micro X-ray fluorescence and mass spectroscopy. The LCN architecture was studied in detail within the interfacial bone of Mg-10Gd implanted in rat tibia in comparison to Ti using SR based transmission X-ray microscopy in conjunction with image-based finite element modelling. Finally, thin sections where cut of the Mg-10Gd and Ti samples and X-ray diffraction and small angle X-ray scattering was performed to assess bone crystal lattice and collagen orientation.

Our analysis revealed that bone surrounding Ti implants displays a higher degree of remodelling at early time points (< 10 weeks) than Mg-10Gd. This was apparent through tissue level parameters such as bone volume fraction, lacunar density, and ultrastructural information such as the crystal lattice spacing, as well as the histological assessment. The LCN analysis showed no differences between Mg-10Gd and Ti in terms of morphology and fluid flow, which may be due to the already achieved similarity in remodelling. At longer healing times, matured bone had formed around all implant types and the degradation layer, which consisted in particular of Ca and P, as well as alloying elements, was in intimate contact with the bone. The rare-earth containing implants showed lower degradation rates and a higher degree of structural integrity in comparison to Ag-containing implants. As the bone formation was not disturbed by the prolonged presence Gd and Y elements and the analysis of organs showed no signifcant increase of Gd levels throughout healing over 8 months it may be concluded that these elements are not detrimental for bone healing or impact animal health overall.

S4.6-O1

Polymer-augmented screws in different bone qualities

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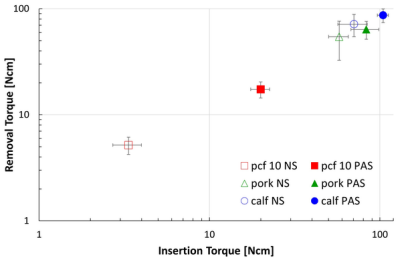
Abstract

Anchoring posterior stabilization systems in vertebrae with reduced bone quality is often a challenging task for the surgeon. One possible solution is the immediate stabilization system (ISS). Its general concept is based on the insertion of a 12mm long polymer-tube into a preprocessed drill hole, to melt the polymer by using an ultrasonic device (Sonotrode®) and to insert the standard implant afterwards into the created denser bony environment leading to enhanced immediate stability [1]. It is a platform technology that could be applied in combination with standard dental, trauma, spinal or orthopaedic implants. This product platform could significantly improve the treatment of osteoporotic bone fractures. In this study biomechanical tests have been performed for investigating the stabilization effect in different synthetic and ex-vivo bone qualities.

Synthetic bone (Sawbone polyurethane foam) and ex-vivo cadaver bones (calf femoral head, porcine tibia) were used to compare different bone qualities. The natural bones were obtained freshly from the local slaughterhouse and cut into 30 mm slices. Polymer sleeves were inserted in predrilled holes and a Sonotrode® was placed and activated to melt the polymer into the porous cancellous bone. A pedicle screw was then driven into the prepared implant bed for the polymer-augmented screw (PAS). For the native screws (NS) control group, the screw was placed without a polymeric treatment.

The biomechanical characterization is performed by removal torque (RT) testing with a defined rotation speed (0.1°/sec). Initial fixation of the implant to the torque meter and subsequent lowering of the bone into a liquid low-melting alloy (T_m=47°C) ensures that the axis of symmetry of the pedicle screw and the drill hole is absolutely collinear with the sensor axis, thus preventing the occurrence of undesirable radial forces. Tab. 1 and Fig. 1 show the measured insertion and removal torques.

sample	Experimental Groups	n	Insertion Torque *IT* [Ncm]	*sign*	Removal Torque *RT* [Ncm]	*sign*
Pork ex-vivo	NS	4	57.7 ± 7.6	0.025	54.5 ± 21.8	0.490
	PAS	4	83.2 ± 15.5		63.7 ± 12.2	
Calf ex-vivo	NS	5	70.5 ± 13.8	0.001	71.5 ± 16.9	0.143
	PAS	5	104.5 ± 7.8		86.8 ± 12.4	
pcf 10	NS	4	3.35±0.64	0.001	5.18±0.96	0.001
	PAS	4	19.98±2.52		17.38±3.00	



Tab. 1: Biomechanical results for IT & RT experiments. Comparison (paired t-test, sign: p-value for significance) within each environment between NS and PAS. Significant differences are highlighted red (p<0.05). Fig. 1: IT plotted against RT.

Proof of the general concept of the ISS method could be provided for pedicle screw-based indications in all bone qualities used. The resulting biomechanical data sets clearly show the increase in primary stability. This project was financially supported by the Swiss Commission for Technology and Innovation under the project number 14929.1 PFLS-LS.

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S4.6-O2

Strong photo-curable thiol-ene based composites for use in the fixation of bone fractures

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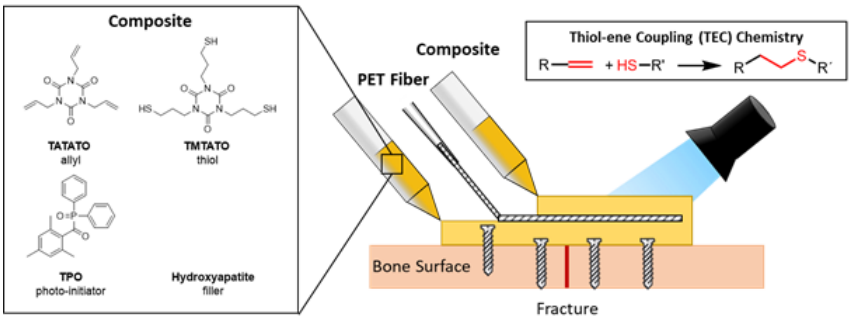
Abstract

Unstable bone fractures typically require surgery and fixation with open reduction internal fixation (ORIF) metal plates and screws. While these plates provide exceptional support to the healing bone they often result in debilitating soft-tissue adhesions and their rigid shape cannot be easily customized. Inspired by the technology behind dental composites, we have designed composites which can be shaped into bone fixation patches directly on the fracture site and then cured in seconds through high-energy-visible-light induced thiol-ene coupling chemistry.[1]

These composites are based on triazine-trione allyl and thiol monomers and contains a large concentration of hydroxyapatite. After curing they display high rigidity and strength; which could be further improved by decorating the hydroxyapatite with alkene functional groups. Water absorption at 37°C was minimal and only resulted in a minor decrease in flexural modulus and strength; important features for a composite intended for use within the human body. *In vivo* testing in rodents[1] and rabbits[2] has shown that our composite does not cause soft-tissue adhesions or adverse effects on surrounding tissues.

The shapeability and biocompatibility of the composite was combined with the anchoring strength and reliability of metal screws to create a new surgically feasible ORIF method, called AdhFix, in which osteosynthesis was achieved by inserting screws into the bone fragments and then constructing a fixation patch with layers of the composite and PET fiber mesh between the screws.[1] Biomechanical testing on fractures in porcine metacarpal bones showed that AdhFix could match the stability and rigidity of ORIF metal plates under low-loading situations. The fabrication of objects using the triazine-trione thiol-ene composites has also been explored with 3D SLA printing.[3]



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S4.6-O3

New metal-based coatings for vertebral prosthesis and customized tolls for the assessment of their antimicrobial and anti-tumor activity

Gabriela Graziani[1,2], Daniele Ghezzi[3], Maria Sartori[1], Raffale Vecchione[4], Martina Cappelletti[3], Brunella Corrado[4], Paolo A. Netti[4,5], Maria Veronica Lipreri[3], Matteo Montesissa[3], Enrico Sassoni[3], Francesca Perut[1], Milena Fini[1], Francesco Valle[6], Donato Monopoli[7], Nicola Baldini[3,1]

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Abstract

Infection and bone metastases are important unmet challenges in spine surgery and they are correlated. To address them simultaneously, we propose the use of silver- and zinc- based coatings. Coatings are obtained by Ionized Jet Deposition (IJD) and are nanostructured, which permits to tune Ag/Zn release, and avoid delamination.

To screen antibacterial and antitumor ability, and select among coatings combinations in a fast and reliable way, we also propose new validation methods. For antibacterial and antibiofilm efficacy, we modified the Calgary Biofilm Device (CBD), to simultaneously screen different coatings and bacteria. For antitumor activity, we developed a gradient-generator microfluidic chip, to optimize the amount of metal to be eluted. The device also permits injection of cells in a gel, for 3D cultures.

Coatings are obtained by Ag, Zn and Ag/Zn-bone apatite composites. Composition, morphology (FEG-SEM/EDS, FT-IR, ICP), biocompatibility (L929, MSCs) are tested. Antibacterial efficacy is shown *in vitro* by standard test, by the CBD assays (E. coli, P. aeruginosa, S. aureus, E. faecalis, reduction of bacterial viability and biofilm formation) and finally *in vivo* (rabbit models, multidrug resistant S. aureus USA 300). Efficacy against bone metastases (MDA-MB-231 cells) is shown for the first time, and is validated *in vitro* in 2D (live/dead assay, FEG-SEM) and using the gradient generator (live/dead assay, MDA and MCSs). Statistical analysis is performed.

All films have the same composition of the target. No cytotoxicity is evidenced for all Ag-based films and for Zn-bone apatite composites.

Remarkable antibacterial and antibiofilm efficacy is found *in vitro* for Zn and Ag films against all gram-positive and gram-negative bacteria. The CBD results shows different efficacy for the two metals against different strains, and among planktonic growth and biofilm formation, and permit to select the optimal coatings. Optimized, silver films show high efficacy *in vivo*, also against MRSA (-99.58% and -86.20% for Ag and Ag-bone).

Ag and Zn coatings show selective efficacy of against MDA-MB-231 cells, with zinc being more effective (100% reduction of MDA viability at 72h). The gradient generator permits to select the optimal zinc concentration and to better mimic the conditions *in vivo*. Indeed, it shows that in the presence of a flux and/or a gel, MDA and, mostly, MSC cells, are less affected by metals, compared to 2D cultures.

The new metal-based films and the *in vitro* systems for their validation show high efficacy and potential application in several fields.

S4.6-O4

Smart pH-responsive coating for orthopedic implants to control bacterial colonization and biofilm growth using antimicrobial peptides immobilization

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Abstract

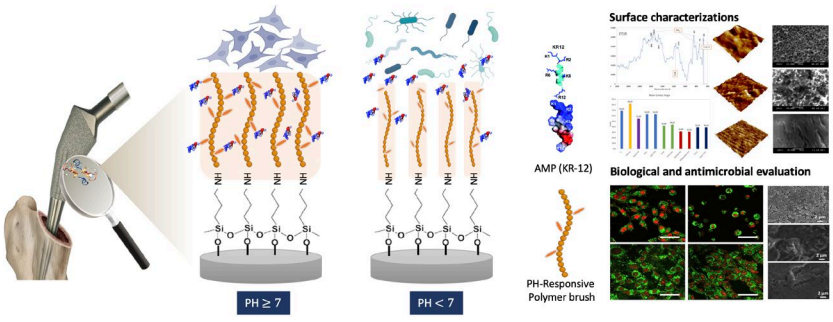
Coating strategies based on covalent immobilization of antimicrobial peptides (AMPs) can substantially tackle infectious problems for orthopedic implants but still developing a technique to selectively eliminate bacterial attachment without affecting the osteoblast cell adhesion and proliferation remains a research precedence.

This study presents a technique to develop a smart antimicrobial coating for titanium surfaces, which later can be applied to a variety of materials for orthopedic applications. Smart coatings with switchable functions to kill attached bacteria can be developed based on pH-responsive polymer brushes. Under normal physiological conditions, the pH-responsive polymer is swelled. The decrease in pH arising from bacterial adhesion leads to the collapse of the pH-responsive polymer layer which results in the exposure of AMPs on the surface to kill bacteria. These polymer brushes directly affect the AMPs' mobility and their antimicrobial activity; also help the AMPs act specifically on bacteria but not osteoblast cells. They also efficiently increase the loading percentage and stability of AMPs.

This approach involves three main steps:

1. Surface functionalization of titanium implants with primary amine groups (–NH2) as anchor points
2. Synthesis of pH-responsive brushes directly on the surface using Dimethyl-aminoethyl-methacrylate.
3. Attachment of AMPs (KR-12) on brushes using EDC/NHS reaction.

XPS, FT-IR, SEM, AFM, and Confocal Raman Spectroscopy (CRS) were used to confirm the successful synthesis of polymer brushes and covalently attachment of AMPs. The XPS and FTIR results revealed that the optimum percentage of the primary amine groups on the surface was 22 % where AMPs can be attached with the highest efficiency without showing any toxicity. Moreover, the CRS and AFM results proved the uniformity of AMPs attachment. The antibacterial tests were performed to evaluate bacterial adhesion and biofilm formation. In contrast with the titanium surface, the samples with polymer-brush-grafted KR-12 killed 98.5% of S. aureus and 99.5% of E. coli. This outstanding antibacterial activity was attributed to the increase in AMP activities after the use of pH-responsive polymers. KR-12 effects on human osteogenic differentiation and proliferation were investigated using CCK-8 assay, flow-cytometry, and Alizarin-red-staining.



The results of CLS microscopy, and SEM proved that KR-12 covalently immobilized on titanium using pH-responsive polymers significantly improved the adhesion and proliferation of osteoblast cells.

In conclusion, the peptide-immobilized based coating, involving pH-responsive polymers, can be fabricated easily and cost-effectively, which provides an opportunity for infection prevention for a variety of orthopedic implants with the improvement of cell adhesion and growth.

S4.7-K1

Photoresponsive hydrogels for multiphoton biofabrication

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Abstract

The development of multiphoton lithography (MPL) as a technique for creating structures of sub-micrometer resolution has opened a new chapter in bioprinting. However, printing tissue-resembling structures in biocompatible hydrogels using MPL has been a challenging journey. At the early stages the biggest efforts were put into developing water-soluble and cytocompatible photoinitiators (PIs) which efficiently start the polymer crosslinking reaction. One of the first PI examples, P2CK, showed no cytotoxicity on its own, but presumably produced cytotoxic reactive species during printing. The issue was partially circumvented by binding the PI to a large inert polymer backbone, which prevented cell entry. Cell viability was drastically improved with the design of diazosulfonate-based PI (DAS), whereby printed gels supported proliferation of embedded cells over a week. Combining optimized PIs with thiol-ene hydrogels further allowed to assess the relevance of different printing parameters for cell survival. The high resolution of the optimized MPL process was then used to introduce microvascular networks into microfluidic chips, offering hope against the long-standing issue of necrotic cores of fabricated tissues. Finally, several strategies were developed to manipulate cell behavior by changing the structural integrity of the hydrogel. o-nitrobenzyl-containing polyethelene glycol-based linkers were used to locally degrade the hydrogel with high precision, enabling cells to fill the created voids, while photografting proved efficient in guiding cell alignment and migration. With the various culturing strategies, material properties and printing parameters described, we finally have the precision necessary to dissect molecular processes in a cell within a 3D environment. Since cell proliferation is an essential process in tissue growth, understanding how the environment affects cell's decision to divide is of highest importance. By following the expression of a classic proliferation marker, Ki-67, we found that cell's position within the hydrogel, as well as the hydrogel stiffness, might play a role in the decision to enter mitosis. Cells in middle and deep hydrogel layers stopped producing Ki-67 five days after encapsulation, while cells in top layers continued. In addition, softer environments appear more permissive for Ki-67 expression, since the expressing zone spreads deeper in soft hydrogels. The observed lack of signal is not attributable to cell death, as all gel layers show high cell viability. Taken together, the innovative MPL approach not only opened up numerous possibilities in the bioprinting field, but it also allows questioning basic molecular mechanisms previously studied in 2D cultures, by bringing them into a 3D context.

S4.7-O1

Towards bedside manufacturing of biodegradable implants: volumetric 3D-printing of thiol-ene crosslinkable poly(ε-caprolactone)

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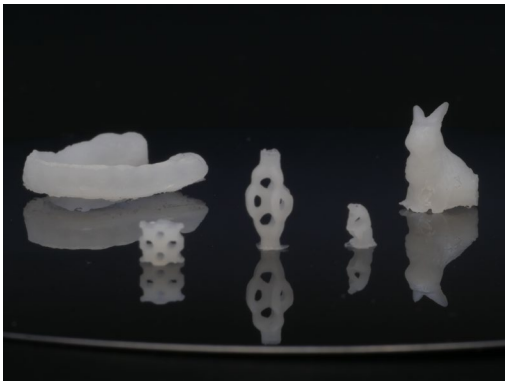
Abstract

Current thoroughly described biodegradable and cross-linkable polymers mainly rely on acrylate cross-linking. However, despite the swift cross-linking kinetics of acrylates, the concomitant brittleness of the resulting materials limits their applicability. Here, we introduce photo-cross-linkable PCL networks through orthogonal thiol-ene chemistry.(1) The step-growth polymerized networks are tunable, predictable by means of the rubber elasticity theory and it is shown that their mechanical properties are significantly improved over their acrylate cross-linked counterparts. Tunability is introduced to the materials, by altering Mc (or the molar mass between cross-links), and its effect on the thermal properties, mechanical strength and degradability of the materials is evaluated. Moreover, excellent volumetric printability is illustrated and we report the smallest features obtained via volumetric 3Dprinting to date, for thiol-ene systems. Finally, by means of in-vitro and in-vivo characterization of 3D-printed constructs, we illustrate that the volumetrically 3D-printed materials are biocompatible. This combination of mechanical stability, tunability, biocompatibility and rapid fabrication by volumetric 3D-printing charts a new path towards bedside manufacturing of biodegradable patient-specific implants.



References: (1) Thijssen, Q.; Quaak, A.; Toombs, J.; Vlieghere, E. De; Parmentier, L.; Taylor, H.; Vlierberghe, S. Van. Volumetric Printing of Thiol-Ene Photo-Cross-Linkable Poly(ε-Caprolactone): A Tunable Material Platform Serving Biomedical Applications. Adv. Mater. 2023, 2210136. https://doi.org/10.1002/ADMA.202210136.

S4.7-O2

Laser-guided bone cell network formation *in vitro* via two-photon microchannel ablation

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Abstract

Bone's structural integrity and metabolic functions depend on a cellular network comprised of osteocytes. By traversing the bone matrix with a multitude of dendrites, the cells form an intricate system of cavities and microchannels to regulate bone formation. However, investigating this naturally inaccessible network poses a major challenge to current bone research. While common hydrogel-based *in vitro* models provide the means to study cell behavior in 3D matrices, biofabrication techniques to manipulate the pericellular environment are lacking. Here, we employ two-photon ablation to erode microchannels in photosensitive hydrogels, guiding 3D outgrowth of human mesenchymal stem cells (hMSC) towards the formation of an *in vitro* bone cell network (Fig. 1a).

To verify the suitability of two-photon ablation for 3D cell cultures, we investigated the ablation efficiency to pattern microchannels in soft cell-laden GelMA hydrogels (G' = 350.9 ± 93.3 Pa). Compared to untreated controls, adding the photosensitizer P2CK[1] resulted in a five-fold decreased energy dosage required for ablation, as demonstrated by a fluorescent tracer assay (Fig. 1b). This reduction in laser dosage enabled cell-compatible patterning of a microchannel grid in the presence of embedded cells. Low-dosage patterning with 100 J/cm^2 was sufficient to ablate 1 µm wide and 30 µm deep microchannels to induce hMSC outgrowth. After 7 days, the cells utilize the microchannels to form a 3D network, aligning with the grid (Fig. 1c). Cell viability shows cellular survival after ablation to be comparable to un-ablated controls in low energy dosages (100 J/cm^2), whereas ablation at high dosages (500 J/cm^2) resulted in 42% cell death (Fig. 1d). Tracing cellular outgrowth in ablated samples demonstrates that low-energy grid patterning significantly increases network formation compared to untreated controls (Fig. 1e). In conclusion, guiding the formation of *in vitro* 3D bone cell networks was achieved by sensitized two-photon ablation, highlighting the potential of laser-assisted biofabrication.

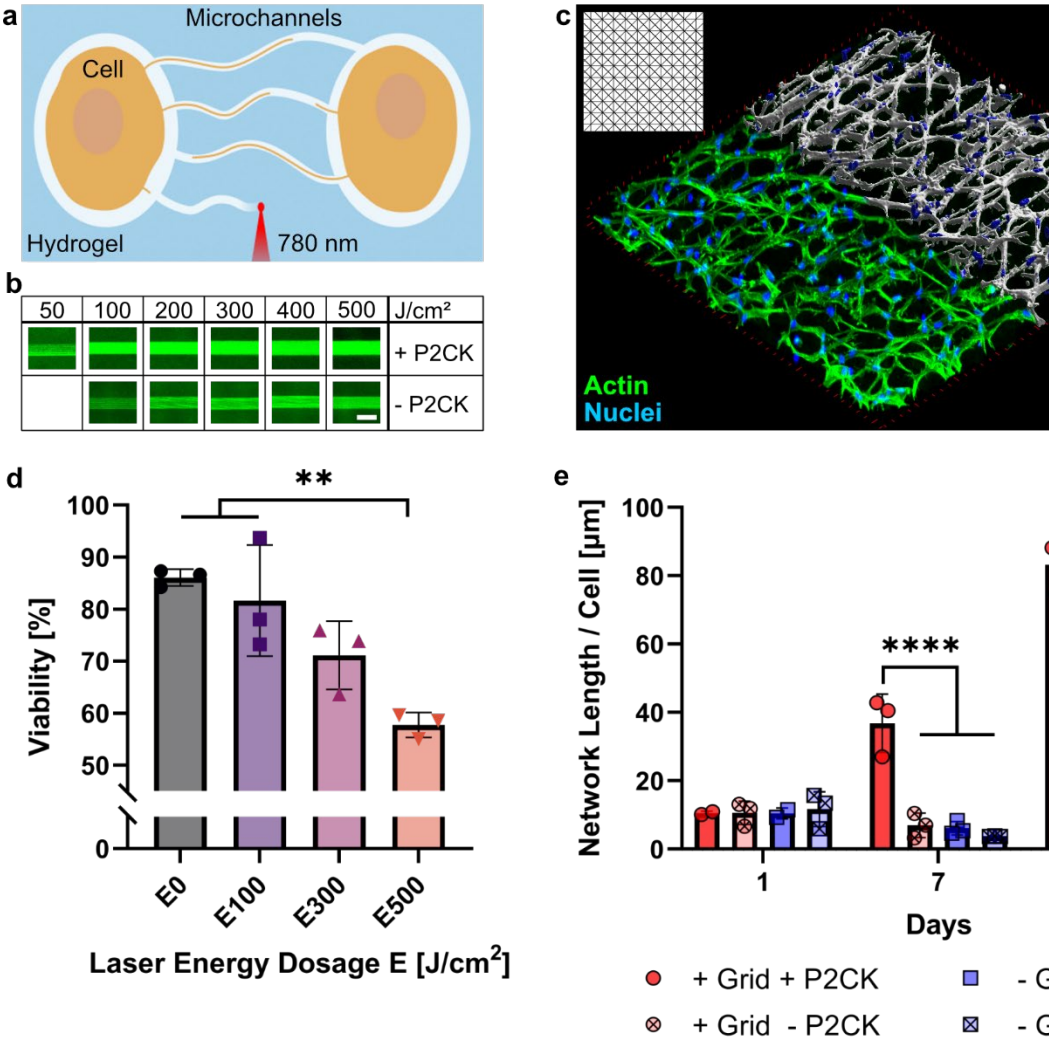

Figure 1: Sensitized two-photon ablation within cell-laden hydrogels. ***a****) Illustrating laser-guided cellular outgrowth into ablated microchannels. b) 5 µm-wide microchannels ablated with varying laser energy dosages (with or without the photosensitizer P2CK) and perfusion with FITC-Dextran. Scale bar, 10 µm. c) Actin and nuclei stained hMSC-derived bone cell networks, following 14 days after sensitized ablation (100 J/cm^2). d) Cell viability at 24h after sensitized ablation with varying dosages (n=3). **p<0.01. e) Effect of sensitized ablation on network formation over time. Network length normalized to the number of cells (n=2-3). ****p<0.001.*

Reference: 1. Li et al. RSC Adv. (2013).

S4.7-O3

Shape memory elastomers for biomedical applications

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Abstract

Among the additive manufacturing techniques, light-based fabrication like Digital Light Processing (DLP) results in high-resolution and fast printing times. These techniques have been recently explored in regenerative medicine, yet the development of customizable biodegradable and biocompatible resins remains a bottle-neck1, 2. Furthermore, the printing of structures with controllable shape, properties or functionality as a function of time has emerged as 4D printing4.We synthesized mechanically customizable resins based on poly(ester-carbonates) (PCT) with tunable mechanical properties and cell adhesion. Depending on the polymer structure, these resins also exhibit thermal properties that are amenable to programmable shape memory. Here, we report the shape memory recovery of printed structures and their interactions with the most abundant serum proteinsOpen box structures were printed and the shape memory properties were explored by a cold-warm cycle. An external force was applied to get the desired configuration (closed box) and it was fixed by cooling. The shape recovery in a water bath near body temperature was achieved in just 5 seconds. So far, the ability of the quick shape recovery, even more than one cycle, and the protein interactions of these scaffolds are promising for tissue engineering applications.

References: 1 C. Mota et al. Chemical Reviews 2020 120 (19), 10547-10607; 2 N. A. Chartrain et al., Acta Biomateralia, 2018, 74, 90–111

S4.7-O4

Spatial-selective volumetric 4D printing and single-photon grafting of biomolecules within centimeter-scale hydrogels via tomographic manufacturing

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Abstract

Bioprinting technologies are extremely powerful tools for biomedical research, given their ability to shape cell-laden biomaterials into highly defined structures. Conventional additive manufacturing and biofabrication techniques are unable to edit the chemico-physical properties of the printed object post-printing. In this study, we are showing a new approach, leveraging light-based volumetric bioprinting as a tool to spatially pattern any biomolecule of interest in custom-designed geometries, even across large, centimeter-scale hydrogels, using single-photon reactions. As a biomaterial platform, a gelatin norbornene (gelNOR) resin is synthesized with tunable mechanical properties, achieved by modifying the length and concentration of the thiolated crosslinker (DTT=~5.4Å or ethyleneglycol dithiol=~11.3Å), resulting in a wide range of stiffnesses (~1kPa-20kPa) and tunable crosslinking kinetics. The bioresin can be volumetrically printed within tens of seconds at high resolution (23.68 µm ± 10.75) using lithium phenyl (2,4,6-trimethylbenzoyl)-phosphinate (LAP) as a photoinitiator. The thiol-ene click chemistry of the gelNOR allows on-demand photografting of thiolated compounds post-printing, from small to large (bio)molecules (e.g., fluorescent dyes or growth factors), in a variety of complex shapes (Figure 1). In this study, PEG_{5000}-Cy3 and PEG_{5000}-Cy5 were used as fluorescent dyes. These molecules are covalently attached into printed structures using volumetric light projections, forming 3D geometries with high spatiotemporal control and ~50 µm resolution. As a proof-of-concept, vascular endothelial growth factor (VEGF) is locally photografted into a bioprinted construct and demonstrated region-dependent enhanced adhesion and network formation of endothelial cells. The endothelial cells showed an angiogenic effect in the structures with grafted VEGF even when there was no VEGF present in the cell medium. This technology paves the way towards the precise spatiotemporal biofunctionalization and modification of the chemical composition of (bio)printed constructs to better guide cell behavior and build bioactive cues gradients. Moreover, it opens future possibilities for 4D printing and for mimicking the dynamic changes in morphogens presentation natively experienced in biological tissues.

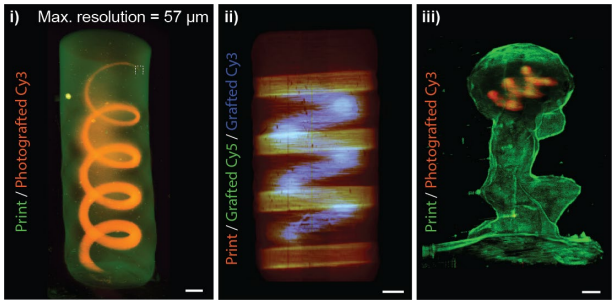

Figure 1. Lightsheet imaging of hydrogels after local editing with customized patterns of fluorescently-tagged molecules. i) resolution measurement, maximum resolution 57 µm. ii) double photografting of two fluorescent dyes (PEG_{5000}-Cy3 and PEG_{5000}-Cy5). iii) Atlas statue with photografted spiral. Scalebars = 1mm.

Acknowledgments. This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No. 949806, VOLUME-BIO).

References. 1. Bernal, P. N. et al. Volumetric Bioprinting of Complex Living-Tissue Constructs within Seconds. Adv. Mater. 31, 1904209 (2019).

S5.2-K1

Biomaterials for Tissue Engineering Meats – challenges and opportunities

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Abstract

The need for future foods for the ever-growing population requires consideration of alternative approaches toward food sustainability, nutrition and security. To address this need, we pursue a cell-based, tissue engineering approach. Our central hypothesis is that sustainable, cost-effective, and scalable cultivated-meat and alternative proteins will provide new food availability options and healthy food alternatives, while decreasing environmental impact. Towards this goal, biomaterials provide key elements to help, from cell carriers to scaffolds, to support muscle and fat cells and tissue formation, offering texture, bulk and stability. However, many factors must be considered for the successful integration of biomaterials into cellular agriculture processes to match cell and tissue biomanufacturing needs. These challenges and opportunities will impact food quality, consumer preferences and economics of scale, all of which will be discussed in the context of this emerging food frontier.

S5.2-O1

Development of bioinks for cultured meat on the basis of polysaccharide hydrogels and plant proteins

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Abstract

Global meat production is on the rise to satisfy the dramatically increased meat consumption of a growing world population. Cultured meat or *in vitro* meat creates the opportunity to produce meat by tissue engineering without the need for raising the whole animal. The needed cells can be grown *in vitro* using stem cells, isolated from a small biopsy of an animal. To create a product that is as close as possible to a conventional whole meat cut, not only cells will be needed but a supporting matrix. These biomaterials should be edible, sustainable, widely available, animal-free, non-toxic, cheap and processable.

Three polysaccharides and two protein isolates were used for preparation of hydrogels. Agarose, gellan and xanthan-locust bean (XLB) gum blend were mixed with pea or soy protein isolates. For all rheological measurements, a rotary oscillating rheometer was used. Next the swelling characteristics were analyzed by the weights of the hydrogels over 7 days. For cell toxicity tests the murine myoblast C2C12 cell line was used. In a next step, the biomaterials were adapted for the use as bioinks in an extrusion-based production process using C2C12 myoblasts and primary bovine adipose derived stem cells (ASCs). Viability and differentiation of the cells were analyzed by different stainings up to 14 days after the printing process.

We successfully built stable hydrogels containing up to 1% pea or soy protein. The gelation temperature range for agarose and gellan blends is between 23–30 °C, but for XLB blends it is above 55 °C. A change in viscosity and a decrease in the swelling behavior was observed in the polysaccharide-protein gels compared to the pure polysaccharide gels. None of the leachates of the investigated materials had cytotoxic effects on the myoblast cell line C2C12. Cell-laden gels were successfully set up for agarose and gellan hydrogels with only a homogenous cell distribution for gellan. For XLB the gelation was not successful at cell-friendly temperatures. This is why we focused on gellan blends (containg up to 4% protein) to create cell-containing bioinks encapsulating either C2C12 or primary bovine ASCs. For both cell types suitable bioink formulations were identified. After the printing process, cells were viable and differentiated in the printed structures for 14 days of culture.

All polysaccharide-protein blends evaluated turned out as potential candidates for cultured meat, with gellan as the most promising for the application as bioink containing muscle and fat progenitor cells.

S5.2-O2

Filamented Light (FLight) biofabrication of centimeter-scale muscle tissue constructs using Pax7-nGFP primary myoblasts

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Abstract

Introduction. Pax7-positive satellite cells are promising for muscle tissue engineering due to their self-renewal and differentiation abilities, which form functional muscle fibers [1]. However, current biofabrication, such as electrospinning and extrusion-base bioprinting, face challenges in production rate and biocompatibility, and often fail to provide three-dimensional (3D) cell guidance cues. Here, we present the next generation of Filamented Light (FLight) biofabrication technology [2], which can create centimeter-scale muscle tissue constructs (lengths up to 3cm) using photocrosslinkable biomaterials. The microstructures within the FLight matrix support cell proliferation, differentiation, and formation of contractile muscle fibers.

Materials and methods. Gelatin-norbornene (Gel-NB) and Thiol functionalized gelatin-thiol (Gel-SH) were mixed with photoinitiators and Pax7-nGFP primary myoblasts (3 x 10^6 cells/mL), and cell-laden constructs were biofabricated using a FLight printer (FLight3D^{TM} Ver. 1.0). The printer uses optical modulation instability to produce microfilaments within the constructs (Figure 1a). The cross-section of the constructs was tailored via a digital micromirror device, while the length of construct was controlled by an auto-feeding system. The constructs were cultured into DMEM medium + 2% horse serum for differentiation before immunofluorescent imaging and electrical muscle stimulation.

RESULTS. The Pax7-nGFP primary myoblasts demonstrated high viability (> 96%). After 1 day of culture, the cells migrated into the microchannels and proliferated. The primary myoblasts become highly aligned and differentiated after 3 days of culture. The immunofluorescent images showed the maturation of aligned multi-nuclear myotubes and the presence of Pax7-positive cells (Figure 1b, c) after 2 weeks of differentiation. The synchronized contraction of myotubes with different frequencies in FLight constructs under electrical stimulation (0.5, 1.0, and 5 Hz frequency, 3V) was confirmed.

Discussion and conclusions. We demonstrated a strategy to biofabricate physiological-scale muscle tissue constructs. The cues in the FLight matrix showed efficient cell guidance properties, enabling the formation of aligned contractile myotubes. The maintenance of Pax7-positive cells after maturation highlighted its possibility for long-term regeneration and biofabrication of muscle tissues, which is promising for the clinical treatment of VML.

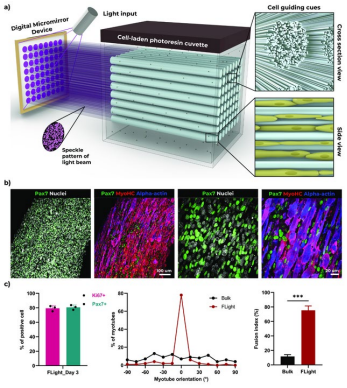

Figure 1. a) Schematic illustration of the FLight biofabrication and microstructures in FLight matrix. b) Immunofluorescent evidence of the formation of aligned multi-nuclear myotubes and maintenance of Pax7-positive cells after 2 weeks of differentiation. c) Quantitative analysis of alignment, maturation of myotubes in bulk and FLight matrix.

References. [1] Christoph *et al., Development* 2011, *138*, 3639; [2] Hao *et al., Adv Mat.* 2022, *34*, 2204301.

Acknowledgements. The authors acknowledge ETH Grant application ETH-38 19-1 for financial support.

S5.2-O3

Injectable and porous hydrogel fate as scaffold in Volumetric Muscle Loss context.

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Abstract

Volumetric muscle loss (VML) lead to fibrosis and loss of muscle function. We hypothesized that an injectable biomaterial that perfectly conforms to the muscle defect shape would enhance muscle repair. Hence, we developed a biocompatible and biodegradable effervescent porous hydrogel from poly-lysine dendrimers and polyethylene glycol, which was previously shown to be suitable for muscle cells *in vitro* and injectable in situ. The porous hydrogel fate and supportive role in muscle regeneration is here assessed by injection in rat VML models of various sizes, through bio-integration, resulting inflammatory response, cell colonization and suitability for muscle cells and regeneration over time.

After creation of 15 and 35 weight% VML in rats tibialis anterior, a porous hydrogel was injected and harvested after 7, 14 and 21 days. Masson's Trichrome allowed to evaluate inflammation, vascularization, hydrogel degradation over time and to quantify cellularization and inflammatory tissue. Emb-MyHC, MyHC and MyoD immunofluorescence staining's were used to characterize muscle cell types and their behavior in the hydrogel.

Hydrogel injection into the VML effectively resulted in porous matrices that perfectly filled the defect without empty spaces. The rats could walk and showed no signs of distress or pain over implantation times.

After 7 days, empty 15%-VML were filled by granular tissue, which was also seen around the injected hydrogels. Important cellularization was observed in the porous structures (Fig. 1F,J) with inflammatory cells and collagen deposition. After 21 days, granular tissue was reduced both in empty defects and around the hydrogel while cellularization was further increased (Fig. 1I). The hydrogel size was reduced (Fig. 1B,D), possibly due to degradation by macrophages (Fig. 1E). Blood vessels were observed within the pores (Fig. 1F) and muscle fibers were in close contact to the hydrogel margins (Fig. 1G), comforting its suitability to chaperone muscle regeneration. Indeed, MyoD+ cells and myotubes (MyHC+) were observed in pores at days 7 (Fig. 2A-B) with also neo-formed fibers (emb-MyHC+) through the hydrogel pores, both at 7 and 21 days.

For 35%-VML, after 14 days we could observe similar cell infiltration in the hydrogel (Fig.1H), although some hydrogels extended outside of the defect, possibly due to swelling.

To conclude, the injectable porous hydrogels can readily be injected into VML of various sizes. Their bio-integration underlines a potential guidance of muscle repair process. On-going characterizations of the various cell types present in the hydrogel over time will further verify this potential.

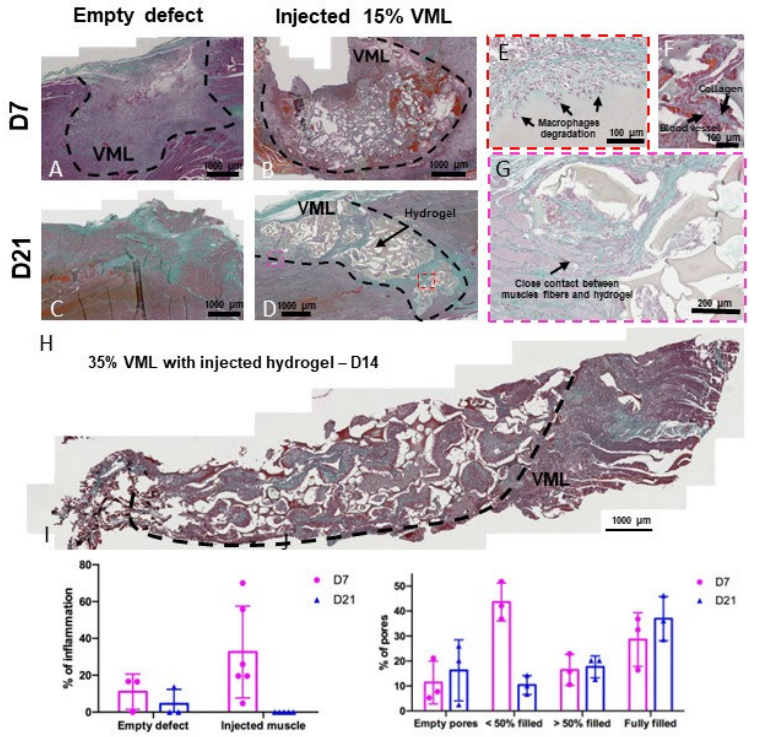

Figure 1 – (A-D) Masson's trichrome of longitudinal section of rat Tibialis Anterior 15% VML, empty or injected with hydrogel, after 7 and 21 days. (E-G) Zoom on hydrogel at D21,(E) observation of macrophages degradation, (F) blood vessel presence and extracellular matrix deposition, (G) proximity of muscle fibers and hydrogel at their interface. (H) Masson's trichrome of 35% VML injected with hydrogel, after 14 days. (I) Quantification of inflammation at defect site after 7 and 21 days (J) Quantification of cell infiltration in hydrogel pores after 7 and 21 days

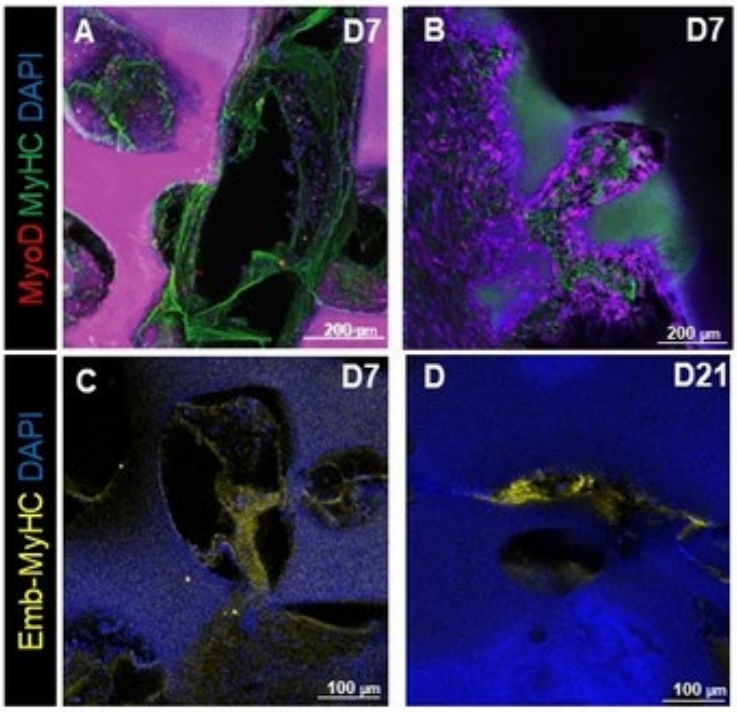

Figure 2 – Immunostaining of muscle section implanted with hydrogel after 7 and 21 days. (A) Observation of MyHC+ cells (myotubes) inside pore. (B) Observation of MyoD+ cells (transcription factor of muscle cell differentiation) at the edge. (C) Observation of emb-MyHC+ cells (*de novo* muscle fibers)

S5.2-O4

Using acoustofluidics for continuous patterning of cells for musculoskeletal tissue engineering

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Abstract

3D encapsulation of cells within hydrogels aims to capture the interaction of cells with the extracellular matrix. However, conventional encapsulation often fails to recreate the cell–cell interactions present in native tissues owing to their structural complexity. To engineer tissues *ex vivo*, it is often critical to mimic these anisotropic cell–cell interactions via spatial organization. For example, myotubes form through muscle progenitor cells (myoblasts) fusion, which requires cell-cell contact. [1]

To address this, we developed an acoustofluidic device to pattern muscle cells within hydrogels. Acoustofluidics is a contactless and label-free technique wherein acoustic waves manipulate small particles, including cells. We engineered a Teflon-in-glass capillary device with a piezoelectric transducer (PT) glued onto a square glass capillary (Figure 1a). [2] The glass capillary combined with PT enabled acoustic patterning, while the Teflon tube ensured smooth and continuous extrusion of patterned cells within the hydrogel fiber.

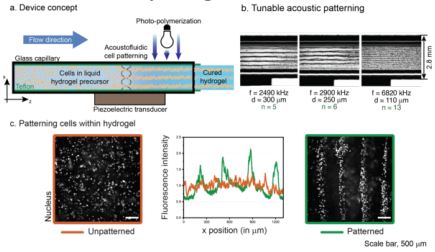

Figure 1. a) Cells in a liquid hydrogel precursor are patterned using an acoustic field, their positions are retained by photo-polymerization of the hydrogel. Continuous flow in the device results in extrusion of the hydrogel fiber with patterned cells. b) Operating frequency variation enables tunable spacing. c) Cell positions compared in unpatterned and patterned samples.

By varying the operating frequency of the device, we defined the spacing between the lines of tracer particles (Figure 1b). Initially, we patterned NIH-3T3 fibroblasts within gelatin methacrylate, which was photo-polymerized (λ = 405 nm). Patterned cells maintained their positions in the hydrogels after extrusion (Figure 1c).

Finally, primary murine myoblasts were patterned in a previously identified hydrogel formulation. [3] Samples were incubated in myoblast growth medium for three days, then transferred to myoblast differentiation medium to initiate myotube formation. Spontaneous twitching was observed from 7 days post patterning, indicating functional myotube formation. Acoustically patterned samples expressed myosin heavy chain (a marker for myotubes) throughout the hydrogel, whereas myotubes were present only on the surface of the unpatterned samples (Figure 2).

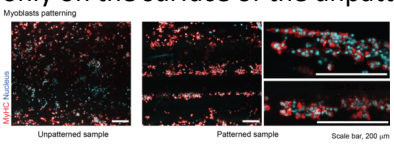

Figure 2. Myotube formation in unpatterned and patterned hydrogel samples. MyHC+ cells were observed in regions of high localized cell density.

Overall, our device enables robust cell patterning to mimic muscle tissue structure and promote myotube formation by increasing local cell density. This label-free technology can potentially be used to engineer other tissues requiring structural anisotropy.

References. [1] Krauss et.al., Cold Spring Harb. Perspect. Biol. 2017; [2] Deshmukh et.al., Adv. Funct.Mater. 2022; [3] Deshmukh et.al., Bioeng. Transl. Med. 2020.

S5.2-O5

Rapid 3D assembly of particles and cells using holographic sound fields

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Abstract

Introduction. Biofabrication includes methods that deposit bioinks (bioprinting) or assemble parts (e.g. cells or spheroids) with the goal of creating functional tissues. Most of these methods require mechanical access (e.g. extrusion printing or aspiration-based pick & place) and work serially (point-by-point or layer-by-layer), which scale poorly to volumetric shapes and applies undue stress on biological cells. Here, we present a technique to direct the assembly without contact and in parallel of particles or biological cells using ultrasound [1].

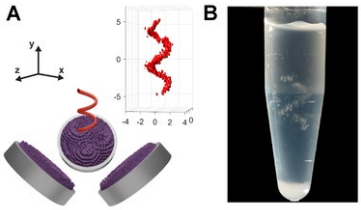

Fig. 1: Acoustic-directed assembly. (A) Schematic of the setup with three sources and helical target. Insert: simulated 3D sound pressure field. (B) Silica gel beads assembled to a helix.

Experimental Methods. We obtain extended 3D trapping fields by superimposing the outputs from multiple acoustic holograms (Fig. 1A). Holograms store the wave front (i.e. a phase distribution) of any wave and can impart it onto the output field of a simple source. For each target shape we optimize wave fields and the associated acoustic holograms using a gradient descent method. We then convert the phase distributions to thickness profiles and fabricate acoustic holograms using a 3D printer (Stratasys Objet Connex 260). The experiments are performed in a large open-topped water tank so that the sample tubes appear at the air-water interface. The particles and cells sediment under gravity and are held by acoustic force when they pass a trapping site. Gelation of the surrounding medium fixes the structures in place.

Results and Discussion. We demonstrate the method by assembling different classes of materials, including silica gel microspheres (70-200µm, see Fig. 1B and Fig. 2A), C2C12 mouse myoblasts (Fig. 2B) and hydrogel beads (gelatin methacrylate, GelMA, 70µm, Fig. 2C). The structures were fixed in a dextran hydrogel (TrueGel, Sigma Aldrich) and observed using a lightsheet microscope (Fig. 2).

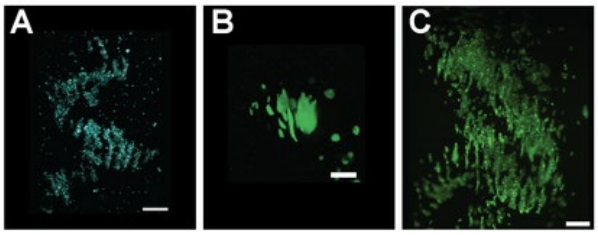

Fig. 2: Different materials can be assembled. (A) silica gel microspheres, (B) C2C12 mouse myoblasts, (C) gelatin methacrylate beads. Scale bars 1mm.

Conclusions. We demonstrate a method for rapid and directed assembly of particles into arbitrary 3D shapes using ultrasound. The technique is contactless and shown to work with standard labware, such as sample tubes, inserts or cuvettes.

References. [1] Melde et al. 9(6) eadf6182 Sci. Adv. (2023)

S5.3-K1

Biofabrication and 3D (Bio)printing Strategies for Musculoskeletal Tissue Regeneration

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Abstract

Our musculoskeletal system has a limited capacity for repair. This has led to increased interest in the development of tissue engineering and biofabrication strategies for the regeneration of musculoskeletal tissues such as bone, ligament, tendon, meniscus and articular cartilage. This talk will demonstrate how different musculoskeletal tissues, specifically cartilage, bone and osteochondral defects, can be repaired using different biofabrication and 3D bioprinting strategies. Increasingly complex strategies will be introduced, beginning with relatively simple examples where emerging additive manufacturing platforms are used to produce cell-free biomaterials capable of directing tissue regeneration *in vivo*, to more complex approaches where cells and/or growth factors are bioprinted into constructs that can be then implanted directly into the body. Finally, more complex biofabrication approaches will be described where biomimetic tissues are first engineered *in vitro* before *in vivo* implantation. The efficacy of these different biofabrication strategies in different preclinical studies will be reviewed, and lessons from the relative successes and failures of these approaches to tissue regeneration will be discussed.

S5.3-O1

Biofabrication and 3D (Bio)printing Strategies for Musculoskeletal Tissue Regeneration

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Abstract

Emulating the properties of native human tissues is a fundamental challenge in tissue engineering and drug discovery. Biomaterials that could accurately recapitulate these properties would be integrated more effectively into damaged tissue and would allow the production of more accurate in vitro models of disease for drug development. In this context, human resources such as blood and perinatal tissues can provide optimal base materials to produce 3D matrices that can achieve a greater degree of biosimilarity.

In this work, decellularized human amniotic membrane (hAM) was processed into hydrogels through supramolecular assembly, in order to grant the material with self-healing capabilities that can mimic the natural ability of tissues to regenerate, while also increasing the processability of the material. The hydrogels were produced by promoting host-guest interactions between the proteins in hAM and a photoresponsive cyclodextrin – mono-acryloyl-β-cyclodextrin (mAc-β-CD). These interactions generate a supramolecular monomer – AMSupraMA – that can be crosslinked through photopolymerization. The interactions between the cyclodextrin and the proteins were characterized by spectroscopic methods, as well as quartz-crystal microbalance analysis. Photopolymerization of the supramolecular monomer generated an ultra-soft matrix suitable for 3D cell culture, which was assessed through the encapsulation of stem cells. It was observed that fractured hydrogels can be reassembled into higher-order structures, confirming the potential of their self-healing capabilities. Even after complete disaggregation of the hydrogels, it was found that the resulting fragments produced an injectable material that could easily be molded into cohesive structures without any additional crosslinking. As such, it is believed that AMSupraMA hydrogels constitute a promising material for modular tissue engineering approaches.

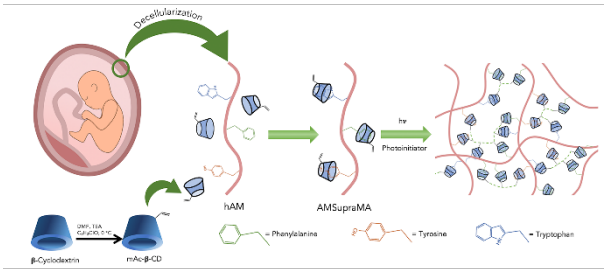

Figure 1. Schematic illustration of the generation of AMSupraMA hydrogels.

Acknowledgements: This work was developed at CICECO – Aveiro Institute of Materials, under the scope of projects UIDB/50011/2020, UIDP/50011/2020 and LA/P/0006/2020. The authors would also like to acknowledge funding by European Research Council grant agreement ERC-2017-ADG-883370 for project REBORN and by the Fundação da Ciência e Tecnologia (FCT) through the individual contract 2020.01647.CEECIND, the doctoral grant 2022.10626.BD and in the scope of project "TETRISSUE" (PTDC/BTM-MAT/3201/2020).

S5.3-O2

Stiffness gradients in skin-derived extracellular matrix (dECM) hydrogel cause phenotypic changes in dermal fibroblasts

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Abstract

INTRODUCTION. Large skin injuries do not heal easy, while the sheer absence of skin such as after burns, cause scarring and tissue fibrosis. One of the consequences of scarring and fibrosis is the high stiffness of excessively deposited extracellular matrix (ECM) due to extensive crosslinking. There is scarce information concerning the role of dermal fibroblasts in dermal scar reversal. We investigated the response of normal human dermal fibroblasts (NHDFs) to stiffness gradients in decellularized dermal ECM (dECM) hydrogels that ranged from normal to fibrotic stiffness.

EXPERIMENTAL METHODS. Porcine skin ECM was decellularized, lyophilized and digested to prepare the pre-dECM gel. Then, an *in vitro* model of dermal scar which has gradient stiffness was made by using a printed versatile mold (Fig. 1a). Diffusion gradients of the photocrosslinker Ruthenium were generated over 3h in the cast gels, after which 405 nm UV light was used to crosslink for 5 min. The mechanical properties of the hydrogel were assessed using LLCT. Correspondingly, the collagen fibre architecture was determined by picrosirius red (PSR) staining. Subsequently, experiments were repeated with seeded NHDF in gradient dermal dECM hydrogels and cell viability, proliferation, morphology, differentiation and matrix remodelling investigated after 4h, 1d and 5d.

RESULTS AND DISCUSSION. Using our designed mold, the Ru0.1x concentration (3mM Ru) produced dECM with an optimal gradient curve and stiffness from 724±424 kPa to 60±20 kPa (1.5 mm - 6.5 mm, Fig. 1b). Corresponding, collagen fibers showed a higher density in stiffer parts compared to soft parts: likely induced by a higher degree of photocrosslinking. NHDF displayed orientated arrangement tends to the direction in which the stress gradient exists and the orientation showed in all of high, middle and low stiffness part in gradient stiffness Ru-dECM while being more randomly distributed in non gradient dECM after 5d 3D culture (Fig. 1c). The NHDF sensed the mechanical signal and then conversely adjusted the hydrogel stiffness by degrading the surrounding ECM especially in high stiff part, which demonstrated by the results of mechanical test and collagen staining after 5d cell culture. The differentiation of NHDF abserved by a-SMA staining also showed differences in the varying stiffness parts of Ru-dECM. This research can help us generate more information about how NHDFs behaviour exhibited in the changed mechanical Ru-dECM.

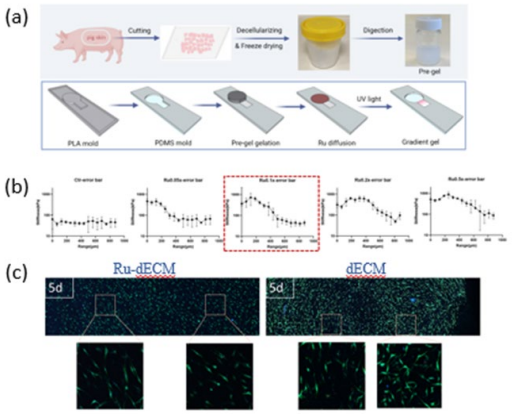

Figure 1. (a) Process for making dECM pre-gel and gradient stiffness dECM hydrogel, (b) Mechanical properties tested by μLLCT, (c) Live-dead staining after 5 days.

S5.3-O3

Development of 3D breast cancer models using decellularized mammary glands bioinks

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Abstract

Introduction. The breast tumor microenvironment (TME) plays a crucial role in the tumor outcome, and it is formed by breast cancer cells (BCCs), stromal cells and extracellular matrix (ECM). 3D *in vitro* models can recreate the main properties of the TME, and it can be even engineered through 3D printing to recreate the cellular organization within the tumor.[1] The importance of the ECM in tumor progression and drug resistances has motivated the synthesis of bioinks that mimic the ECM complexity. In this regard, decellularized tissues-derived matrices (TDMs) can recapitulate many native tissue biological cues. However, its inadequate mechanical properties prevent their bioprinting as well as to mimic the tumor stiffness. The aim of this work is to develop a 3D *in vitro* model of breast cancer using breast TDM-derived bioinks.

Methodology. Bioinks were prepared by mixing decellularized and enzymatically digested porcine breast tissue, gelatin-methacrylamide (GelMA), and alginate (TGA).[2] Collagen 1 (Col1), overexpressed in breast cancer, was also incorporated (TGAC). Breast cancer cells (BCCs) or mesenchymal stem cells (MSCs) were suspended in the bioinks, and scaffolds were bioprinted and crosslinked. Printability, mechanical properties, cellular survival, proliferation, morphology, invasion, expression of adhesion molecules, malignancy and drug resistance were studied.

Results and discussion. TGA and TGAC bioinks could be bioprinted in the absence of a sacrificial material and they recreated tumor stiffness' once crosslinked. BCCs and hAMSCs were able to proliferate in the scaffolds, forming spheroids and cell networks. BCCs showed lower expression of e-cadherin, and a high expression of fibronectin, VEGF, multidrug resistance protein 1 and high resistance to doxorubicin (Fig. 1A). BCCs showed a matrix metalloproteinase-dependent invasion in TDM scaffolds (Fig. 1B). 3D *in vitro* models of MSCs and BCCs could be successfully bioprinted with TDM bioinks.

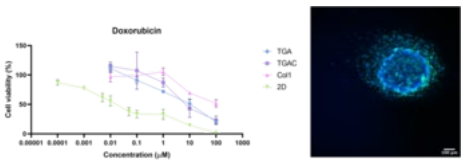

Figure 1. (A) Doxorubicin efficacy in cell-laden scaffolds, collagen 1 and in 2D. (B) MDA-MB-231 invasion in TGA hydrogels after 3 days.

Conclusions. Taken together, we have shown that TDM bioinks could be used to bioprint breast cancer models, closely recreating the tumor ECM.

Acknowledgement. European Union's Horizon 2020 (Marie Skłodowska-Curie 712754), Spanish Ministry of Economy and Competitiveness (SEV-2014-0425, CEX2018-000789-S), FEDER and Spanish Ministry of Science, Innovation and Universities (RTI2018-096320-B-C21; MAT2015-68906-R), Spanish Ministry of Economy, Industry and Competitiveness (EUIN2017-89173), European Commission (JTC2018-103).

References. [1] Blanco-Fernandez et al. J. Adv. Sci. 8: 2003129, 2021; [2] Blanco-Fernandez et al. ACS Appl. Mater. Interfaces 14: 29467 2022.

S5.3-O4

Synthetic and extracellular matrix (ECM)-based hydrogels enable translational precision medicine for patient-derived breast cancer organoids

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Abstract

3D organoid model technologies have led to the development of innovative tools for cancer precision medicine. However, the current gold standard culture system (Matrigel) lacks the biophysical manipulation required to model various cancer microenvironments, due to undefined biochemical composition, and batch-to-batch variability. Tuneable hydrogel matrices provide enhanced capability for drug testing in breast cancer (BCa), due to their improved mimicry of the physicochemical characteristics of the native extracellular matrix. In this study, we analysed the formation and therapeutic response of patient-derived BCa organoids in bioprinted polyethylene glycol (PEG), and gelatin-based hydrogels of varying crosslinking chemistry (gelatin methacryloyl; GelMA crosslinked via photoinitiation, and thiolated-gelatin; GelSH crosslinked via click-chemistry reaction with PEG-4MAL). Briefly, surgically-resected tumour specimens were collected from consenting donors (4 female patients, aged 32-76) and enzymatically digested to recover cells. Following monolayer cell expansion, cells were encapsulated in drop-on-demand bioprinted PEG-based matrices (+/-peptides; RGD, GFOGER and DYIGSR) using the RASTRUM bioprinter from Inventia Life Sciences, photocrosslinkable (GelMA) supplied by Gelomics or click-crosslinkable gelatin (Gel-SH) developed in-house[1] and now patented under Patent No. 2022903674. Organoid formation as well as chemotherapy treatment response was evaluated. Within ranges of BCa stiffnesses (1–6 kPa), GelMA, GelSH and PEG-based hydrogels successfully supported the growth and organoid formation of HR+or−/HER2+or− primary cancer cells for at least 2–3 weeks, with superior organoid formation within the GelSH biomaterial (up to 268% growth after 15 days)[2]. BCa organoids responded to doxorubicin, EP31670 and paclitaxel treatments with increased IC50 concentrations compared to 2D cultures, and highest IC50 for organoids in GelSH. Cell viability after doxorubicin treatment (1 µM) remained >2-fold higher in the 3D gels compared to 2D and doxorubicin/paclitaxel (both 5 µM) were ~2.75–3-fold less potent in GelSH compared to PEG hydrogels. The data demonstrate the potential of hydrogel matrices as easy-to-use and effective preclinical tools for therapy assessment in patient-derived breast cancer organoids. The results of this study indicate that PEG- and particularly click-crosslinkable-gelatin-based hydrogel matrices serve as an economical, tuneable, consistent, and translatable culture system for patient-derived BCa organoids, surpassing the limitation of the gold standard Matrigel®.

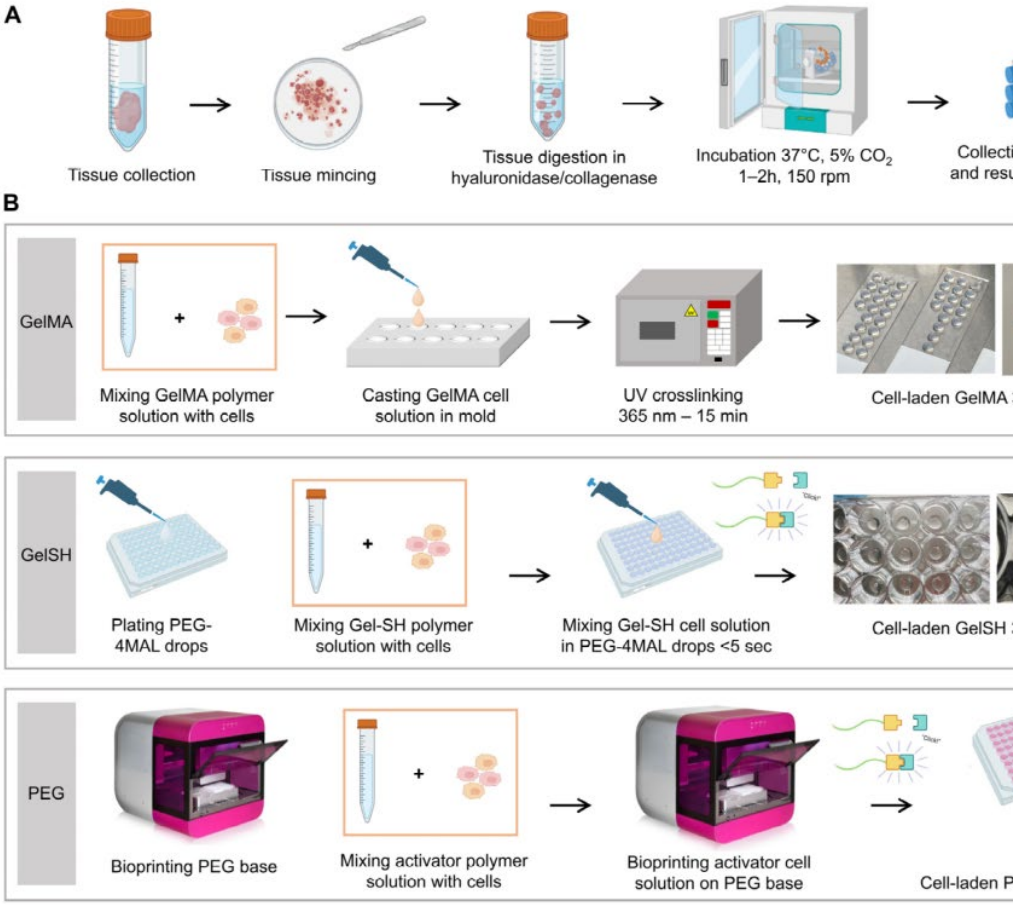

Figure 1. Schematic representation of tissue processing from tumor collection and preparation (A) to encapsulation into 3D hydrogels (B). GelMA = gelatin methacryloyl. GelSH = Gel-SH/PEG-4MAL, PEG = polyethylene glycol. Figure partly created with BioRender.com

References. [1]Hipwood L, et al. Gels 8, 821, 2022; [2]Bock N, et al. Pharmaceutics 15, 261, 2023.

S5.3-O5

Decellularized nucleus pulposus-based hydrogel: from decellularization scale-up to biocompatibility evaluation

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Abstract

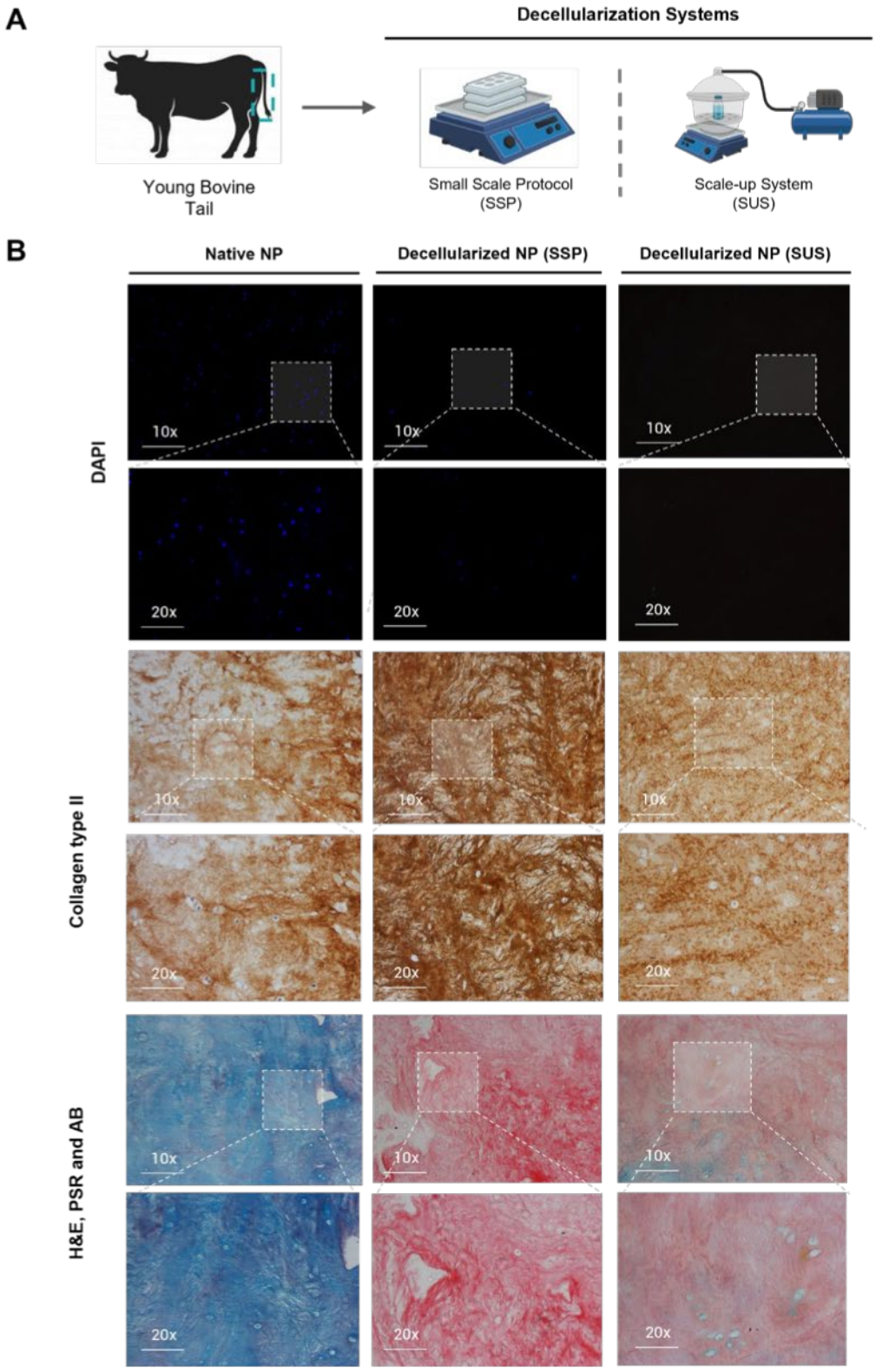
Hydrogels based on decellularized nucleus pulposus(dNPs) from intervertebral disc(IVD) constitute a promising approach for IVD regeneration since they mimic the native microenvironment and can be injected. Nevertheless, decellularization can be tricky in tissues with high density of extracellular matrix(ECM).

In this study, we aimed to develop a scale-up decellularization method that could provide optimal dNPs to be used for the production of a stable, biocompatible and easily injectable hydrogel. For that, bovine NPs (1-yr old) were decellularized with a SDS-based method1 using: 1) agitation system(AS); 2) vacuum-assisted perfusion(VAP); or 3) vacuum-agitated system(VAS). Decellularization efficiency was analyzed by DNA quantification and ECM preservation. An hydrogel based on dNP was obtained by pepsin digestion2. Hydrogel injectability through different needles (21,25,30G) was evaluated. Biocompatibility studies with bovine NP cells were conducted.

The results showed that the VAS method demonstrated to be more efficient, with higher DNA removal and higher sGAGs retention, compared to the AS and VAP methods. The hydrogel pre-solution based on dNP was easily injected through the different needle diameters, with a time increasing from 2 to 6s with the decrease of needle diameter (from 21 to 30G). Swelling and degradation rates of the hydrogel showed that hydrogels remained stable for 7 days in PBS, with residual/negligible disintegration. Results from hydrogels extracts revealed a slight decline in NP cell viability (22% reduction with 100% extracts, but still above the 70% baseline accepted by ISO10993-5). Alterations in cell morphology were visible (cells became rounded). LDH quantification revealed a toxicity below 30%. In direct contact assays, NP cells were seeded on the top of the hydrogel and cell metabolic activity decreased from 70 to 50% after 24h of culture and significantly increasing to 70% up to day 7, suggesting NP cell proliferation and recovery. LDH assay revealed cytotoxicity below 30%. DAPI staining confirmed increasing cell numbers with time. Cell distribution showed higher cell concentration at the gel borders at day 1 that progressively became evenly distributed after 7 days, indicating cell migration towards the gel center.

In conclusion, this work showed the development of a stable in-situ gelling biomaterial from dNP with promising biological features. Envisioning further product development, the scale-up decellularization system was successfully validated, achieving an optimal compromise between cell removal and retention of ECM components, comparable to small-scale systems (Figure 1).

Reference. 1 Fiordalisi M, Biomat Adv, 2022; 2 Wasch, Spine J, 2017


A
Decellularization Systems
Young Bovine Tail
Small Scale Protocol (SSP)
Scale-up System (SUS)
B
Native NP
Decellularized NP (SSP)
Decellularized NP (SUS)
DAPI
Collagen type II
H&E, PSR and AB
10x
20x

S5.4-K1

Scaffolded spheroids for modular bone and cartilage tissue engineering

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Abstract

Assembly of modular building blocks is an increasingly popular strategy in tissue engineering (TE). In particular multicellular aggregates, also referred to as spheroids or pellets, have been shown to exhibit improved biological properties with regard to regenerative capacity. However, they are also not without challenges. For example, prolonged culture of spheroids often results in rather inhomogeneous size and shape distribution, while their compaction and fusion can lead to substantial volume loss. High-resolution 3D printing enables realization of specialized microscaffolds, which can host individual spheroids and be used as building blocks for assembly for larger tissue constructs. We have previously demonstrated that such microscaffolds help to reduce the compaction of the spheroids, as well as maintain their shape and roundness over extended cell culture periods. Furthermore, the presence of the microscaffolds does not hinder fusion of scaffolded spheroids, produced from human adipose-derived stem cells (ASCs). They show high viability and preserve chondrogenic and osteogenic potential of ASCs. These findings indicate that the microscaffolds carrying high density of cells are promising building blocks for cartilage and bone TE facilitated by bottom-up assembly. Furthermore, this approach provides a possibility to recreate more complex tissues by combining scaffolded spheroids differentiated towards different phenotypes, as demonstrated on the example of osteo-chondral construct. In this contribution our recent progress, as well as perspectives for further TE applications of scaffolded spheroids, will be discussed.

S5.4-O1

Fabrication of scaffolds for tissue regeneration using melt electrowriting of PEOT-PBT copolymers

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Abstract

Introduction: Melt electrowriting (MEW) is a promising technique for fabricating scaffolds that enables the precise deposition of molten polymers into organized fibers with diameters in the range of a few to tens of micrometers. However, the number of printable polymers for MEW is limited, with polycaprolactone (PCL) being the most commonly used. To address this challenge, we investigated the use of poly(ethylene oxide terephthalate)-poly(butylene terephthalate) (PEOT-PBT), an elastomeric multiblock copolymer with water-absorbing properties. We also compared the biological activity of PEOT-PBT and PCL scaffolds in terms of their ability to support tissue healing by culturing fibroblasts.
Methodology: PEOT-PBT (PolyVation, the Netherlands) and PCL were printed using a MEW machine (Spraybase, Ireland). The mechanical properties of the fabricated scaffolds were studied using tensile testing. Biocompatibility was analyzed by culturing NIH3T3 cells on the different designs of the printed scaffolds (fig:1), and various cell-based assays were performed. Statistical significance was determined using a two-way ANOVA method.

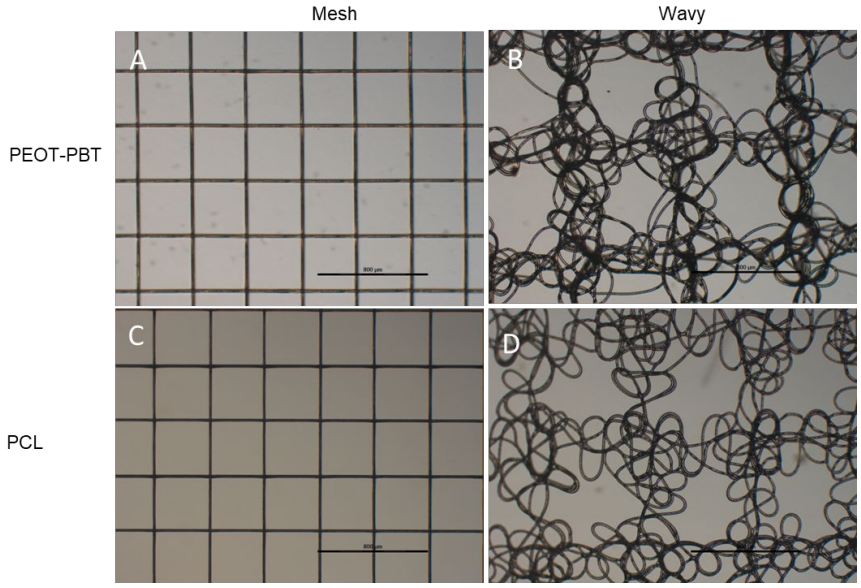

Figure 1. Brightfield images of MEW scaffolds

Results and Discussion: We showed that PEOT-PBT block copolymer is suitable for MEW, showing the ability to easily manipulate fiber diameter and scaffold design by changing printing parameters. The mechanical analysis revealed ultimate tensile strength in the range of 0.15 to 0.4 MPa and strain at break of 1 to 10, depending on the printing design.
Biological analysis revealed good biocompatibility of both PCL and PEOT-PBT scaffolds. Immunofluorescence study showed positive staining for collagen-I and alpha-smooth muscle actin (α-SMA) on all types of scaffolds after 14 days of culture (fig:2). Fibroblast proliferation was faster with

higher contractile activity in PEOT-PBT Mesh scaffolds. Furthermore, after 28 days of culture, PEOT-PBT Mesh scaffolds have a significantly higher cell number compared to PCL scaffolds. These results suggest PEOT-PBT mesh scaffolds may exhibit faster tissue healing or regeneration behavior.

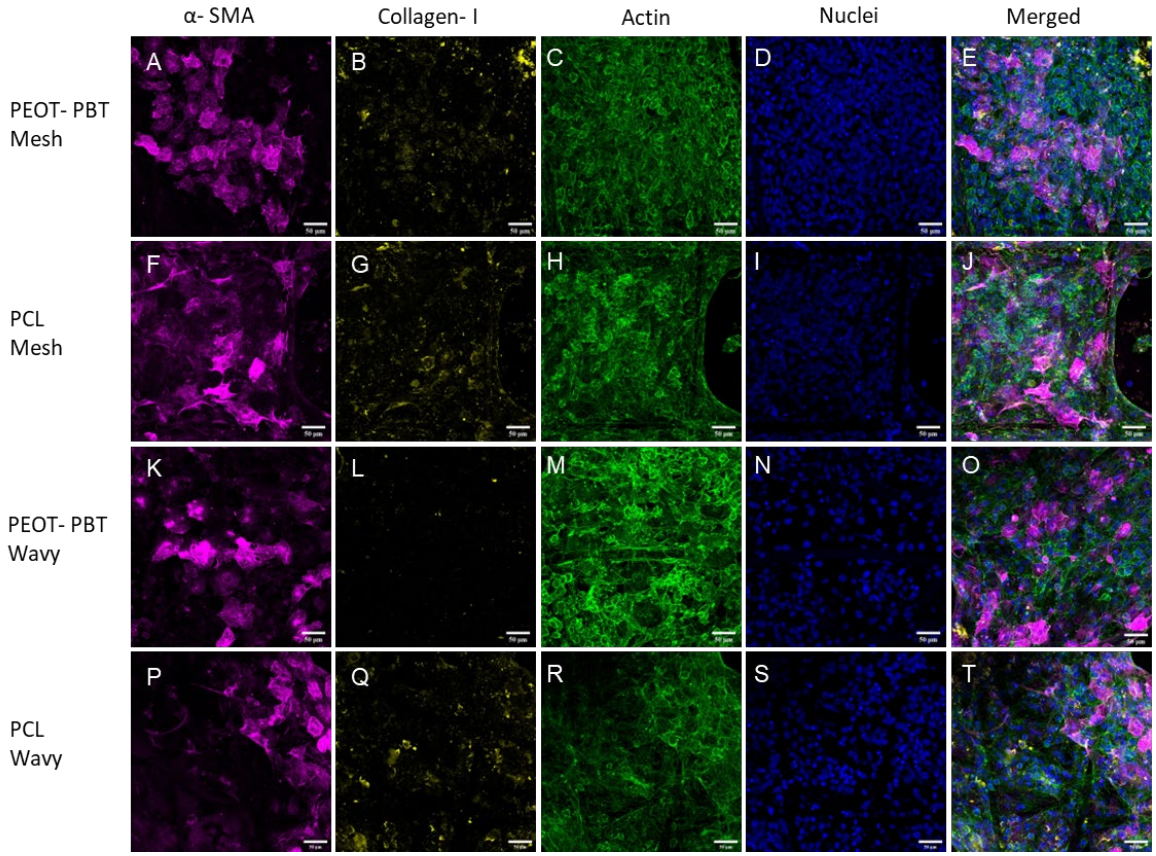

Figure 2. Immunocytochemistry after 14 days of culture. Scale bar = 50 µm.

Conclusion: Our study highlights the potential of MEW using PEOT-PBT as a printable material for scaffold fabrication in biomedical applications. PEOT-PBT scaffolds demonstrate good biocompatibility, surpassing PCL at longer time points. Additionally, the mechanical properties of the printed scaffolds are within the required range for tissue engineering applications. The softer nature of PEOT-PBT compared to other commonly used polymers in MEW provides greater flexibility in tailoring the mechanical properties of the MEW scaffolds to suit specific tissue engineering applications, opening up new possibilities for regenerative medicine.

S5.4-O2

Multifunctional hybrid organic-inorganic porous silica nanoparticles and 3d assemblies with hierarchical porosity for controlling delivery of small to large molecule therapeutics

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Abstract

Porous silica nanoparticles (PSiNPs), with their high porosity and surface area and biodegradability are attractive for drug delivery. However, multiple functionalisation and purification steps are typically required to impart desirable functionalities for biomedical applications. We have recently developed a simple and highly versatile one-pot polyelectrolyte complex (PEC) templated method to obtain monodisperse and multifunctional PSiNPs for anticancer drug delivery, obviating the need for any post-synthetic modifications.[1,2] In our first study, PECs formed from oppositely charged L-arginine (Arg)/poly(acrylic acid) (PAA) were used to template silane mineralisation, giving monodisperse PSiNPs ranging from 20-180 nm (Figure 1A-C).[1] Importantly, PAA retention in the PSiNPs electrostatically enhanced doxorubicin (Dox) loading and promoted pH responsive release in acidic environments. Concomitantly, the surface presentation of Arg conferred intrinsic cancer targeting and increased uptake of the PSiNPs by primary glioblastoma cells but not in non-tumourigenic neural progenitor cells. In a subsequent study, the versatility of the system was further demonstrated using a different type of PEC composed of poly(ethyleneimine) (PEI)/L-glutamate (Glu).[2] Here, monodisperse popcorn-like PEI-functionalised PSiNPs were obtained which could electrostatically crosslink with hyaluronic acid (HA) to give self-healing hydrogels with tuneable mechanical properties (Figure 1D-E). Importantly, the cationic PEI-PSiNPs enhanced loading of the anionic anticancer drug methotrexate and provided sustained drug release. Lastly, we explored the directional freezing of PSiNPs to give fibrous 3D assemblies with hierarchical porosity. By increasing the size of the PSiNPs (25, 50, 100, and 150 nm) used to form the 3D assemblies, the interparticle porosity could be increased, giving a faster rate of drug release from the fibres (Figure 1F-G). Furthermore, the unique hierarchical porosity enabled co-loading of higher molecular weight lysozyme in the interparticle pores and smaller molecular weight Dox inside the PSiNP micropores. Taken together, these novel systems are highly promising as post-surgical implants for delivering a wide range of small to large molecule therapeutics in cancer treatment.

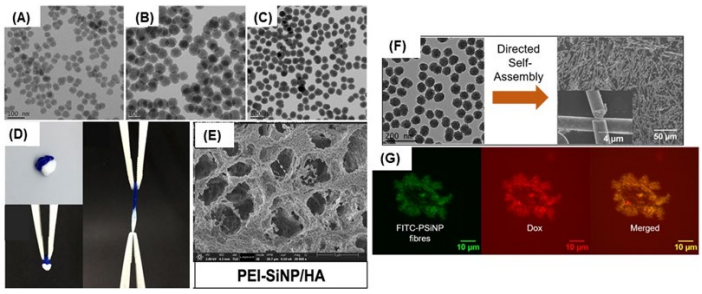

Figure 1. (A-C) Highly uniform Arg/PAA-PSiNPs with tuneable size, cancer targeting, and pH responsive properties. (D-E) Self-healing hydrogels formed from PEI/Glu-PSiNPs and HA. (F-G) Directional freezing of PSiNPs into fibres with hierarchical porosity which permits Dox loading into the pores of the PSiNPs and between the PSiNPs for tuning drug release.

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S5.4-O3

Proteolytic remodeling of 3D bioprinted hydrogel structures in breast cancer models

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Abstract

Hydrogels have gained widespread use in tissue engineering for the three-dimensional (3D) culture of cells. The advent of 3D bioprinting using hydrogel-based bioinks has opened new avenues for the biofabrication of tissue-like constructs, enabling detailed studies of cell-matrix interactions and intercellular crosstalk in complex 3D environments. Bioprinted tumor models hold great promise for revolutionizing cancer research by providing model systems for investigating tumor progression, invasion, and facilitating drug screening. The complex tumor microenvironment (TME) can be replicated by combining various types of cells and extracellular matrix components in a spatially defined manner. Using bioprinting, we created a 3D breast cancer model that included breast cancer cells and fibroblasts to investigate the interactions between cells and extracellular matrix (ECM)-mimicking materials. We employed combinations of protease-degradable and non-degradable bioinks to conduct systematic studies and elucidate the roles of proteases in the TME. The bioinks were prepared by conjugating cyclooctyne bicyclo[6.1.0]nonyne (BCN) to hyaluronic acid, which was later crosslinked with either a protease-degradable or a polyethylene glycol (PEG)-based crosslinker containing azide groups. The bioprinting process was initiated by bioprinting MCF-7s using protease-degradable and non-degradable bioinks, followed by covering the bioprinted structures with primary human fibroblast-containing bioinks that were either protease-degradable or non-degradable.Following a 14-day culture period, we observed that the presence of fibroblasts in a bioprinted structure significantly influenced the formation of MCF-7 spheroids. No MCF-7 spheroids were seen in the absence of fibroblasts, whereas encapsulation of MCF-7 cells in the bioprinted structure containing fibroblasts led to spheroid formation in the size range from 100 to 50,000 μm^2. Notably, the size of MCF-7 spheroids was highly dependent on the protease degradability of the bioink. Protease degradability of the bioink was associated with a significant increase in the size of spheroids formed within the bioprinted structure. The formation of MCF-7 spheroids in the bioprinted structure was consequently influenced by both the bioink properties and the presence of surrounding cells. Protease degradability of the bioink in combination with the presence of fibroblasts, the most abundant stromal cells in the TME, resulted in a significant increase in MCF-7 spheroid size. These findings highlight the synergistic effects of extracellular components and surrounding cells on spheroid formation within the 3D microenvironment. Our study demonstrates that 3D bioprinting using meticulously engineered bioinks can be utilized as a valuable tool for systematic investigations into the intricate events of the TME that could potentially influence cancer progression.

S5.4-O4

Acoustic holographic bioassembly for tissue engineering

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Abstract

Acoustic holograms, which are phase plates designed to modulate wavefronts of sound waves, enable the control of acoustic fields with exceptional intricacy and show potential as a tool for biological research. One acoustic hologram's application of particular interests for tissue engineering is the assembly of biological components to build tissue constructs from the bottom up. Comparing with magnetic and electric fields that can also drive bioassemblies, acoustic fields confer superior biocompatibility, scalability of wavelength and intensity, as well as low attenuation in most media. Direct, contactless, and label-free acoustic manipulation of various biological building blocks such as cells, cell-encapsulated particles, spheroids, and organoids is therefore feasible under a range of experimental conditions, enabling rapid and one-step assemblies. Coupling single or multiple acoustic holograms to ultrasonic transducers, our group have showcased patterning of cells into complex shapes within a Petri dish [1] as well as three-dimensional assembly of hydrogel beads and cells in common labware [2]. Advancing from the two prior works, we have recently developed an acoustic holographic bioassembly platform to assemble cells within a cell culture insert into centimeter-scale tissue constructs that are of arbitrary shapes and physiologically relevant cell densities, without relying on the presence of an air-liquid interface. By demonstrating the viability, proliferation, and migration of the acoustically assembled cells over time, we show that this bioassembly platform has the potential to be employed in developing *in vitro* models for tissue-wide biological studies. Additional applications in acoustically-enhanced bioprinting and tissue graft fabrication can be envisaged as well. Offering a unique set of advantages, acoustic holographic bioassembly is expected to be applied more broadly in the field of tissue engineering in the future, both as a biofabrication method on its own and as a complement to other methods.

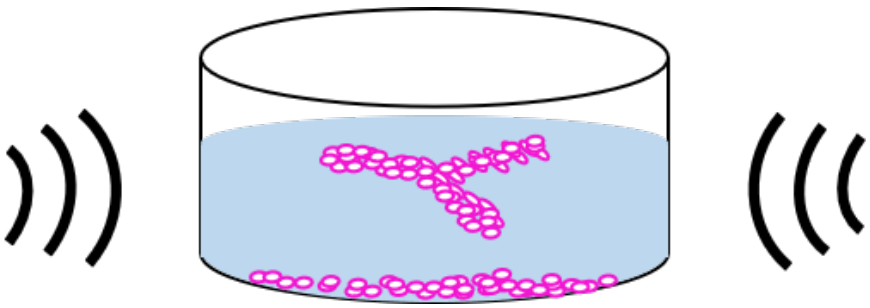

Figure 1: Illustration of rapid and one-step acoustic holographic cell assembly within a cell culture insert into a centimeter-scale tissue construct of arbitrary shape and physiologically relevant cell density.

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S5.4-O5

Self-assembly driven smart biomimetic 3D-printed hybrid aerogel-based theragenerative scaffolds for bone regeneration and bone cancer therapy

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Abstract

The rational design of the theragenerative[1] (combining therapy and regeneration) 3D printed smart hybrid aerogel scaffolds has shown great promise in regulating cell-matrix interactions with enhanced osteoconductivity, osteoinductivity, antibacterial and antitumoral properties. Profiting from the ease of surface modification of silk fibroin (SF) biopolymer and its self-assembly capability, we recently fabricated a range of 3D printed bio-inpsired aerogel-based nanocomposites with controlled microstructure, macroscopic shapes, mechanical properties and bioactivity[2, 3, 4]. These biomimetic scaffolds were developed by incorporation of various functional inorganic nanoparticles, namely hollow mesoporous silica nanoparticles, black bioactive glass mesoporous nanofibers, titanium carbide (Ti_3C_2, MXene) 2D nanosheets, and bismuth sulfide (Bi_2S_3) nanobelts[4] into ligand modified SF matrix prior to self-assembly driven by robust covalent and bio-inspired non-covalent supramolecular interactions.

Both the therapeutic and bone regeneration potential of developed composite aerogels have been governed by biochemical properties of loaded functional micro and nanoparticles in the aerogel network as well as by its highly porous interconnected network. Intriguing examples in this regard are development of 3D printed multi-functional aerogels through incorporation of photothermally active MXene 2D nanosheets and black bioactive glass nanofibers (BGNF, 75%SiO_{2-x}, 25%CaO, 5%P_2O_5) inside the SF biopolymer matrix via mussel-inspired chemistry mechanism. With this study, we demonstrated that while 3D printed composite scaffolds could mediate the *in vitro* growth, proliferation, and differentiation of human mesenchymal stem cell lines, they also showed a strong anti-osteosarcoma through photothermal ablation of bone cancer cells as well as self-healing with strong bone adhesion performances.

The biomimetic dual functional aerogel-based hybrid 3D printed scaffolds and chosen therapeutic techniques are thought to render a significant breakthrough in biomaterials' future clinical applications.

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S5.5-O1

Polydopamine nanoparticles-based hyperthermia and chemotherapy for the treatment of liver cancer

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Abstract

Introduction. Polydopamine (PDA) is a polymer originating from the self-polymerization of dopamine monomers; during the polymerization process, spherical nanoparticles are formed (PDA NPs). These nanostructures show high drug encapsulation capacity, easy and versatile surface modification, the ability to convert near-infrared radiation (NIR) into heat, as well as strong antioxidant properties [1]. In this study, PDA NPs have been proposed for a combined anti-cancer strategy via hyperthermia through remote NIR excitation and chemotherapy, by loading the chemotherapy drug sorafenib (SFR-PDA NPs), specifically effective against liver cancer (Figure 1) [2]. In order to exploit homotypic targeting, membranes isolated from liver cancer cells (HepG2) have been used as nanoparticle-coating agents (CM-SRF-PDA NPs) [3].

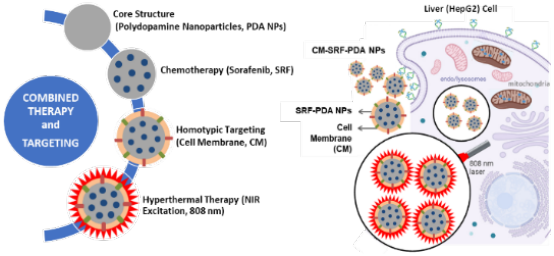

Figure 1. Schematic representation of the CM-SRF-PDA NPs-based therapeutic approach.

Results

Methods. Transmission electron microscopy (TEM) images, size distribution, and ζ-potential assessments of SRF-PDA and CM-SRF-PDA NPs show their excellent colloidal stability (Figure 2). The presence of phosphorus just in the structure of CM-SRF-PDA NPs, analyzed by X-ray photoelectron spectroscopy (XPS), indicates the successful CM coating. The data obtained with a single-mode NIR laser (808 nm) show a consistent temperature increment from 37°C to 54°C.

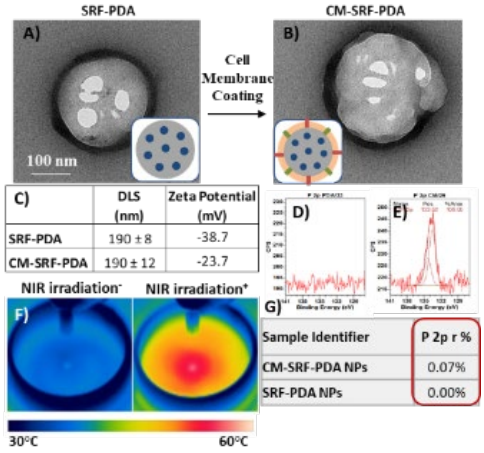

Figure 2. Representative TEM images (A and B) and a table (C) to present size distribution, ζ-potential measurements, and XPS analysis (D and E) of SRF-PDA and CM-SRF-PDA NPs. Representative thermo-images of NIR-stimulated CM-SRF-PDA NPs (F).

Confocal images of HepG2 cells show intracellular (per-nuclear) localization of CM-SRF-PDA NPs at 30 and 90 min of incubation (Figure 3). The flow cytometry results show higher internalization of CM-coated NPs. Eventually, we demonstrated as the metabolic activity and viability of CM-PDA- and CM-SRF-PDA-treated cells were significantly reduced in the case of NIR irradiation.

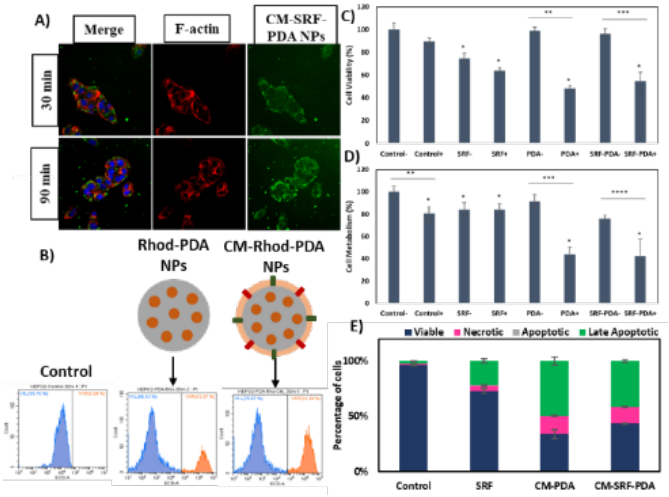

Figure 3. Confocal images (A) of CM-SRF-PDA NPs-treated HepG2 cells (f-actin, red; nuclei, blue; NPs, green); cellular up-take investigation by flow cytometry (B). Effects on cell viability (C), metabolism (D), and apoptotic phenomena (E).

Discussion. The combined chemotherapy and hyperthermia therapy show promising improvement in the reduction of cancer cell viability as well as liver cancer targeting via a homotypic approach. The obtained data encourage further studies on complex *in vitro* models, before *in vivo* translation.

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S5.5-O2

Exploring the limits of enhanced permeability-retention effect in triple-negative breast cancer

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Abstract

Introduction. The enhanced permeability-retention (EPR) effect often is a key strategy for nanoparticle (NP) delivery to tumors, yet true extent of this effect and its relationship to NP size are not clear due to rapid opsonization-induced clearance. We developed non-opsonizing NPs to investigate the limits of EPR effect and size dependence in a triple-negative breast cancer (TNBC) model.
Methodology. Heavily PEGylated bottle-brush polymers (18-55 nm) were prepared as NP surrogates and fluorescently labeled. They were characterized by dynamic light scattering, and resistance to non-specific opsonization was studied using fluorescence correlation spectroscopy (FCS) in full serum. NP concentrations in blood and tissue homogenates were measured and pharmacokinetics (PK) and biodistribution (BD) were assessed in healthy Balb/c mice and TNBC-bearing mice.
Results and Discussion. Long plasma residence time is crucial for the EPR effect but is typically hindered by rapid opsonization. Our NPs showed no changes in size after one week incubation in full serum, confirming resistance to opsonization. Clearance half-lives increased with decrease in NP size, reaching 3.5 days in case of NP with D_H of 18nm. Extended circulation enabled NPs to extensively probe extravasation sites, including tumor, and test the full potential of EPR effect. We observed no accelerated NP clearance in TNBC-bearing mice compared to healthy ones, suggesting tumors were not effective NP scavengers. Highest concentrations of NPs of all sizes were found in tumors. Tumor/liver selectivity was only moderate and was increasing with a decrease in nanoparticle size reaching a value of 1.7 for 18 nm particles. Interestingly, tumor presence significantly reduced NP accumulation in the spleen, most notably for 55 nm NPs. Biodistribution to other organs remained unaffected by tumor presence.
Conclusions. Non-opsonizing NPs enabled evaluation of the extent of EPR effect in TNBC model. The presence of tumor did not appreciably accelerate NP clearance, rejecting long-standing opinion that tumors act as a sink for NPs due to EPR effect. Although highest concentrations of NPs of all tested sizes were found in tumors, the tumor/liver selectivity was only moderate and increased with a decrease in NP size. Overall, we conclude that passive targeting via EPR effect cannot be a sufficient mechanism for NP-based delivery to TNBC tumors and the developed materials can serve as a tool to study the EPR in other cancer types.

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S5.5-O3

The therapy enhancing effect of noble metal nanoparticles in proton therapy is driven by the surface functionality

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Abstract

Proton therapy (PT) is a modern form of radiation therapy, particularly useful when treating cancer in sensitive areas like brain, eyes, or in developing organs of children. *In vitro* and *in vivo* studies already demonstrated that metal nanoparticles (NP) can function as radiosensitizers in PT [1]. Here, it was believed that reactive oxygen species (ROS) generated in the presence of the NP are a main contributor [2]. However, the mechanism of ROS generation during PT in the presence of NP is still underexplored, particularly when clinically relevant radiation doses of 2-5 Gy are used.

In this work, we studied the effect of colloidal ligand-free Pt and Au nanoparticles on ROS generation in water phantoms after irradiation with protons. ROS formation was monitored directly using fluorescent dyes and indirectly by a complementary assay detecting breakage of DNA by gel electrophoresis. Here, initially ligand-free sterilizable[3] NP are particularly useful model materials to pave the way towards tuning of NP properties for maximum efficiency in PT, particularly as sodium citrate, a stabilizer commonly utilized in chemical NP synthesis negatively affects ROS formation. We found a linear dependence of the ROS generation on irradiation dose and particle surface concentration [4]. However, we were also able to deduce a higher efficiency of smaller 5 nm AuNP in contrast to their 30 nm counterparts even at the same total surface area, which points at additional effects driven by surface chemistry e.g. the density of structural defects. This is complemented by the fact that PtNP were significantly more active than AuNP, which we believe to be linked to the higher catalytic activity of Pt surfaces in generation of ROS [4]. Finally, we also examined the impact of common biological stabilizers like albumin on PT efficiency and transferred our model to hydrogel-based phantoms to better emulate conditions in target organs like brain or eye as another step towards clinical applicability of the nanoparticle-based sensitizers in proton therapy.

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S5.5-O4

Development of tunable hydrogel with Fenton-like activity for natural killer cells and self-assembled antibody nanoparticles delivery to suppress postoperative malignant glioma recurrence

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Abstract

Malignant brain tumors are one of the leading cancer causes of death in young adults and adults. Approximately 40,000 people were diagnosed with malignant brain tumor every year. Among these patients, 15,000 cases are glioblastoma (GBM) with very poor prognosis; even after aggressive surgical resection and adjuvant chemo/radiotherapy, the median survival is only 14.6 months. The major reason for poor prognosis is the blood-brain barrier preventing the therapeutic agent from delivering to the target area. Therefore, there are only a few choices of drugs available for brain tumor treatment. To date, temozolomide (TMZ) and Gliadel wafer have been clinically used as standard chemotherapy agents after brain tumor resection, however, the drug resistance for TMZ and the poor drug diffusion rate of Gliadel wafer still result in ineffective suppression of residual cancer cells. To overcome the above-mentioned difficulties for clinical brain tumor treatment, this project will develop a tunable hydrogel using fibrinogen, thrombin, and alginic acid as backbone, which can be easily switched to sprayed hydrogel or injectable hydrogel with natural killer cells (NK cells) and Fenton-like dual-antibody self-assembly nanoparticles loading to form sprayed immune hydrogel or injectable immune hydrogel as novel brain tumor treatment system. The surgeons can apply the sprayed immune hydrogel on the wall of intracranial cavity immediately after surgery, then deliver the injectable immune hydrogel twice (once every two weeks) by convection-enhanced delivery (CED) two weeks later for combination therapy (NK cell therapy, chemodynamic therapy, immune checkpoint blockade therapy) to prevent brain tumor recurrence. Our results showed that the delivery of immune hydrogel through CED infusion twice can significantly prolong the median survival to over 60 days compared with the control group (median survival: 28 days) and pure hydrogel group (median survival: 31 days). In summary, this synergistic therapeutic hydrogel has the opportunity to become the most reliable adjuvant therapy after clinical malignant brain cancer surgery to greatly improve the survival rate. With this innovative approach, the potential for further clinical application is highly expected.

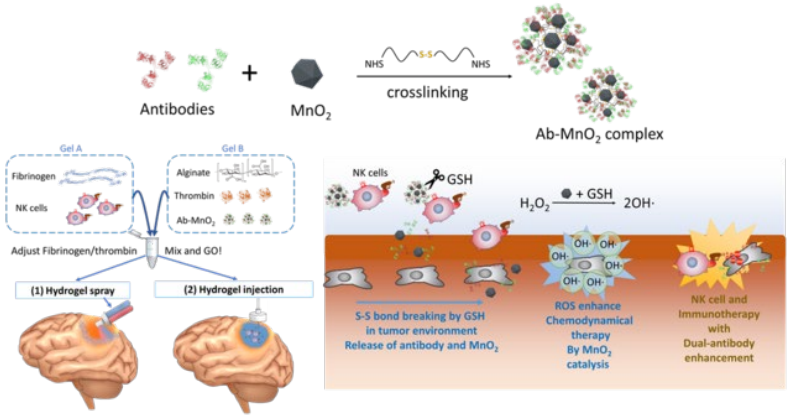

Figure 1. Schematic illustration of the developed immune hydrogel applied to the postoperative treatment of malignant brain cancer.

S5.5-O5

Thermoresponsive dicarboxy hyaluronan/phenanthriplatin nanogels with very high carrier capacity and anticancer efficacy

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Abstract

One of the major drawbacks of hyaluronan (HA) is the low density of -COOH groups, which are the most convenient target for both drug attachment and other modifications. Functional modifications of HA thus often have to be performed at expense of drug carrier capacity and vice versa.

Here, a novel approach how to circumvent this issue is presented and the synthesis of thermoresponsive nanogels with very high carrier capacity for phenanthriplatin (PhPt), a next-generation platinum anticancer drug, is performed. Initially, poly(N-isopropyl acrylamide), a thermoresponsive polymer undergoing a sol/gel transition change around 32°C, is grafted to HA carboxylic group using DMTMM. Next, regioselective sequential periodate/chlorite oxidation, only recently described by us for unmodified HA,[1] is performed. Selectivity of the oxidation towards vicinal diol ensures that it runs only at C2 and C3 of the glucuronate unit of HA and grafted pNIPAM is not impacted. The resulting pNIPAM-grafted 2,3-carboxy hyaluronan (DCH-P) features a pair of carboxy groups in perfect distance and orientation for the chelation of platinum anticancer complexes. DCH-P is soluble at laboratory temperature, but, when loaded with PhPt and heated to 37°C, it self-assembles into nanogels containing up to 60 wt.% of PhPt, nearly twice the maximum capacity of unmodified HA. pNIPAM sol/gel transition also slows the PhPt release rates compared to the unmodified carrier and protects the carried drug.

The *in vitro* testing revealed greatly improved anticancer efficacy of PhPt-loaded DCH-P nanogels compared to a free drug (more than 10x lower IC_{50} for the A2780 ovarian cancer cell line; 4x lower IC_{50} for the MiaPaCa-2 pancreatic cancer cell line) and showed a moderate migrastatic potential.

To summarize, sequential regioselective oxidation, which essentially triples the amount of carboxylic group in HA, can be used in tandem with other modifications of HA to prepare various functional derivatives. The DCH-P nanogel prepared by this method combines both thermoresponsivity and high drug carrier capacity into a highly promising drug delivery system with improved anticancer efficacy, thus demonstrating the potential of this method.

Acknowledgment: The authors would like to thank the Ministry of Education, Youth, and Sports of the Czech Republic project DKRVO (RP/CPS/2022/007) for financial support.

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S5.5-O6

A self-assembly combined nano-prodrug to overcome Gemcitabine chemo-resistance of pancreatic tumors

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Abstract

Introduction. Gemcitabine (GEM), as a first-line chemotherapy drug for pancreatic ductal adenocarcinoma (PDAC) treatment, still faces several clinical challenges, restricted by instability in blood circulations, low tumor selectivity and acquired nature characteristics of chemo-resistance. To solve these challenges, the rational design of combination therapy with GEM and other therapy modalities for PDAC treatment is imperative.

Results and Discussion. Herein, we develop a small molecular self-assembly GSH-responsive nano-prodrug, which can achieve the co-delivery of GEM, Ferrocene (Fc) and nutlin-3a on the achievement of GEM-induced apoptosis with ferroptosis (Figure 1). In this nano-prodrug, the disulfide linkage not only acts as a GSH-responsive trigger but also plays an important role in self-assembly behavior of nanoparticle that can load nutlin-3a. Interestingly, the nutlin-3a could not only effectively sensitize cells to ferroptosis of Fc by inhibiting cystine uptake, but also promote apoptosis by elevating p53 expression. To further enhance the drug accumulation on PDAC tumors and maintain stability in systemic circulations, this nano-prodrug is then encapsulated into plectin1 receptor-targeting phospholipid micelles (DSPE-PEG-PTP). The structure of FSSG was characterized by ^{1}H nuclear magnetic resonance and high-resolution mass spectrometry. The size distribution of Nut@FSSG and Nut@FSSG-PTP were ~184.7 nm and ~209 nm with conspicuous Tyndall effects (Figure 2A, 2C). To prove the successful loading nutlin-3a, the element of S, Fe, F and Cl simultaneously detected in one nanoparticle and overlapped good distribution (Figure 2B). The drug loading efficiency (DLE) of Nut@FSSG-PTP loaded with nutlin-3a was about 11.77% determined by HPLC. The DLE of GEM and Fc were 29.84% and 26.11% respectively (Figure 2D). The cytotoxicity of Nut@FSSG was higher than that of FSSG NPs due to the combination therapy of apoptosis and ferroptosis. The cytotoxicity of Nut@FSSG-PTP was the highest cytotoxicity, indicating that the DSPE-PEG-PTP modification could actively target Panc02 cells (Figure 2E). The results of western bolt suggested that the released nutlin-3a simultaneously achieved the up-regulation of p53 expression and then down-regulation of SLC7A11 expression (Figure 2F). In addition, the cells treated with FSSG NPs, Nut@FSSG and Nut@FSSG-PTP showed obvious swelling of mitochondria and the crista of mitochondrion was ruptured which was recognized as a typical characteristic of ferroptosis (Figure 2G). We are looking forward to positive results *in vivo* which could confirm the inhibition of tumor growth and good biosafety on different mice models.

Conclusion. Our findings provide new insights into the combination therapy of GEM with ferroptosis for reduced chemo-resistance on PDAC treatment.

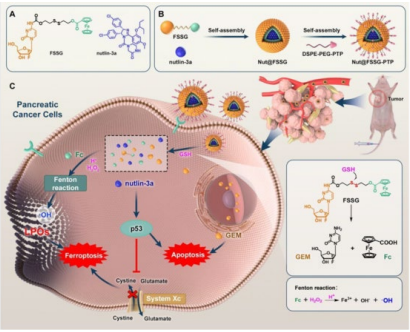

Figure 1. Schematic illustration for self-assembly active targeting nano-prodrug that can co-deliver Fe catalyst (Fc), chemotherapeutics (GEM) and cystine inhibitor (nutlin-3a: p53 activator) to achieve the combination of enhanced ferroptosis and apoptosis therapeutic effect on PDAC tumor inhibition.

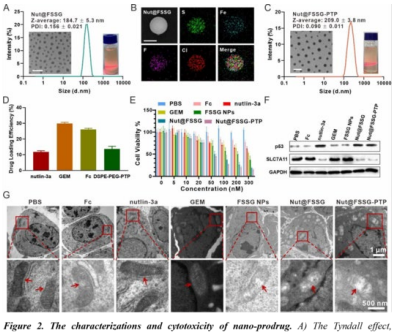

Figure 2. The characterizations and cytotoxicity of nano-prodrug. *A) The Tyndall effect, hydrodynamic diameters (Z-Average), PDI and transmission electron microscopy image (TEM) of Nut@FSSG, Scale bar: 500 nm. B) Element mappings of Nut@FSSG, Scale bar: 100 nm. C) The Tyndall effect, Z-Average, PDI and TEM of Nut@FSSG-PTP, Scale bar: 500 nm. D) The drug loading efficiency of different formulations of Nut@FSSG-PTP. E) The cell viability of Panc02 cells treated with different formulations. F) The intracellular p53 protein and SLC7A11 protein expression were determined by western blot in Panc02 cells. G) Morphology of Panc02 cells treated with different formulations. The red arrows indicate the positions of the mitochondria.*

S5.5-O7

Retinoic acid nanoparticle formulation for in situ haematopoietic stem cell niche modulation.

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Abstract

Treatment of Acute Myeloid Leukemia (AML), a blood cancer, would benefit from an integrated approach enabling the elimination of resistant leukemia stem cells (LSCs) at the bone marrow (BM), through chemotherapy, and at the same time promote the hematopoietic stem cell (HSC) engraftment for the reestablishment of long-term normal hematopoiesis. In this study, we show that light-activatable nanoparticles (NPs) encapsulating all-trans-retinoic acid (RA+NPs) are able to do this. *In vitro*, we have observed that mouse AML cells transfected with RA+NPs give origin to antitumoral M1 macrophages through a mechanism mediated by RIG.1 and OASL gene expression. *In vivo*, RA+NP-transfected-AML cells are able to engraft the BM niche in AML mouse models. Here, RA+NPs can be photo-disassembled inside the grafted cells by blue laser and trigger their differentiation towards the macrophage lineage. Together with this macrophage activation there is a systemic anti-leukemic response within the BM compartment in animals where transplanted cells were carrying RA+NPs, when compared with animals transplanted with cells carrying empty NPs.

In another set of experiments, we show that normal HSCs can benefit from RA+NPs release of retinoic acid inside the BM for better engraftment in the niche. This is the first time that retinoic acid has been shown to promote long-term hematopoietic reconstitution using an *in situ* activation approach, without any exogenous priming or genetic conditioning prior to transplant. Overall, this work opens new windows of opportunities of dual intervention in consolidation therapy inside the BM niche: tackling resistant leukemia and increasing HSC engraftment at the same time.

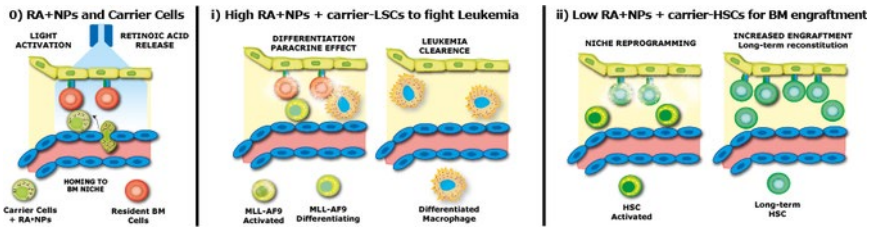

Figure: 0) Cells with a natural tropism for BM are transfected with RA+NPs and transplanted into a mouse model. After BM engraftment, monitored by intra-vital microscopy, retinoic acid release is triggered by light; i) in the context of leukaemia, a high concentration of RA+NPs can be used to trigger the differentiation of leukemia stem cells into anti-tumoral M1 macrophages; ii) in the context of normal HSCs BM engraftment, a low concentration of RA+NPs can be used to enhance long-term hematopoietic reconstitution.

Acknowledgements: This work was financed by the COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia, under project[s] PTDC/05946/2022 and UIDB/04539/2020, UIDP/04539/2020 and LA/P/0058/2020

S5.6-K1

ESB-EORS: unmet challenges and new biomaterials in spine

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Abstract

The talk will address investigations addressing mechanisms of age-dependent degeneration using various mouse models and the contribution of cell senescence to this process. I will discuss findings on the contribution of p16INK4A to SASP maintenance rather than for the onset of senescence in the disc. I will also discuss recent studies that showed that treatment of mice with a cocktail of Dasatinib and Quercetin, known senolytic molecules, reduces the burden of senescent cells in disc compartments and improve aging-related outcomes, clearly establishing causality between cell senescence and disc degeneration. The talk will conclude with biomaterial-based delivery strategies to deliver senolytic compounds to the disc.

S5.6-K2

Nature inspires bioactive, multifunctional 3-D scaffolds for spinal bone regeneration

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Abstract

The regeneration of critical size bone defects still remains a largely unmet clinical need with steadily growing societal and economic impact. Particularly, treatment of diseased spine conditions such as disc degeneration and low-back pain interest a large fraction of the population. Spinal surgery entails invasive procedures, also burdened by high infection rates, and further exacerbated by the ageing of the population and the occurrence of degenerative diseases such as osteoporosis.
Increasingly boosted by recent results, it is widely accepted that bone regeneration should be supported by bioactive scaffolds, able to promote and sustain the natural biological processes at the cell level, accompanied by progressive resorption of the scaffold in compliance with the natural bone turnover. Bioactivity is strongly related with biomimicry of composition and structure of bony tissues and able to comply with biomechanical demands. In this respect, nanostructured, porous scaffolds based on nanocrystalline, ion-doped calcium phosphates are largely recognized as relevant targets. On the other hand, nanocrystalline materials possess chemically active surface permitting the link with various antibiotics or anticancer drugs for local administration with controlled and sustained release profiles. However, intrinsic limitations in fabrication processes make the development of nanostructured, bioactive apatites in the form of 3D scaffolds is still a challenge. The present work illustrates recent results and perspectives offered by biomimetic scaffolds obtained by nature-inspired processes such as biomineralization and biomorphic transformations. Biomineralization reproduces the natural processes occurring *in vivo* during new bone formation, permitting the achievement of hybrid collagen-apatite constructs mimicking the immature bone tissue, thus characterized by excellent regenerative ability. Such scaffolds can be implanted by mini invasive procedures and clinical studies already demonstrated effectiveness in spinal fusion. On the other hand, biomorphic transformation processes are based on heterogeneous reactions occurring in the 3D state by which natural structures mimicking the bone architecture can be converted into nanostructured, ion-doped apatites with damage-tolerant properties, relevant to comply with biomechanical demands acting at the spine level. The active surface, nanostructure and multiscale porosity of these scaffolds also allow the chemical bond with various antibiotic and anticancer drugs which are released in a prolonged and modulable manner permit the loading with various drugs and sustained release modulated by the scaffold bio-dissolution and the diffusive processes related to nano- and micro-scale porosity. Bio-inspired scaffolds thus hold great promise for new spinal regeneration therapies, even in the case of impairing conditions such as infections, osteoporosis or tumors.

S5.6-O1

Anisotropic collagen/hyaluronan 3D printed hydrogels as novel model of Annulus Fibrosus

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Abstract

Intervertebral disc (IVD) degeneration is not completely understood yet and requires the development of novel 3D *in vitro* models. The Annulus Fibrosus (AF) is the outer part of IVD, that confines the Nucleus Pulposus. The latter consists of anisotropic lamellae made of collagen I and glycosaminoglycans (GAG) with fibroblast-like cells located between. Collagen/GAG biomaterials are difficult to synthesize by 3D printing because of the strong interactions between both polymers in solution that leads to the formation of polyionic complexes (PICs).

In this study, we first developed a novel bioink consisting of type I collagen and tyramine functionalized hyaluronic acid (THA) in the appropriate pH and NaCl conditions to inhibit PICs formation. THA gelling was performed either by enzymatic crosslinking (HRP/H_2O_2) or by photo-crosslinking to determine the most efficient crosslinking pathway. The physicochemical study revealed that PICs formation was inhibited when collagen/THA solutions at pH 5.5 and 400 mM NaCl were used. Homogenous collagen hydrogels were formed and fibrillogenesis was not inhibited. The THA enzymatic crosslinking was ineffective while photo-crosslinking led to hydrogels with high mechanical properties (Fig.1A). PICs were inhibited and collagen fibrils were formed since the ionic strength and the pH were close to the collagen isoelectric point (Fig.1B).

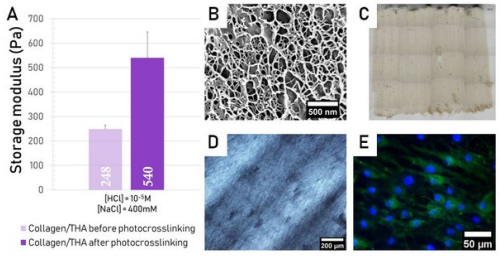

Figure 1 : (A) Mechanical properties of diluted collagen/THA photocrosslinked hydrogels at a ratio of 1:1 ; (B) Cryo-SEM of dense collagen/THA ink at pH5.5 and 200mM of NaCl ; (C) Macroscopic observation of the printed constructs reconstituted after optical microscopy (x4) ; (D) Collagen alignment on photocrosslinked THA impregnated collagen hydrogels observed by polarized light optical mcroscopy (x20) and (E) NHDF morphology and alignment after 7 days captured by fluorescence microscopy (green : actin filaments, blue : nucleus).

Then, this bioink was used to fabricate a novel composite collagen I/THA anisotropic hydrogel by 3D printing. For this purpose, a dense composite bioink consisting of 30 mg.mL-1 collagen and 7.5 mg.mL-1 THA was printed in a PBS bath to trigger a rapid gelling and then photo-crosslinked using eosin and green light. Alternatively, pure collagen dense solutions were printed, gelled, impregnated with THA and crosslinked. 3D printing parameters, were optimized (85 kPa, extrusion, 4.5 mm/s speed and 80% fill-in percentage) to generate anisotropic layers made of collagen or collagen/THA thanks to appropriate shearing and gelling conditions within a PBS and NaOH bath (Fig.1C). Collagen alignment was confirmed by polarized light microscopy (Fig.1D). THA crosslinking in composite hydrogels or after THA impregnation increased the mechanical properties by 3.

Finally, generated constructs were cellularized with fibroblasts to obtain a novel 3D model of Annulus Fibrosus. Several layers were printed with fibroblasts seeded between them. Fibroblasts viability, proliferation and morphology were then studied over 21 daysand revealed that cells adhered to layers, spread, proliferate and aligned along the axis of printed layers (Fig.1E). Hence, our model is a promising biomaterial to study the AF degeneration.

S5.6-O2

In situ forming macroporous hydrogel for nucleus pulposus cell delivery

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Abstract

Introduction. Degeneration of the intervertebral discs of the spine is a common source of chronic pain. Degeneration most commonly initiates in the nucleus pulposus (NP); consequently, regenerating degenerated NP via cell implantation is of clinical interest. The objective was to create an in situ forming, NP cell supportive, microporous, enzymatically degradable hydrogel, with a modulus similar to human NP tissue. Thus, a biohybrid ABA dimethacrylate triblock copolymer was synthesized, possessing an LCST below 37 ºC containing an MMP-degradable peptide flanked by polycarbonate (P(TMOE-3)) blocks to provide mechanical resilience and thermoresponsiveness (Figure 1). This triblock copolymer was then used to form NP cell-laden hydrogels, with or without thiolated chondroitin sulfate via redox initiated free radical polymerization.

Methods. The α-vinyl sulfone, ω-methacrylate thermoresponsive P(TMOE-3) was synthesized and characterized as in [1]. This polymer was then reacted with the MMP-cleavable peptide GCGPQG↓IWGQCG in a 2:1 molar ratio to form the ABA triblock. Thiolated chondroitin sulfate (SH-CS) was prepared as in [2]. The ABA triblock ± SH-CS was combined with 10^6 P1 bovine NP cells/mL in medium containing ammonium persulfate/sodium bisulfite (5 mM) and 25 µL transferred to a cylindrical mold and crosslinked at 37ºC for 10 min. The cell-laden hydrogels were cultured in complete medium under hypoxic conditions (5% O_2). At 1, 7, 14, and 28 days, hydrogel porosity, volume and modulus, cell number, collagen I and II, and sulfated GAG content were measured.

Results and Discussion. Following crosslinking, the hydrogels maintained their volume and phase separation induced by the P(TMOE-3) blocks created macropores throughout. The hydrogels had modulus within the range reported for human NP (2-10 kPa), which was maintained throughout the study. NP cell viability was ~ 80% after crosslinking, and the hydrogels supported cell growth with NP cells homogeneously distributed throughout CS-free hydrogels. Collagen II and sulfated GAG accumulation was apparent with no collagen I, and the NP cells effectively degraded the peptide block to remodel the network.

Conclusion. The CS-free hydrogels are promising as am NP cell supportive scaffold. Further studies under more physiologically mimetic conditions, including dynamic loading, are required to assess their potential.

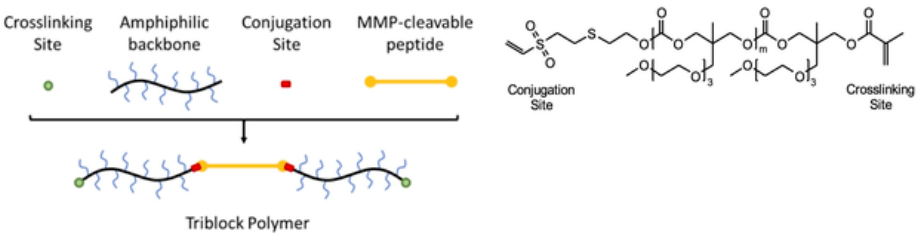

Figure 1. Schematic of ABA triblock copolymer structure (left) and vinyl sulfone-P(TMOE-3)-methacrylate (right).

References. [1] A. J. Brissenden, B. G. Amsden. Journal of Polymer Science 58 (2020) 2697-2707; [2] A. Köwitsch, M. J. Abreu, A. Chhalotre, M. Hielscher, S. Fischer, K. Mäder, T. Groth,. Carbohydrate polymers 114 (2014) 344-351.

S5.6-O3

Artificial extracellular matrices to regulate nucleus pulposus regeneration

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Abstract

Reestablishing the gelatinous nucleus pulposus (NP) matrix is pivotal to restoring intervertebral disc function following herniation and subsequent discectomy. Although current nucleus replacement strategies aim to maintain disc height and its cushioning function, they largely fail to regenerate or reverse the pathogenesis at the cellular level, thus leading to future reherniation events. Generally, disc degeneration is characterized by the extensive degradation of matrix proteoglycans, namely aggrecan. These unique bottlebrush-like polyelectrolyte structures bearing chondroitin sulfate (CS) side chains render hydration to NP and its unique ability to resist compression. Incidentally, the decline in aggrecan content drives vascular and nerve growth in an otherwise avascular and aneural healthy disc, thus marking the early event of discogenic pain. We hypothesized that the inhibitory effect of aggrecan is mainly conferred by its highly charged CS side chains. Hence, spatial presentation of CS chains within the matrix could endow anti-inflammatory, anti-neurogenic, and anti-vasculogenic abilities. Accordingly, we developed a hydrogel matrix incorporating aggrecan-mimicking bottlebrush structures with varying chondroitin sulfate side chains.

Hyaluronic acid (HA) was chosen as the base matrix, and it was functionalized with methacrylic anhydride to confer light-promoted crosslinking and tunable viscoelastic properties. The matrix was further tethered with linear polysaccharide, pullulan, that can serve as a core for subsequent tethering of CS bristles via Schiff-base reaction. The molar concentrations of CS (22kDa) were varied to obtain hydrogel constructs with increasing CS side chains, without varying pullulan and HA content. The successful grafting of CS to pullulan and its subsequent conjugation to HA was confirmed using Fourier Transform Infrared Spectroscopy, Fluorescamine, and Dimethyl methylene blue assay. Rheological characterization showed a faster stress relaxation in the hydrogel with increasing CS content without any change in the elastic moduli. Concordantly, the anabolic effect in response to varying CS content was elucidated using primary bovine nucleus pulposus cells encapsulated within the composite hydrogel by an increase in mRNA and protein expression level for Aggrecan and Type II Collagen. Further, the role of CS aggregates in inhibiting endothelial and neural invasion was demonstrated by co-culturing the 3D encapsulated bovine NP cells with either endothelial (HUVECS) or neuroblastoma (SH-SY5Y) cells separated by transwell insert. Altogether, these findings provide critical insights into the significance of incorporating matrix-bound glycosaminoglycan aggregates in hydrogel therapeutics for degenerative disc disease, specifically to regulate catabolism, inflammation, angiogenesis, and neurogenesis that are typically associated with the etiology of lower back pain.

S5.7-O1

Hexagonal boron nitride quantum dots embedded biopolymer for peroxidase-assisted colorimetric detection of pathogens

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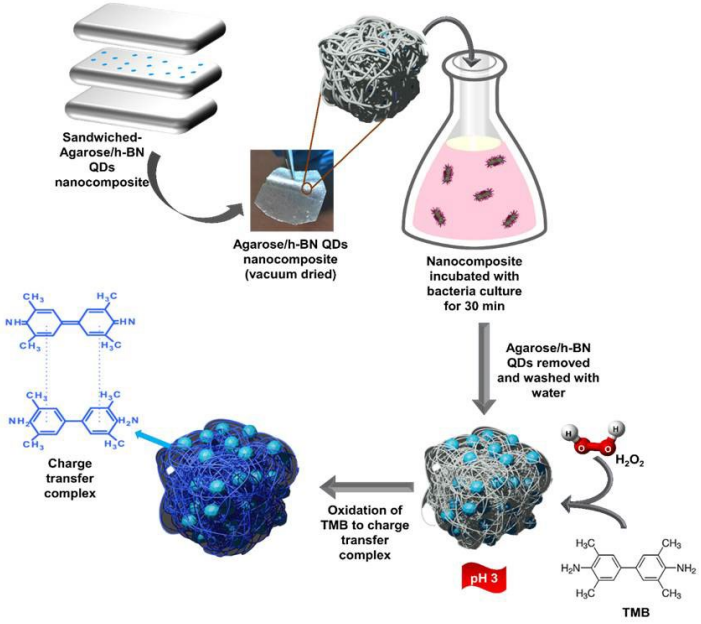
Abstract

Pathogen detection has become a major research area all over the world for water quality surveillance and microbial risk assessment. Therefore, designing simple and sensitive detection kits plays a key role in envisaging and evaluating the risk of disease outbreaks and providing quality healthcare settings. In this regard, nanozyme-based colorimetric analysis has attracted considerable attention from researchers all over the globe. Herein, we have designed a facile and low-cost colorimetric sensing strategy for selective and sensitive determination of β-galactosidase producing pathogens through hexagonal boron nitride quantum dots (h-BN QDs) intercalated with agarose biopolymer as a solid substrate based on the peroxidase-like catalytic activity. The h-BN QDs was established as nanozyme that showed prominent peroxidase-like activity, which catalyzes 3,3',5,5' -tetramethylbenzidine (TMB) oxidation by H_2O_2. The β-galactosidase enzyme then partially degrades β-1,4 glycosidic bonds of agarose polymer resulting in accessibility of h-BN QDs on the solid surface. This assay can be conveniently conducted and analyzed by monitoring the blue color formation within 30 min. After optimization, the limit of detection was calculated to be ~$5.0x10^2$ and ~$3.0x10^3$ for *Escherichia coli* (E. coli) and *Klebsiella pneumonia* (K. pneumonia), respectively. Furthermore, this novel sensing approach is an attractive platform that was successfully applied to detect E. coli in spiked water samples with good accuracy, indicating its practical applicability for the detection of pathogens in real samples. Based on these interpretations, we anticipate that this colorimetric pathogen detection technique on solid surfaces using the biopolymer and peroxidase mimic activity of h-BN QDs may provide a new way for on-site detection of pathogens present in water.



Scheme 1. Schematic representation of TMB oxidation by agarose/h-BNQDs incubated with pathogens.

S5.7-O2

Cryopreservation of Red blood cells in absence of toxic cryoprotectants via ice templating

Kankan Qin, Christophe Hélary, Francisco M Fernandes

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Abstract

In most conditions freezing threatens the integrity and the viability of biological entities. Paradoxically, cryopreservation is the single available solution to extend the lifespan of living cells and to preserve tissues and biomolecules. In recent years, the field of cryopreservation has increasingly relied upon toxic cryoprotectant agents (CPAs) such as dimethyl sulfoxide (DMSO) or glycerol[1]. However, the limitations imposed by toxic CPAs still hamper the widespread use of cryopreservation in blood products for most transfusion purposes. In this communication, we discuss the relevance of a materials science strategy, ice templating (or directional freezing), to obtain high yield of functional red blood cells (RBCs) after freezing and thawing in absence of toxic CPAs, a strategy that could revolutionize cell therapies.

Ice templating has evolved from a materials processing technique to an increasingly relevant technique in biomaterials[2,3]. Using a home-built ice templating setup[4] (Fig. 1a) we controlled the ice front velocity of sheep RBC suspensions. After freezing, cell samples were kept at -80°C for days 1 and 100 to assess cell recovery by hemolysis, flow cytometry (esterase activity), scanning electron microscopy and by 3D confocal microscopy.

Multiple concentrations of RBCs were frozen using ice templating between 10 and100 µm.s^{-1} to determine the optimal cryopreservation conditions in absence of toxic CPAs. Higher ice front velocities and BSA contents provided the best cell recovery rates (c.a. 90%, Fig. 1d) and esterase activity fractions measured under flow cytometry[5,6]. Results between 1 and 100 days storage at -80°C showed no significant difference in cell activity and integrity, suggesting the method is adapted for long-term cell preservation.

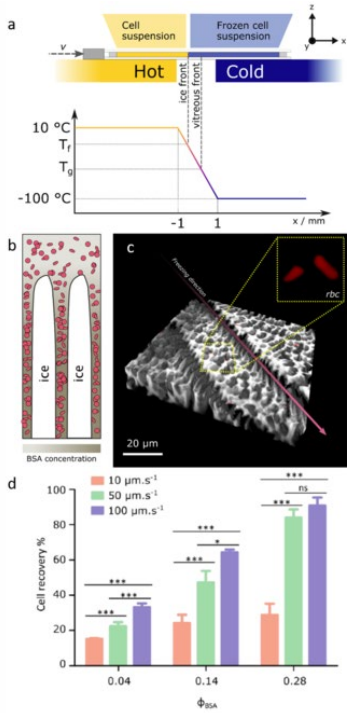

Figure: a) Freezing setup and temperature profile of the cryopreservation process. b) Encapsulation of RBC in between ice crystals. c) Confocal microscopy of RBCs encapsulated in BSA. d) RBC recovery as a function of ice front velocity.

Because of control over the cell environment formed within the interstitial space formed by ice crystals, ice templating of living cells allows addressing the challenges of cryopreservation under a new perspective. Beyond RBCs, this approach is currently being explored to the preservation of other cell types, relevant from fundamental biology research labs up to the clinic.

References. [1] Smith AU, Lancet 1950;256:910–911. [2]. Wegst UGK, Philos. Trans. A. 2010; 368:2099–2121. [3]. Qin K, Mater. Chem. B 2021; 9:889–907. [4]. Qin K, J. Phys. Chem. Lett. 2020;11,7730–7738. [5]. Qin K et al. PCT/FR2022/05530 6. Qin K et al, in preparation.

S5.7-O3

In situ formed ROS-responsive hydrogel with STING agonist and gemcitabine to intensify immunotherapy against pancreatic ductal adenocarcinoma

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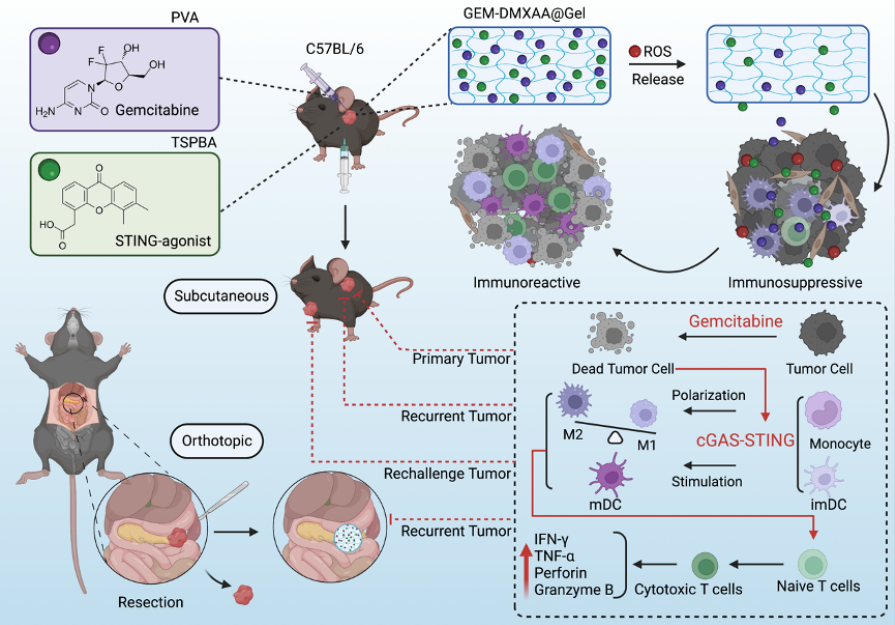
Abstract

Introduction. Pancreatic carcinoma is an aggressive solid malignancy with a dismal survival rate. Gemcitabine alone could alleviate the condition of most cases, but unfortunately with very limited prolongation of the 5-year survival rate. Stimulator of interferon (IFN) genes (STING) has emerged as one of the most important adjuvants to boost cancer immunotherapy. Injectable hydrogel as a powerful drug delivery platform has been extensively explored. Here, we designed a reactive oxygen species (ROS)-responsive drug-delivery hydrogel to co-deliver gemcitabine and the STING agonist DMXAA.

Experimental Methods. The ROS-responsive hydrogel was prepared by the crosslinking reaction between diols of PVA and benzoboric acids on the ROS-labile linker TSPBA. The hydrogel carrying both DMXAA and gemcitabine was prepared by adding the equal volume of DMXAA-containing TSPBA solution to the gemcitabine-containing PVA solution. The therapeutic effect of the GEM-STING@Gel *in vivo* was investigated by an immunocompetent mice model with subcutaneous and orthotopic pancreatic cancer model.

Results and Discussion. The TSPBA linker was synthesized and confirmed by 1H-NMR (Fig. 1A,B) and the ROS-responsive hydrogel was prepared successfully (Fig. 1C). This hydrogel-based strategy, delivering GEM alone, DMXAA alone, or their combination, all showed notable therapeutic effects compared to free drugs. Remarkably, GEM-DMXAA@Gel resulted in a complete therapeutic response with no detectable tumor tissue, demonstrating the excellent therapeutic synergy of the chemo-immunotherapy combination (Fig. 1D,E). To validate the recurrence-preventing ability of the therapeutic hydrogel, we established an orthotopic model mimicking the clinical scenarios of postoperative recurrence (Fig. 1F). Interestingly, the mice treated with single-dose of postoperativetherapeutic hydrogel showed no obvious recurrence and increased infiltration of the CD8+ T cells (Fig. 1G,H).

Conclusion. We successfully developed an ROS-responsive hydrogel loaded with gemcitabine and STING agonist to achieve an integrative strategy of both chemotherapy and immunotherapy, which can enhance antitumor immune responses synergistically.



Scheme. Illustration of immunotherapy strategy using an ROS-responsive hydrogel.

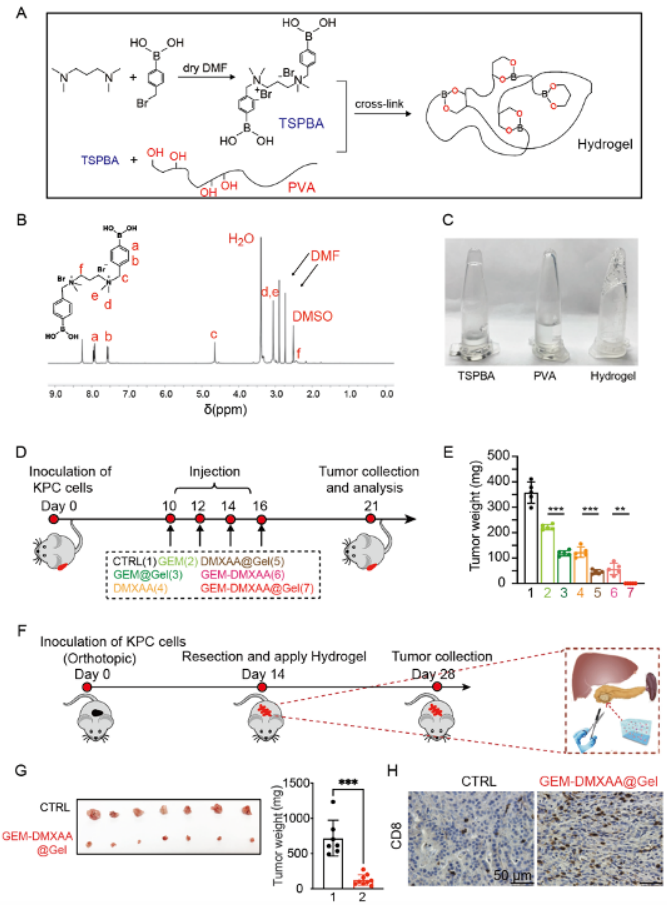


Figure 1. Characterization and application of GEM-DMXAA@Gel. A) Synthesis route of TSPBA linker and PVA-TSPBA hydrogel. B) ^{1}H-NMR spectrum of TSPBA linker. C) Hydrogel formation by mixing TSPBA linker (5%, w/w) with PVA matrix (5%, w/w). D) Schematic illustration of the subcutaneous tumor model in vivo experimental assay. E) Measurements of tumor weights in treated mice at the end of the assay. F) Schematic illustration of the orthotopic tumor model . G) The bright field photos and the weights of the tumors. H) IHC studies marking CD8 staining of the tumor sections after indicated treatments.

S5.7-O4

An ionic liquid ablation agent for local ablation and immune activation in pancreatic cancer

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Abstract

Introduction. Pancreatic carcinoma, featuring early metastasis and poor survival, is likely to become the second leading cause of cancer related death in the next decade.[1] The immunosuppressive microenvironment and desmoplastic stroma in pancreatic carcinoma might contribute to the lack of therapeutic efficiency of systemic therapy.[2–4] Herein, an ionic liquid ablation agent (LAA), synthesized from choline and geranic acid, which triggers necrosis-induced immunotherapy by remodeling an immunosuppressive “cold” tumor to an immune activated “hot” tumor is described.

Experimental Methods. LAA was synthesized via an ionic metathesis reaction between choline bicarbonate and geranic acid at a molar ratio of 1:1 (Fig. 1A). The tumor ablation ability and immune activation ability were demonstrated by *in vivo* studies up to now.

Results and Discussion. LAA displayed a greater diffusion-based ablative ability in subcutaneous xenograft pancreatic cancer in C57BL/6 mice, which was significantly larger than that after absolute ethanol treatment (Fig. 1B, C, D). The effect of IL ablation on TME remodeling was mainly characterized by increasing the infiltration of CD8 T cells as well as reducing the infiltration of immunosuppressive macrophages (Fig. 2A, B, C, D, E).

Conclusion. LAA, a type of chemical ablation agent, to initiate and amplify the immune response against immunosuppressive solid tumors, providing a potential solution to immunotherapy inefficiency.

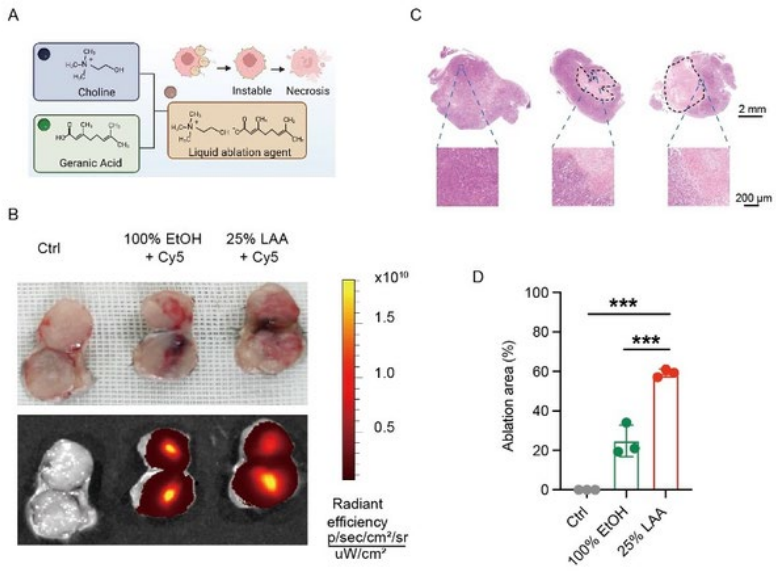

Figure 1. LAA effectively ablates solid tumors. A) Schematic illustration of IL-induced antitumor effect. B) Representative images of in vivo Cy5 fluorescence distribution, with bright-field photos and fluorescence images of the tumor cross-sections in the subcutaneous pancreatic tumor at 24 h after intratumoral injection of Saline plus Cy5 (Ctrl), EtOH plus Cy5, or LAA plus Cy5. C) Histological study of the treated tumors showing necrotic areas after ablation (marked by dashed lines) . D) Comparison of the ablated areas in the treated tumors.

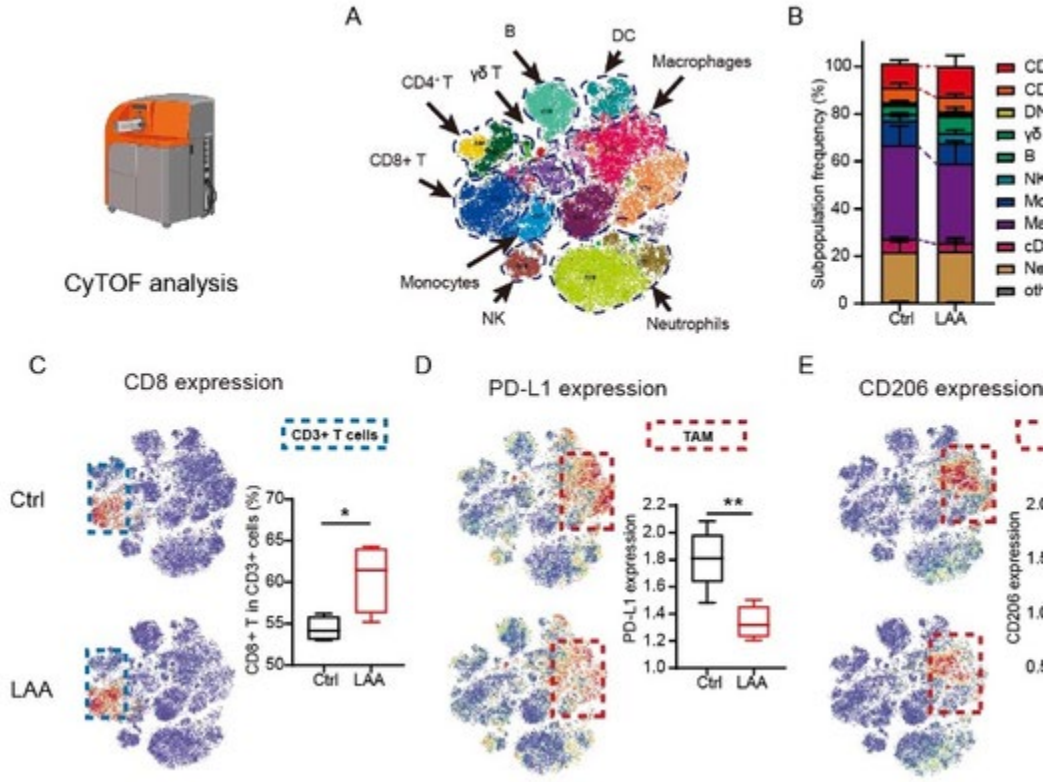

Figure 2. LAA ablation alters the tumor immune microenvironment. A) A t-distributed stochastic neighbor embedding (tSNE) plot via nonlinear dimensionality reduction showing the immune cell clusters in the LAA-treated tumors. B) Frequency of the immune cell clusters in the treated tumors. C) Color-coded tSNE plots showing CD8 expression in CD3+ cells with quantitative comparison. T-SNE plots showing expression levels of D) PD-L1 and E) CD206 in TAMs with corresponding quantitative comparisons.

S5.7-O5

Development of tuneable hyaluronic acid hydrogels for transcranial drug delivery

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Abstract

Introduction. The passive diffusion of biomolecules through the skull could deliver drugs to the cortex 1. Continuous delivery through the skull requires a sustained release of drugs over a long period1. Hydrogels are a cross-linked network of polymers capable of entrapping therapeutic moieties and controlling their release, advantageous for treating neurodegenerative diseases 2. We designed a hyaluronic acid (HA) hydrogel multimodal system loaded with molecules of different molecular weights. The physicochemical properties and cytocompatibility of the system were characterised and optimised then the transcranial diffusion was studied ex-vivo and in-vivo.

Methods. The hydrogels were tuned and developed by crosslinking various quantities of HA (1MDa) with four arm- Polyethylene glycol–amine 2000 MW (4-arm PEG2K-NH2) at varying ratios in the presence of (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM). The transcranial diffusion was tested *ex vivo* using skulls of rats aged 8-10 weeks and fluorescent-tagged molecules of various molecular weights as shown in Figure 1 B. It was further studied *in vivo* by placing Manganese chloride on mice's skulls and scanning Magnetic Resonance Imaging (MRI).

Results and discussion. Tuneable HA hydrogels were prepared, as shown in Figure 2. Several molecules with various molecular weights were tested ex-vivo dyes of low molecular were able to diffuse through the skull for 24-48 hours. The concentration of DMTMM influenced the swelling behaviour of hydrogels and the degradation.

MRI scans showed a slow diffusion of the loaded molecules over time *in vivo* as shown in Figure 3. The solution of Manganese chloride diffused quickly, and MRI scans show that it reached sub-arachnoid space within 1 hr of application and subsequently the cortical regions, while when placed in hydrogel Manganese chloride diffused slowly over time. Further studies are ongoing to determine the diffusion rate and factors affecting it.

Keywords: Hydrogel; Hyaluronic acid; Transcranial diffusion

Acknowledgment. This project received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant agreement No. 813263. It is also supported in part by a grant from SFI and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073_P2.

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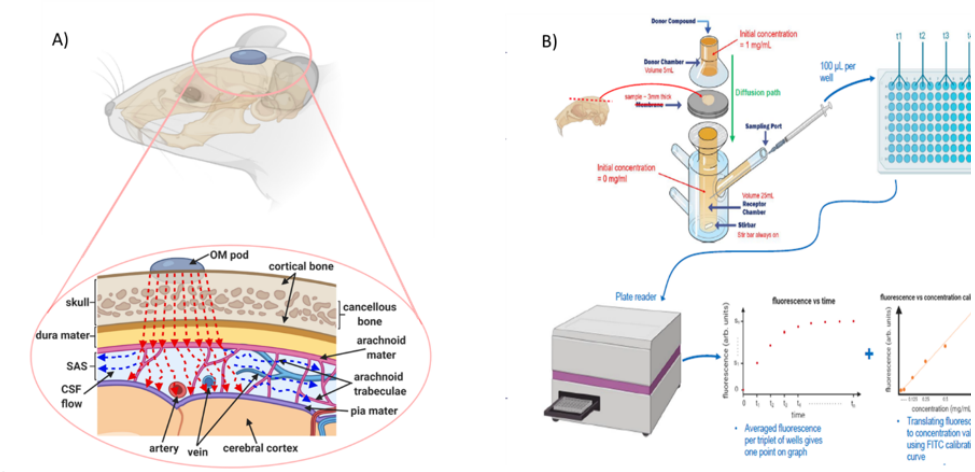

Figure 1. *Schematic summary of the project. A) Schematic representation of the transcranial diffusion in which a pod is placed over the skull of a rat. The pod releases its contents over time through the skull into the subarachnoid space (SAS). B) Schematic representation of the method used to quantify the diffusion of the molecules ex-vivo using Franz Cell and fluorescent molecules of various molecular weights.*

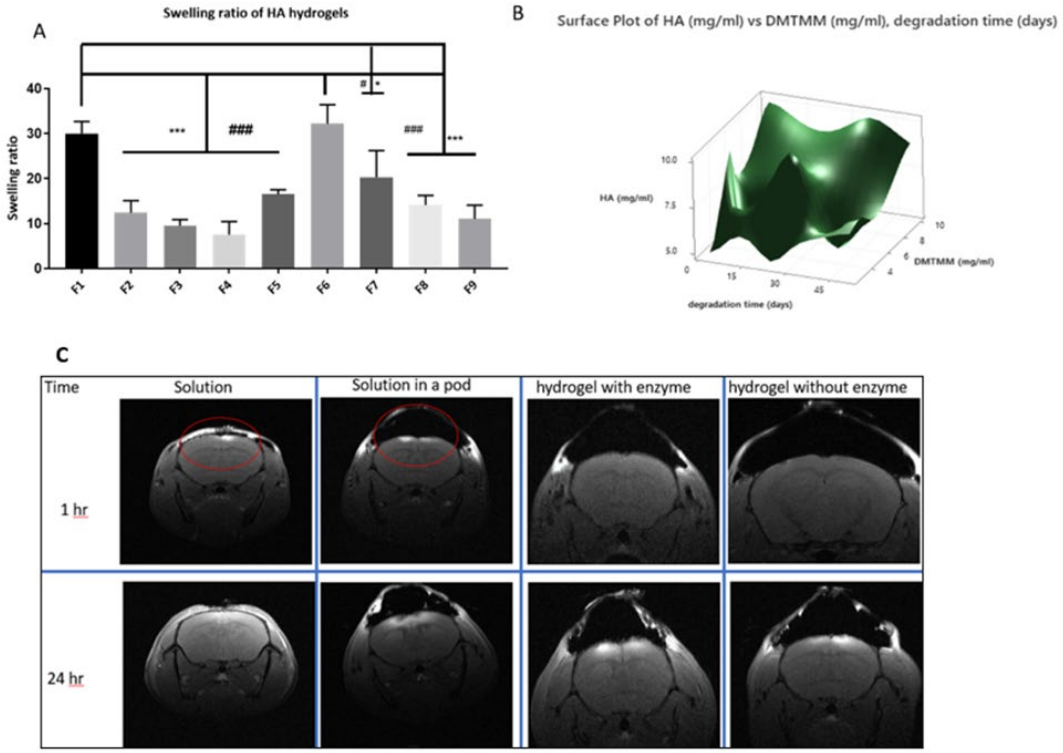

Figure 2. ***A)*** *The swelling ratio of HA hydrogels in artificial cerebrospinal fluid after 48 hours. Data presented as mean ± SD (one-way ANOVA) (n = 3).* ***B)*** *Surface plot of the effect of HA concentration vs DMTMM concentration on the degradation time of hydrogels.* ***c)*** *Representative qualitative images of MRI scans which show the diffusion of manganese chloride through the skull of mice. The mice were scanned 1 hr after the application and 24 hrs later. The manganese chloride solution diffuses through the skull within an hour while when placed in hydrogel manganese chloride takes longer to diffuse.*

S5.7-O6

Delivery of GSDMD-N mRNA with engineered PBAE nanoplatforms to boost anticancer immunity as pyroptosis induced *in situ* tumor nano-vaccine against pancreatic cancer

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Abstract

Introduction**.** *In situ* cancer vaccine is capable of leveraging the immune system to attack tumors by utilizing the whole repertoire of TAA (tumor associated antigens) at tumor site. Pyroptosis caused by N-terminal of GSDMD (GSDMD-N) could be adopted to extensively release TAA *in situ*, while its delivery to tumor is of great challenge. Highly-branched poly(β-amino ester)s (hPBAE) is known as a gene vector with high transfection efficacy, and its multiple terminal groups promised it tailorable for certain functionality enhancement.

Experimental Methods. We identified a hPBAE formulation exhibiting powerful immune-stimulating function with certain side alkyl chain and end capping monomer, which further assemble with pancreatic cancer targeting peptide modified lipid-PEG (DSPE-PEG-PTP), forming a gene delivery nanoplatform IA-hPBAE^{PTP} functioning also as an immunoadjuvant. With GSDMD-N mRNA encapsulated inside, we proposed a novel pancreatic cancer immunotherapy strategy, Pyroptosis-induced And Immune-stimulating *in situ* cancer Vaccine (PAIVAX) with high biocompatibility, accurate targeting, efficient transfection and immune-activating ability (Fig. 1a).

Results. The immune-stimulating ability of IA-hPBAE was validated by increased extracellular TNF-α and IFN-β and phosphorylation of STAT3, NF-κb and STING after incubation with THP-1 cells (Fig. 1b,c). PAIVAX nanoparticle was successfully prepared according to TEM (Fig. 1d). Elevated GSDMD-N expression and pyroptotic morphology of KPC cells were observed after treatment with PAIVAX (Fig. 2a,b). Notably, PAIVAX resulted in significant shrinkage of tumor and activation of anti-tumor immunity, demonstrating strengthen of pyroptosis-induced immune responses by IA-hPBAE^{PTP} as immunoadjuvants in flow cytometry and RNA-seq (Fig. 2c-f). Orthotopic recurrence model reflected efficient inhibition of tumor recurrence and enhanced anti-tumor memory (Fig. 2g-h).

Conclusion. Our designed *in situ* cancer vaccine PAIVAX has achieved boosted antitumor immune responses profited from GSDMD-N as pyroptosis inducer and gene vector as immunoadjuvants.

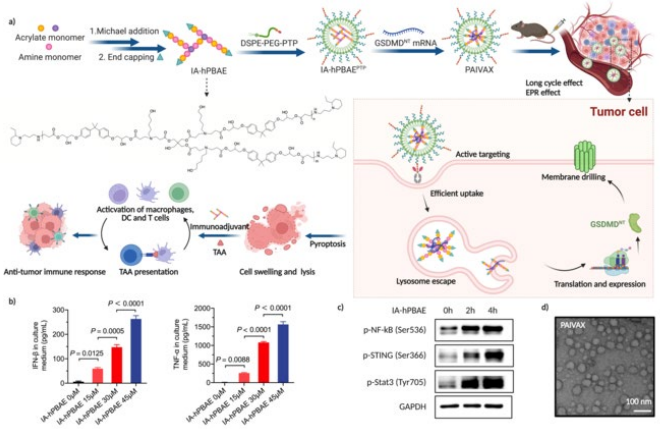

Figure 1. a) Schematic illustration. b) Dose escalation of IA-hPBAE incubation with THP-1 and corresponding TNF-α and IFN-β levels. c) Western blot of kinetics of activation of STAT3, NF-κb and STING in THP-1 stimulated with IA-hPBAE. d) TEM image of PAIVAX (IA-hPBAE^{PTP}@GSDMD-N).

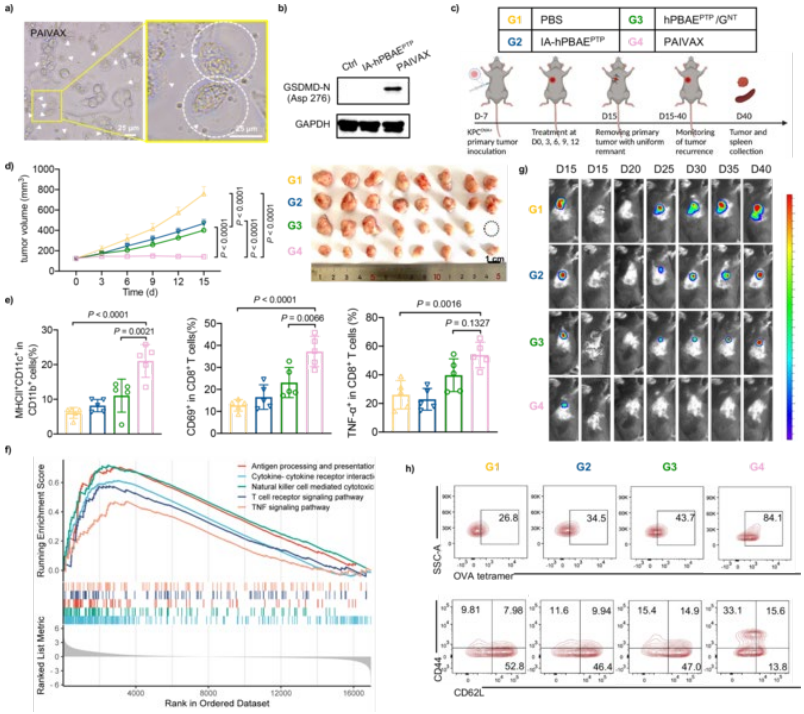

Figure 2. a) Pyroptotic morphology and b) western blot of GSDMD-N in KPC cells after PAIVAX treatment. c) Schematic illustration of tumor treating and recurrence model. d) Tumor growth curve and photograph of tumors with indicated treatments. e) Flow cytometry regarding tumor-infiltrating activated APC and CTL. f) GSEA results of PAIVAX versus ctrl based on tumor RNA-seq. g) Monitoring of tumors in recurrence model and h) representative flow cytometric analysis of CTL in spleen.

S5.7-O7

Heparin-RBD complex in multilayered polymeric particles enhances induction of serum antibodies and induces protective immunity against SARS-CoV-2

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Abstract

Multilayered polymeric particles work as carriers of bioactive compounds with controlled release triggered by the disassembly of the layers. It also allows chemical stability of the compound (embedded into the polymer layer) along with transport through the bloodstream and tissues—an increasingly interesting application in vaccine development.[1,2] Multilayered polymeric particles can also recruit immune cells, promote the internalization of antigens and, thus, exert strong adjuvant effects.[3] Here, we present a versatile multilayered polymeric particle approach carrying a recombinant form of the receptor binding domain (RBD) of the SARS-CoV-2 (PLTP-H-RBD). Our particle core is composed of poly-DL-lactide (PDLLA) coated with polyethyleneimine (PEI, a positive polyelectrolyte), and prepared by nanoemulsion/solvent evaporation method. Triton X-100 was applied to match the interface between the hydrophobic PDLLA surface with the charged layer of PEI. Next, we functionalized the core using a Layer-by-Layer technique with heparin as a negative polyelectrolyte (PLTP-H), an outer layer responsible for anchoring the RBD protein produced in HEK293T cells. The PLTP-H particles presented low toxicity and reached a complexation efficiency of 95% for the RBD, showing a final hydrodynamic diameter of 140 nm with a Zeta Potential of -46 mV. To answer whether the PLTP-H-RBD particles were able to enhance specific antibody responses against the SARS-CoV-2, K18 mice were subcutaneously immunized with three doses of 10 µg of PLTP-H-RBD and challenged 14 days after the third dose with 5x104PFU of the Wuhan strain (GenBank MT350282.1). The immunization with PLTP-H-RBD particles promoted a strong adjuvant effect and led mice to induce high levels of serum IgG antibodies capable of neutralizing pseudoviruses and the SARS-CoV-2 viable particles in culture conditions. In addition, immunization with the PLTP-H-RBD particles conferred enhanced protective immunity to the challenged animals, resulting in a significant reduction of viral load in the lungs of mice infected with SARS-CoV-2. These findings highlight the vaccine potential of the PLTP-H system and represent a proof of concept for the nanoparticle platform as a delivery/adjuvant strategy for recombinant antigens aiming at the induction of neutralizing antibodies against SARS-CoV-2.

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S6.1-K1

Mechanically-tunable wholly-cellular bioinks for large-scale scaffold-free biomanufacturing

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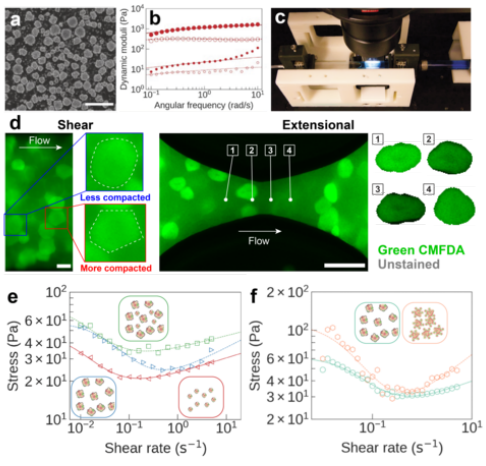
Abstract

Bioprinting pluripotent human induced pluripotent stem cells (hiPSCs) has shown great potentials for therapeutic-scale and on-demand biofabrication. Particularly, wholly-cellular bioinks consisting of compacted hiPSC aggregates (hAs) have been recently used for creating functional tissue constructs with optimal cell density and more native extracellular environments. However, two engineering challenges impede the advent of practical biofabrication from such bioinks: (1) the complex fluid dynamics of the wholly-cellular bioinks have not been fully characterized during and after printing, thus precluding optimal trace-stability and cell functionality, and (2) limited techniques are available to optimize the mechanical properties while retaining the autologous nature. To address these challenges, we developed non-invasive methods to probe the mechanics of wholly-cellular bioinks (Fig. 1a), which exhibit yield-stress fluid rheology that can be described by the fractional Jeffrey model (Fig. 1b). We further captured the dynamics and structural variations of the bioink in a bespoke capillary rheometer that allows for accurate measurements of flow properties through customized channel geometries, and simultaneous visualization of the spatiotemporal evolution (Fig. 1c). We demonstrated the hA morphology under practical shear and extensional flows and distinguished the flow-induced deformation from the viscoelasticity-induced self-relaxation in the hAs, elucidating a complex flow behavior arising from hA interactions dominated by different timescales (Fig. 1d). Based on these findings, we explored two xeno-free methods to engineer the rheology of bioinks by enhancing the topological interactions: (1) via multimodal hA size distributions in the bioink, and (2) via modified hA shapes into non-convex and self-lockable patterns. The modified bioinks were rheologically characterized and compared with single-modal, spherical-organoid bioinks. In both methods, we observed significant increase in the yield stress (Fig. 1e and Fig. 1f), showing reinforced bioink strength for large-scale biomanufacturing.



Characterization and tuning of wholly-cellular bioinks. *(a) WTC-11 hAs at day 3. Scale bar: 400 µm. (b) Frequency responses of the storage (solid) and loss (hollow) moduli for hAs (large makers) and single-cell (small markers) bioinks. (c) Visualizable InExpensive Wireless Rheometer (VIEWR) mounted on an inverted microscope for the visualization of bioinks during flow measurements. (d) hA morphology under shear (left) and extensional (right) flows from VIEWR. Scale bars: 200 µm (left) and 1 mm (right). (e) Flow curves of single-modal large (blue) and small (red) hAs, as well as bimodal (green) hA bioinks. (f) Flow curves of spherical (teal) and 6-arm shaped (orange) hA bioinks. Solid lines in (e-f): Fitting lines from a modified Herschel-Bulkley model.*

S6.1-O1

Volumetric printing across melt electrowritten scaffolds fabricates multi-material living constructs with tunable architecture and mechanics

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Abstract

Introduction: Major challenges in biofabrication revolve around capturing the complex, hierarchical composition of native tissues, including biochemical and biomechanical cues. Volumetric bioprinting (VBP), an ultra-fast, light-based technique sculpts cell-laden hydrogel bioresins into convoluted three-dimensional structures in a layerless fashion, overcoming some of the key limitations of conventional 3D bioprinting approaches. However, the cell-friendly, hydrogel-based bioresins commonly used in VBP lack the mechanical stability that is, for a large number of organs and tissues, essential to replicate native-like functions. Herein, for the first time, the possibility to converge VBP with melt electrowriting (MEW), which excels at patterning microfibers, is shown for the fabrication of tubular hydrogel-based composites with enhanced mechanical behavior.

Methods: MEW of tubular structures was carried out using polycaprolactone, and VBP was performed using gelatin methacryloyl bioresin with photoinitiator lithium-phenyl-2,4,6-trimethylbenzoyl-phosphinate. First, the light attenuation was characterized followed by the assessment of the mechanical properties of the hybrid VBP-MEW constructs, tensile, burst pressure and bending properties were measured for the different pore architectures and increasing layer heights. Finally, the ability to create complex, native-like architectures using this hybrid printing approach was explored. As proof-of-concept, a tri-layered cell-laden vessel model was fabricated.

Results and Discussion: Tubular MEW scaffolds with different pore architectures (rhombi and hexagons) were successfully incorporated into the VBP process (Fig.1A). Light attenuation studies revealed 34° rhombi (91.6±1.6 %) as the most permissive, also showing low deviations in print accuracy (<3%) at 40 layers. Tensile, burst and bending (Fig.1B) mechanical properties were tuned, altering the electrowritten mesh design resulting in complex, multi-material tubular constructs with customizable, anisotropic geometries that better mimic intricate biological tubular structures. The ability to precisely print over and within MEW scaffold was exploited to create perfusable, engineered vessel-like features (valves, branches, fenestrations) (Fig.1C). Finally, a tri-layered cell-laden vessel model was developed through sequential VBP printing, exhibiting distinct compartments as observed in native vessel structures (Fig.1D).

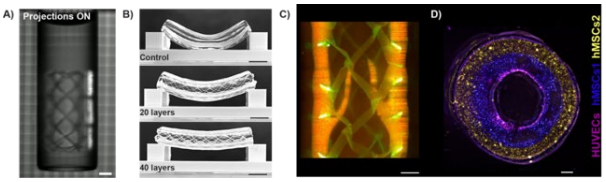

Figure 1: A) VP printing of tubular MEW mesh. B) Bending Test with and without meshes C) Functional venous valve C) Proof-of-concept multi- material and cell vascular structure.

Conclusion: For the first time, we demonstrated the combination of reinforcing melt electrowritten structures with the ultra-fast volumetric bioprinting approach. Despite the incorporation of opaque components into the printing process, the design freedom characteristic of this approach was maintained and exploited to create engineered vessel-like structures. This multi-technology convergence offers a new toolbox for manufacturing hierarchical and mechanically tunable multi-material living structures.

S6.1-O2

An architectured mechano-hybrid-scaffold induces endogenous healing of critical size bone defects in small and large animals

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Abstract

Until today, no pure biomaterial strategy for bone defect regeneration exists, even though associated low costs, off the shelf availability and a low risk of side effects are clear advantages. We have previously shown that a soft, collagen-based biomaterial with a channel-like pore architecture is able to induce development-like endochondral ossification for the healing of critical size bone defects (Petersen et al., Nat Commun 2018). Here, we report on the incorporation of a 3D printed support structure for the mechanobiological optimization of the biomaterial and its evaluation in small and large animal models.

Support structure architectures were designed in SolidWorks (Dassault Systems) and produced from medical grade polycaprolactone (PCL) by selective laser sintering. Following a design optimization, mechano-hybrid-scaffolds (MHS) were produced by introducing the support structure into the fabrication process of the collagen-based guiding structure. Mechano-bioreactors were used to characterize cell recruitment into MHS and to analyze the stability of the support structure under *in vivo*-like loads. MHS were first implanted into 5mm critical size defects in the rat femur and subsequently evaluated in a 3cm defect in the sheep tibia stabilized by two locking compression plates. No bioactive molecules, cells or bone grafts were added. Bone defect healing was studied via x-ray, µ-CT and (immune)histology.

The strong cell recruitment potential of the collagen-based guiding structure was not impaired in MHS. Optimized sintering parameters for the support structure resulted in MHS that did not show fatigue during cyclic loading (>$1*10^6$ compression cycles). Implantation of MHS into the critical-size femoral bone defect in rats revealed a robust induction of endochondral ossification. Compared to pure collagen scaffolds, the integrated support structure in MHS further improved the linear alignment of extracellular matrix fibers and stabilized the endochondral healing process. Nine weeks after implantation, three out of six animals were in the process of bony bridging as verified by µ-CT and histology. Even in the 3cm tibia defect in sheep, MHS induced a linear alignment of collagen fibers across the entire bone defect. While macro-porous implants have shown limited ability to enhance bone healing in large animals in the past, guided mineralization across the bone defect was observed in MHS.

The results of this study verify the potential of the architecturally and mechanobiologically optimized MHS to induce the regeneration of critical size bone defects. The use of clinically approved and fully degradable materials are regarded beneficial for a future translation to the clinics.

S6.1-O3

Influence of acid type and pH on lyophilised collagen I scaffold architecture

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Abstract

Introduction. Collagen is an excellent biomaterial for creating scaffolds, offering tuneable degradability and stiffness alongside cell recognition sites. Porous collagen scaffolds can be formed through freeze-drying of an acidic collagen slurry, with architectural parameters closely controlled by the applied thermal profile and mould properties. However, there is inconsistency in literature regarding the effects of slurry acidity and its composition on resulting structures 1,2. This work aimed to deconvolute pH and acid type to evaluate their impact on architectural properties.

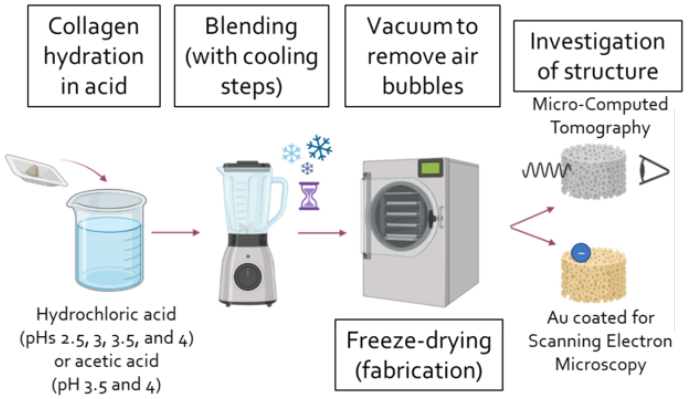

Figure 1: Fabrication of samples for subsequent structural investigation. Collagen I (bovine tendon) was hydrated in the appropriate acid and blended with sufficient cooling steps to prevent denaturing. Degassed slurry was freeze-dried to form scaffolds, which were subsequently investigated via Micro-Computed Tomography (μ-CT) and Scanning Electron Microscopy (SEM).

Methods. Collagen I was hydrated in hydrochloric acid (HCl), pHs 2.5, 3, 3.5, and 4, or acetic acid (AcA), pH 3.5 and 4, then blended with appropriate cooling steps to prevent denaturing. A vacuum was applied to remove air bubbles and each sample was subjected to the same freeze-drying protocol with a final freezing temperature of -20°C. Sample structures were probed using Micro-Computed Tomography (μ-CT) and Scanning Electron Microscopy (SEM).

Results and Discussion. As illustrated in Figure 2, pH changes altered the architectural properties. Lower pH values (2a, b) resulted in a smaller structure separation (2c; analogous to pore size), for both acids (from 120 μm to 65 μm for HCl samples, and 85 μm to 45 μm AcA). This occurred at different pH values for the two acids, suggesting dependence on the concentration and nature of the conjugating base.

The finer structural architecture corresponded to a smaller object size at 50% interconnectivity, decreasing from 180 μm to 60 μm (HCl), and from 144 μm to 36 μm in AcA. This will result in a significant difference in permeation throughout the scaffolds, particularly for seeded cells.

Micrographs (2e, f) obtained *via* SEM also illustrate a unique fibrous, string-like morphology in HCl samples prepared at pH 2.5 (2e), with features disrupting pore walls compared with smooth pore walls for all other samples (*e.g.* Figure 2f).

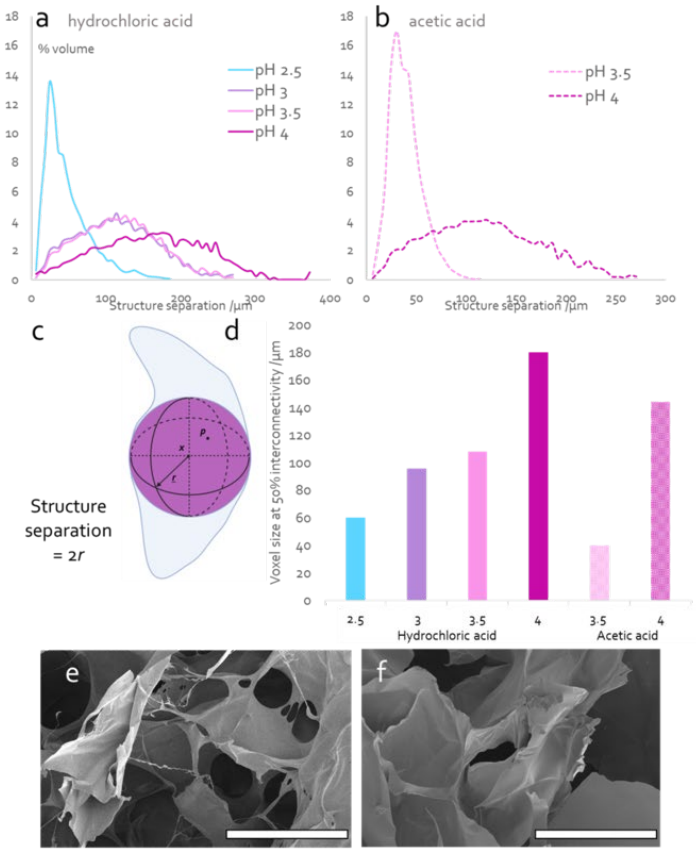

Figure 2: Data illustrating the structural changes with acid and pH variation.
a, b, c – For both acids, a decrease in pH resulted in a narrower distribution of pore sizes and a smaller average size (a, b), characterised here using structure separation (c), the largest sphere able to fit into each pore. The mean structure separation decreased from 120 µm to 65 µm in hydrochloric acid (pH 4 to 2.5), and from 85 µm to 45 µm in acetic acid (pH 4 to 3.5).
d – The impact of these finer structural features will have consequences for the scaffolds' applications to tissue engineering, such as the ability of cells and other materials to travel through them. 50% of the scaffold was accessible to objects varying from 60 µm (pH 2.5) to 180 µm (pH 4) in hydrochloric acid and from 36 µm (pH 3.5) to 144 µm (pH 4) in acetic acid.
e, f – Micrographs of the lowest pH conditions obtained using SEM. It was observed that samples prepared using hydrochloric acid at pH 2.5 resulted in a fibrous microstructure, whereas all others had similar morphologies with predominantly smooth pore walls (e.g. f, prepared using acetic acid at pH 3.5). Scale bar = 100 µm.

Conclusion. The work presented here demonstrates the effectiveness of adjusting the initial slurry pH as an additional parameter to control the final scaffold structure, which is infrequently reported in literature discussing freeze-drying. This could offer a valuable extra parameter to control final scaffold properties, which could include mechanical properties and cell migration.

Acknowledgements. This work was funded by Cambridge Display Technologies, Ltd.
References. [1] M. Madaghiele, *et al.*, J. Biomed. Mater. Res. A, 2008 85A, 3, 757–767, doi: 10.1002/jbm.a.31517; [2] M. Kuberka, *et al*., Int. J. Artificial Organs 2002 25, 1, 67–73, doi: 10.1177/039139880202500111.

S6.1-O4

New understanding of type I collagen dense phases for the elaboration of biomimetic materials

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Abstract

The extracellular matrix (ECM) of soft and hard tissues is mainly composed by collagens, in particular type I collagen. Biosynthesis of collagen is mediated by cells that secrete the molecular units, control their self-assembly into fibrils, and their release in the extracellular environment.
A large variety of biomaterials relies on collagen as a central building block to mimic the ECM. However, comprehension limits in the underlying parameters that control fibril formation from the precursor acid-soluble state *in vivo*, restrain the reconstitution of the native fibrillar motifs in tissue-engineering. Key factors have been identified, such as concentration, temperature, osmotic pressure, and pH. Still, most biomaterials do not reach the concentration of collagen in native tissues, and rely heavily on chemical crosslinking rather than self-assembly [1]. We herein propose a new constitutive phase diagram of type I collagen both in acidic and fibrillar conditions, to guide the elaboration of collagenous materials for clinical applications.
Our lab has developed an expertise in fibrillar dense collagen gels obtained *in vitro*, with a wide variety of applications in tissue engineering and regenerative medicine, using different processing methods (ice templating, wet extrusion, 3D bioprinting, etc.). A broad range of highly concentrated collagen films, from 40 to 900 mg.mL-1 were prepared in acidic conditions. Collagen films were studied in solution and after fibrillogenesis, using two methods of self-assembly to assess the influence of pH and osmotic pressure. The physico-chemical properties were investigated to assess: fibrils size and arrangement (AFM, TEM, SAXS/WAXS), liquid-crystal organization (polarized-light optical microscope), and phase transitions as functions of the concentration and temperature (differential scanning calorimetry, dynamic mechanical analysis) (Fig. 1).

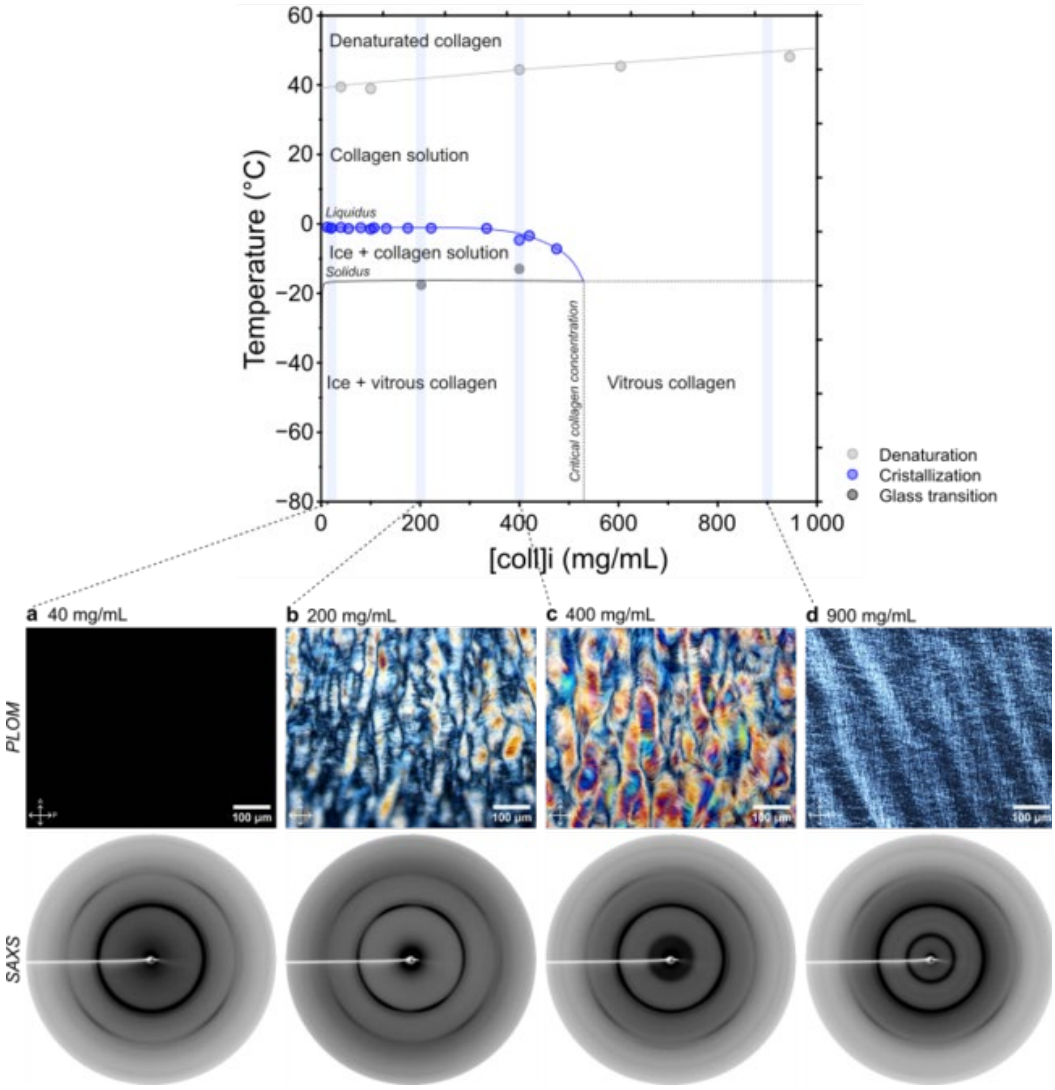

Fig. 1: Extended phase diagram of type I collagen in acid-soluble form.

The construction of this phase diagram demonstrates that it is possible to reach the organization and concentration range of native fibrils, by controlling the temperature and initial concentration. The fibrillogenesis pathway determines the arrangement of collagen at the fibrillar and sub-molecular levels, which allows to tailor the mechanical and biological properties [2]. This phase diagram extends the understanding of collagen, and drives forward the outstanding possibilities of this biomolecule to mimic living tissues.

References. [1] Giraud-Guille, M. M. *et al*. Liquid crystalline properties of type I collagen: Perspectives in tissue morphogenesis. *Comptes Rendus Chimie* **11**, 245–252, (2008); [2] Martinier, I. *et al*. In prep.

S6.2-K1

Vascularized models of *in vitro* tissues and organs and their generation from pluripotent cells

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Abstract

Vascular networks play a crucial role in *in vitro* tissue and organ models by providing a means to meet the metabolic needs of cells and replicate the mechanical environment found in living organisms. These networks can be created through various approaches, including self-assembly of suspended cells to form natural microvascular beds. Our research group has employed this method for a wide range of applications, such as developing models for primary and metastatic cancer, studying transport across endothelial cells, and simulating the human blood-brain barrier to investigate drug delivery and neurodegenerative diseases. In this presentation, we showcase the methods utilized to culture and modify vascular networks, as well as techniques to characterize their structure and morphology. One of the main challenges in these models lies in obtaining a consistent cell source for all the required cell types, especially as the complexity of the model increases. Therefore, we introduce several methods to modulate vascular morphology during network formation or when the networks are fully established. Additionally, we highlight our neurovascular platforms, which serve as valuable tools for modeling neurodegenerative diseases. Furthermore, we will discuss strategies to derive all cell types from human pluripotent cells, aiming to enhance model consistency and ultimately develop fully patient-specific models. Overall, this presentation will provide insights into the growth and manipulation of vascular networks, with a particular focus on their application in disease modeling and the future prospects of personalized medicine.

S6.2-O1

Sound patterning of microcapillary networks for establishing a guided peripheral neurovascular system model

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Abstract

In the peripheral neurovascular (NV) system, blood vessels and nerves are coupled together to ensure physiological functioning and homeostasis of the tissues. Particularly, it has been observed that the neurites growth is influenced by the presence of endothelial cells (EC) which are able to attract axons projections[1]. However, due to the lack of accessible *in vitro* models, there is limited knowledge on the interaction between vessel and nerves in healthy and pathological conditions such as peripheral neuropathies caused by diabetes or after traumatic events. Here we show the biofabrication of a 3D *in vitro* model in fibrin which recapitulates the peripheral NV system by coupling a pre-patterned and spatially orchestrated capillary network with an embryonic dorsal root ganglion (DRG) explant. The use of sound patterning allowed to fabricate a reproducible hierarchical organized microcapillary network within a multi-wells platform where neurites could outgrowth from the DRG body following the microenvironmental cues. The sound patterning process[2,3] was used to pattern ECs and pericytes in a ring-shaped pattern within fibrin by enhancing the local cell density of the single cell suspension. The microcapillary network (Figure 1) self-assembled and maintained the pre-patterned ring shape with a diameter of 1781 ± 142 µm and a thickness of 416 ± 124 µm. After 24 hours from the patterning, the freshly explanted embryonic DRG was positioned inside the well, above the pre-patterned microcapillary network. Neurites projections from DRG explant showed to be directed in privileged directions traveling through the fibrin and establishing contact with the EC. In contrast, in absence of microcapillary network, the neurites outgrowth (Figure 2) was isotopic showing lack of directional guide. In this *in vitro* model, the presence of a prepatterned microcapillary network offer a physiochemical guide for the neurites outgrowth. Thus, the alignment and parallel maturation of the NV axis could be easily quantified by image analysis after immunofluorescent staining. This model allows to gain more knowledge about the NV axis development and to study the peripheral neuropathies which often result in NV dysfunctions.

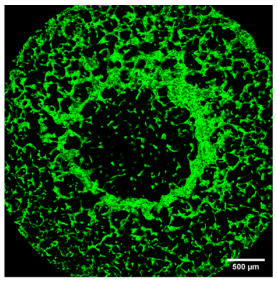

Figure 1. Sound patterned microcapillary network. Green = GFP-HUVEC

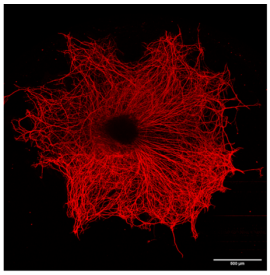

Figure 2. Neurites outgrowth from DRG explant. Red = BIII tubulin

References. [1] J. M. Grasman et al. Scientific reports, 2017; [2] N. Di Marzio et al. Materials Today Bio, 2022; [3] D. Petta et al. Biofabrication, 2020.

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S6.2-O2

Microfluidic platforms to optimize granular hydrogel microenvironments for tissue regeneration

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Abstract

Introduction. Granular hydrogel materials may be particularly suitable for tissue healing purposes, as suggested by promising *in vivo* studies[1]. However, only few *in vitro* platforms to assemble granular hydrogel building blocks[2] and to assess the impact of their properties on cellular and tissue responses under highly reproducible conditions have been described. Here, we report the development of microfluidic platforms to reproducibly confine and immobilize microgels without the need for secondary crosslinking and engineer a novel tissue healing model.

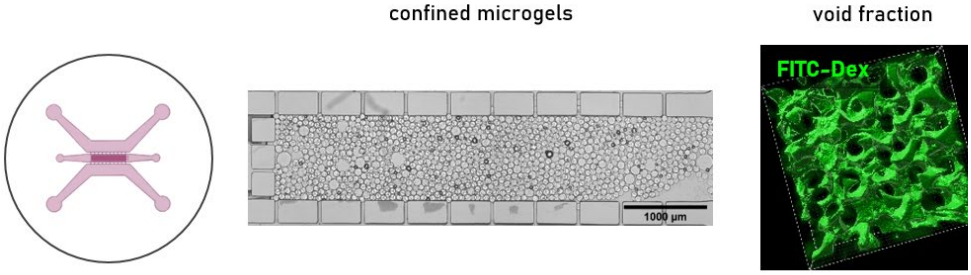
Methods. We designed microfluidic devices featuring a “confinement chamber” with or without an adjacent “tissue chamber”, flanked by two medium channels. Platform characterization was done using previously described norbornene-functionalized poly(ethylene glycol) (PEG-NB) microgels produced via microfluidic droplet formation as granular material building blocks. The microgels featured an RGD adhesion motif and had a diameter of 75 µm. The medium channels were separated from the confinement chamber by pillars spaced 25 µm apart to prevent microgel escape and allow for their concentration on the device. The resulting void space was visualized by perfusing FITC-dextran (500 kDa). Human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) were co-confined along with the microgels to assess their performance within the granular environment or encapsulated in an enzymatically crosslinked PEG hydrogel in the tissue chamber. Cell number, spreading, extracellular matrix deposition as well as migration within and invasion into the granular environments were assessed via immunofluorescence, time-lapse imaging and image-based quantification.

Results. Microgels were reproducibly confined on the devices from diluted microgel suspension, yielding a void fraction of 20-25%. With varying initial cell suspensions co-confined with the microgels, the final cell count within the chamber could be reproducibly tuned. hBM-MSCs spread around the microgels as early as 3 hours after co-confinement, migrated in the 3D porous environment and showed deposition of the ECM components fibronectin, collagen and laminin after 3 days of culture . hBM-MSCs encapsulated in a bulk hydrogel formed a stromal-line tissue, from which they invaded and colonized the granular tissue created by the confined microgels within the neighboring confinement chamber.

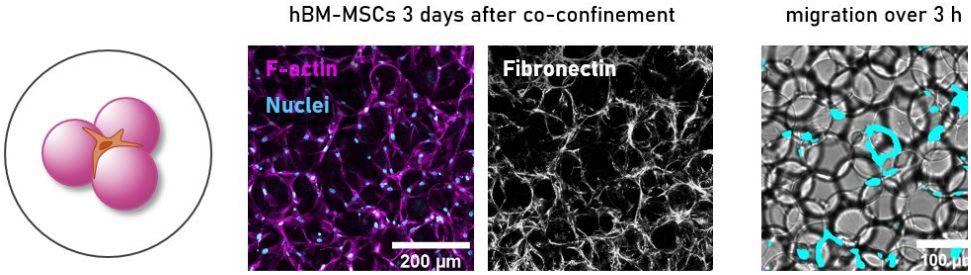
Conclusion and Outlook. Different microfluidic platforms were designed and used to assess cell behavior in a granular material environment. Further experiments are focusing on evaluating vascularization and immune cell interactions in the created constructs. Together, we envision these platforms to be of relevance as a pre-clinical tool for the faithful comparison of different granular materials for tissue regeneration.

References:[1] https://doi.org/10.1038/nmat4294; [2] https://doi.org/10.1021/acsbiomaterials.0c01612

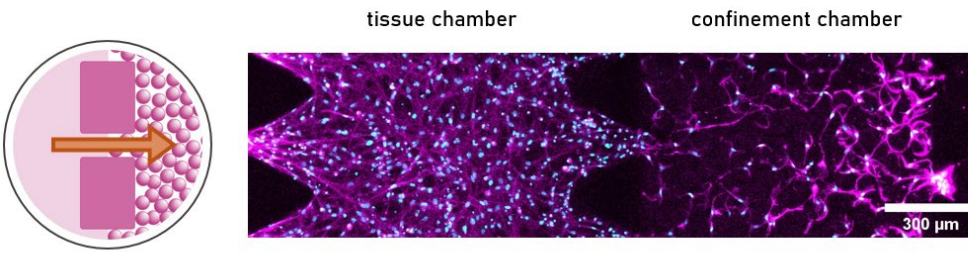
Microgel confinement within microfluidic device



Assessing cell behavior in co-confinement



Tissue healing/invasion assay



S6.2-O3

3D printing-based vascularized *in vitro* skin equivalent for investigating diabetic chronic wounds

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Abstract

Poorly regulated blood glucose and insulin resistance lead to decreased elasticity and narrowing of blood vessels, which causes insufficient blood flow and impaired healing at diabetic wound sites, but also limits the efficiency of the current clinical treatments that include debridement and administration of drugs that stimulate revascularization. In order to develop more advanced strategies for treating diabetic chronic wounds, better models are required, as animal testing is associated with high costs and ethical concerns, whereas even sophisticated *in vitro* 3D models have limited value due to the lack of vascular system. 3D printing, especially stereolithography, has attracted great interest for the construction of complex 3D features such as branched vessel-like structure, and is thus a promising approach to develop physiologically more relevant vascularized *in vitro* 3D skin equivalents.

In the current work, 3D skin equivalents with perfusable vasculature are developed using norbornene-functionalized poly vinyl alcohol (nPVA) and cysteine-containing matrix metalloproteinase-sensitive peptides as cell-responsive bioink for fabrication of the vascular structure, but also as biomimetic matrix for the creation of dermal equivalents. By adjusting the concentration of photoabsorber tartrazine, a micro-scaled branched structure was printed via stereolithographic printing, achieving good resolution and perfusable channels (Fig.A). To mimic diabetic skin, human dermal fibroblasts (HDFs) and diabetic HDFs were encapsulated within nPVA hydrogel, where significantly higher production of IL-6 was observed in dHDFs group compared to the HDFs group at both d1 and d10 (Fig.B). Notably, dermal equivalents with dHDFs showed a lower cell density and production of collagen type I compared to the ones with HDFs at d7 and d14 (Fig.C). These results indicate that the hallmarks of chronic wound, such as persistent pro-inflammation, impaired proliferation and matrix remodeling were presented in diabetic dermal equivalents. Incorporation of the printed vascular structure within the diabetic skin equivalent, and the introduction of hyperglycemic conditions by perfusing medium with high glucose levels through the vessel-like structure will allow us to build an *in vitro* platform for the assessment of developing advanced strategies against diabetic chronic wound.

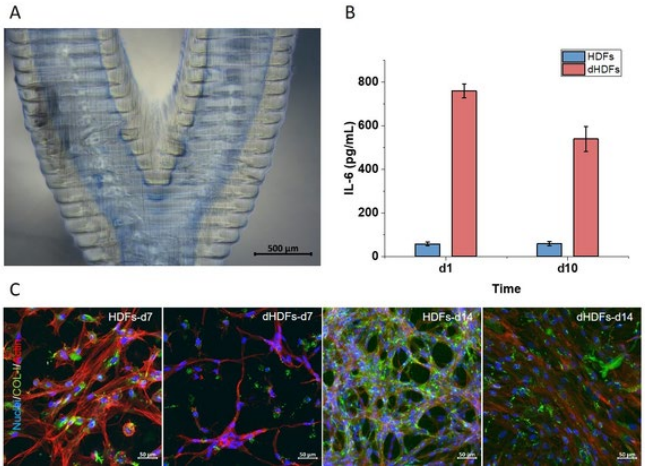

Figure. A) A micro-scaled branched structure printed by the nPVA-based bio-ink on stereolithographic printer and perfused by brilliant blue (scale bar, 500µm). B) The production of IL-6 in the culture medium for HDFs and dHDFs encapsulated within nPVA hydrogel measured by ELISA. C) Confocal images of cells stained for nuclei/COL-I/actin (blue/green/red) encapsulated within nPVA hydrogel cultured for 7 and 14 days (scale bar, 50µm)

S6.2-O4

Printing the intervertebral disc: a hyaluronan-collagen bioink analogue of the nucleus pulposus

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Abstract

Background. Intervertebral disc (IVD) degeneration and its treatment are poorly understood, despite its high prevalence and considerable healthcare costs. This is in part due to overly simplistic and poorly representative models. With bioprinting, precise control over the cell microenvironment makes it possible to recreate the disc's complex composition, structure, and load-bearing capacity. We present a composite bioink for the fabrication of structures biochemically and mechanically representative of the gel-like nucleus pulposus (NP) of the IVD.

Methods. Tyramine-functionalized hyaluronic acid (THA) was combined with fragmented type I collagen and gelatin microgels. The collagen component was produced via a pH-increase induced fibrillogenesis, followed by extrusion through a 32G needle. Gelatin microgels were produced by exploiting temperature-dependent solubility of gelatin in an ethanol/water mixture. An extrudable paste of 3%, 2%, and 5% respectively of THA, collagen, and gelatin was reinforced after printing with a visible light-initiated ruthenium-catalysed cross-linking reaction to produce stable constructs with tunable stiffnesses. Shear and compressive mechanics were characterized. Filament formation and gap bridging capabilities were evaluated, and simple multi-layer structures printed. Cytocompatibility was evaluated via live/dead fluorescent microscopy of embedded bovine NP cells.

Results. Shear-thinning behaviour and elastic recovery were observed (Figure 1). Extruded filaments were uniform and capable of bridging >8 mm gaps. Stable multi-layer structures were produced with the use of photocrosslinking. Material stiffness was easily tuneable in the range of human nucleus pulposus. Constructs remained dimensionally stable during culture, with embedded cells demonstrating good viability and a round morphology as found natively (Figure 2).

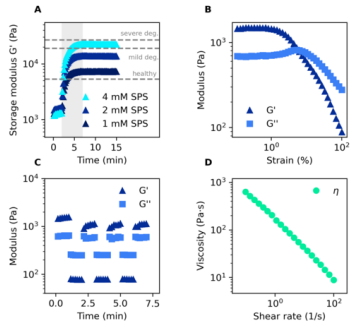

Figure 1: A) Storage moduli after light crosslinking. Light exposure and approximate moduli at various stages of tissue degeneration marked. B) Amplitude sweep. C) Elastic recovery (0.1%/100% shear strain cycles). D) Shear-thinning with increasing shear rate.

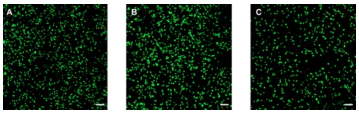

Figure 2: Embedded NP cells viable >7 days and maintain native-like rounded morphology. Scale = 100 µm. Calcein AM and ethidium homodimer-1 staining.

Conclusions and Outlook. We developed a composite bioink resembling human nucleus pulposus. The bioink is composed of biochemically suitable components representative of native tissue and approaches its high concentrations. Due to the material tuneability and the bottom-up approach afforded by bioprinting, the presented bioink is a flexible tool for the development of tailored, reproducible *in vitro* models of the IVD, which can offer new insight into the treatment of disc degeneration.

Acknowledgements. Funded by the Swiss National Science Foundation (189310) and the French National Research Agency (ANR-19-CE06-0028).

S6.3-K1

Bioinspired polyelectrolyte materials: from biomedical adhesives to inks for 3D bioprinting

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Abstract

The threads produced by velvet worms are remarkably sticky and stiff; the beak of a jumbo squid is extremely hard; and spider silk is incredibly tough. The extraordinary material properties found in these natural systems have been of interest to researchers for a long time. However, only recently, biologists discovered that a crucial element in the processing of many of these materials are coacervates. Complex coacervation is an associative liquid-liquid phase separation phenomenon driven by electrostatic attraction between oppositely charged macro-ions (e.g. polysaccharides, proteins etc.) and counterion release, resulting in a polymer rich aqueous phase in equilibrium with a polymer poor phase. For a given polyelectrolyte couple, depending on the salt concentration of the medium, a complex coacervate either behaves as a free-flowing viscoelastic fluid or a rigid polyelectrolyte complex solid or anything in between. This outstanding versatility has made polyelectrolyte complexes good candidates for a wide range of applications.
In the Kamperman group we are dedicated to improve and engineer complex coacervates to introduce novel advanced functional materials. In this presentation I will focus on our efforts to develop coacervate-based medical adhesives and inks for 3D bioprinting.

S6.3-O1

Complex Coacervates of oppositely charged, intrinsically disordered polypeptides with LCST behavior

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BIOFORGE Lab, CIBER-BBN, University of Valladolid, Valladolid, Spain

Abstract

Previous studies with intrinsically disordered proteins (IDPs) have shown that the active interaction of different molecular interactions unlocks the possibility of new and more complex nanostructures. Collectively, they play key roles in cellular organisation and biomaterials science knowledge. For this purpose, we study the spinodal decomposition leading to self-assembled nanostructures and microstructures of two synthetic amphiphilic IDPs with opposite charges on their hydrophilic block and a common neutral, thermosensitive hydrophobic block. Their mixtures at different charge ratios are also studied. The synthetic polypeptides to be examined are Elastin-Like Recombinamers (ELRs) based on motif repeats found in the intrinsically disordered regions of tropoelastin. The two ELRs are dissolved in cold water, and characterized at physiological temperature by different techniques such as isothermal titration calorimetry (ITC), circular dichroism (CD), differential scanning calorimetry (DSC), dynamic light scattering (DLS), confocal and Cryogenic transmission electron microscopy (Cryo-TEM). On their owns, they are characterised by retaining the phase behaviour of natural elastin at lower critical solution temperature (LCST), undergoing a reversible phase transition (known as inverse temperature transition (ITT)), above a characteristic transition temperature (Tt), and exhibiting random coils and beta-turns conformations. When the two synthetic amphiphilic IDPs are mixed, their inverse transition temperatures and random coils conformation decrease as the molar ratio converges to equal charge numbers. Contrarily to what happens when they are alone (spherical micelles with hydrodynamic diameters between 100 and 200 nm), their mixtures generate larger and more complex self-organised structures. Through microscopy techniques, we have witnessed the formation of much larger worm-like structures due to the combination of hydrophobic and ionic pairing that takes place in these coacervates (Figure 1). The build-up of these more complex and ordered structures becomes more accentuated as the system reaches charge neutralisation. Our work shows the importance of the interplay between different self-organising forces, as well as the order-disorder balance in the spontaneous emergence of complex hierarchical structures from IDPs. These results provide a better understanding of the self-organising nature of biological macromolecules and liquid-liquid phase separation (LLPS). They also have implications for the elaboration of protein-based supramolecular materials.

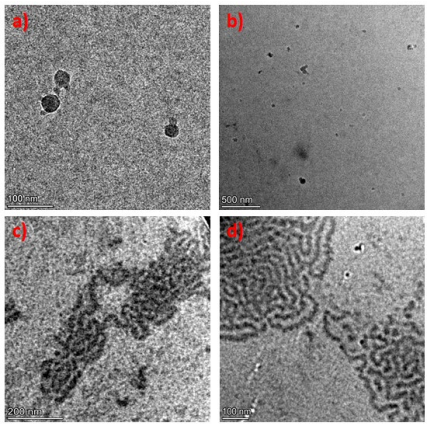

Figure 1. Cryo-TEM images from Complex Coacervates between two oppositely charged diblock recombinamers: 25 µM of the final concentration in water at pH=7 and 37°C: (a) Negatively charged diblock ELRs. (b) Positively charged diblock ELRs. (c & d) Complexes structures formed by mixing the two diblocks under charge-neutralised conditions.

S6.3-O2

3D Printable dynamic hydrogel: As simple as it gets!

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Abstract

3D Printing technology offers a vast range of applications for tissue engineering applications. Over the past decade a vast range of new equipment has been developed; while, 3D printable biomaterials, especially hydrogels, are investigated to fit the printability requirements. The current candidates for bioprinting often requires post-printing cross-linking to maintain their shape. On the other hand, dynamic hydrogels are considered as the most promising candidate for this application with their extrudability and self-healing properties. However, it proves to be very difficult to match the required rheological in a simple material. Here, we present for the first time the simplest formulation of a dynamic hydrogel based on thiol-functionalized hyaluronic acid (HA-SH) formulated with gold or silver ions. Rheological studies demonstrated that the dynamic hydrogel prepared at 1.5 wt% HA-SH with 1 mol% of Ag+ or Au+3 resulted in a hydrogel with the appropriate viscosity to be used as printable material with a 3D printer. In addition, strain-sweep experiments demonstrated that biomaterial kept its instantaneous and full self-healing properties, thanks to the permanent dynamic exchange between Metal-thiolate species and disulfide bonds used to cross-link the hydrogel. Such properties are crucial as the material can recover its 3D structure after being injected, offering a material that can be used for 3D printing without the use of external stimuli. The best printing characteristics were obtained with a needle with 0.41 mm diameter. 3D construct with a relatively good precision and up to 24 layers, corresponding to 10 mm high, was achieved. While the materials proved to be cytocompatible, the dynamic hydrogel prepared with silver ions showed interesting antibacterial properties. In addition, the incorporation of collagen type I did not affect the rheological and printability properties. On the contrary, the bioactivity of the dyanmic hydrogel was improved, facilitating cell adhesion. As far as we know, this material is the simplest 3D printable hydrogel and its mixture with cells and biological compounds is expected to open a new era in 3D bioprinting.

S6.3-O3

Instructing immune system via structurally programmable tyramine-modified self-assembling β-sheet peptides and hyaluronic acid hydrogels

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[1]AO Research Institute Davos, Davos, Switzerland. [2]Diamond Light Source, Harwell Science and Innovation Campus, Fermi Avenue, Didcot, United Kingdom. [3]ETH Zürich, Zürich, Switzerland. [4]New York University Abu Dhabi, Abu Dhabi, UAE. [5]Mines Saint-Étienne, Univ Jean Monnet, INSERM, Saint-Étienne, France

Abstract

Generating or manipulating a complexity of custom immune instructive cellular signals during regenerative processes is one of the greatest challenges of tissue engineering.[1] One solution to this is to design immunomodulatory biomaterials that controllably interact with the surrounding immune cells,[1] for example by molecular weight directly influencing the polarization state of macrophages.[2]
In this work we explored the immunomodulatory potential of selection of tyramine-modified self-assembling β-sheet peptides (SAPs) and tyramine-modified HA (THA) hydrogels. For this, we designed a selection of peptide sequences (DABACABACD) based on the alternation of hydrophobic (A: F phenylalanine or Y tyrosine) and hydrophilic (B/C: K lysine or E glutamic acid) amino acids, and synthesized THA of two molecular weights (280 kDa and 1640 kDa). Self-assembly and gelation of all designed sequences were evaluated using titrations, oscillatory rheology, FTIR, TEM and SAXS.

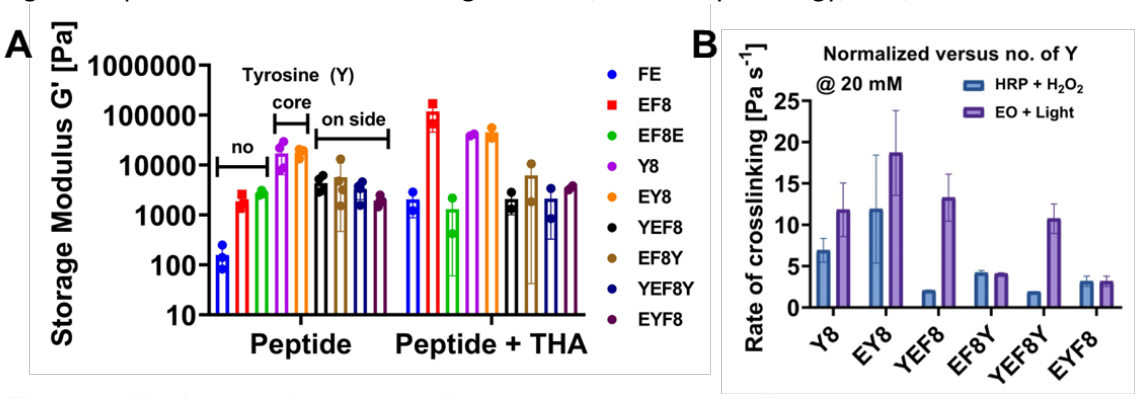

Figure 1. ***(A)*** *Storage shear moduli for peptide and peptide – THA composite physically mixed hydrogels obtained at 1 Hz frequency and 0.2% strain. Hydrogels were prepared at 20 mM peptide concentration and 20 mg mL^{-1} of THA with M_w = 1.64 MDa.* ***(B)*** *Rate of in-situ crosslinking reactions (enzymatic and light crosslinking) of tyrosine-containing peptide hydrogels measured by an oscillatory rheology.*

A parametric study was carried out on the designed selection of DABACABACD peptides to verify the effect of peptide sequence design on final physicochemical and biological properties of SAPs and peptide-THA composite hydrogels. D residues were rationally varied between Y or E amino acids to modulate the interactions' ability of formed β-sheet edges and shell with: i) other peptide fibres, ii) THA, and iii) with macrophages. Self-assembly, rheological properties and printability of both SAPs and SAP-THA hydrogels were controlled by the choice of primary peptide sequence, concentration, and fabrication technique (Fig. 1).

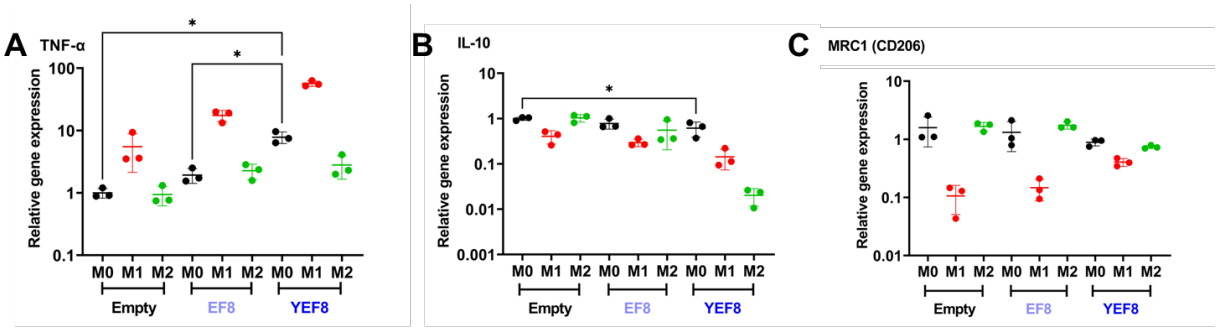

Figure 2. ***(A)*** *TNF-α,* ***(B)*** *IL-10,* ***(C)*** *MRC1 (CD206) gene expression measured by qPCR for cells without any material conditioning (empty), or encapsualted with 2 mM of peptide EF8, or YEF8, which vary by the addition of the edge-tyrosine. Data is represented for three cases: i) M0 without any additional stimulation, ii) M1 pre-stimulated by the addition of TNF-α and IFN-γ and iii) M2 pre-stimulated by the addition of IL-4, prior to the peptide conditioning. For easier identification, each polarization state has been marked by distinct colour on all graphs in the following way:* ● - **M0** ● - **M1** ● - **M2**. *Gene expression is represented as fold change compared to control using RPLP0 as a housekeeping gene. Data represent mean ± SD of 3 independent experiments, for single donor. Analysis by one-way ANOVA with Sidak multiple comparisons test, *p < 0.05 for comparison between M0 groups are plotted where signifiance occurred. Note comaprisons of other groups indicate statistical significances, however these are not plotted to clearly indicate the effect of peptide alone on the polarization capacity of macrophages.*

Here we also demonstrate the polarization effects of the SAPs/THA on macrophages. M1-like and M2-like polarization modulation was found to depend on the edge amino acid (Fig. 2) and on molecular weight of THA, as unravelled by the immunocytochemistry, gene expression analysis and ELISA. We also studied the effect of microgravity on the structure and stability of SAPs/THA using a random positioning machine to provide better understanding for their future biofabrication in space.

In summary we uncover the link between basic molecular interactions driving self-assembly of tyramine modified biomaterials and demonstrate their capabilities as extrudable platforms for immunomodulatory tissue engineering.

Acknowledgements. This work was supported by the European Union's Horizon 2020 (H2020-MSCA-IF-2019) research and innovation programme under the Marie Skłodowska-Curie grant agreement 893099 — ImmunoBioInks and by the Leading House for the Middle East and North Africa for Research Partnership Grant 2022 "Space ImmunoBioInks" (RPG-2022-38).

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S6.3-O4

Optimization of hydrogel crosslinking chemistry for developing hyaluronic acid-based bioink

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Abstract

Background: 3D bioprinting is a revolutionary technology that allows the fabrication of complex, cell-laden structures for tissue repair and disease modeling. Despite significant progress in developing hydrogel-based bio-inks, there is still a need for materials that mimic the cellular microenvironment, have suitable viscosity and stability for printing, as well as biocompatibility of the printed cells. To address this problem, we developed a combination of disulfide and thiazolidine chemistry to develop a bio-ink for the 3D printing of stem cell-laden scaffolds. For this purpose, we synthesized hyaluronic acid (HA) derivatives having 1,2 aminothiols and aldehyde functional groups, using EDC chemistry as optimized previously in our group. Taking advantage of reduced pKa of thiols in 1,2 aminothiols as in cysteine, we combined disulfide chemistry with thiazolidine chemistry (1,2 aminothiol reaction with aldehyde) to fine-tune the hydrogel properties that allow shear thinning properties and improved cell viability after injection.

Method: HA-cysteine and HA-aldehyde were synthesized according to our previously published protocols. The hydrogel formation process involved crucial steps to ensure optimal results. Firstly, HA-aldehyde was dissolved in phosphate-buffered saline (PBS, pH 7.4), while HA-cysteine was dissolved in degassed, acidic buffer (PBS pH 5.0), with the final solid content, being 2% while the ratio between HA-cysteine and HA-aldehyde were varied (100:00, 75:25, and 50:50) to form hydrogels containing, disulfide, disulfide and thiazolidine together and just thiazolidine crosslinking. Once completely dissolved, the pH was then adjusted to 7.4 using NaOH before forming a hydrogel and hMSCs added to the HA-cysteine solution. After 10 minutes of complete mixing of the two compounds, the mixture was loaded into the 3D printer syringe with a needle size of 25G and evaluated for cell viability.

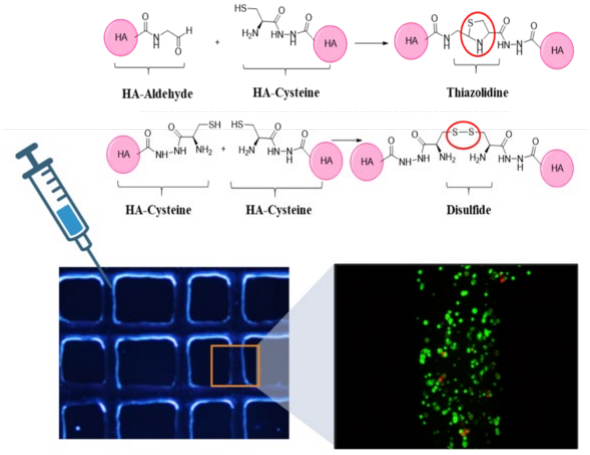

Figure 1. Design of 3D printed HA hydrogels having thiazolidine and disulfide linkages.

Results: Incorporation of different ratios of HA-cysteine and HA-aldehyde concentrations revealed that the best combination for the 3D printing of hydrogels containing human mesenchymal stem cells is to have 50% thiazolidine and 50% disulfide linkages (Figure 1). Such bio-ink having a combination of two chemistries (disulfide and thiazolidine) provided a superior ink for 3D printing when compared to hydrogels containing just disulfide or thiazolidine linkages. The fast reaction kinetics of the thiazolidine reaction improves the initial viscosity of the matrix, while disulfide formation enhances the structural stability and stiffness. Interestingly, our cell studies also revealed that this hydrogel effectively protects cells from anoikis during extrusion, as compared to extrusion in culture media.

S6.4-K1

Peri-implant infections in the oral environment and implant surface functionalization.

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Abstract

Biological complications around implants are frequent, with about 20-25% of patients suffering from an advanced form, i.e., peri-implantitis, involving crestal bone loss. Current non-surgical therapies have been rather unsuccessful in combating the peri-implant infection, resulting in very large rates of recurrence within a year. Therefore peri-implantitis treatment often involves surgery, which is associated with high costs and patient suffering; in addition, systemic antibiotics are often prescribed, contributing to the burden of antibiotic resistance. Importantly, treatment success is difficult to maintain, and recurrence of disease is common.

In this context, modern 2nd and 3rd generation dental implants have modified/structured surfaces, that enhance the osseointegration process, decrease healing time, and subsequently shorten the time required for implant loading. However, these surfaces – when exposed to the oral environment – are associated with increased bacterial attachment and biofilm formation, compared to the 1st generation machined/turned implant surfaces; in addition, modified/structured surfaces seem to increase bacterial virulence. Indeed, although it has been difficult to capture any negative impact of in terms of peri-implantitits prevalence, pre-clinical *in vivo* studies have clearly demonstrated higher rates of peri-implantitis progression at implants with modified/structured surfaces compared with turned implants.

One possible approach to reduce the risk of peri-implantitis is by implant surface manipulation, to develop surfaces that can interfere with bacterial colonization and/or exert bactericidal activity. For example, titanium functionalization with Strontium (Sr) has recently been assessed for its antimicrobial potential in a series of *in vitro* studies. The results indicated that Ti surfaces functionalized with Sr - in the form of a nanostructured coating comprised of Ti, Sr, and oxygen (O), produced using magnetron sputtering – possess antimicrobial potential against bacteria commonly associated with implant-related infections. In this context, Sr is especially interesting due to its known anabolic effect on bone formation; in particular, modified implants carrying the Ti-Sr-O surface, mentioned above, achieve larger amounts of osseointegration, relative to implants with a benchmark SLActive surface.

Thus, it seems that Sr functionalized implants, may not only possess greater osseointegration potential, but also bear a reduced risk for peri-implantitis.

S6.4-O1

Dual-function peptide decorated nanoparticles to fight infection and promote angiogenesis in skin chronic wounds

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Abstract

Skin chronic wounds affect, approximately, 40 million patients worldwide (US market: 25 billion dollars). Moreover, their prevalence is expected to increase, due to rising rates in diabetes and obesity.[1] Chronic wounds heal slowly (>12 weeks) and show persistent inflammation, hindering both epidermal and dermal cells from responding to reparative stimuli. Exposure to the external environment facilitates bacterial infection, further perpetuating inflammation.[2] Antiseptics/antibiotics have limited residence time and may induce antimicrobial resistance, which is less likely when using antimicrobial peptides. However, due to high enzymatic load in wounds, the latter may be better protected from degradation if covalently conjugated onto biomaterials. Herein, the highly efficient photoclick thiol-ene chemistry is used to conjugate an antimicrobial peptide, Dhvar-5 (LLLFLLKKRKKRKY-spacer-C), onto norbornene-modified chitosan nanoparticles (NorChit NP).[3] Furthermore, chronic wound healing is also impaired by deficient angiogenesis. Therapies based on vascular endothelial growth factor (VEGF) delivery are equally susceptible to enzymatic degradation.[4] Thus, a VEGF-mimicking peptide, QK (C-spacer-KLTWQELYQLKYKGI), was also conjugated to NorChit NP. NP production and peptide conjugation occurred in a microfluidics device coupled with UV LEDs (λ=365 nm). NorChit and peptide solutions containing the photoinitiator VA-086 were perfused through the device and resulting NP were washed in 50-kDa Amicon® filter units. Peptide was quantified both in the supernatant and in the NP. Dhvar5-NorChit NP were tested on *Staphylococcus epidermidis* (ATCC 35984) for 6h in 50% Mueller-Hinton broth, in dynamic conditions. Moreover, a 3-day proliferation assay using Human Umbilical Vein Endothelial Cells (HUVEC) was performed to test QK-NorChit NP. Produced NP (~100 nm; 10^9 NP/mL) had 40% and 70% of initial Dhvar5 and QK, respectively. Dhvar5-NorChit NP (10^8 NPs/mL) showed bactericidal effect, but even bare NorChit NP were able to cause a 1-log reduction in culturable bacteria, suggesting that free norbornene groups also have antimicrobial activity. Regarding proliferation, both bare and NorChit-QK NPs doubled the metabolic activity of HUVEC compared to control, while free QK peptide did not induce such increase, hinting that conjugation favors peptide activity. These results confirm that NorChit NP conjugated with bioactive peptides can tackle two hallmarks of chronic wounds - bacterial infection and impairment of endothelial cell proliferation-, showing potential to improve current therapies.

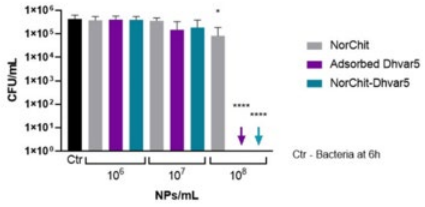

Figure 1 – *Staphylococcus epidermidis* CFU counts after 6h incubation with bare norbornene-chitosan nanoparticles (NorChit) and NorChit nanoparticles with adsorbed and conjugated Dhvar5. Data are presented as mean ± standard deviation (Kruskal-Wallis non-parametric test: *p<0.05; ****p<0.0001).

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Financial support: FCT-MCTES through "Bio2SkinAdvanced" (NORTE-01-0247-FEDER-047225) and LAQV-REQUIMTE (UIDB/50006/2020). National (FCT/Norte 2020) and/or European Union funds (ESF – European Social Fund) support doctoral grants SFRH/BD/145471/2019 (PMA) and SFRH/BD/146890/2019 (DRF).

S6.4-O2

Biocompatible photothermal-responsive plasmonic nanocomposites for near infrared-activated bacterial eradication

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Abstract

In recent years, novel strategies and approaches to develop antimicrobial biomaterials have attracted increasing attention, targeting multi-functional systems to eliminate bacteria from membranes, surfaces, medical devices, infected sites, contact lenses, etc. More specifically, eradicating bacteria (both resident and exogenous) at the wound site is crucial to guarantee fast and effective wound healing without complications, while sterilization of personal protective equipment (*e.g.,* face masks) makes it possible the safe re-use.[1,2] In this frame, photothermal therapy holds great potential since it can kill pathogenic bacteria with minimal invasiveness.[3]

In this study, plasmonic nanoparticles have been combined with biopolymers to provide the system with bactericide functions. More in detail, plasmonic gold nanorods (AuNRs) are encapsulated into electrospun matrices made of poly(lactic-co-glycolic acid) or polyacrylonitrile by loading into the polymeric solution prior to electrospinning or spraying on the already spun material to obtain the final composites (Figure 1A). The photo-thermal properties of the incorporated AuNRs are exploited to activate the near infrared (NIR)-mediated temperature response upon exposure to NIR light. By reaching a temperature > 55°C, the eradication of 99.5% of bacteria is achieved (Figure 1B), while the stability of the composite materials is maintained. Additionally, *in vitro* biocompatibility tests performed by culturing fibroblast cells onto the proposed systems show suitable biological properties with no toxic or inflammatory reactions. Taking into account the results, the biocompatible photothermal-responsive nanocomposites reveal their potential in photothermal therapy as a wound dressing and face mask coating.

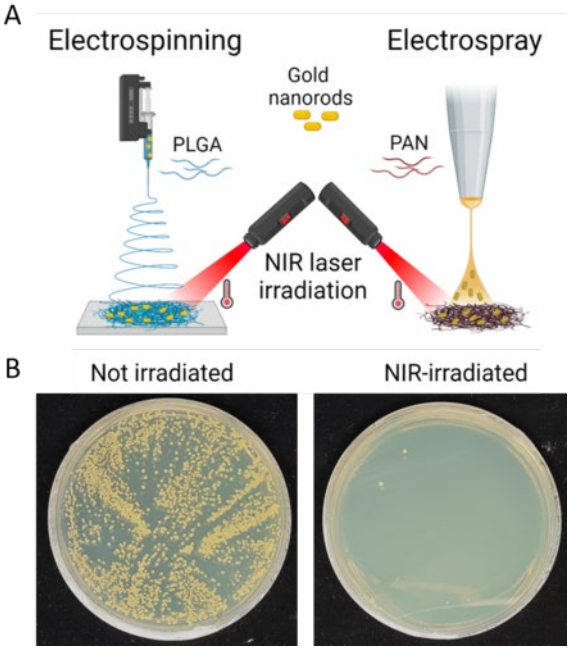

Figure 1. Photothermal-responsive composites. A) Schematic illustration of composition and production of the plasmonic nanocomposites. B) Bacterial eradication upon NIR irradiation.

Acknowledgments. This study was supported by the National Center for Research and Development (WPC2/NanoHealer/2021). Figure 1A is created with BioRender.com.

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S6.4-O3

Antibacterial properties of multiple antigenic peptides (MAP) based on polyarginine: from experiments to molecular dynamic simulations

Eloïse Lebaudy[1], Lauriane Petit[1], Engin Nihal Vrana[2], Florent Barbault[3], Philippe Lavalle[1,2]

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Abstract

Every day, 20% of hospital patients in Europe develop Healthcare associated infections (HAIs). To prevent the infection risks, current strategies are based on administration of antibiotics. However, many bacteria have developed resistance against antibiotics and the design of innovative antimicrobial drugs is mandatory[1].

Our studies demonstrated the strong antibacterial properties of homopolypeptides like polyarginine (PAR)[2]. Bacteria are not able to acquire resistance towards this polycation. Moreover, this polycation, in combination with a natural polysaccharide like hyaluronic acid, can be used to build an antibacterial coating on every kind of surfaces. The antibacterial activity of PAR in the coatings is related to the number of arginine residues on the chain[2-4].

From these results, we wondered if the structure of the PAR has also an impact on the antibacterial properties. Thus, we studied multiple antigenic peptides (MAP) composed of arginine residues that are attached in a branched structure. MAP is composed of several PAR arms with different lengths. Some MAPs have surprisingly higher antibacterial activity

To understand the difference of the antibacterial properties of peptides observed *in vitro*, molecular dynamic simulations were developed. These simulations were used to see the interaction between the bacterial membrane of gram-negative bacteria *P. aeruginosa* or gram-positive bacteria *S. aureus* and the peptides Modelling results identical to experimental results were obtained. Finally, MAPs are promising tools for the design of new antibacterial materials for medical applications.

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S6.4-O4

Assessment of biomaterials with new antimicrobial surfaces for dental applications

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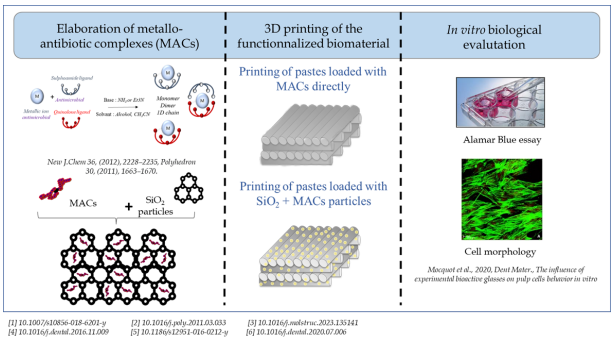
Abstract



Antimicrobial dental materials are developed with the premise that restorations should not only serve the purpose of reconstructing damaged dental mineralized tissue, but also to induce suitable biological behaviour that prevent secondary caries occurrence, without having adverse toxic effects on the surrounding host cells and tissues. Combining both antimicrobial activity and biocompatibility remains a real technical and clinical challenge. In this context, the present study focuses on developing and accessing new restorative dental biomaterials with textured antimicrobial surfaces. The methodology used here is to synergize both the effect of metallo-antibiotic complexes and the effect of the optimized surface achieved by additive manufacturing (robocasting) [1]. The synthesis of metallo-antibiotic complexes is based on the combination of metals ions, such as silver, zinc and copper, with antibiotics as ligands, such as sulphonamides and quinolones commonly used in biomedical applications [2][3]. Briefly, antibiotic ligands are deprotonated in a mixture containing metal salt and ammoniac/ethanol. The formation of a precipitate by the excess of ammoniac is observed and single crystals are formed after 2-5 days of slow evaporation. The formed complexes are characterized through single-crystal and powder X-ray diffraction, FTIR and NMR analysis. The obtained functionalized biomaterial would be able to inhibit the initial biofilm formation at its surface and enhance the active antimicrobial molecules release. Several complementary approaches will be also studied regarding the release kinetics: the direct incorporation of antibacterial complexes inside the biomaterial (calcium phosphate cement) or the encapsulation of these complexes inside mesoporous silica particles before incorporation inside the biomaterial [4]. These will act as a carrier to preserve the active molecules degradation and to enhance their efficiency and their release kinetics [5]. *In vitro* biological assessment of the functionalized antimicrobial biomaterials and their components will be performed on the targeted dental cells according to the ISO 10993. The effect of both the topographical surface and the antimicrobial molecules released will be evaluated in cell adhesion, proliferation and colonisation. Alamar Blue assay and confocal imaging will be used for the assessment [6]. From fundamental and clinical perspectives, this study provides original biomaterial models to assess the antibacterial complexes release kinetics within a robocasted functionalized biomaterial. It also provides new strategies for 3D implanted biomaterials with high performances for dental and biomedical applications.

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S6.5-K1

Biomaterials for bone tissue engineering and lesions treatment – scaffolds, matrices and templates

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Abstract

Bone has considerable ability to regenerate; however, there are injuries, e.g. of critical size or infected ones that do not heal spontaneously and need special therapies and/or tissue engineering approach. Biomaterials supporting the treatment of such bone lesions should, in principle, be designed to kill pathogenic bacteria, followed by facilitating osteogenic cell signalling and bone tissue expression processes. They should accommodate cells within an environment that replicates, as much as possible, the environment in which those cells normally reside, to provide them with the conditions under which they express new tissue. This is a difficult endeavour, and several strategies are considered, including, e.g., design of scaffolds and templates that mimic bone extracellular matrix (bECM), applying surface and bulk modifications, or supplementation with drugs or biologically active molecules.

Our research shows that there are benefits to modifying both degradable porous polymeric scaffolds [1] and nondegradable ceramic scaffolds [2] with the components present in native bECM. We found that calcium phosphate deposition on poly(L-lactide-co-glycolide) (PLGA) scaffolds promotes the healing of osteochondral defects in the rabbit model [1]. The surface deposition of collagen, chondroitin sulphate and sulphated hyaluronan was beneficial for the osteogenic differentiation of human mesenchymal stem cells (hMSC), which were even improved if scaffolds contained bimodal porosity [3].

Moreover, we have addressed the problems of bone infections, which might be targeted by local antibiotics delivery systems consisting of degradable polymeric micro-/nanoparticles loaded with antibiotics. More recently, we developed a method to change the chemical state of hydrophilic antibiotics by hydrophobic ion-pairing, to make them more compatible with the hydrophobic polymer matrix [4]. It resulted in almost 100% encapsulation efficiency, allowing us to better control the delivery of the drug to the required place. These drug-loaded particles can be used to obtain injectable formulations intended to treat osteomyelitis locally [4-5]. They can also be immobilised on the scaffold pore walls, to design implantable medical devices, when mechanical support is particularly essential [6-7]. The developed biomaterials were cytocompatible and released the drugs in a controlled manner to be adapted to the clinical needs.

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S6.5-O1

NFFA-Europe Pilot: a great research and innovation opportunity for the European and worldwide biomaterials community!

Flavio Carsughi

Forschungszentrum Jülich, Jülich, Germany

Abstract

NFFA-Europe is an open-access resource for experimental & theoretical nanoscience to perform multidisciplinary research at the nano and the microscale. NFFA-Europe offers a seamless series of harmonized, interoperable and integrated science services enabling research with an impact on science and innovation. It integrates into one comprehensive research infrastructure distributed throughout Europe, advanced cutting-edge facilities specialized on growth, lithography, characterization, theory, simulation and fine-analysis with Synchrotron, FEL and Neutron sources.

The pan-European NFFA-Europe consortium is composed of 23 partners with a core of 13 co-located nanofoundries and LSFs (Synchrotron, FEL and Neutron). More than 600 state-of-the-art instruments are currently available free of charge to European and international researchers, thanks to the Horizon 2020 NFFA-Europe PILOT project.

NFFA-Europe PILOT aims to further consolidate the sustainability of the research infrastructure through the optimization of good practices and the training of a new generation of researchers getting used to formulate their science objectives by taking full advantage of all that NFFA-Europe has to offer. Moreover NFFA-Europe PILOT plans to upgrade and develop new innovative tools for nanoscience such as real-time observation and control in microscopy and spectroscopy, X-Ray Wavefront Metrology and pattern transfer methods. A safe-by-design platform for nanomaterials and a FAIR approach to data are further features of the new NFFA-Europe PILOT project.

Users can request access to this comprehensive research offer by submitting their proposals through the www.nffa.eu single entry point portal. Proposals are evaluated by an international peer-reviewed panel, and approved projects are granted free access to the best suited instruments, competences and technical support. Furthermore, a financial contribution for travel, accommodation and subsistence costs is available to the applicant team.

S6.5-O2

Surface morphology and dynamic changes in the element distribution in the corrosion layer induced by direct monoculture and co-culture models of osteoblasts (OBs) and osteoclasts (OCs) on pure Mg and WE43 alloy

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Abstract

As absorbable metals, magnesium (Mg) and its alloys are being researched due to their biocompatibility and mechanical properties close to the bone, which makes them promising temporary orthopedic implant materials. The *in vitro* corrosion behavior and material-cell interaction need to be characterized to predict how Mg-based implants would behave in the human body. When Mg is immersed in an aqueous environment, a degradation product/layer is formed. This corrosion layer acted as the interface between the body fluids and the Mg implant surface *in vivo*. Previous research has focused on studying the interface between cells and Mg degradable material using single monoculture *in vitro* tests. Nevertheless, since osteoblasts (OBs) and osteoclasts (OCs) interaction have an important role in bone remodeling, the co-culture with both bone cells (OB-OC) is required to achieve a better understanding of the material-cell interaction and the impact on Mg degradation for its potential use as a biomedical material.

This study investigated the corrosion performance and element distribution in the corrosion layer of pure Mg and WE43 alloy using direct monoculture and co-culture of OBs and OCs. Monoculture and co-culture of pre-differentiated OBs and OCs (used at a ratio of 1:2 OB: OC) were cultured for 7 and 14 days on both pure Mg and WE43 discs (**Fig-1a**). Live/dead staining assays were used to evaluate cytocompatibility, whereas actin cytoskeleton staining was used to examine changes in adhesion. The surface topography and cell morphology were analyzed by electron microscopy (SEM). Cross-section images and the distribution of elements composing the corrosion layer at the material-cell interface were analyzed by FIB/SEM/EDX.

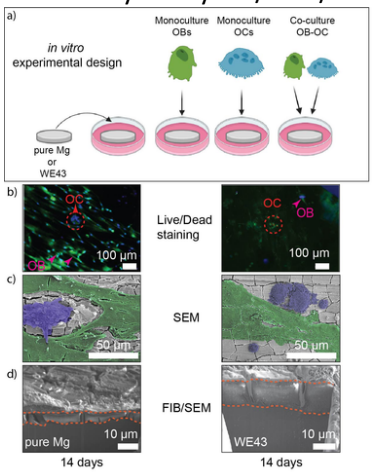

Figure 1. *In vitro* direct monoculture and co-culture of osteoblasts (OBs) and osteoclasts (OCs) model on pure Mg and WE43 alloy. a) experimental design; b) cell viability by Live/Dead staining; c) Color-enhanced SEM micrographs of the cell morphology of OB (green) - OC (blue) co-culture; d) FIB/SEM cross/section showing the corrosion layer formed beneath OB-OC co-culture after 14 days of culture.

The results revealed better cell attachment and the vitality of differentiated OBs and OCs on pure Mg surface when compared with WE43 in direct cell monoculture and co-culture models after 14 days. The corrosion layers formed beneath OB-OC coculture were enriched with P and Ca on WE43 after 7 days and with pure Mg after 14 days of *in vitro* cell culture (**Fig-1b-d**). These results demonstrated that OB-OC co-culture influences the corrosion layer composition differently in the tested Mg materials. To better understand the role of the corrosion layer in the material-cell interaction, additional research is required to develop *in vitro* corrosion test systems that reasonably simulate the *in vivo* corrosion behavior of biodegradable Mg alloys.

Acknowledgements*:* The research has received funding from the European Training Network within the framework of Horizon 2020 Marie Skłodowska-Curie Action (MSCA) No. 811226.

S6.5-O3

Biomimetic micro- and nano-scale surface features produced by femtosecond laser-texturing enhance TiZr implant osseointegration *in vitro* and *in vivo*

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Abstract

Introduction: In modern oral maxillofacial surgery, long-term implant stability is intrinsically linked to the quality of osseointegration. The stochastic nature of commonly used implant surface modification techniques, including sandblasting and acid etching, precludes precise control over the uniformity and consistency of the resulting implant surface features. Femtosecond laser-texturing has emerged as an up-and-coming alternative that enables the production of designer surfaces with micro- and nano-scale surface features defined in size and arrangement, which would allow to mimic the multiscale structures of trabecular bone. The aims of this study were therefore to develop a biomimetic laser-textured implant surface, to investigate the influence of laser-texturing on its physicochemical properties, to evaluate its blood-material interaction, and to assess its osseointegration capacity *in vitro* and *in vivo*.

Methods: TiZr samples (discs and rods) were laser-textured (TiZr laser) using a femtosecond laser system and boiled in water to tune their wettability. Sandblasted and acid etched TiZr (TiZr SLActive) served as reference. All samples were incubated with blood and the adsorption of fibrinogen and fibrin network formation was assessed using confocal laser scanning microscopy (CLSM). Bone progenitor cells (HBCs) were seeded on samples pre-incubated with blood, and mineralization was evaluated using calcium quantification assays (n=5). Biomechanical pull-out tests were performed using a rabbit model of osseointegration 4 weeks after implantation (n=8), and complemented with an in-house developed abiological pull-out test using a dental resin to decouple the influence of surface architecture and biological response (n=3).

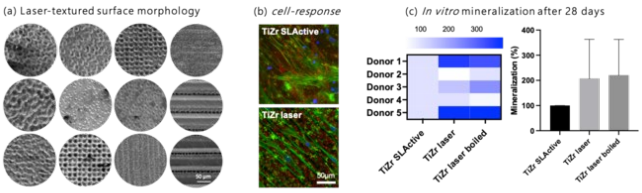

Fig. 1a) Selection of laser-textured TiZr (TiZr laser) implants featuring micro- and nano-scale features, b) HBC attachment and spreading on blood-preincubated surfaces after 24h of culture and c) enhanced mineralization on TiZr laser in comparison to sandblasted and acid etched TiZr (TiZr SLActive)

Results and discussion: After fabricating a wide variety of laser-structured surface morphologies (Fig.1a), a surface with an organized trabeculae-like microarchitecture superimposed with nano-scale laser induced periodic surface structures was selected for biological evaluation. TiZr laser supported fibrin network formation to the same extent as TiZr SLActive, but only after boiling treatment. Notably, HBCs showed preferential alignment only on TiZr laser (Fig.1b) and TiZr laser also outperformed TiZr SLActive in terms of supporting *in vitro* mineralization (Fig.1c). In line, biomechanical pull-out tests confirmed that implant osseointegration was ~2.5-fold enhanced in rabbits treated with TiZr laser in comparison to TiZr SLA. The abiological pull-out tests on the other hand showed an inferior response of TiZr laser implants, suggesting that the enhanced osseointegration observed with laser structured TiZr was mainly driven by the biological response to the surface.

Conclusion: This study presents a lean manufacturing alternative to traditional sandblasting and acid etching, for the production of TiZr implants with enhanced osseointegration capacity.

S6.5-O4

Interpenetrating network hydrogels for studying the role of matrix viscoelasticity in 3d osteocyte morphogenesis

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Abstract

During bone formation, osteocytes are embedded in the osteoid tissue and form an extensive 3D dendritic network within the mineralizing bone matrix. This environment has made the study of osteocyte biology *in vivo* challenging. Despite immense efforts, a functional 3D interconnected osteocyte network has not been achieved *in vitro* so far. Since the native osteoid tissue is a collagen-rich material exhibiting viscoelastic stress-relaxation properties, we set out to investigate the role of matrix viscoelasticity in 3D osteocyte morphogenesis *in vitro*.

Here, we report a new 3D culture of murine osteocyte-like IDG-SW3 cells embedded in soft alginate-collagen interpenetrating network (IPN) hydrogels with comparable stiffnesses (4.4-4.7 kPa, Figure 1b) but varying stress-relaxation times ($t_{1/2}$, 1.5-14.4s, Figure 1c). The IPN hydrogels consist of an ionically crosslinked alginate network to tune stress relaxation as well as a permissive collagen network to promote cell adhesion and matrix remodeling (Figure 1a). IDG-SW3 cells were pre-differentiated on collagen-coated tissue culture flasks under osteogenic conditions to drive osteoblast-to-osteocyte transition. After 14 days, ca. 60% of the cells became Dmp1-GFP-positive osteocyte-like cells as indicated by their fluorescence. The mechanosensitivity of these cells to fluid shear stress (2 Pa) was confirmed by live-cell calcium imaging. After embedding in the IPN hydrogels, cells remained highly viable following 7 days of 3D culture. After 24h, osteocytes in the fast-relaxing hydrogels showed the largest cell area and longest dendritic processes. During the two-week culture, increased osteogenic gene expression and matrix mineralization indicated the progression of tissue maturation. However, a significantly larger increase of some osteogenic markers (Dmp1; ALP, Figure 1d) as well as intercellular connections via gap junctions were observed in slow-relaxing hydrogels at the two-week end point. Our findings imply that fast-relaxing IPN hydrogels promote early cell spreading, whereas slow-relaxation favors osteogenic differentiation. In the future, we plan to extend our studies to human bone cells and their morphogenesis in the described viscoelastic IPN hydrogels. These findings may further advance the development of 3D *in vivo*-like osteocyte models to better understand bone mechanobiology.

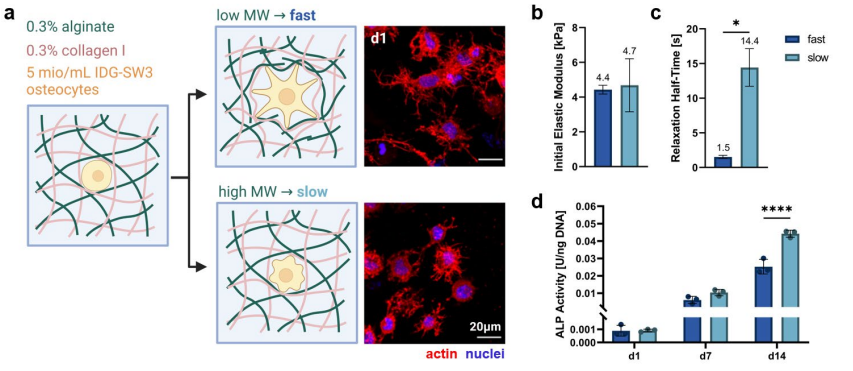

Figure 1 a) Schematic of the spreading IDG-SW3 osteocytes embedded in fast- and slow-relaxing alginate-collagen hydrogels and their different morphologies after 1 day as shown by actin-nuclei staining. b-c) Hydrogel mechanical properties determined by unconfined compression testing: b) initial elastic modulus and ***c)*** *stress-relaxation half-time. d) Colorimetric ALP enzyme activity quantification from cellular samples during two weeks of culture. * p = 0.05 to 0.01, **** p ≤ 0.0001.*

S6.6-K1

Electro conductive biomaterials - application of conductive polymers and 2d nanomaterials in tissue and sensor applications

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Abstract

Electroconductive biomaterials are gaining attention for regeneration in tissues where electrical functionality is of crucial importance, such as myocardium, neural, musculoskeletal, and bone tissue. Conductive fillers such as graphene, carbon nanotubes, metallic nanoparticles, and MXenes and conjugated polymers such as polyaniline, polypyrrole, and poly(3,4-ethylendioxythiophene) can possibly achieve optimal electrical conductivities f with appropriate suitability for tissue engineering approaches. Many studies have focused on the use of these materials in multiple fields, with promising effects on the regeneration of electrically active biological tissues such as orthopedic, neural, and cardiac tissue. In this talk, I present my groups work in the development of electroconductive platforms in both coating and scaffold form using 2D nanomaterials and electroconductive polymers for both culture and iPSC-Cardiomyocyte differentiation. Conductive biohybrid platforms were engineered by blending collagen type I and 2D MXene (Ti3C2Tx) to harness the biofunctionality of the protein component and the increased stiffness and enhanced electrical conductivity (matching and even surpassing native tissues) that two-dimensional titanium carbide provides. These MXene platforms were highly biocompatible and resulted in increased proliferation and cell spreading when seeded with fibroblasts. Conversely, they limited bacterial attachment (Staphylococcus aureus) and proliferation. When neonatal rat cardiomyocytes (nrCMs) were cultured on the substrates increased spreading and viability up to day 7 was studied when compared to control collagen substrates. Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were seeded and stimulated using electric-field generation in a custom-made bioreactor.

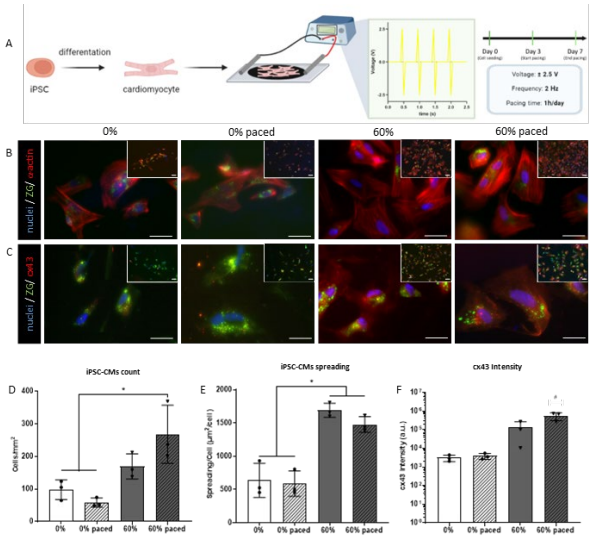

Figure 1. A) Schematic illustration highlighting the electric stimulation parameters; B) Nuclei/α-actin staining (scale bar: 10 µm) at day 7; C) Nuclei/Connexin43 (cx43) staining (scale bar: 10 µm) at day 7; D) Quantification of viability (cells mm-2) at day 7. E) Cardiomyocytes spreading quantification (µm2 cell-1); F) Quantification of cx43 expression (a.u.). Statistical analysis was performed using a 1-way ANOVA with Tukey's multiple comparison test, t $p< 0.05$ is considered significant.

The combination of an electroconductive substrate with external electrical field enhanced cell growth, and significantly increased cx43 expression (Figure 1). This *in vitro* study convincingly demonstrates the potential of this engineered conductive biohybrid platform for cardiac tissue regeneration.

Acknowledgements. This work was supported through the Advanced Materials and Bioengineering Research (AMBER) Centre (SFI/12/RC/2278_P2, partly supported by the European Regional Development Fund). The SEM imaging for this project was carried out at the Advanced Microscopy Laboratory (AML), Trinity College Dublin, Ireland.

S6.6-O1

Bio-orthogonal double-crosslinked alginate-gelatin/mxenes hydrogels as biomimetic viscoelastic and electroconductive substrates supporting cardiac regeneration

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Abstract

Myocardial infarction is one of the leading causes of death worldwide and represents a major clinical challenge [1]. In recent works, cell culture hydrogel substrates enhanced miRNA-mediated direct reprogramming efficiency of human cardiac fibroblasts (HCFs) into induced cardiomyocyte (iCMs) [2][3]. Notably, these hydrogels were purely elastic or highly soft, whereas several studies highlighted the importance of ECM-like stress-relaxing viscoelastic hydrogels in regulating cell behavior [4]. Furthermore, electroconductive hydrogel substrates enhanced the maturation of cardiac contractile cells [5]. Herein, alginate-gelatin/MXenes hydrogels based on bio-orthogonal click-chemistry, with tunable viscoelastic and electroconductive properties were developed with the aim to support cardiac regeneration by enhancing direct reprogramming of HCFs into iCMs.

Firstly, a solution of alginate-azide and gelatin-azide conjugates, was mixed with a 4-arm-PEG-DBCO solution to form spontaneous hydrogels via bio-orthogonal strain-promoted azide-alkyne click reaction (SPAAC). Double-crosslinked hydrogels were obtained, through additional ionic cross-linking by Ca2+ ions. Furthermore, MXene quantum dots (MQDs) were incorporated into hydrogels, to impart electrical conductivity. Physicochemical properties of hydrogels were deeply investigated. *In vitro* cell viability and adhesion were performed by embedding HCFs into hydrogels.

Bio-orthogonal alginate-gelatin hydrogels were successfully obtained through SPAAC click reaction. By varying azide:DBCO molar ratio, physicochemical properties of hydrogels were modulated. At increasing azide:DBCO ratio, hydrogels showed increased mechanical stiffness and more pronounced elastic response, mimicking healthy cardiac tissue. All hydrogels showed physiological rates of stress relaxation. Particularly, substrate viscoelasticity could be tuned by double-crosslinking strategy, which enabled viscous stress dissipation by unzipping of ionically packed molecules. MQDs could be finely dispersed within the hydrogel network enhancing substrate electroconductivity. Thanks to SPAAC bio-orthogonality, all hydrogels supported cell viability, adhesion and spreading. The study of *in vitro* direct reprogramming of HCFs into iCMs in contact with the developed hydrogels is ongoing.

As a conclusion, bio-orthogonal double-crosslinked alginate-gelatin/MXenes hydrogels developed in this study are promising candidates as biomimetic substrates for cardiac direct reprogramming applications, deserving future investigations.

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S6.6-O2

Conjugated polymers enable optical fine tuning of intracellular reactive oxygen species in Human Umbilical Vein Endothelial Cells

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Abstract

Therapeutic angiogenesis represents a promising strategy to treat ischemic diseases by stimulating blood vessel growth to rescue local blood perfusion. Intracellular Reactive Oxygen Species (ROS) represent a crucial player in the regulation of vascular functions, e.g. vasorelaxation, migration, proliferation and angiogenesis. [M.R.Antognazza et al., *Ox. Med. Cell. Longev.*, 2019]
In particular, physiologically safe amounts of ROS can induce neovessel formation through an increase in intracellular Ca^{2+} concentration and TRP Vanilloid 1 channel activation. [S.Negri et al., *Cells*, 2020]
The use of physical stimuli to finely manipulate intracellular signaling pathways, including ROS-related ones, offers important advantages over chemical and pharmacological approaches. In particular, the possibility to stimulate cells by light provides high spatial and temporal resolution, while reducing invasiveness. Since living cells are not responsive to visible light, it is necessary to employ optogenetic tools or light-sensitive materials as photo-transducers that guarantee a minimally invasive, gene-less approach.
Our group recently demonstrated that is possible to modulate the angiogenic process by employing the light-sensitive, photoelectrochemically active low band-gap polymer poly(3-hexylthiophene-2,5-diyl) (P3HT) in the form of thin films. [F.Lodola et.al., *Sci.Adv.*, 2019] In general, the ideal material used for photostimulation should satisfy properties such as high photo-electrochemical efficiency, stability in an aqueous environment, biocompatibility and long-term implantability. [N. Martino et al., *Sci. Rep.*, 2015]
In this work, we aim at optimizing the efficiency of the photo-transducer material, and we do that in two different ways: optimizing P3HT efficiency by adding functional groups and proposing a new strategy based on two low band-gap conjugated polymers commonly employed in high performance organic solar cells, namely poly[[4,8-bis[5-(2-ethylhexyl)-2-thienyl]benzo[1,2-b:4,5-b']dithiophene-2,6-diyl]-2,5-thiophenediyl[5,7-bis(2-ethylhexyl)-4,8-dioxo-4H,8H-benzo[1,2-c:4,5-c']dithiophene-1,3-diyl]] (PBDB-T), Poly({4,8-bis[(2-ethylhexyl)oxy]benzo[1,2-b:4,5-b']dithiophene-2,6-diyl}{3-fluoro-2-[(2-ethylhexyl)carbonyl]thieno[3,4-b]thiophenediyl}) (PTB7). We report the fabrication and the optoelectronic characterization of polymer thin films and nanoparticles, focusing on the investigation of the phototransduction mechanism and the assessment of in-vitro biocompatibility in Human Umbilical Vein Endothelial Cells (HUVECs). Then we demonstrate that the illumination of a polymer thin film or polymer nanoparticles leads to spatially and temporally precise control of intracellular ROS at non-toxic levels.
Results of this work represent an important step forward the development of new tools for precise, non-invasive and on-demand modulation of intracellular ROS, opening the way for investigation of ion channels activation, intracellular Ca^{2+} signaling and gene expression, aiming at in-vitro and in-vivo angiogenesis modulation for therapeutic purposes.

S6.6-O3

Controlling the architecture of freeze-dried collagen scaffolds with ultrasound-induced nucleation

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Abstract

Introduction: Freeze-drying, a common fabrication route to produce collagen scaffolds for tissue engineering applications, relies on the sublimation of ice from a frozen slurry. The scaffold architecture is largely determined by the arrangement of ice crystals, and therefore the freezing protocol has a direct influence on scaffold pore size and anisotropy. However, even within the same batch, scaffold architecture can vary significantly, due to the stochastic nature of ice nucleation (Fig.1). The aim of this study is to use ultrasound to trigger nucleation in supercooled collagen slurries to control the nucleation event and directly regulate scaffold architecture.

Experimental: 1 wt% collagen slurry was prepared from type I bovine dermal collagen. To record the thermal profile of the slurry during freezing, type K thermocouples were fixed inside the cell culture plate. 4 ml of collagen slurry was pipetted into each well, and the plate was placed into a pre-cooled ultrasonic bath (40 kHz, 0.2 Wcm^{-2}) inside a -20 °C freezer. The first group of samples underwent random nucleation. For the second group, ultrasound was switched on for 3 seconds at a particular temperature to induce nucleation. Then, the frozen slurry was dried in a freeze-dryer. Scaffolds prepared with ice nucleating at different temperatures were characterised under SEM and micro-CT.

Results & Discussion: Ultrasound was shown to be an effective trigger of ice nucleation, causing an instantaneous increase in slurry temperature to the equilibrium freezing temperature, as latent heat of crystallisation was released. Ultrasonic triggering was relevant to the localised high pressure and flow stream around cavitation bubbles (Hickling, 2015; Zhang, Inada and Tezuka, 2003). As shown in Fig.2, scaffolds exhibited smaller average pore size and narrower pore size distribution with ultrasound, compared with random nucleation at the same temperature. We propose that ultrasound induces the formation of more ice nuclei simultaneously across the entire slurry, forming smaller and uniform ice crystals. In addition, for both nucleation methods, scaffolds were more isotropic and had smaller percolation diameters when ice nucleated at a lower temperature.

Conclusions: Ultrasound was found to be an effective method to induce ice nucleation in collagen slurries. Ultrasound-triggered scaffolds tended to have smaller average pore size and narrower pore size distribution than from random nucleation. Ultrasound-assisted freeze-drying has the potential to enable fine tuning of scaffold architecture and to improve reproducibility.

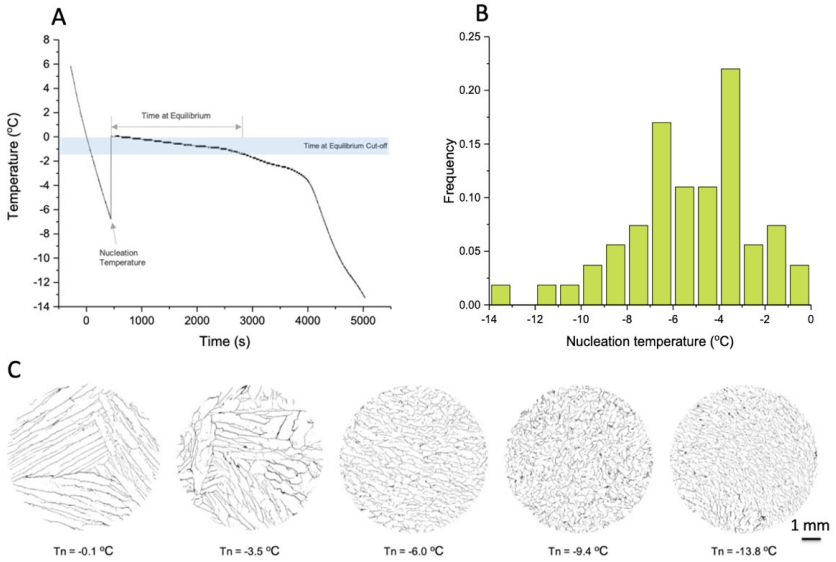

Figure 1 (A) Typical thermal profile of collagen slurry during freezing. (B) Distribution of ice nucleation temperatures in 1 wt% collagen slurry. (C) Horizontal cross-sections of scaffolds fabricated at different ice nucleation temperatures (Tn) from micro-CT.

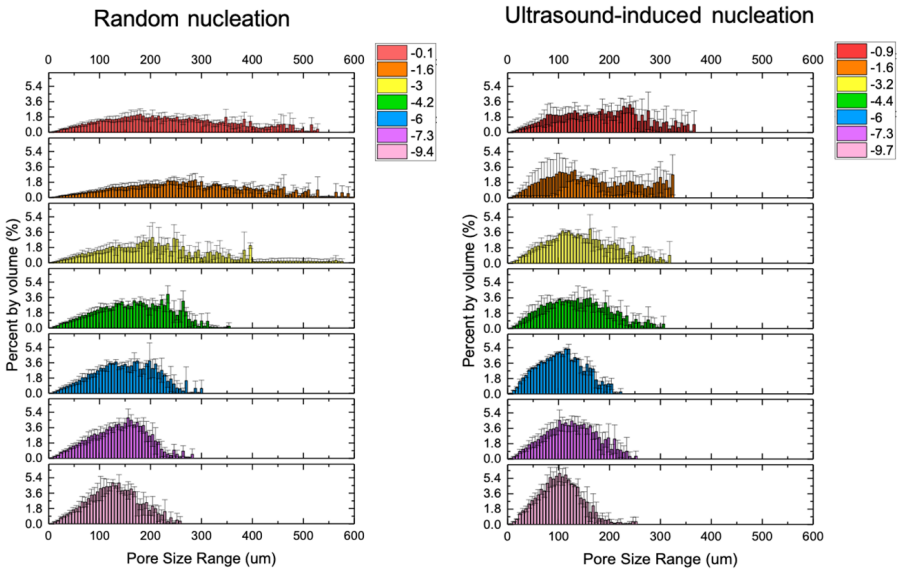

Figure 2. Pore size distribution of scaffolds underwent random (left) and ultrasound-induced nucleation (right) at different nucleation temperatures (℃).

Acknowledgement: Funding from a Benefactors scholarship, St John's College, Cambridge (XS) and the EPSRC (MP) is gratefully acknowledged.

S6.6-O4

Rapid regeneration of a neoartery with elastic lamellae in a tropoelastin-polyglycerol sebacate small-diameter vascular graft

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Abstract

Coronary heart disease is the leading cause of death worldwide. Autologous or synthetic grafts can be used as coronary artery bypass conduits, but these often fail to provide long-term patency and display limited regeneration. Specifically, the regeneration of organized elastin such as elastic lamellae (EL) remains the key challenge in the vascular graft field. Towards this goal, we have fabricated vascular grafts from a combination of tropoelastin (TE), the soluble precursor to elastin, and polyglycerol sebacate (PGS), a highly elastic degradable biomaterial.

Scaffolds were fabricated by electrospinning into a spectrum of microstructures from TE fiber-reinforced PGS matrix to pervasive TE-PGS fiber-networks, resulting in scaffolds with mechanical properties comparable to the human coronary artery. The addition of TE promoted the attachment and proliferation of vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs). When cultured *in vitro* on the scaffolds VECs formed a monolayer and expressed the functional endothelium markers VE-Cad, eNOS, and vWF, while VSMCs changed from rhomboid to spindle shape and expressed contractile phenotype markers such as alpha-SMA and SM-MHC, which have been shown to contribute to reducing the risk of hyperplasia. Grafts implanted in mouse aortas underwent rapid degradation and remodeling, resulting in the formation of a neoartery with a spatial arrangement of collagen, elastin, VSMCs, and VECs highly similar to the native aorta: with mature collagen located in the adventitia, long, continuous EL sandwiched between circumferentially aligned α-SMA$^+$ and smoothelin$^+$ VSMC layers in the intima-media and a lumenal monolayer of CD31$^+$ VECs (Figure 1A). The *de novo* generated medial ELs were roughly half the thickness of the native EL (Figure 1B) with twice numbers (Figure 1C), giving a similar total elastin fraction (Figure 1D). Furthermore, we observed a thick internal elastic lamina (IEL) regenerated next to the VECswith thickness similar to the native IEL. These results demonstrate the capacity of TE-PGS scaffolds to create functional blood vessels with appropriate elastic laminae *in vivo*.

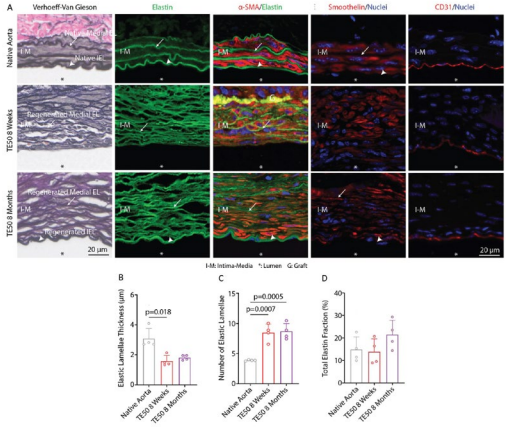

Figure 1. De novo regeneration of elastic lamellae (EL) within the intima-media region of the TE-PGS graft. A) Representative images of A) Verhoeff–Van Gieson staining showing organized EL (black), autofluorescence of EL (green), immunoflouresence staining of α-SMA (red), smoothelin (red), CD31 (red). Quantification of B) EL thickness, C) EL number, and D) total elastin fraction. n = 4 for all quantitative experiments.

S6.7-K1

Magnetic tissue engineering

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Abstract

The emergence of multifunctional inorganic nanohybrids providing multiples functions recently paved the way to tailor-made therapeutic prescriptions and theranostic functionalities. In cancer therapy, they have raised the prospect of thermal treatments that have few if any adverse effects, which we extensively explored this last decade.
However, the magnetism of iron oxide - based nanoparticles also provide cells with sufficient magnetization to manipulate them. Magnetic nanoparticles thus appear as a promising tool for tissue engineering opening up challenging perspectives. We developed magnetic-based methods to manipulate cells, towards the goal to provide magnetic artificial tissue replacements, that can be stimulated on demand. For instance, it could induce mechanically stem cells differentiation. Similarly, it allows to magnetically compress cancer spheroids alongside their genesis or drug testing and even nanoparticles-mediated therapy, then in an all-in-one actor/probe action of the magnetic nanoparticles.
The therapeutic use of nanoparticles in cancer therapy or regenerative medicine application still raises the more general issue of intracellular nanoparticle long-term fate. Cell spheroids models and magneto-thermal tools will be introduced, as tools to monitor long-term nanomaterials intracellular integrity. It evidenced a massive intracellular degradation, which could be prevented by a polymeric coating or an inert gold shell. Remarkably, human cells could also biosynthesize their own magnetic nanoparticles, with longer persistence, and limited toxicity.

S6.7-O1

Mechanotransduction in Magnetic Hydrocups: a hollow magnetic electrospun scaffold for hydrogel stiffening

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Abstract

Introduction

Cells mechanically respond to their local microenvironment, and actively remodel the mechanical properties of the extracellular matrix. Mechanotransduction is often used to direct cell fate, regulate tissue regeneration, and plays a key role in pathologic changes.1 However, these dynamic behaviors are extremely challenging to replicate in matrices for in vitro study. We develop a new approach by electrospinning, where hydrogels are injected into a hollow electrospun scaffold with magnetic responsiveness to enable dynamic, spatiotemporal and remote control over the mechanics of the matrix.

Materials and Methods

Magnetic hollow scaffolds were produced by mixing a polymer solution of 300PEOT55PBT45 (PolyVation) with a magnetic Fe3O4 nanoparticle solution, followed by an electrospinning process. The mixture was fed to a charged needle between 12 – 18 kV at 3000 µL/h, and the mandrel with a diameter of 1.5 mm was charged with -1.5 kV. A biomimetic polyisocyanide2 (PIC) hydrogel was formed by heating or covalently crosslinked by a linker DBCO-PEG4-DBCO (DPD). Alginate hydrogels were ionically crosslinked with calcium ions. Magneto-rheology was used to study the magneto-rheological properties.

Results

Hydrogels were injected into a hollow scaffold, named Hydrocups. For stress-stiffening PIC gels, we hypothesized that the magnetic hydrocups would apply external force to hydrogels under a magnetic field, inducing gel stiffening. We used magneto-rheology to test magnetic hydrogels to quantify the stiffening effects. The absolute stiffness of covalently crosslinked PIC (0.2 wt%) with particles (15 wt%) increased up to 23.3 kPa, whereas particles alone was 6.3 kPa. As a control, magnetic alginate (0.6 wt%) only increased up to 3.6 kPa.

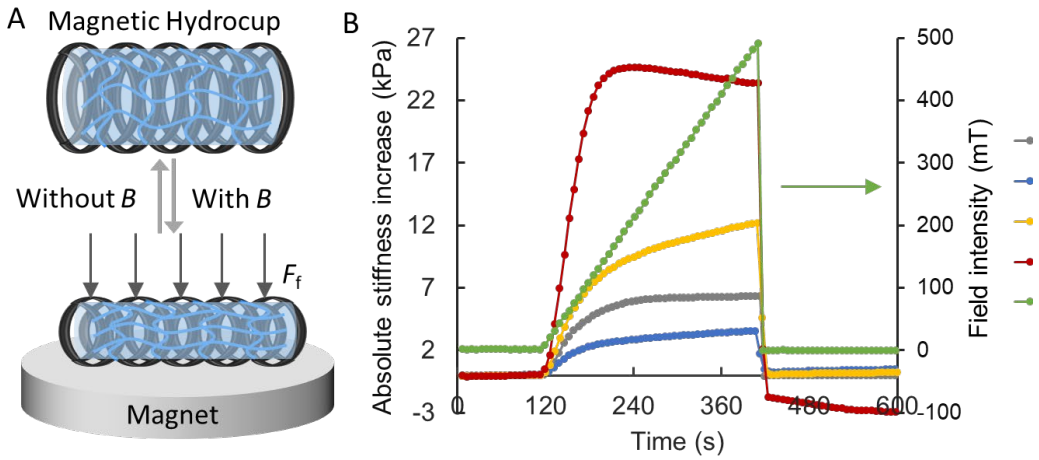

Figure 1. (A) Scheme of the magnetic responsive Hydrocups. (B) Absolute increase of storage modulus of magnetic gels in the presence of an external magnetic field.

Conclusions

We developed a highly stiffening scaffold and we believe magnetic Hydrocups are promising for the cellular mechanostransduction studies. Currently, we are investigating the potential of these new magnetoresponsive constructs on cell activity.

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Acknowledgements

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S6.7-O2

Melt electrowriting of magneto-active fiber scaffolds for skeletal muscle cell stimulation *in vitro*

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Abstract

Current therapies for muscular dystrophies have not successfully restored myotube alignment, which is crucial for muscle contraction. Moreover, mechanical stimulation of injured skeletal muscle has shown to facilitate functional tissue regeneration. Here, we hypothesized that magneto-active scaffolds with well-organized fiber architectures could guide myotube growth and remotely stimulate muscle contraction. To achieve this, we used melt electrowriting (MEW), a unique technique for fabricating highly ordered microfiber scaffolds, to create magneto-active scaffolds from polycaprolactone (PCL) blends with partially reduced graphene oxide nanoplatelets (rGNP) modified with iron oxide nanoparticles (ION).

Medical-grade PCL was melt-blended with ION-decorated rGNP (rGNP@), yielding magneto-active composites (rGNP@ content: 2–20wt.%). Processing was systematically assessed based on key MEW parameters in an in-house built device [1]. Well-organized fiber scaffolds with hexagonal micro-structures were fabricated, and their assembly into cartesian or polar grid macrostructures was explored. The effect of rGNP@ content and MEW parameters on scaffold morphology, magnetic properties, and mechanical performance was studied. MEW/Matrigel/collagen scaffolds were seeded with C2C12 immortalized mouse myoblasts. Cell viability, differentiation efficacy, and fusion were assessed.

ION deposition on rGNP yielded well-dispersed rGNP@ particles with sizes >0.5µm and low aspect ratio. Organic solvent-free melt-blending yielded homogeneous PCL/rGNP@ composites with high magnetic filler content, up to 20wt.%. MEW allowed to design magnetic hexagonal scaffolds with tunable fiber diameter, modularity, and zonal distribution of magneto-active and nonactive material. Hexagonal scaffolds (400µm thickness, 600µm pore size, fiber diameter: 18–22 µm) displayed elastic deformability under tension, mitigating limitations of high filler content. External magnetic fields <300mT triggered out-of-plane reversible deformation in scaffolds with filler content ≤10wt.%. Myoblast culture showed that magnetic particles did not significantly affect viability and differentiation rates after 8 days under static conditions.

Our study demonstrated the reproducible fabrication by MEW of highly-ordered microfiber scaffolds based on PCL/rGNP@ blends, allowing for controlled deposition of zonal magneto-active materials with custom geometries. This allowed to design actuating scaffolds that support skeletal cell growth and differentiation *in vitro* and magnetically-triggered mechanical stimulation, providing new perspectives towards reliable soft-robotic muscle microtissues.

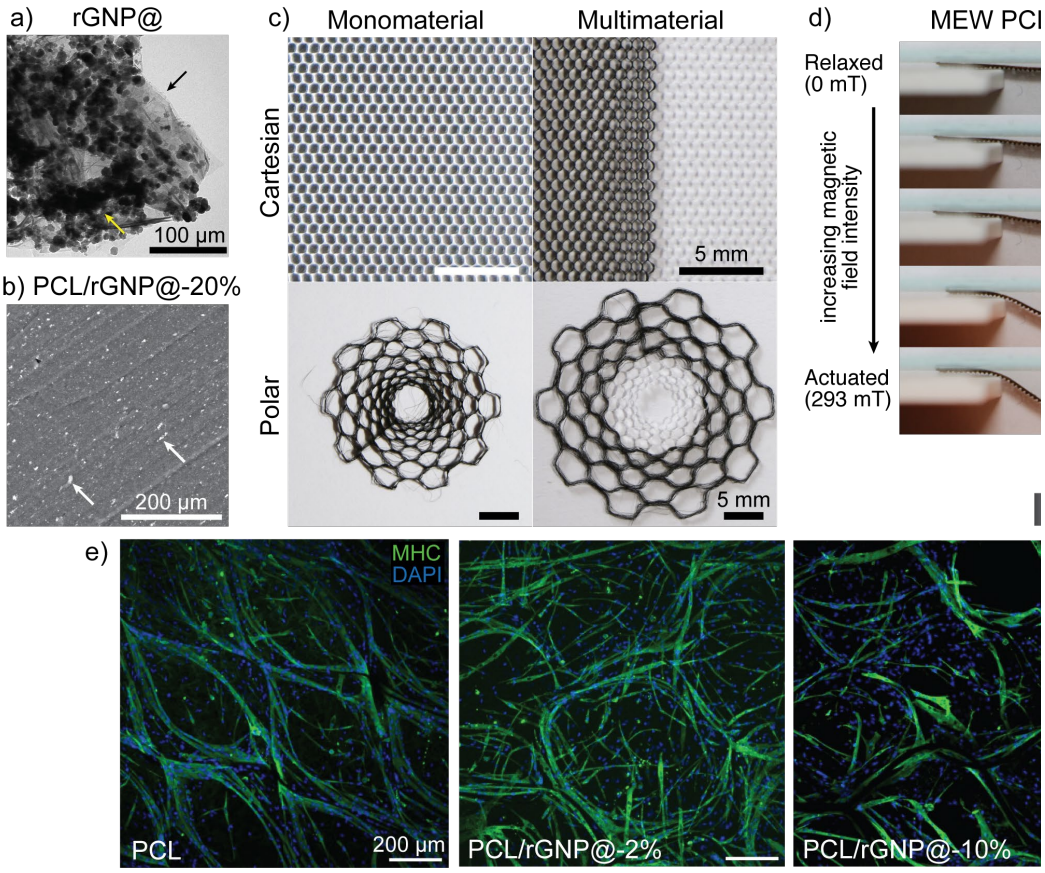

Figure 1. a) TEM of rGNP@; rGNP (black arrow); ION (yellow arrow). b) SEM of PCL/rGNP@-20% filament cross-section; rGNP@ (arrows). c) Modular MEW scaffolds based on PCL (white) and PCL/rGNP@ (black). d) PCL/rGNP@-10% scaffold deformation under magnetic fields. e) Immunofluorescence of C2C12-seeded Matrigel/collagen/MEW scaffolds after static culture, 8 days. MHC (myosin heavy chain); DAPI (nuclei).

References: [1] Castilho M. Adv.Funct.Mater.28:1803151,2018.

S6.7-O3

Magnetic implants for tissue engineering: novel therapeutic strategy for *in situ* bone regeneration

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Abstract

The physiological and functional outcomes of tissues and cells are greatly impacted by the physical microenvironment they inhabit. This microenvironment activates a variety of signaling pathways via mechanostransduction mechanisms, playing an essential role in organogenesis, homeostasis and cellular functions. Particularly, magnetic fields have been applied to manipulate cellular surroundings, exhibiting promising outcomes in the field of tissue engineering. Encouraged by the knowledge that internalized magnetic nanoparticles can remotely manipulate receptors at the cell surface thereby activating critical signaling pathways within tissues, we herein propose a novel therapeutic strategy that uses magnetic tissue implants with an architecture similar to native tissues to ensure proper tissue integration, while utilizing the superior external trigger of a magnetic field to regulate cell fate *in situ*. In this study, we investigated the efficacy of a cyclic magnetic field (CMF) in stimulating magnetized tissues composed of human adipose-derived stem cells (hASCs). After 21 days of CMF exposure, *in vitro* osteogenic differentiation of the tissues was observed, as evidenced by the presence of key osteogenic indicators such as an enhanced collagen matrix, osteopontin and hydroxyapatite nodules. The expression of relevant osteogenic genes also substantiated the differentiation into an osteoprogenitor tissue. The potential of this new therapeutic approach was validated *in vivo* using mice models. In these trials, the presence of the CMF accelerated the implant's integration into the host tissue, while triggering its osteogenic differentiation with minimal local tissue inflammation. Our findings suggest that this wireless magnetic stimulation can be a promising approach for i*n situ* bone regeneration, highlighting the potential of magnetic tissue engineering for clinical applications.

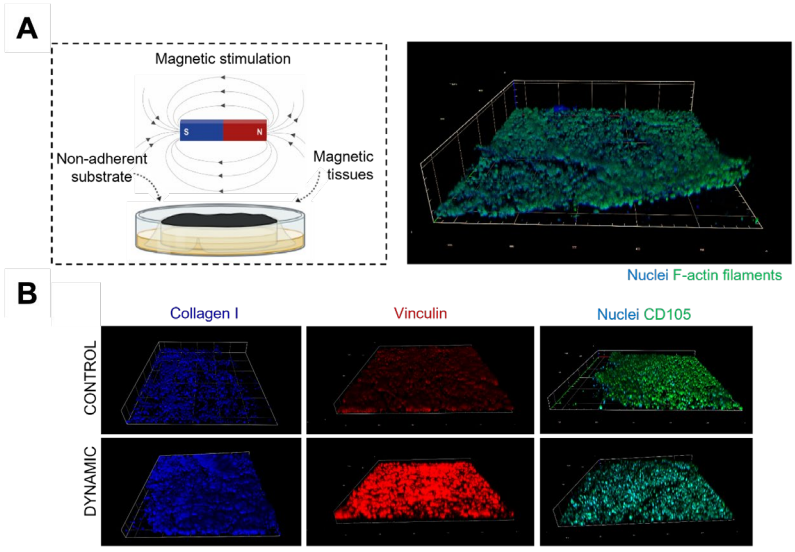

Figure 1 – Characterization of the magnetic tissue. A. Confocal microscopy of the magnetic tissue. F-actin filaments (green) and cell nuclei DAPI (blue). B. Immunostaining images of collagen I, vinculin, and CD105 expression.

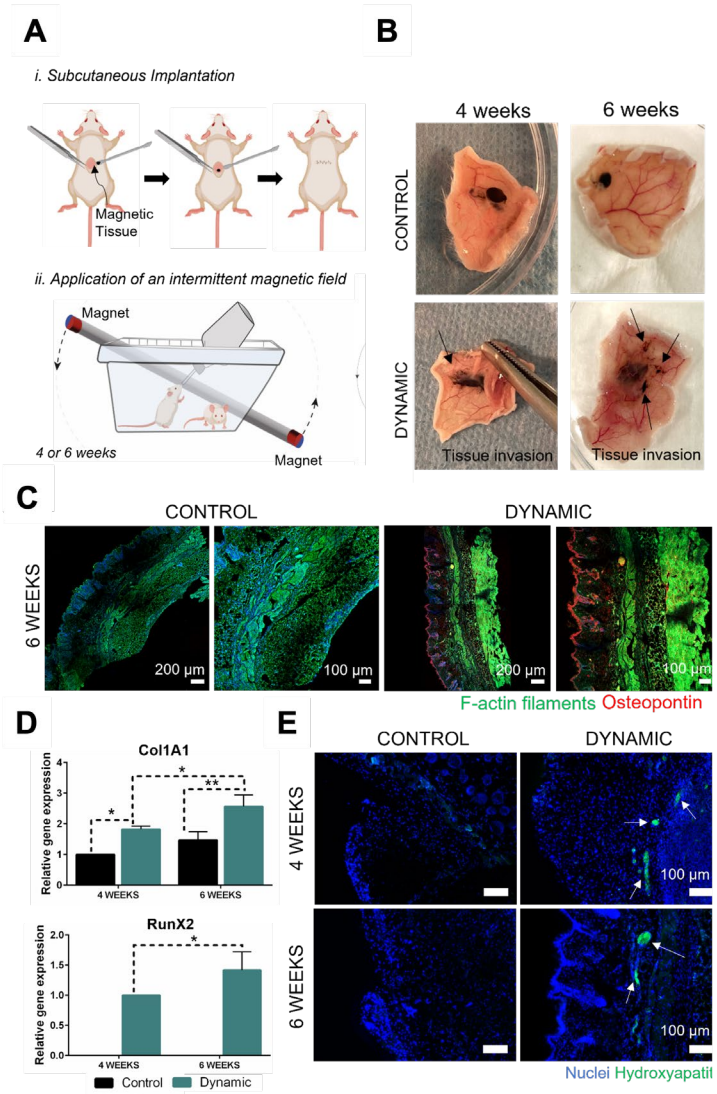

Figure 2 – In vivo magnetic stimulation. A. Experimental design. B. Photographs of the implant and surrounding tissue at 4 and 6 weeks post-implantation. C. Osteopontin (red) at 4 and 6 weeks post-implantation. F-actin filaments (green) and cell nuclei - DAPI (blue). D. Relative expression of collagen I and Run X2. E. Implant mineralization upon magnetic stimulation. OsteoImage™ Mineralization Assay: cell nuclei - DAPI (blue) and hydroxyapatite (green).

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S6.7-O4

Designing a hydrogel for mimicking nervous tissue: development, optimization and application

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Abstract

When developing a hydrogel for applications in contact with very soft tissues, e.g. neuronal tissue, different requirements must be considered. Firstly, the material must be equipped with mechanical properties, especially the very low stiffness, of such tissues. To serve as a matrix for the 3D culture of neuronal cells the hydrogel material must further be biocompatible and allow cell adhesion and migration through the network. Additionally, the matrix should be degradable over a timespan of several weeks, but stable during the first weeks of incubation.[1] For the application as organ or tissue-phantom, the hydrogel construct should not degrade for several months and maintain its mechanical and handling characteristics during this time. Further, the material should be 3D printable to allow versatile sample fabrication for specialized applications. Hyaluronic acid is a biodegradable, biocompatible and non-immunogenic polysaccharide native to the human body extracellular matrix.[2] Through an oxidation process aldehyde groups are formed in the polysaccharide chain which can be used to covalently crosslink the oxidized hyaluronic acid to amino groups in proteins, such as gelatine by Schiff base formation.[2] Gelatine is synthesized from collagen, which is an abundant component in the mammalian body.[3] In this work we present an enzymatically crosslinked hydrogel system based on periodate oxidized hyaluronic acid and gelatine with similar mechanical properties to native neural tissue. After comprehensive characterisation of material properties, the hydrogel system was optimized towards ideal stability and degradation behaviour *in vitro* and under storing conditions to allow different forms of applications, including organ/tissue phantoms and nervous tissue engineering. The printing behaviour was examined and improved by pre-crosslinking to allow the fabrication of complex structures. Finally, different applications of the developed hydrogel matrix were examined. After confirming the positive behaviour of encapsulated fibroblasts and primary neurons, the hydrogel was considered as a filler material for a nerve conduit structure, combined with a shell structure, fabricated by melt-electrowriting PCL and further, the applicability as brain phantom material was demonstrated.

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S7.1-K1

Shining a new light on cells and materials: Biofabrication of multi-component synthetic and functional tissues

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Abstract

Conventional additive manufacturing via layer-by-layer biofabrication technologies, has led to significant advances in the generation of complex 3D tissue constructs. However, in layer-wise approaches, many complex architectures are not accessible without printing dedicates support materials, and often require long printing times, potentially detrimental for cells when aiming to build centimeter-scale, cell-laden constructs of clinically relevant size. In recent years, volumetric bioprinting (VBP), emerged as an ultra-fast, light-based biofabrication approach, to resolve virtually any 3D geometrical patterns in less than 20 seconds. This is possible by projecting multiple visible light tomographic patterns of the intended object onto a vial containing a cell-laden photosensitive hydrogel, causing the material to rapidly photocrosslink in a volumetric, layer-less fashion. However, as for most vat-polymerization technologies, introducing multi-material and heterocellular features, typical of native living tissues and organ, remains challenging. In this work, we addressed this limitation via converging VBP with extrusion-based printing. By developing a microgel-based bioresin, with shear-thinning rheological behavior, multiple materials, and cell types can be loaded and injected in desired regions of the bioresin via suspended printing. Next, VBP allows to sculpt the whole constructs across centimeter-scale in a matter of seconds. Further, mechanical reinforcement can be provided either via co-printing stiff polymeric inks or embedding reinforcing meshes, i.e. produced via melt electrowriting (MEW). The potential of these strategies is shown in the context of pancreatic and vascular tissue engineering. Optimizing the mechanical and optical properties of gelatin-based microparticles enables their use as support bath for suspended extrusion printing, in which features containing high cell densities can be easily introduced. Upon shaping with VBP, the microgel bioresins formed centimeter-scale, hierarchically porous, complex constructs. Inter-particle microporosity enhanced differentiation of multiple stem/progenitor cells (vascular, mesenchymal, adipose), otherwise not possible with conventional bulk hydrogels. As proof-of-functionality, constructs produced combining extrusion bioprinting and VBP was applied to create complex synthetic biology-inspired intercellular communication models, where adipocyte differentiation is regulated by optogenetic-engineered pancreatic cells. Moreover, additional, spatial-specific chemical functionalization can be performed utilizing a secondary VBP process to graft bioactive proteins in specific locations within constructs. Finally, the inclusion of MEW scaffolds within VBPrinted structures facilitates handling of otherwise soft and mechanically unstable hydrogel-based objects. Introducing anisotropic, multicellular/multimaterial patterns within volumetrically bioprinted construct enables the biofabrication of free-form tissue models that can more closely mimic the complex biochemical and structural composition of native tissues, to more precisely guide cell fate and maturation.

S7.1-O1

Acoustic patterning of three dimensional osteo-inductive constructs.

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Abstract

Acoustic assembly is emerging as a contactless and rapid strategy to fabricate relevant structures. This technique directs the assembly of biologically active components through the emergence of specific fluid patterns (i.e., pressure fields, surface instabilities/waves) that generates spatially specific hydrodynamic forces which direct the organization of micron-sized particles. These forces can be directly controlled by external excitatory stimuli (i.e., frequency and amplitude of chamber vibration). Among the available hydrodynamic phenomena, Faraday Ripples (FR) demonstrated to be particularly appealing for the easy generation and the large-scale applicability. FR emerges at the interface between two fluids (e.g., water/air). The resultant hydrodynamic forces can assume a wide range of different patterns according to the frequency applied by the vertical vibrator. FR has been applied to fabricate a variety of 2.5D constructs where biological or inanimate particles are embedded in a hydrogel-based matrix. However, these forces are intrinsically constrained to a plane transverse to the vibration direction. To date, little has been done to translate this technology to three-dimensional architecture to unlock the full potential of hydrodynamic assembly. We propose a rapid approach to precisely fabricate 3D constructs through a successive hydrodynamic assembly of osteo-inductive tricalcium phosphate particles (d = 35-70 µm, TCP) in a gelatin methacryloyl matrix (50 % degree of substitution, 5 % weight / volume, GelMA). Hydrodynamic forces are applied to TCP particles suspended in the GelMA prepolymer solution to form a first structure in a 2D plane. Therefore, the formed TCP@GelMA structure is frozen by a light-induced partial cross-linking, and a second layer of patterned TCP is created on top of the previous. Theoretically, this process could be iterated an infinite number of times, and solely the experimental errors introduced by users do set a practical limit on the possible number of iterations (i.e., thickness of the construct). Once several layers are assembled (typically 3-4), a final cross-linking step is applied to the entire structure to obtain a monolithic TCP@GelMA structure. Following this methodology, we create a precise 3D construct with bio-inductive features or cellular spheroids precisely organized along every spatial dimension. The constructs are characterized by quantitative analysis of X-Ray computed tomography and optical microscopy images. Overall, we present a methodology able to fabricate 3D bio-inductive constructs with features spatially organized along every spatial direction. This work paves the way for the simultaneous 3D assembly of cellularized and acellularized building blocks to fabricate the next generation of cellularized scaffold.

S7.1-O2

Liver-inspired artificial cells for communication and bioprinting

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Abstract

Artificial cells (ACs) mimic the structure and function of living cells. Most often, the focus is on mimicking a selected cellular feature, rather than the entire complexity of the living cell. Further, the mimicry of cellular features can draw inspiration from specific tissue functions. There is a rising interest in interfacing ACs and living cells and the fabrication of semisynthetic multicellular assemblies. Here, communication becomes a central feature when considering tissue-level organization of artificial and natural cells. We explore different approaches to integrate ACs with liver cells. Specifically, hydrogel-based ACs equipped with metalloporphyrins (MPs) are used. MPs are enzyme mimics of the natural cytochrome P450 (CYP450) enzymes, which play a vital role in the phase I metabolism of the liver.

First, MP-functionalized ACs are used to demonstrate a one-way signal transfer to liver cells (Figure 1a). Two types of ACs were fabricated using MPs that catalyse a dealkylation or hydroxylation reaction, exemplified by the production of two different fluorescent molecules. The fluorescent products diffuse into neighbouring HepG2 cells to demonstrate a one-way signal transfer. This observation demonstrated that MP-functionalized ACs function in a biological setting and established a first important step towards reciprocal communication.

Second, the MP-functionalized ACs were used as microgels in bioprinted structures (Figure 1b). HepG2 cell aggregates and ACs were suspended in a gelatin/alginate-based ink, and the bioink was printed in lattices. The HepG2 cells were shown to integrate in the printed structures and maintain cell viability and CYP450 activity for at least 5 weeks. Further, the MP-functionalized ACs were found to boost the function of the hepatocytes when the CYP450 activity of the prints was measured.

Together, these efforts highlight the use of ACs with liver-like function, and their successful interfacing with natural liver cells.

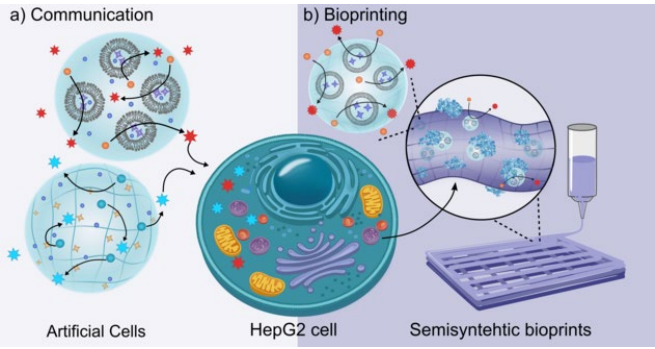

Figure 1. a) Two types of MP-functionalized ACs catalyze the production of two fluorescent compounds, which can diffuse across the cell membrane of HepG2 cell to facilitate a one-way signal transfer. b) Cell aggregates of HepG2 cells and ACs are suspended in a gelatin/alginate-based ink, and the resultant bioink is used to print semisynthetic bioprints with boosted CYP450 activity.

S7.1-O3

PEG/graphene oxide hydrogels: from 2D material characterization to a FRESH approach on 3D printing vascular grafts

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Abstract

Patients with severe coronary/peripheral artery disease have to undergo a bypass surgery, where vascular grafts are implanted to allow blood flow. The gold standard are autologous vessels, which still have disadvantages, notably failure due to thrombosis. Hence, there is a need for synthetic small-diameter vascular grafts. Here, we propose the use of PEG hydrogels as anti-adhesive, antithrombogenic, antimicrobial hydrogels, reinforced with graphene oxide (GO) to have appropriate mechanical properties for vascular grafts.

Hydrogels of 15wt% PEG-dimethacrylate (8kDa), with 0 or 4wt% GO were produced by APS/SMB-initiated chemical crosslinking. Regarding tensile properties, PEG/4%GO hydrogels presented 163 kPa Young's modulus, 218 kPa tensile strength and 126% elongation at break – 6x stiffer, 14x stronger and 1.7x more stretchable than PEG-only. PEG/GO hydrogels have low water contact angle (~20°) and are anti-adhesive against HUVECs (phalloidin/DAPI staining), human blood platelets and *Staphylococcus aureus* (both by SEM).

To produce vascular grafts, we aim to 3D print PEG/GO dispersions using the FRESH technique, where a microparticulate support bath allows for shape retention of low-viscosity inks. Various support baths – 22.5% Pluronic F-127, 0.5% agarose fluid gel, 1.2% Carbopol 974P, gelatin slurry, and gelatin microparticles – were tested. The gelatin microparticle bath showed the best self-healing properties (rheology) and filament structure (extrusion tests), contrary to Pluronic. The gelatin microparticle bath was selected for further experiments. Rings (ø=4mm, L=2mm, WT=0.25mm) were printed (3kPa, 3mm/sec) with PEG/GO dispersion. The support bath allowed for shape retention (impossible when printing "in air"), and constructs were easily removed after printing, although filaments were microscopically irregular. To increase ink viscosity, PEG dispersions with 4wt% GO + 5wt% PEO (MW 900 kDa) or with 6wt% GO were used to print rings, showing better filaments than control PEG/4%GO. To decrease ink diffusion into support bath, baths were washed before printing with APS, $CaCl_2$ or Tween20. Rings printed in baths with Tween20 presented larger, smoother filaments (WT=0.89mm), while those of $CaCl_2$ bath showed thinner filaments (WT=0.64mm) – both yielding better results than APS bath (WT=0.91mm), where significant ink dispersion was observed. Hence, printing in $CaCl_2$-washed baths, using low pressures/speeds, allows for PEG/GO rings closer to designed.

PEG/GO hydrogels show great potential for use as anti-thrombogenic, antimicrobial biomaterial. FRESH 3D printing is a promising technique for extrusion of PEG/GO dispersions, paving the way for applying this biomaterial in vascular grafts.

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S7.1-O4

Injectable hydrogels reinforced with structures obtained through melt electrowriting

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Abstract

Introduction. The field of cartilage tissue engineering faces significant challenges in repairing chondral and osteo-chondral defects. The limitations of hydrogels, such as poor mechanical properties, raise the need for reinforced hydrogels. One of the most investigated approaches involves printing fibrous scaffolds through melt electrowriting (MEW) technology, which are then filled with hydrogels [1]. However, this approach requires an open surgery procedure for implantation. Injection of reinforced hydrogels via minimally invasive procedures, such as arthroscopic ones, can overcome this limitation.
In this work we investigated the injectability of a hydrogel reinforced with polycaprolactone (PCL)-based fibrous structures fabricated using the MEW technology. The fibrous structure geometry was varied. Three different pore sizes were achieved and correlated with the extrusion force needed for successful injection.

Methods. GMP-grade PCL was extruded through a custom-made MEW printer [2]. Structures were printed with square (GRID) and hexagonal (HEX) pores, varying their pore sizes (0.6,0.8,1.0mm). The structures were punched (diameter:8mm), mechanically characterized, and embedded into alginate-gelatin blend (A3G3, 3%w/v each). These reinforced hydrogels were injected using three different needles (18G,16G,14G) to evaluate extrusion forces. Finally, the structure showing the smallest injection force was mechanically compared with the bare hydrogels after ionically crosslinking with calcium-chloride (1%w/v).

Results&Discussion. The printing process produced well-defined GRID and HEX fibrous structures with well-stacked intersection points in both designs (Figure1a). The compressive modulus did not show statistically significant differences between GRID and HEX structures at any pore sizes (Figure1b), while the tensile modulus of HEX structures was higher than GRID ones (Figure1c). The evaluation of injection forces showed statistically significant differences depending on pore sizes in both design structures (Figure1d-f). Moreover, HEX structures showed higher injection forces than GRID ones. The lowest injection force was recorded for GRID structures with 1.0 mm pore size through 14G-needles. Finally, this MEW-reinforced hydrogel showed superior compressive modulus to bare hydrogels (Figure1g).

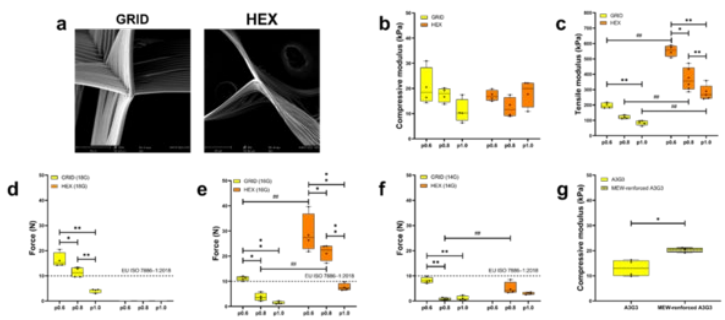

Figure1. Results on MEW structures characterizations.

Conclusions. This study represents the first evaluation of the injectability of MEW structures embedded into hydrogels. The MEW-reinforced

hydrogel shows good injectability properties, and enhanced mechanical properties when crosslinked with respect to the bare hydrogel. This concept could be extended to other hydrogel types, procedures, and tools, such as arthroscopic procedures, and for a wide range of applications beyond orthopedics, such as cardiovascular ones.

Acknowledgement. The work received funding from the EU's Horizon2020 research program, grant agreement N.814413, project ADMAIORA. References. [1]Visser,2015; [2]Eichholz,2022.

S7.2-K1

Multivalency as Geometric Puzzle: Engineering (super)selectivity at the biointerface with DNA

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Abstract

Understanding and manipulating precise interactions between materials and biology – the biointerface – is key to ensure optimal performance of diagnostics and therapeutics. Functional materials for biological applications, e.g. vaccines or implants, work best when their interaction with cells is precise. If not, side effects and toxicity might occur. Interactions are labeled superselective, when they happen only in a very specific (cellular) context and as such, present a strategy to enhance the therapeutic effect of bioactive materials.

Selective multivalent interactions are traditionally engineered with a focus on the balance of valency and affinity, and often a good amount of structural flexibility is present. In my laboratory, we hypothesized that rigidity at the nanoscale could be a strong determinant of super-selectivity. We combine insights from biophysics and tools from DNA nanotechnology to engineer materials with a controlled flexibility/rigidity balance which allows to present molecules and organize interactions in precise spatial patterns. I will show how structural mechanical properties on the nanoscale determine the selectivity of interactions between DNA and lipid membranes, in immune activation pathways, and how they are critical for super-selective Multivalent Pattern Recognition (MPR). Exploiting programmable flexibility within the well-defined DNA molecule, our research presents a new engineering strategy to investigate the impact of nanorigidity in functional soft matter, surface order and communication with life.

S7.2-O1

Hydrogel biointerfaces with spatiotemporal control over gel properties

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Abstract

Cells sense and adapt to forces and physical constraints imposed by the extra cellular matrix. Such mechanotransduction plays a crucial role in cell function, differentiation and cancer. The ECM mechanical rigidity and distribution of ligands are both sensed and modulated through the contractile and adhesive molecular machinery in the cells. Previous technological developments have provided interfaces with well-defined patterns, chemistry or stiffness, which revolutionized how biological questions can be answered. Current research is expanding these parameters to exploring viscoelastic and strain stiffening materials, dynamic materials and controlled display/delivery of multiple signals.

In our research group, we are developing materials to achieve spatiotemporal control over mechanical properties. For example, we have developed a simple projection method to pattern the elastic modulus of GelMA1 and we have developed viscoelastic gels where the mechanical properties of polyacrylamide gels are tailored by polymerizing a second network within the polyacrylamide gel2. The results highlight the importance of interactions between the two networks, where interacting networks give rise to a higher dissipation. By using light to polymerise the second network, it is possible to create gradients in dissipation, without changing the elastic modulus. We have also developed a range of conductive hydrogels, that provide tools for both electrical and mechanical stimulation of cells3, 4. These conducting hydrogels can be oxidised and reduced, leading to large actuation and a small, but significant, change in the Young's modulus. Such conductive hydrogels also have the potential to be used to encapsulate and release drugs. Our data demonstrates excellent control over the release of a small model drug, upon reduction of the conducting hydrogel. In addition, larger protein drugs can be loaded into the gel for passive release.

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S7.2-O2

Scalable biomimetic bone marrow model promoting stemness and expansion of hematopoietic stem cells

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Abstract

Hematopoietic stem cell (HSC) transplantation to treat haematological disorders is greatly restricted by the low availability of cell numbers and donors. *Ex vivo* expansion of HSCs is often used to overcome this limitation but has shown limited success so far due to the loss of stem cell properties in culture. Therefore, modelling a culture scaffold that mimics the physiological properties of the bone marrow (BM) in a scalable format is an important target to enable sufficient cell numbers to be produced for therapeutic applications. Here, we report a scalable system that enables expansion of HSCs in co-culture with mesenchymal stem cells (MSCs). Beyond cellular and biochemical components (e.g., matrix and growth factors), an important element of the BM microenvironment is its architecture, dense in adipocytes, with relatively limited matrix and anisotropic mechanical properties. To capture this context, we propose the use of bioemulsions[1,2] in which oil droplets and associated mechanical anisotropy recreate important architectural features of the hematopoietic niche. The architecture and mechanical anisotropy of this environment are characterised by fluorescence, electron and force probe microscopy, as well as interfacial rheology, confirming our ability to closely mimic the properties of the BM. In addition, MSCs grown at the surface of such bioemulsion remodelled this environment, assembling an interstitial ECM network that resembled the BM microenvironment composition. MSCs secreted biochemical signals that mimicked the crosstalk between stromal cells and HSCs in the native environment. In turn, in co-cultured HSCs were able to adhere to MSCs and expand significantly. Cytokine analysis showed that both SCF and CXCL12 secreted by MSCs were internalised by HSCs, confirming the crosstalk between the two cell types. Flow cytometry analyses after 15 days in culture showed that HSC populations in co-culture retained stemness features and, importantly, a sub-population of long-term repopulating HSCs was identified in co-culture but not on HSC monoculture. Co-cultures were scaled to a small bioreactor system able to produce 2M cells, with retention of HSC stemness and proliferation. The differentiation potential of HSCs cultured in this artificial BM confirmed their ability to commit to myeloid and erythroid lineages, despite long-term co-culture. Overall, we developed an innovative 3D platform that can closely mimic the hematopoietic niche and be adapted to current bioreactor formats to enable HSC expansion.

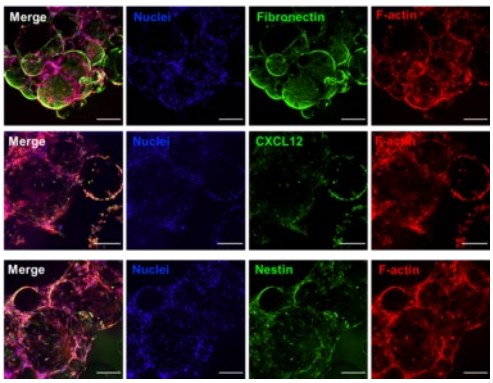

Figure: Immunofluorescence confocal images of MSCs growing on bioemulsions.

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S7.2-O3

Biomimetic platforms for *in vitro* cell growth and biomedical applications: towards precision medicine

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Abstract

The design of powerful *in vitro* cell culture platforms in precision medicine can contribute to predict patient's response to diseases treatment, including cancer and wound healing[1]. Electrospun nanofibers manufactured in a controlled manner possess a dual ability to support *in vitro* cell growth by mimicking the extracellular matrix (ECM) and to control drug release[2]. Here, we established an electrospinning method to produce biocompatible fibrous scaffolds of different starting materials and with several applications. First, we developed and characterized blended polyvinyl-alcohol (PVA)/ gum arabic (GA) ECM-like nanofibers capable of delivering nanocomposites for biomedical application (Figure 1a)[3]. Next, we used heat treatment to crosslink the nanofibers that were functionalized with GA-gold nanoparticles (GA-AuNPs) and we evaluated their cellular delivery. We found that GA-AuNPs were effectively delivered the nanocomposites to metastatic melanoma cells. In addition, we produced polycaprolactone nanofibers loaded with iron magnetic nanoparticles as heat mediators to introduce a multi-therapeutic approach based on the combination of the magnetic hyperthermia with the chemotherapeutic effects of doxorubicin (Figure 1b)[4]. The drug-free magnetic fibers showed high biocompatibility when used as substrates for growing fibroblast cells, whereas the fibers containing both magnetic nanocubes and doxorubicin showed significant cytotoxic effects on cervical cancer cells following the exposure to magnetic hyperthermia. In addition, we developed fibrous scaffolds for dermal applications based on thermally annealed propolis-loaded zein nanofibers[5]. We investigated the physical-chemical properties of the zein/propolis nanofibers and their interaction with cell membrane of murine fibroblasts (Figure 1c). Also, the delivery of propolis to cells were successfully tested *in vitro*. Therefore, zein nanofibers containing propolis are promising for the development of wound-healing patches – one of propolis main applications.

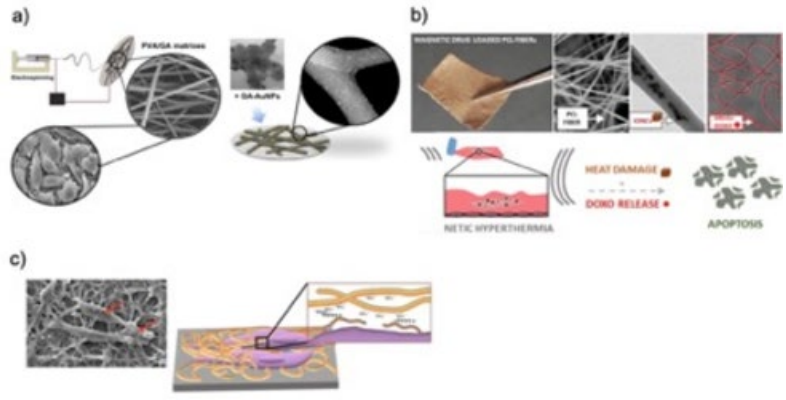

Figure 1. Schematic illustration of three different type of biocompatible ECM-like nanofibers a) PVA/gum arabic (GA) nanofibers capable of delivering gold nanocomposites to metastatic melanoma cells; b) PCL nanofibers co-loaded with iron oxide nanocubes (IONCs), as heat mediators to be selectively activated under alternating magnetic field, and doxorubicin, as a chemotherapeutic drug; c) thermally annealed zein-propolis nanofibers as wound-healing patches.

Acknowledgments: The research leading to these results was supported from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 759959, ERC-StG "INTERCELLMED"). References. [1] Turetta M et al, Curr.Med.Chem. 2018; 25,4616-4637; [2] Jin L et al, Macromol. Mater.Eng, 2017;302,1–9; [3] Serio F et al, Int J Biol Macromol. 2021; 188:764-773; [4] Serio F et al, J Colloid Interface Sci. 2022;607 (Pt 1):34-44; [5] Hochheim S et al, Macromolecular Bioscience, 2023.

S7.2-O4

Modelling and experimental characterisation of auxetic mesh geometric parameters for tissue engineering substrate straining

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Abstract

Introduction. Tissue engineering substrates should, ideally, recapitulate the native cell niche in terms of biochemistry, architecture and mechanical behaviour. However, due to the complex equipment required, effects of applied scaffold mechanics in cell culture environments are often overlooked. Auxetic meshes exhibit a negative Poisson's ratio (ν), where uniaxial straining results in biaxial expansion. Here, we describe our work to characterise the behaviour of a specific auxetic re-entrant honeycomb mesh in uniaxial tension with the aim of inducing physiologically relevant biaxial strains to mimic tissue environments (e.g. the lung). Modelling was undertaken to evaluate the effects of auxetic mesh unit cell geometry and boundary constraints. The outputs were then validated experimentally with a mesh containing a collagen scaffold.

Methods. Fig.1 shows the re-entrant honeycomb unit cell structure [1]. To recapitulate realistic uniaxial straining, finite element analysis was carried out for polylactic-acid (PLA) meshes of varying unit cell geometry strained to 3% where both ends of the mesh were bounded. The mesh geometry was varied such that the theoretical ν remained at -1 according to the analytical solution [2]. Ice-templated collagen scaffolds were then embedded in 3D-printed PLA meshes and the ability of the mesh to apply biaxial straining to a substrate was assessed.

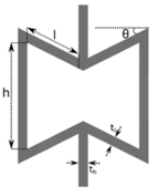

Figure 1. Unit cell of a re-entrant honeycomb mesh demonstrating the geometry defining parameters.

Results & Discussion. Boundary constraints had a strong influence on the biaxial expansion of the meshes (Fig.2a). Fig.2b summarises the effects of h/l, t_h/h, and t_l/l and shows the geometrical parameter space for all simulated meshes that create a "surface" with a theoretical ν of -1. The projected colour map illustrates the actual measured ν and shows that the simulated meshes approached the theoretical value only in the low h/l, t_h/h, and t_l/l corner of the surface. The geometry of the 3D-printed mesh was therefore selected accordingly. Fig.2c illustrates the straining device embedded with a scaffold. The 3D-printed mesh had a ν of -0.79 under bounded conditions. Uniaxial straining of the mesh ($\varepsilon_{x,\,mesh}$) led to biaxial straining of the scaffold (Fig.2d). The ratio of the induced transverse ($\varepsilon_{y,\,substrate}$) and longitudinal strains ($\varepsilon_{x,\,substrate}$) at 3% $\varepsilon_{x,\,mesh}$ was 0.71, consistent with ν.

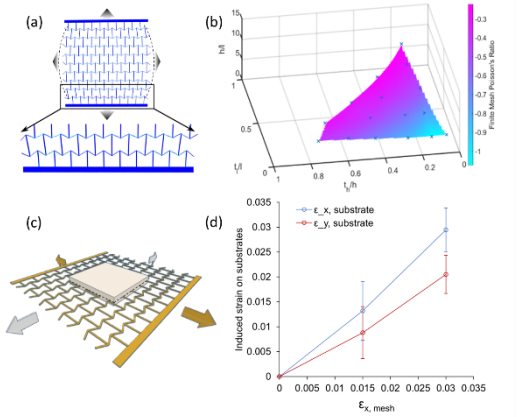

Figure 2. (a) Simulated bounded mesh under strain showing restricted auxetic behaviour. (b) The theoretical -1 Poisson's ratio surface rendered with a colour map representing the actual Poisson's ratio of the simulated meshes. (c) Schematic of the device where a substrate is embedded in the auxetic mesh. (d) Uniaxial straining of the mesh resulted in biaxial straining of the embedded scaffold.

Conclusion. The results show that auxetic meshes offer a predictable and reproducible method to convert simple uniaxial actuation to biaxial strain to provide physiologically appropriate mechanical cues in collagen substrates.

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S7.3-K1

Responsive hydrogels for high resolution 3D printing

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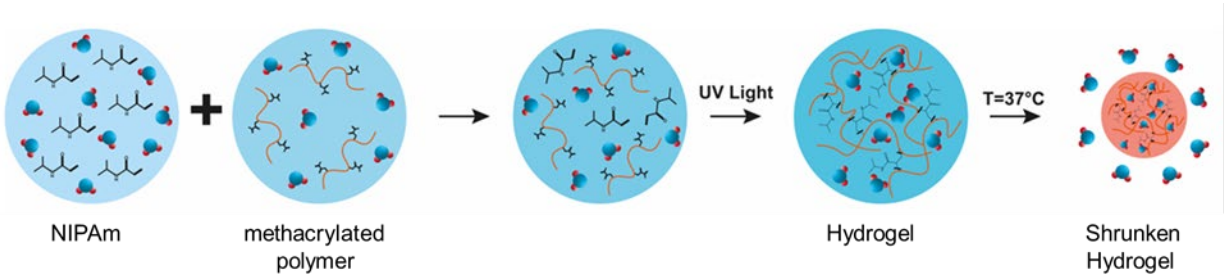
Abstract

Shape plays an important role in the function of cells in a 3D scaffold. Printing high resolution scaffolds with intricate geometries and small sizes (< 100 µm) is thus of high interest in biofabrication for the regeneration of complex tissues, yet remains a fundamental challenge especially when soft hydrogels are used as biomaterials for tissue culture. Resolution enhancement in bioprinting is challenged by the presence of living cells that can handle only a certain amount of stress and pressure during fabrication. Therefore, instead of a printer-engineering approach, we have developed high resolution prints using a new materials design-based approach. We developed multiple hydrogel shrinking techniques to decrease the dimensions of a 3D printed structure post-printing, while retaining the same shape. Our first shrinking technology was based on water expulsion upon complexation of charged polymers.[1]
In our current studies, we designed an alternative shrinking technology based temperature as external trigger. This thermosensitive shrinking method for printed hydrogels makes use of the lower critical solution temperature (LCST) behavior of poly N-isopropyl acrylamide (pNIPAm) to expel water from the network. Thermoresponsive hydrogels have been prepared using either fully synthetic polymers or biopolymer derivatives (silk fibroin and gelatin). These materials were used to print 3D constructs with complex geometries capable of reducing their size by a temperature increase to 37 °C with a 2-3 times reduction in size.
Overall, this study provides a new material-based technology to realize a significant resolution enhancement by shrinking of 3D-printed hydrogels, with high potential in complex tissue engineering applications.



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S7.3-O1

3D microfluidic bioprinting of foamed fibres for hierarchical fabrication of skeletal substitutes

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Abstract

Mimicking the structural heterogeneities of native tissues is of tremendous relevance for the biofabrication of reliable 3D tissue models. Specifically, the trabecular architecture of bone tissue plays a key role for physiological tissue development affecting both mechanical properties and the biological response.

Recently, microfluidic-based systems have emerged as advanced tools to control micro-porosity through the production of tailored emulsions and foams[1]. In particular, the combination of microfluidic devices with 3D printing systems allows for unprecedented micro- and macro-architectural control of 3D constructs[2].

In this work, we report a new strategy for 3D printing fibre-based scaffolds with controllable intra-fibre porosity (Fig. 1(a)) harnessing a flow focusing microfluidic chip combined with a novel extrusion-based microfluidic printhead. The upstream flow focusing chip allows for modulation of the physical features of the produced foams (i.e. size of bubbles and gas volume fraction) which are then deposited as porous fibres using a microfluidic printhead with core-sheath microchannel configuration.

The characterisation of the foaming process showed the potential to form air bubbles up 2 kHz with a size ranging from 24±1 to 73±3 µm (Fig. 1(b)). By modulating the biomaterial ink flowrate between 25 and 40 µL/min, the air volume fraction inside fibres can reach up to 60% of the total volume (Fig. 1(c)). The foamed biomaterial ink includes alginate and gelatine combined with nano-silicate particles (Laponite XLG) that have been found to enhance printability and hold osteoinductive properties[3].

Notably, we reported the printing of single- and multi-porosity scaffolds along with a thorough morphologically (confocal microscopy, optical coherence and micro-computed tomography), mechanical (compression and degradation test) and functional (release kinetics and mineralisation) characterization. To verify the differentiation of bioprinted human bone marrow stromal cells (HBMSCs) towards osteogenic lineage, alkaline phosphatase (ALP) staining and histological staining (e.g. Alizarin Red, Von Kossa) were carried out. Finally, Chorioallantoic Membrane Assay (CAM) was performed to investigate angiogenic properties of scaffolds according to different levels of porosity. Altogether, we propose an innovative approach for engineering bone tissue substitutes using a novel microfluidic-assisted biofabrication system, enabling a precise control in 3D space over fibre micromorphology to control (i) mechanical properties, (ii) cell proliferation and (iii) vascularisation.

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[2]M.Marcotulli et al.,Adv.Mater.Technol.2201244,1(2022).
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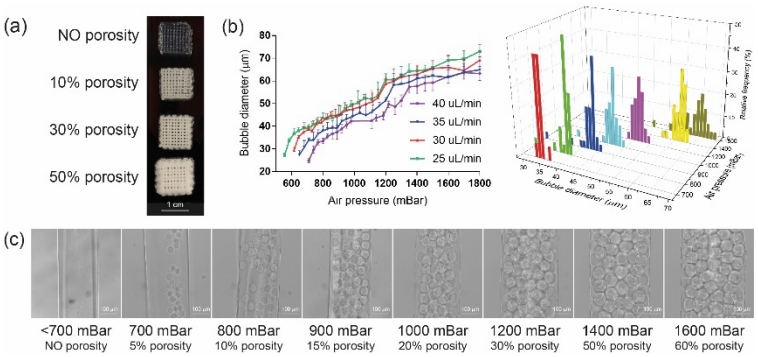

Figure 1. a) 3D printed scaffolds. b) Bubble diameter vs bioink flowrate and bubble diameter distribution for 35 µL/min. c) Foamed fibres at 35 µL/min.

S7.3-O2

Mimicking the extra-cellular matrix of arteries: an ice-templating approach

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Abstract

Arteries and especially small-sized arteries, lack of biocompatible replacement solutions[1]. Despite providing similar shape and mechanical properties to native tissues, current grafts fail to reach their complex biological composition and organization. The arterial wall is composed of an assembly of glycosaminoglycans and proteins, mainly type I collagen[2], assembled into the extra-cellular matrix (ECM) that surrounds cells. When decellularized, the ECM left reveals a porous 3D collagenous structure, whose reconstitution has remained elusive despite the advances in biofabrication.

Our group has pioneered the elaboration of porous materials using biologically derived polymers. In particular, we have developed a successful method to control the hierarchical structure of 3D dense collagen materials using ice templating[2,3]. Such biomaterials recapitulate the structural, biological and functional features of cells' microenvironment. Highly concentrated collagen solution were ice-templated to allow ice crystals growth and the subsequent segregation of collagen molecules within the interstices formed in-between the crystals. The ice crystals were then slowly melted at low temperature to reveal the porous network, while collagen compaction is maintained and its self-assembly induced by ammonia vapors (Figure 1). Versatile structures can be obtained by tailoring the freezing parameters, resulting in various mechanical and biological properties.

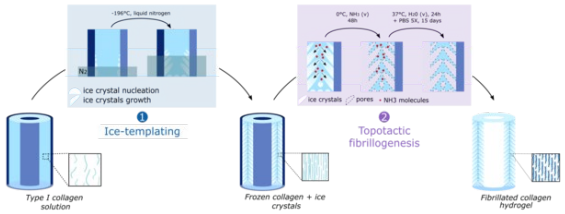

Figure 1. Biofabrication process of collagenous materials.

Here we report the use of ice nucleation and growth as tools to shape collagen-based biomaterials. Our approach enables to tailor: the textural aspects of the materials' interfaces to direct its interactions with relevant cells (endothelial cells and mesenchymal stem cells), the number of structural layers to reproduce the native architecture of arteries (from one to three layers), and the various mechanical features (compliance and axial moduli) of native arterial tissues (Figure 2). These results open an exciting pathway to tackle the current limitations of small diameter arterial replacement using new biomimetic matrices. Large preclinical animal model experiments are ongoing[4].

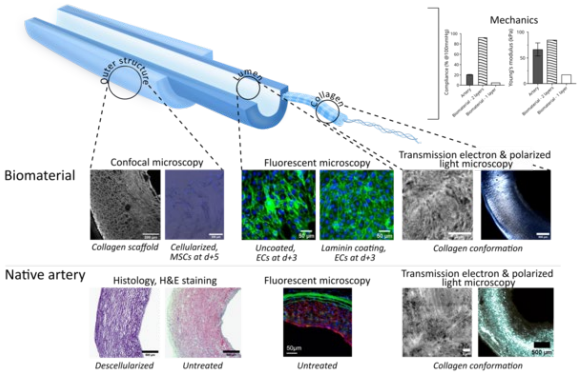

Figure 2. Mimicking arteries' ECM through ice-templating and innovative collagen self-assembly.

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S7.3-O3

Instructing engineered living microcomposite tissues from within using stimuli-responsive cell-adhesive micromaterials

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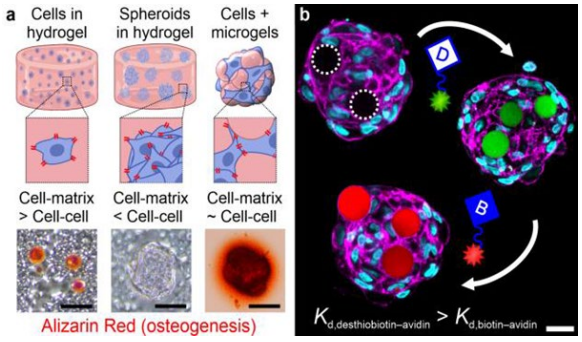
Abstract

Modular tissue engineering exploits the 3D self-assembly of cells and micromaterials to create larger tissue constructs with higher complexity and resolution. However, current modular tissue engineering strategies have near-exclusively relied on static non-responsive, micromaterials, whereas functionality of native tissues is dictated by their inherently dynamic nature. Here, we report on in situ tunable microbuilding blocks of which the biochemical and biophysical properties can be altered via orthogonal crosslinking strategies.

Methodology. Microgel production: Microgel precursor droplets composed of 5% dextran-tyramine-biotin (Dex-TAB; ~1 mM biotin) and 22 U/mL horseradish peroxidase in phosphate-buffered saline were emulsified in Novec 7500 Engineered Fluid with 2% Pico-Surf 1 using a microfluidic droplet generator, and subsequently crosslinked via controlled microfluidic supplementation with H2O2. Dex-TAB microgels were consecutively incubated with 1 µM neutravidin, washed, incubated with 1 µ M biotinylated or desthiobiotinylated molecule-of-interest (e.g., biotinylated c(RGDfK)), and washed again. Tunable modular tissue engineering: Microgels were homogeneously co-seeded with cells into non-adherent 3% (w/v) agarose microwell chips at a density of ~50 units per microwell. The mechanical properties of microgels could be modified post-synthesis by exploiting visible-light-induced crosslinking of tyramine moieties using 1 mM ruthenium and 2.5 mM sodium persulfate as initiators. Chemical post-modification of the microgels was achieved via competitive supramolecular complexation of avidin and biotin analogs.5 Adipogenic and osteogenic differentiation of mesenchymal stem cells (MSCs) was analyzed microscopically following Oil-Red-O and Alizarin Red staining, respectively.

Results. Reactive tyramine and biotin moieties in Dex-TAB microgels could be enzymatically post-crosslinked and/or functionalized with avidin/biotin analogues, respectively, in an orthogonal manner. This enabled virtually independent in situ tuning of the microgels' mechanical and chemical properties. Coating Dex-TAB microgels with biotinylated c(RGDfK) enabled integrin-mediated self-assembly with cells, even without serum. In contrast to conventional bulk encapsulation of cell spheroids in hydrogel, incorporating cell-sized microbuilding blocks within engineered tissue uniquely supported cell-material mechanotransduction, which was essential for osteogenic differentiation of 3D stem cell cultures (Figure 1a). Material stiffness-induced lineage programming of modular 3D stem cell/microgel spheroids was dependent on the size, stiffness, and number ratio of microbuilding blocks, as well as the timing of material stiffness-induced mechanical cues. In situ biochemical control over cell-building block aggregates was shown by temporally endowing the building blocks with (desthio)biotinylated fluorophores (Figure 1b), as well as bone morphogenetic protein (BMP)7 neutralizing antibodies, which showed temporal control over the cellular response to BMP7 using a BMP-reporter cell line.



S7.3-O4

Improving mesenchymal stem cell function and survival via silencing tissue factor genes or extracellular matrix coating

Oommen P Oommen, Vignesh Kumar Rangasami

Tampere University, Tampere, Finland

Abstract

Stem cell therapies hold promise for treating diseases or conditions with limited treatment options. However, clinical trials attempting to harness the immunosuppressive and regenerative potential of MSCs have yielded disappointing results, largely due to poor viability and thrombotic activation of the transplanted cells. To overcome this challenge, we have developed two distinct strategies: (a) a method for efficiently silencing the Tissue Factor (TF) gene in MSCs using pluronic-based nanocarriers,[1] and (b) a technique for coating stem cells with polyelectrolyte polymers.[2]

Our first strategy involved designing a novel pluronic-based nanomicelle system to deliver siRNA targeting the TF gene. We anticipated that silencing of TF gene (CD142) would mitigate the activation of the coagulation cascade after allotransplantation. The pluronic nanomicelles were able to efficiently silence the target gene (72%) without producing any adverse toxicity. Silencing of TF in human bone marrow-derived MSCs (BMSCs) significantly reduced the incidence of instant blood-mediated inflammatory reactions (IBMIR) by inhibiting platelet aggregation and thrombin-antithrombin complex formation. In addition, TF knockdown enhanced the differentiation potential and paracrine signaling of BMSCs, as evidenced by increased stimulation upon exposure to endotoxin.[1]

Our second strategy focused on leveraging the natural anticoagulant and anti-inflammatory properties of heparins to address thrombosis challenges. We coated individual cells with gelatin and heparin polyelectrolyte polymers using a layer-by-layer approach.[2] We found that MSCs coated with heparin and gelatin polymers were protected from complement and coagulation cascade activation, and exhibited decreased IBMIR in human whole blood. This was evident from lower platelet aggregation and thrombin anti-thrombin (TAT), C3a, and s-C5b9 generation in the coated MSCs (cMSCs) when compared to the uncoated MSCs. The coated MSCs also retained their ability to suppress inflammation and undergo differentiation.

Thus, by silencing TF in BMSCs or coating MSCs with polyelectrolyte polymers, we were able to improve stem cell survivability and enhance immunosuppressive properties. These approaches offer a potential solution to the inherent stem cell survivability issue encountered upon transplantation, increasing the safety and efficacy of BMSC-based therapies.

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S7.4-K1

Soft colloidal building blocks to overcome limitations in tissue engineering

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Abstract

We apply polymeric molecular and nano- to micron-scale building blocks to assemble into soft 3D biomaterials with anisotropic and dynamic properties. Microgels and fibers are produced by technologies based on fiber spinning, microfluidics, and in-mold polymerization [7,8]. To arrange the building blocks in a spatially controlled manner, self-assembly mechanisms and alignment by external magnetic fields are employed. Reactive rod-shaped microgels interlink and form macroporous constructs supporting 3D cell growth [1,4,6,8]. On the other hand, the Anisogel technology offers a solution to regenerate sensitive tissues with an oriented architecture, which requires a low invasive therapy. It can be injected as a liquid and structured in situ in a controlled manner with defined biochemical, mechanical, and structural parameters. Magnetoceptive, anisometric microgels or short fibers are incorporated to create a unidirectional structure. Cells and nerves grow in a linear manner and the fibronectin produced by fibroblasts is aligned [2,3]. Regenerated nerves are functional with spontaneous activity and electrical signals propagating along the anisotropy axis of the material. Another developed platform is a thermoresponsive hydrogel system, encapsulated with plasmonic gold-nanorods, which actuates by oscillating light [9]. This system elucidates how rapid hydrogel beating affects cell migration, focal adhesions, native production of extracellular matrix, and nuclear translocation of mechanosensitive proteins, depending on the amplitude and frequency of actuation. The time spent in the *in vitro* gym seems to affect myoblast differentiation and fibrosis [5].

S7.4-O1

The “shuttles” to neuroprotection: Biodegradable dendrimers as delivery vectors in the context of stroke

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Abstract

Stroke is the 2nd cause of death worldwide and the 1st in Europe. Ischemic stroke, the most prevalent type, occurs when blood supply to the brain is occluded, resulting in neuronal death. Nevertheless, current therapies are not applicable to most patients and only mitigate the effects of occlusion by restoring the blood-flow, lacking neuroprotective properties. Several neuroprotective agents have been explored for stroke, tough they have failed in clinical trials, mainly due to the lack of an effective delivery vehicle. Here, we propose the use of our proprietary fully-biodegradable dendrimers (PEG-GATGE) as delivery vectors of neuroprotective therapeutics that aim to: (A) improve neuronal survival by delivering mRNA encoding for the brain-derived neurotrophic factor (BDNFmRNA), and (B) reduce glutamate excitotoxicity by delivering the LC domain of Botulinum Neurotoxin type A (BoNT/A LC) (Fig.1).

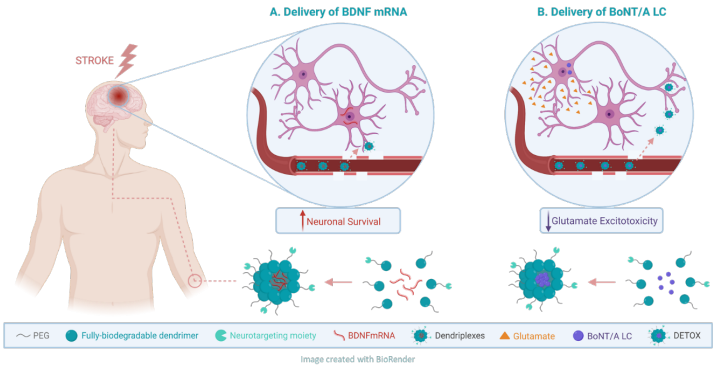

Figure 1. Fully-biodegradable dendrimers (PEG-GATGE) as delivery vectors of neuroprotective therapeutics.

The resulting benzylamine-terminated dendrimer-BDNFmRNA nanoparticles (dendriplexes) already showed suitable physico-chemical properties for the proposed strategy, with sizes <100nm, PdI <0.3, positive net charges, and mRNA complexation efficiencies >90%. Also, in-vitro studies revealed dendriplexes excellent internalization capacity, without causing cytotoxicity in primary cortical neurons. The system hemocompatibility was also demonstrated, which is significant for a systemic administration. In-vivo, dendriplexes were able to reach the brain, especially the infarct area. And excitingly, preliminary data showed an increased expression of BDNF in the ipsilateral side (Fig.2), resulting in a decreased infarct volume. This demonstrates the great ability of the dendrimer to protect and effectively deliver the mRNA to the lesioned brain after systemic administration.

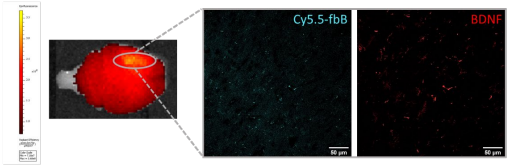

Figure 2. Cy5.5-labeled benzylamine-terminated dendrimer (Cy5.5-fbB) in complexation with BDNFmRNA was IV administered in stroke compromised animals (pMCAO). IVIS Lumina III images showed a higher presence of dendriplexes in the ipsilateral side, and an increased expression of BDNF was also verified in the lesion area by IHC.

For the dendrimer-BoNT/A LC nanoparticles (DETOX), amine-terminated dendrimers were explored. The recombinant domain (50 kDa) was successfully produced with ~99% purity. Importantly, LC catalytic activity was demonstrated in primary cortical neurons by immunodetection of the cleaved form of SNAP-25 (the LC neuronal target) after treatment of the cells with the protein. Concerning DETOX formation, we are presently investigating the best conditions regarding buffers and dendrimer:LC ratios, to obtain optimized therapeutic complexes in terms of size, charge and stability. Subsequently, their in-vitro and in-vivo biological performance will be evaluated. Together, these results point to the versatility of the explored dendrimers as promising drug delivery systems towards the promotion of neuroprotection in stroke. Due to their excellent biocompatibility and capacity to cross the BBB, their extrapolation to the treatment of other neurological conditions can be foreseen.

Acknowledgments. FCT for project PTDC/BTM-MAT/4156/2021, as well as M.T. (SFRH/BD/146754/2019) and S.D.S. (DL57/2016/CP/CP1360/CT0013) contracts, and BiotechHealth doctoral program (ICBAS, UP).

S7.4-O2

Multicellular dorsal root ganglion system assembled using hydrodynamic forces to study disc nerve ingrowth

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Abstract

Introduction: Chronic low back pain (LBP) is associated with the intervertebral disc (IVD) nerve ingrowth. [1] The cell bodies of these nerve fibers locate in the dorsal root ganglion (DRG), where nociceptive/pain signal is firstly processed. We developed a multicellular system of DRG *in vitro* using a mild sound assembly process based on contactless hydrodynamic forces. [2] This results in a densely packed cell-cell contact mimicking the native DRG tissue and a controlled AF-neuron distance. In this system, the effect of IVD tissue on sprouting of nerve fibers was investigated.

Methods: Explants of outer annulus fibrosus (AF) tissues (2*2*2 mm) from bovine caudal IVDs were primed with IL-1β and TNF-α (each 10 ng/mL) for 24 h. AF without cytokine priming served as control. Bovine DRG micro-tissue unites were assembled into concentrical rings around the AF tissue in a collagen-based extracellular matrix (0.5 mg/mL). The DRG calcium signals were analyzed using calcium imaging (Fluo4). The morphology of satellite glia cells (SCGs) was evaluated using immunofluorescent staining of glial fibrillary acidic protein (GFAP). In the DRG-AF co-culture system, nociceptor nerve fibers were labelled by calcitonin gene-related peptide (CGRP). The axon morphology was evaluated using 'imageJ SNT' and 'R'.

Results: In the DRG multicellular system, calcium signals were observed to transmit from one neuron to other attaching neurons at day 4. SCGs self-organized to an aligned structure which guided the axonal directions at day 8. The cytokine-primed AF tissue increased the length of CGRP(+) axons by 52% compared to non-cytokine control at day 2. In the cytokine-primed group, 86.7% of CGRP(+) neurons had majority of their axons turning towards the AF, while this proportion was only 50% in non-cytokine control. These effects were only significant when neurons were positioned within 1.3 mm from AF tissue (n=12 and 15 neurons, n= 119 and 236 axons for control and cytokine treated AF, respectively).

Discussion: We have successfully fabricated a multicellular DRG system. This multicellular morphology is essential for intercellular crosstalk and structural cell self-organization. We demonstrated that the AF tissue-mediated nerve sprouting depends on the AF-neuron distance which can be tuned by sound-based hydrodynamic forces. This is the first *in vitro* IVD nerve ingrowth model using large animal tissues. This model may advance our understanding of LBP pathophysiology and may help in finding novel LBP treatments.

References: [1]. Freemont et al., Lancet, 1997; [2]. Petta et al., Biofabrication, 2020

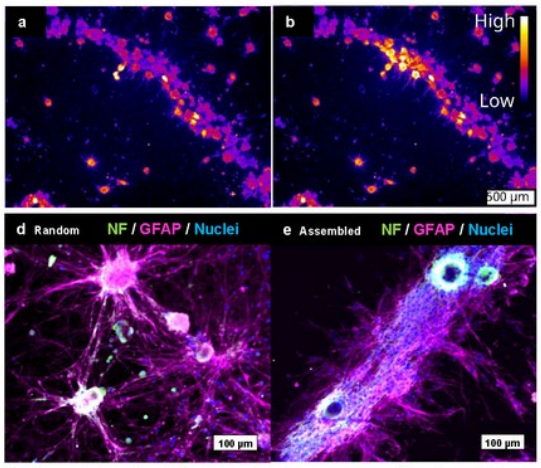

Figure 1. a-b) *The calcium signal is transmitted from one cell to its neighbours in the assembled multicellular DRG system.* ***c)*** *In the random culture, the self-organization of satellite glial cells was limited, although they exhibited a small trend to connect cell aggregates at day 8.* ***d)*** *The multicellular assembly obtained via hydrodynamic forces accelerated the cells' self-organization.*

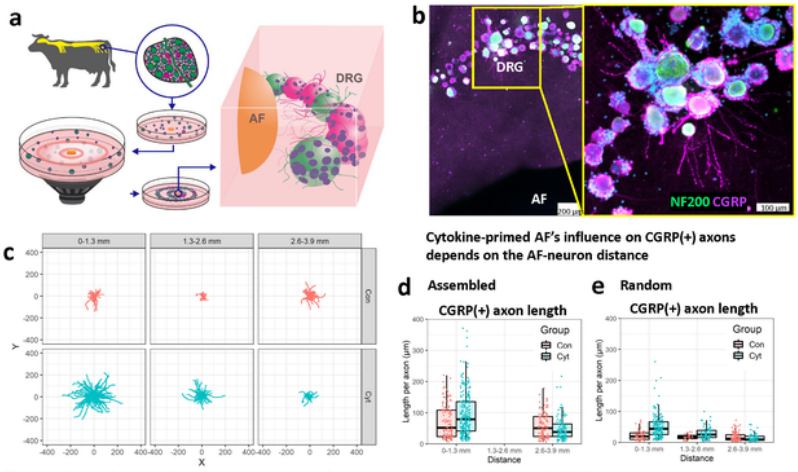

Figure 2. a) *Schematic of hydrodynamic forces-induced assembling of DRG neurons surrounding an anulus fibrosus (AF) tissue explant to investigate AF nerve ingrowth.* ***b)*** *Immunofluorescent staining (CGRP) of axons in the assembled multicellular system.* ***c)*** *Axonal morphologies are shown as a function of the distance between annulus fibrosus (AF) tissue and cell body of nociceptors. XY axis unit is micron. Cytokine-primed AF (Cyt group) showed a distance-dependent promoting effect on the CGRP(+) axonal length in both SIM-assembled multicellular system* ***(d)*** *and random culture* ***(e)****.*

S7.4-O3

Self-Assembling glycopeptide hydrogels for neuronal differentiation

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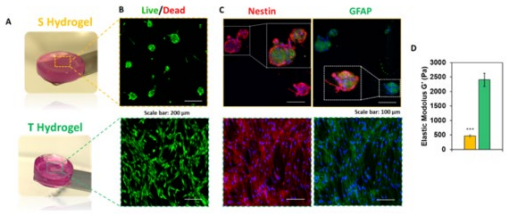
Abstract

Supramolecular hydrogels generated by unidirectional self-assembly of short biofunctional peptides mimic the morphological and biochemical features of the extracellular matrix (ECM).[1-2] Importantly, most ECM proteins are glycosylated -the attached glycans can code bioinformation, affect the conformation of core protein, and modulate cellular response. As an example, in the central nervous system, heparan sulfate (HS) proteoglycans are present in the ECM, being essential in neurogenesis by guiding axon growth and synaptogenesis.[3] In this context, we designed a minimalistic HS-mimicking glycopeptide amphiphile, i.e. Fmoc-diphenylalanine-glucosamine-6-sulfate (Fmoc-FF-GlcN6S), that self-assembles into nanofibers (copycatting the ECM) and gels in aqueous media.1 We used two distinct procedures to generate supramolecular gels from Fmoc-FF-GlcN6S: temperature (T) or solvent (S) switch methods. In the T method, the glycopeptide was dissolved in water at a temperature of ~90°C, and gelation was induced by cooling the solution to room temperature. In the S method, the glycopeptide was dissolved in DMSO, followed by its dilution into water, inducing self-assembly and gelation. Both methods resulted in the formation of entangled meshes of nanofibers (AFM). While the non-glycosylated peptide, i.e. Fmoc-FF, assembled into supramolecular β-sheets (CD and FTIR), the glycosylation (Fmoc-FF-GlcN6S) altered the nanofibers' secondary structure to α-helices. The preparation method influenced the stiffness of the hydrogels: G'(T)~2.4kPa > G'(S)~0.5kPa (both within the range of neural tissues, i.e. between 0.5-3.0kPa)[4] and their self-healing capacity - after an applied force, gels obtained by the T-method recovered better than the hydrogels generated by the S-method. Fmoc-FF-GlcN6S hydrogels were non-cytotoxic towards human adipose-derived stem cells (hASC). Interestingly, a homogeneous hASC distribution and spreading was observed for the T-hydrogels, while cellular clusters were formed on the S-hydrogels. qPCR and immunostaining characterization showed that both hydrogels induced an overexpression of GFAP and Nestin by hASCs at day 3 of culture, and MAP2 and βIII-tubulin at day 9. Of note, hASC cultured on S-hydrogels showed a neurosphere-like cellular organization, i.e. nestin-positive cells on the sphere surface and GFAP-positive ones in the core. The developed glycopeptide hydrogels successfully copycat the ECM of neural tissue and induced differentiation of hASC into neural lineages - features that make these gels promising materials for neural tissue engineering, regeneration and healing.



A. Macroscopic images of the hydrogels obtained from the Fmoc-FF-GlcN6S glycopeptide (10mM, culture medium) using solvent-switch (S) or heating-cooling (T) procedure. **B.** Confocal microscopy images of human adipose-derived stem cells (hASC) cultured on glycopeptide (Fmoc-FF-GlcN6S) hydrogels for 3 days and stained with calcein AM (green) and ethidium homodimer-1 (red). **C.** Immunostaining for Nestin and GFAP (hASC at 3 days of cell culture). **D.** Elastic modulus (G', 37°C) of the hydrogels prepared from the glycopeptide using the S (yellow) and T (green) method.

Acknowledgments. We acknowledge FCT and M-ERA.Net for financial support (PTDC/CTM-REF/0022/2020-OncoNeoTreat, M-ERA-NET3/0007/2021-RePark, PD/BD/135256/2017, COVID/BD/152018/2021 and CEECINST/00077/2018). References. 1.Levin, A.; Nat.Rev.Chem. 2020, 4(11), 615-634. 2.Sato, K.; Chem.Soc.Rev 2018, 47(20), 7539-7551. 3.Soares da Costa, D.; Annu.Rev.Biomed.Eng 2017, 19,1-26. 4.Murphy, M.C.; Neuroimage.Clin,2016,10,283-90.

S7.4-O4

The effect of bioactive scaffolds with enhanced supramolecular motion on neuronal regeneration and modeling

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Abstract

Over the past decades, biomaterials have been continuously tested as key players in a variety of central nervous system (CNS) strategies. Current biomaterial approaches are inconsistent, cost inefficient, and ultimately fall short in their ability to promote neuronal maturation and repair. This is due in part to the lack of synergistic cues derived from the architecture, chemical composition, and molecular dynamics of the native extracellular matrix. Supramolecular polymers have emerged as new materials that can have ordered structures and tunable dynamics, in contrast to conventional hydrogels, which are typically based on cross-linked covalent polymers. We report here on peptide-amphiphile supramolecular nanofibers that contain distinct bioactive signals on their surface and differ in the intensity of molecular motion within the fiber. By mutating the monomers of their non-bioactive domains, we enhanced the motions of the molecules within the scaffolds, resulting in enhanced bioactivity in an iPSC-derived motor neuron model as well as in a mouse model of spinal cord injury (SCI) (Figure 1). Proteomic, biochemical and functional assays show that scaffolds with highly mobile molecules lead to increased mature electrophysiological activity of neurons. When tested in a mouse model of SCI, this resulted in remarkable differences in vascular growth, axonal regeneration, and functional recovery. Our work highlights the importance of supramolecular motion in the design of bioactive scaffolds to improve function and dysfunction in the CNS.

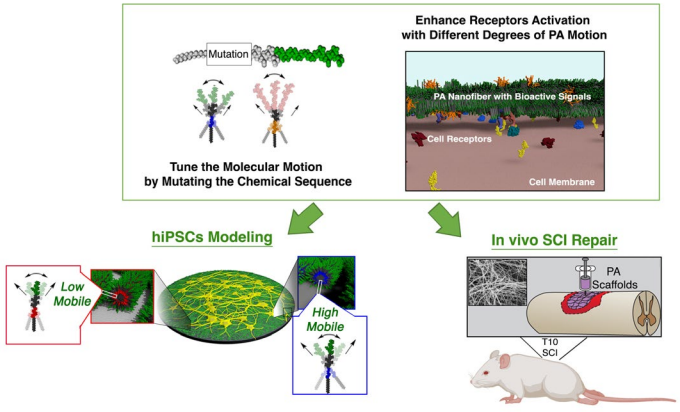

Figure 1. Schematic of peptide-amphiphile molecules with tuned motion resulting in enhanced cell bioactivity in a human induced pluripotent stem cell (hiPSC)-derived motor neuron model and a mouse model of spinal cord injury (SCI).

S7.5-K1

Drivers of early peri-implant endosseous healing

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Abstract

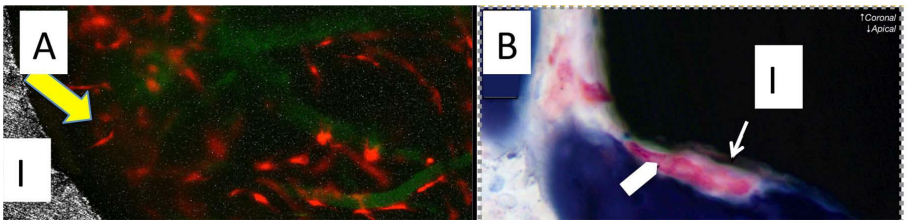

Figure: (A) Neo-vessels (green) with peri-vascular mesenchymal cell ingress into the peri-implant compartment (yellow arrow) [Khosravi et al Biomaterials 273 (2021 120837] (B) a single TRAP+ multinucleate cell (arrow head) tunneling through bone juxtaposed to the implant surface. Note the thin seam of bone (arrow) on the implant surface. I = implant. Field Widths 1.2mm and 237µm respectively.

The experimentally proven sigmoidal increase of endosseous secondary implant stability illustrates that osseointegration comprises three distinct phases—lag, growth and plateau—which represent the biologic phenomena of acute inflammation, bone formation and bone remodeling respectively. The implant surface has a profound effect on contact osteogenesis. More recently, we have shown that implant topography can drive the pattern of peri-implant angiogenesis and the ingress of mesenchymal progenitors into the peri-implant compartment. But this begs the question: “What other important lag-phase biology may also be influenced by implant surface design?” This presentation attempts to address this question based on our recent work and also evidence from other areas of inflammation and bone research.

Multiple studies have shown that implant surfaces have a profound effect on platelet activation generating knock-on effects in both neutrophils and macrophages, all of which secrete VEGF to stimulate endothelial cells during angiogenesis. Indeed, invading neutrophils, act cooperatively with monocyte/macrophages to effect the acute immune response, and facilitate macrophage polarization to an M2 phenotype. Nerve growth factor (NGF) secreted by both neutrophils and macrophages, plays a key role in the bidirectional signals between the nervous and immune systems and NGF expression precedes osteogenesis. Intramuscular administration of NGF to diabetic rats has resulted in increased osseointegration, although clinical translation is limited.

Macrophage/monocytes, play multiple roles including the creation of vascular mimicry channels that serve as highways for the directed migration of endothelial cells. On the contrary, their multinucleate fusion progeny serve as yet undefined roles in peri-implant healing. Our most recent work has shown that both osteoclasts and MNGCs in the peri-implant compartment exhibit varying levels of tartrate-resistant acid phosphatase (TRAP) staining. This may be important since TRAP+ cells have been shown to play an important role in linking angiogenesis and osteogenesis by driving the formation of type-H vessels and their surrounding committed osteogenic perivascular cells.

This review explores other early “lag phase” biology that may be influenced by implant surface design and thus, by extension, could inform novel therapeutic targets.

S7.5-O1

A redox active hydrogel with antioxidant, ROS scavenging and osteogenic properties for osteoporotic bone repair

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Abstract

Introduction: Osteoporosis, characterised by depleted bone mass due to bone resorption exceeding bone formation, is intrinsically linked to age-related hormone deficiency which increases reactive oxidative species (ROS) induced oxidative stress, inducing premature osteoblast apoptosis2. Cerium has both antioxidant and osteogenic properties but its use in repairing osteoporosis-impaired bone is relatively unknown. The aim of this study was to formulate a chitosan/collagen(CS/Coll) hydrogels functionalised with Ce nanoparticles(Ce-NPs), as a radical scavenger to protect mesenchymal stem cells (MSCs) from ROS-mediated inhibition and promote bone-formation.

Methods: Ce-NPs were prepared by co-precipitation method3. Molar solution of cerium nitrate hexahydrate was prepared in distilled water with 2M sodium-hydroxide as a reducing agent. CS/Coll hydrogels functionalized with Ce-NPs were prepared by mixing acid solubilized CS with Coll and Ce-NPs in β-glycerophosphate at pH~7.40. MSCs derived from healthy and ovariectomized (OVX) rats were cultured on the hydrogels under oxidative stress (20mM, H2O2).The antioxidant potential of the hydrogels was measured using DCFDA-Cellular ROS Detection Assay. MSC viability and proliferation was measured using Alamar-Blue and Pico-Green assays respectively. A calcium-assay and RT-qPCR was used to assess the osteogenic potential of hydrogels over 21 days.

Results: An oxidative stress environment was successfully created whereby MSC viability (Fig 1a) and proliferation (Fig1b) was significantly reduced after 30mins exposure to H2O2. Ce-NPs functionalized hydrogels successfully reduced H2O2-induced intracellular-ROS in MSCs after 24hrs (Fig1c&d). A greater antioxidant effect was observed in hydrogels cultured with OVX MSCs compared to healthy. Hydrogels successfully rescued MSC viability (Fig 2a) and proliferation (Fig 2b) after exposure oxidative stress. All hydrogels showed the presence of a hydroxyapatite layer on the surface after 1 day of incubation in osteogenic media, indicating their biocompatibility. A significant increase in calcium mineralization over 21-day culture-period was observed in the hydrogels containing 0.1M Ce-NPs compared to hydrogel without Ce-NPs (Fig. 2(c)).

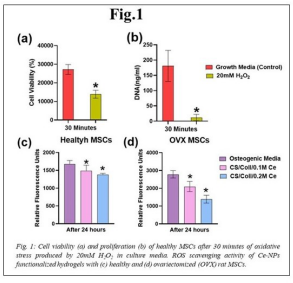

Fig. 1: Cell viability (a) and proliferation (b) of healthy MSCs after 30 minutes of oxidative stress produced by 20mM H_2O_2 in culture media. ROS scavenging activity of Ce-NPs functionalized hydrogels with (c) healthy and (d) ovariectomized (OVX) rat MSCs.

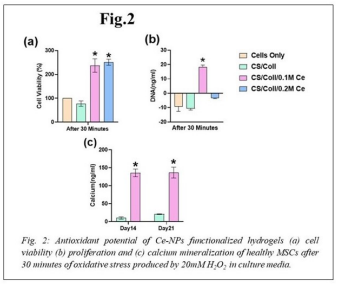

Fig. 2: Antioxidant potential of Ce-NPs functionalized hydrogels (a) cell viability (b) proliferation and (c) calcium mineralization of healthy MSCs after 30 minutes of oxidative stress produced by 20mM H_2O_2 in culture media.

Discussion: We formulated antioxidant thermoresponsive hydrogels and demonstrated the protective effects of Ce-NPs against ROS-inhibition of osteoporotic MSCs *in vitro*. We showed that H2O2 increased the intracellular ROS production and MSC apoptosis, which was then alleviated by Ce-NPs functionalized hydrogels. Furthermore, Ce-NPs significantly enhanced MSCs osteogenic differentiation and matrix mineralization. The present findings provide a basis for applying the Ce-NPs as a novel agent to relieve the oxidative damage in the process of osteoporotic bone repair.

References: 1. Pinna et al., Acta Biomater, 2021; 2. Wu et al., J. Mater. Chem. B, 202

S7.5-O2

3D cryoprinted sodium alginate scaffolds impregnated with plasmid dna for enhanced bone regeneration

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Abstract

Tissue engineering has recently undergone significant advancements, moving beyond the utilization of biomimetic scaffolds that facilitate regenerative processes, towards more complex matrices loaded with biotherapeutic agents that enhance and accelerate tissue repair. These constructs are intended not only to fully match the damaged tissue area but also to serve as a platform for the targeted delivery of therapeutic agents stimulating the differentiation of host cells.

In this study, sodium alginate (SA) based hydrogel scaffolds impregnated with plasmid DNA (pDNA) are formed via the original three-dimensional (3D) cryoprinting technique. pDNA condensed with polyethyleneimine (PEI) are known to form polyplexes for safe and effective transfection of mesenchymal stem cells (MSCs), leading to the expression of bioactive therapeutic proteins. The implementation of our 3D cryoprinting technique using low temperatures and divalent ions as crosslinking agents allows for the fabrication of porous 3D scaffolds of a given architectonics without additional complications, ensuring the structural safety and integrity of the genetic material embedded in the hydrogels.

SA aqueous solution with plasmid DNA incorporated prior to 3D printing was extruded onto the cooling (up to -10°C) platform according to the 3D computer model and transferred to the solution of Ca^{2+} or Ba^{2+} for subsequent gelation. Crosslinked with barium ions scaffolds possessed enhanced mechanical properties but lacked biocompatibility, compared to calcium ions. pDNA, encoding *EGFP*, was used to evaluate the release kinetics of genetic constructions and transfection kinetics of cells. Gene-activated scaffolds (GASs) were shown to provide a sustainable polyplex release sufficient for the transfection of cells and gene expression for 21 days. When using pDNA, encoding *BMP2*, MSC osteogenic differentiation was investigated and the increase in gene and protein expression was detected for the main osteogenic differentiation markers (Runx2, Alpl, Opn). By means of micro-CT and histomorphometry GASs with pBMP2/PEI were demonstrated to provide the new bone volume increase by 5 times compared to blank scaffolds.

The newly developed gene-activated SA scaffolds were shown to provide effective transfection followed by cell differentiation *in vitro* and enhance target protein expression and bone regeneration *in vivo*. The 3D cryoprinting technique of manufacturing highly effective GASs is hailed as a promising new approach in regenerative medicine applications.

This research was funded by the Ministry of Science and Higher Education within the State assignment FSRC «Crystallography and Photonics» RAS in part of hydrogel scaffolds 3D cryoprinting development, the State assignment RCMG in part of plasmid DNA production and characterization.

S7.5-O3

3D-printed composite scaffolds combining hyaluronan, collagen and osteoinductive calcium phosphate to promote bone regeneration

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Abstract

Introduction: Large bone defects are a tremendous clinical challenge due to insufficient self-healing, and the gold standard treatment, autografting, has severe limitations due to donor site morbidity. Current tissue engineered bone graft substitutes lack spatial control over scaffold architecture to anatomically match complicated bone defects. Therefore, this study aims to develop a composite biomaterial-ink of tyramine modified hyaluronic acid and collagen type I (THA-Col) with osteoinductive calcium phosphate particles (CaP), to 3D-print patient-specific bone graft substitutes that provide control over shape, architecture, and composition (**Figure 1A**).

Methods: The biomaterial-ink comprises 35 mg/mL THA combined with 5 mg/mL rat tail Col mixed 1:1, supplemented with 0.5 U/mL horseradish peroxidase (HRP) and 0.01% w/v Eosin Y blended with a range of 0-30% w/v CaP (size 45-63 or 45-106 µm). By adding 0.085 mM H_2O_2 and 6 mM NaOH a 3D-printable gel was formed. Post 3D-extrusion, matrices were fully cured by visible light crosslinking (505 nm). Composites were characterized by rheology, swelling, and compressive modulus. Next, scaffolds were evaluated *in vitro* for cytotoxicity by seeding human mesenchymal stromal cells (hMSCs) on the matrices and assessing viability with live/dead assay for up to 7 days. The osteogenic potential *in vitro* of hMSCs on the composites was analyzed by alkaline phosphatase (ALP) production after 14 days, by gene expression analysis (RUNX2, SOX9, ALPL, IBSP, SPP1, MMP13, COL1A1) at 14 and 28 days and osteoprotegerin (OPG) production over 4 weeks of differentiation.

Results: Rheology showed that THA-Col has viscoelastic and shear-thinning properties suitable for 3D-printing. THA-Col had the highest swelling ratio, and this was reduced by adding CaP, which is favorable for preserving 3D-printed structures. Compression tests showed increasing compressive moduli with increasing CaP content. *In vitro* analysis of hMSCs revealed high viability on all composites on day 7 (**Figure 1B**). *In vitro* osteogenic differentiation resulted in a more intense ALP staining on THA-Col alone followed by THA-Col + 10% of both CaP sizes (**Figure 1C**), also confirmed by ALP activity assay. OPG secretion confirmed these results, with highest production in THA-Col alone (**Figure 1D**). Gene expression analysis also showed similar trends.

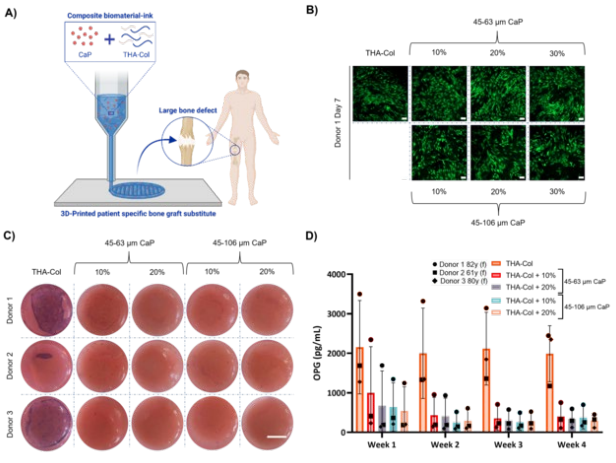

Figure 1: A) Composite biomaterial-ink combining THA-Col and CaP for bone regeneration. B) Live (green) and dead (red) staining of hMSCs at day 7, scalebar = 100 µm. C) ALP staining (blue) after 14 days of hMSCs osteogenic differentiation, scale bar = 5 mm. D) OPG secretion of hMSCs during osteogenic differentiation.

Conclusion: Here, a 3D-printable biomaterial-ink of THA-Col and CaP was engineered, holding significant potential as patient-specific bone graft substitute for the regeneration of large bone defects.

S7.5-O4

Modulation of environmental osmolality as cue to guide mesenchymal stromal cell osteogenic differentiation in 3D viscoelastic hydrogels

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Abstract

Cells can sense and be influenced by extracellular matrix (ECM) biophysical properties (e.g., mechanics, osmolality). Viscoelasticity was shown to regulate spreading, proliferation, and fate decision of mesenchymal stromal cells (MSCs) in our earlier work. We engineered a controlled drop in environmental osmolality mimicking human bone fracture and the associated switch in marrow fluidic niches. Consequently, we asked how a biomimetic osmolality drop affects ECM mechanics and MSC osteogenic differentiation at the onset of healing.

Mechanical characterization was performed for *ex vivo* tissue or hydrogels by uniaxial compression (TestBench LM1 system, BOSE), osmolality measurements (*ex vivo* fluids, media) by freezing-point osmometry (OSMOMAT Auto, Gonotec), characterization of cell behavior by cell viability (live/dead), spreading (actin), proliferation (KI-67), or early osteogenic differentiation (ALP) using histological staining. Imaging was performed using confocal microscopy (Leica SP5) and image analysis by ImageJ. Gene expression was analyzed by RNA sequencing, and alterations in chromatin-condensation by heterogeneity of nucleus staining (DAPI). Alginate hydrogels (Pronova UP, DuPont) were adhesion-ligand-functionalized using RGD peptides (GGGGRGDSP, Peptide 2.0).

Fracture hematoma viscous properties were enhanced in hypo-osmolality. Using hydrogels that resembled these characteristics revealed that hypo-osmolality increased proliferation (~5-fold), spreading (~25%) and osteogenic differentiation (~2-fold). Gene expression was altered two hours after the osmolality drop, and with characteristics distinct from effects of mechanics. Chromatin condensation was decreased 15 minutes after the osmolality drop, which aligned with GO-term analysis of differentially expressed genes.

Our data suggest that a quick drop of osmolality acts as instant stimulus for MSCs in their mechanical niches. The osmotic change causes alterations in cell shape, and nucleus structures with associated alterations in gene expression. Our results underline the potential relevance of instant osmotic changes as a potential trigger of regeneration cascades in post-injury tissue.

S7.6-K1

Engineered extracellular vesicles for tissue regeneration

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Abstract

Extracellular vesicles (EVs) are nanosized lipid vesicles that contain a cocktail of bioactive factors, such as proteins, mRNAs and non-coding RNAs. Evidence that EVs are an important component of the paracrine effects induced by transplanted cells in cell-based therapies has generated considerable interest of the scientific community in using these vesicles as therapeutic agents, namely for regenerative purposes. Despite positive data achieved in preclinical studies with EVs in their native form, issues related to therapeutic efficacy and accumulation in the target site still need to be overcome. In the last years, we have focused on the development of EV-based therapeutics for brain and heart regeneration, particularly in the context of ischemia. We combined different engineering strategies for the modification of EVs surface with controllable amounts of targeting moieties and for the modulation of EVs bioactive content in order to enhance brain targeting, accumulation in ischemic cells and, ultimately, maximize their biological effect.

S7.6-O1

Injectable thermosensitive hydrogel for cardiac delivery of therapeutics

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Abstract

Many genes and pathways have been indicated to play essential roles during heart regeneration and manipulation of these pathways using mRNAs or small molecules have been shown to be promising therapeutic strategies. For this, a dual delivery system composed of mRNA polyplexes and micelle-containing thermosensitive hydrogel, previously complexed with CHIR99201 (a small hydrophobic GSK3 inhibitor and Wnt agonist), was designed allowing local sustained release of mRNA as well as small molecular weight drugs (Fig.1) [1,2]. In this study, the synthesis of mPEG-pDMAEMA (PD) diblock copolymer was optimized via RAFT polymerization and was used as polymeric carrier for mRNA condensation. Condensed mRNA polyplexes showed an average size of 146±11 nm (N/P charge ratio 10) with a positive zeta potential (10.1 ± 0.3 mV). Subsequently, the loading of mRNA polyplexes into a thermosensitive pNIPAM-PEG-pNIPAM (NPN) hydrogel was evaluated to facilitate local and sustained mRNA release. The NPN triblock copolymer synthesized by ATRP polymerization, was used as a loading carrier for CHIR99021 (CHIR) by forming flower-like micelles that encapsulate the hydrophobic drug via heat-shock procedure. The CHIR-NPN gel was then formulated by increasing the CHIR-NPN polymer content, reaching the final concentration of 25% w/w. After 15 days, NPN hydrogels were fully dissolved while drug-loaded hydrogels exhibited much longer degradation times (up to 54 days). This proves that the presence of CHIR affects the stability of the hydrogel, presumably due to its interaction with the dehydrated pNIPAM blocks. Experimental studies revealed a sustained release of the drug over 54 days, demonstrating release kinetics mainly governed by hydrogel erosion. NPN and CHIR-NPN gels showed temperature-sensitive behavior with a gel point below 37 °C, proving their injectability and in situ gelation upon administration, as previously demonstrated. The final dual delivery system, composed of mRNA polyplexes-CHIR-NPN hydrogel, showed the same mechanical properties as the CHIR-NPN gel and demonstrated injectable features at room temperature. *In vivo* pericardial injection of the dual delivery system in healthy mouse hearts was performed thanks to the thermosensitive properties of the hydrogel. After 4 days of treatment, it was shown that the hydrogel was able to trigger the proliferation of cardiomyocytes (Fig. 2). Encouraged by these data, follow-up studies are planned to evaluate the ability to induce proliferation of cardiomyocytes in infracted hearts upon administration of mRNA-CHIR-NPN hydrogel.

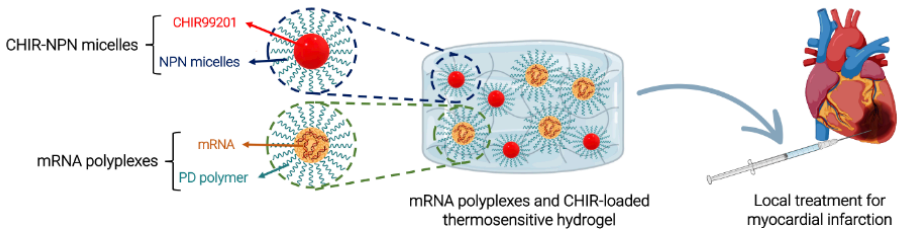

Figure 1. *Schematic overview of the designed dual delivery system for heart regeneration.*

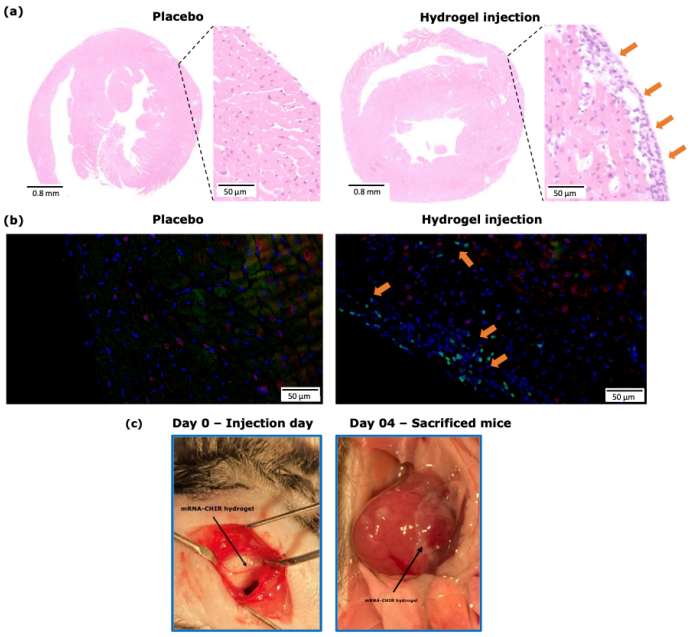

***Figure 2**. Bioactivity of mRNA polyplexes loaded into CHIR-NPN hydrogel (a) Representative images of H/E staining of mouse heart sections treated with placebo (PBS) or hydrogel 4 days after injection (n = 2 per group). Scale bar = 0.8 mm for whole heart view, scale bar = 50 µm for higher magnification view. Note, the higher magnification of H/E images on the right indicated the emergence of the massive cells on the surface of the epicardium treated with the hydrogel (orange arrows). (b) Immunofluorescence staining 4 days after injection. Representative images of Ki67(green)/PCM1(red)/DAPI(blue) staining in both groups (magnification 20x). Hydrogel treatment promotes pericardial cells proliferation compared to placebo group (orange arrow). (c) Optical images of the mouse heart at the day of the injection (left) and at day 4 when the mice were sacrificed (right). The milky substance visible indicates the presence of the hydrogel after 4 days of treatment (black arrow).*

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S7.6-O2

Combining MSC exosomes and HA for TMJ-OA: A promising rabbit study

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Abstract

Introduction: Temporomandibular joint osteoarthritis (TMJ-OA) is a condition characterised by the progressive degradation of cartilage, remodelling of subchondral bone, synovitis, and chronic localised pain. In a rat TMJ-OA model, weekly injection of mesenchymal stem cells (MSC) derived exosomes showed promising therapeutic potential for cartilage repair and pain relief. This study aims to evaluate the efficacy of MSC exosomes, in combination with hyaluronic acid (HA), for treating TMJ-OA in a rabbit model.

Experimental methods: MSC exosomes were isolated from the conditioned medium of human MSCs. OA of bilateral TMJs was induced in 6 rabbits using monosodium iodoacetate (MIA) injection. Additional 3 rabbits received needle pricks at the TMJ area and served as healthy control. After 2 weeks of OA induction, 200µl PBS with 50µl HA or 0.2mg exosomes in 200µl PBS with 50µl HA were delivered to the OA joints weekly for the 3 consecutive weeks. Rabbits in the healthy control group received only needle pricks. The effect of treatments on TMJ OA pain was assessed weekly by measurements of the head withdrawal threshold using an algometer. At the 10th week post-intervention period, the rabbits were euthanised. And the dissected TMJs were examined using gross examination, histological assessment and micro-computed tomography (micro-CT) evaluation.

Results and discussion: Compared to the TMJs treated with HA, the TMJ treated with MSC exosomes and HA demonstrated improvements in head withdrawal threshold and significantly better outcomes in the macroscopic score ($P < 0.05$), OARSI histologic score ($P < 0.01$) and synovial membrane inflammatory score ($P < 0.01$). Micro-CT evaluation also showed a higher ratio of bone volume over total volume (BV/TV) in the latter group as compared to the former ($P < 0.01$). Osteoarthritic TMJs treated with MSC exosomes and HA demonstrated macroscopic and radiographic features (BV/TV and trabecular thickness), cartilage and synovial health that approximated that of the healthy control ($P > 0.05$).

Conclusion: MSC exosomes with HA demonstrated a superior therapeutic effect over HA alone for treating TMJ OA in a rabbit model in terms of reducing pain, promoting condylar cartilage and subchondral bone restoration and suppressing synovial inflammation.

Acknowledgments: The authors would like to thank Ministry of Education, Singapore (Grant no: A-0002093-00-00), National University of Singapore (Grant no: A0002938-00-00), National Medical Research Council, Singapore (Grant no: A0002218-00-00), and Agency for Science, Technology and Research, Singapore for providing financial support to this project.

S7.6-O3

Engineering extracellular vesicles to cross the blood-brain barrier

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Abstract

The scientific community is making significant efforts to discover and develop drugs to treat neurological disorders, which are the leading cause of disabilities worldwide. However, getting therapeutics to the brain is challenging due to the presence of the blood-brain barrier (BBB), a dynamic interface between cerebral vessels and parenchyma. This complex structure successfully prevents dangerous molecules to affect the brain, but it also impedes the drugs to reach the target cells. In order to address this problem, our research aimed to temporarily open the BBB by taking inspiration from the changes in the barrier that occur during ageing. In particular, we employed miRNAs related with ageing to modulate plasma extracellular vesicles (EVs) derived from umbilical cord blood and we then incubated the enriched EVs with a human BBB model obtained by co-culturing endothelial cells and pericytes. Our results showed that a combination of one of the tested miRNAs with the EVs could significantly increase the BBB paracellular permeability in a reversible manner by decreasing the expression of claudin 5, a protein involved in the tight junctions of the BBB. We therefore investigated the target genes of the miRNA of interest, and this helped us to explain the weakening of the tight junctions of the BBB. Finally, we explored the internalization and the effects of the modulated EVs on the endothelial cells of a microfluidic system-based human BBB model. In this case, flow parameters and shear stress were set to mimic the physiological conditions in the cerebral vessels. Our findings suggest that miRNA-engineered EVs have potential as a strategy for the temporary BBB opening, making it easier for drugs to reach the brain when injected into the bloodstream.

Acknowledgements**:** The authors would like to express their gratitude to the European Commission for the funding through the Marie Skłodowska-Curie Innovative Training Network “NANOSTEM” (n. 764958) and through the RESETageing project (H2020 Widespread Research Executive Agency (REA) - Grant Agreement n. 952266), and to FCT for the funding through the projects “Polymeric nanoparticles loaded with CRISPR/dCas9 for direct cell reprogramming” (ref 2021.06297.BD) and “A therapeutic strategy to fight blood-brain-barrier (BBB) ageing” (ref: PTDC/BTM-SAL/5174/2020).

S7.6-O4

Local delivery of si-TNF-α-laden exosomes using a core-sheath 3D-bio-printed scaffold as a fast-degradable wound dressing

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Abstract

Although there have been advancements in wound care, treating chronic wounds remains difficult for a number of reasons. One of these reasons is the excessive secretion of TNF-α, which can delay the healing process. Therefore, the therapies that target TNF-α or its downstream effectors may hold promise for the treatment of chronic wounds. si-TNF-α as a short interfering RNAs has been applied to target and suppress TNF-α expression in chronic wounds. The use of exosomes as delivery vehicles for siRNAs holds great potential for the development of novel wound dressings. By incorporating exosomes into the 3D-bio-printed scaffolds, they can be released in a controlled manner, promoting localized delivery. Additionally, the scaffold provides a supportive environment that protects exosomes from degradation and enhances their stability. Core-sheath 3D-bio-printing allows the creation of highly customized constructs with precise control over the spatial distribution and composition of multiple materials. Alginate and Carboxymethyl cellulose hydrogels can be used as a biocompatible and effective carrier to deliver exosomes into wound sites, promoting wound healing by stimulating cellular communication and reducing inflammation. The use of alginate lyase to degrade alginate fast in wound dressings can improve the ease of dressing changes and reducing the risk of bacterial infection and inflammation.

In this study, a precipitation method was used to isolate exosomes from NIH/3T3 cells. Following morphological and molecular characterizations of exosomes, si-TNF-α were loaded into exosomes via the electroporation method. Furthermore, si-TNF loading and functionality were confirmed by qPCR and ELISA. Finally, si-TNF-α laden exosomes have been loaded into a core-sheath 3D-bio-printed scaffold for delivery to the wound. By this formulation, si-TNF-α laden exosomes were incorporated into alginate, while carboxymethyl cellulose combined with alginate lyase formed the sheath. The sheath bio-ink was added with alginate lyase to speed-up the degradation of wound dressings and enable fast delivery of si-TNF-α laden exosomes. The final wound dressing was characterized morphologically, physically, *in vitro* and *in vivo*.

In vitro, it was shown that si-TNF-α containing exosomes could be delivered functionally from a 3D-bioprinted scaffold with a validated morphology of isolated exosomes and a core-sheath structure. When used *In vivo*, the core-sheath scaffold loaded with the si-TNF-α laden exosomes showed significantly better performance compared to the scaffold loaded with the bare exosomes and the negative control. This study suggests that an extracellular matrix-mimicking fast-degrading scaffold with the capability to deliver si-TNF-α-laden exosomes could be considered as an efficient wound dressing.

S7.7-K1

Frontier platforms for experimental cell modelling

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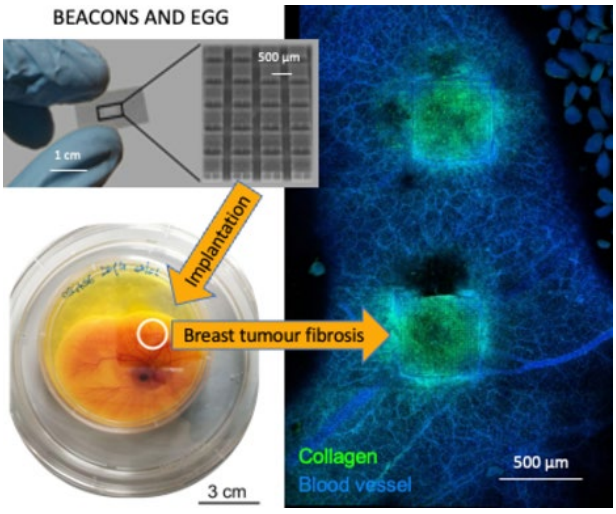
Abstract

Introduction. This talk provides the fundamental concepts behind the design of frontier platforms for experimental cell modelling that I have invented in the last two decades.

THE MOAB. One concept is miniaturisation associated to full-thickness optical accessibility, to recreate and monitor in real time and in long term culture, the interstitial perfusion process of 3D natural tissues such as brain, bone marrow and lymph node tissues. Cell models based on this concept proved able to recapitulate *in vitro* several slowly-developing biological processes, such as the formation of a bone metastasis by breast cancer cells, the instruction of adaptive immune cells in a lymphnode and the neuroprotective effect of mesenchymal stem cell secretome on pathological neurons. I have then connected these models to recapitulate mechanisms involving multiple body compartments, such as the microbiota-gut-brain axis in neurodegeneration and the bone marrow-lymph node axis in leukemic transformation.

THE NICHOID. Another concept is miniaturisation of the 3D cell scaffold. In the aim to better control and monitor stem cell function, I miniaturised the culture substrates in my models by applying a microfabrication technique called two-photon laser polymerization. Using these 3D micro scaffolds, I was able to condition mesenchymal stem cells, neural precursor cells and embryonic stem cells towards maintenance of a greater multipotency or pluripotency, compared to conventional 3D scaffold culture.

THE MICROATLAS. Another revolutionary concept is implanting a cell model in a living organism, to regenerate a microvascular network anastomosed to the host, allowing for studies involving interactions of the cell model with the host immune system. My group has recently replicated the human microvascular niche and relevant druggability *in vivo* in a chick embryo model. These results are opening the way to the application of micro-optics implanted *in vivo* for optical inspection of biological processes.



Future developments. We are starting to use human breast cancer cells adhering to 3D polymeric micro scaffolds to create arrays of tumour micro environments. We will implant the arrays *in vivo* in the chorioallantoic membrane of an embryonated avian egg, to elicit a foreign-body fibrotic reaction. We will vary the micro scaffolds geometry to condition tumour infiltration by the host's vessels and cells. We will predict mass transport of solutes and anticancer agents by computational modelling. To validate this platform, we will quantify *in vivo* the dose-dependent efficacy and cancer specificity of therapeutic agents whose success is known to depend on the fibrotic stage of tumours.

S7.7-O1

Unlocking the potential of amphiphilic cyclodextrin nanoparticles: Tailoring molecular composition for controlled drug release

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Abstract

Cyclodextrins (CDs), a group of naturally occurring oligosaccharides, are well known pharmaceutical excipients. They form inclusion complexes with drugs, resulting in increased drug solubility, stability, absorption, and permeability across biological barriers. Grafting of aliphatic chains onto native CDs renders them amphiphilic and enables self-assembly into supramolecular drug nanocarriers (NCs), such as micelles, vesicles, and particles, having the potential to protect drugs from biodegradation, to improve their solubility, and to control their release. Since the choice of aliphatic chain will determine the extent of hydrophobic interactions during self-assembly, drug encapsulation, and drug release, tailor-made engineering of physicochemical NC properties, release kinetics, and biological interactions is possible and amphiphilic cyclodextrin conjugates hold a great potential to comprise a drug delivery platform.

In this study, we synthesized a library of amphiphilic CD derivatives and systematically investigated their self-assembly into NCs encapsulating model drugs with different physicochemical properties as well as molecular weights. Ester derivatives $CDOC_6$ and $CDOC_{12}$ were synthesized by conjugation of acyl chlorides to the primary hydroxyl groups of native β-CD. The thioether derivative $CDSC_6$ was obtained from native β-CD by bromination and subsequent nucleophilic substitution with 1-hexanethiol. Further conjugation of hydroxyethyl groups to the C2 position yields the PEGylated derivative $CDSC_6OH$ (Fig 1A).

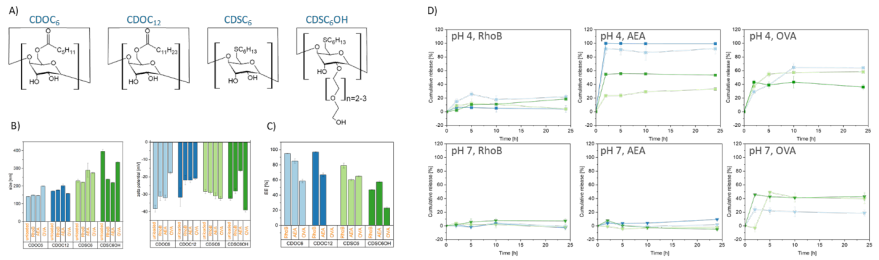

Figure 1: Amphiphilic cyclodextrin nanocarriers as drug delivery platform. *Chemical structures of amphiphilic CD derivatives (A). Size and zeta potential of CD nanocarriers (B). Encapsulation efficiency (EE) of model drugs anandamide (AEA), rhodamine B (RhoB) and ovalbumin (OVA) into CD nanocarriers (C). Drug release profiles of model drug from CD nanocarriers at pH 4 and 7 (D).*

Upon nanoprecipitation, all CD derivatives spontaneously self-assembled into nano-sized carriers with a uniform size distribution (PDI>0.2) and a negative surface charge (Fig. 1B). Further, CD nanocarriers were able to accommodate different model drugs with a broad range of physicochemical profiles as well as molecular weights. Size, surface charge, and encapsulation efficiency were not only dependent on the physicochemical properties of the drug molecule but also on the molecular composition of the CD derivative (Fig 1B and C). Similarly, a synergistic effect of molecular composition of CD derivative and physicochemical properties of the drug molecule on release profiles, was observed (Fig 1D).

In conclusion, amphiphilic CD derivatives provide a platform for designing self-assembling nanocarriers with tailor-made control over physicochemical properties, drug encapsulation and release profiles for a broad range a drug molecules with different molecular weights and physicochemical profiles.

S7.7-O2

Design of novel ionizable amino-polyester lipid nanoparticles for potent tissue-selective mRNA delivery

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Abstract

Messenger RNAs (mRNAs) are a new class of biologics with the potential to revolutionize the treatment of many diseases. Unlocking the full potential of mRNA therapies requires efficient intracellular delivery. While lipids are the most clinically advanced platform for mRNA delivery to liver hepatocytes, polymeric nanoparticles display the potential to facilitate delivery beyond the liver. However, current cationic polymers face a number of challenges for clinical translation related to their broad molecular weight dispersity, biocompatibility and poor mRNA delivery efficacy. To address this challenge, we developed ionizable amino-polyesters (APEs) synthesized via controlled ring-opening polymerization of tertiary amino-alcohols and lactones. The composition of the two components allows to control different characteristics of the polymer, including its hydrophobicity and ionization constant (pK_a) which can affect the physicochemical properties of the nanoparticles. We previously showed that APEs co-formulated with lipid excipients into nanoparticles (APE-LNPs) could elicit tissue and cell-type selective mRNA expression in the lungs. In the current work, we set out to understand a structure-activity relationship (SAR) between the polymer composition and APE-LNPs mediated mRNA delivery efficacy *in vitro* and *in vivo*.

We rationally designed, synthesized, and characterized a library of 36 novel ionizable APEs focusing on tertiary amines content, length of the lactone side chain and polymer structure (branched vs linear). The polymers were formulated into NPs using microfluidic mixing together with lipid excipients, including 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy-(polyethyleneglycol)2000] (C14PEG2000) to encapsulate Firefly luciferase (FLuc) mRNA. The potency of mRNA delivery by the APE-LNPs was evaluated in cancer, immune and endothelial cell lines, identifying polymers capable of efficiently delivering the mRNA. We observed that mRNA transfection efficacy and pKa of the APE-LNPs were predominantly governed by the composition of the tertiary amino-alcohol, while the length of the lactone side chain significantly impacted the polymer physical properties (glass transition) and influenced APE-LNPs size, encapsulation efficacy and surface charge. Our results showed that optimal polymer design is required to promote stable APE-LNP formation and efficient mRNA delivery. We also investigated the role of the lipid excipients in the APE-LNPs formulation and found an important function of phospholipids, such as DOPE, for maintaining APE-LNP structure, cellular uptake and mRNA delivery efficacy. Ongoing work is focused on investigating the SAR for selected APEs in C57BL/6 mice to better understand the influence of the polymer design on the APE-LNPs biodistribution and the potency of mRNA delivery to different tissues.

S7.7-O3

The potential of polydopamine nanoparticles as a treatment against hepatic steatosis

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Abstract

Hepatic steatosis is the world's most common liver disease affecting over 25% of the adult population.[1] Lipid metabolism in the liver is finely controlled by enzymes and hormones; however, an imbalance in this enzymatic machinery can lead to the onset of liver steatosis.[2] Some of the consequences of liver steatosis involve systemic inflammation, injury and loss of liver functionality, and cardiovascular impairments that can even lead to the patient's death.

In recent years antioxidant molecules have been investigated in the treatment of liver steatosis due to the generally high level of oxidative stress typical of the disease and linked to lipid peroxidation.[3] Polydopamine nanoparticles (PDNPs) are a class of biodegradable nanomaterials characterized by several interesting properties for biomedical applications such as a relatively high antioxidant capacity.[4] In this work, we investigated PDNPs as a potential tool against hepatic steatosis by testing them on an *in vitro* model of the disease. Our *in vitro* model was developed by treating HepG2 cells with oleic acid (OA). The model was analyzed by exploiting absorbance measurement (Oil Red O staining), fluorescence analysis, and confocal imaging of cells (AdipoRed™ staining). PDNPs were synthesized by a Stöber process[4] and characterized through dynamic light scattering measurements, zeta potential analysis, and scanning electron microscopy imaging (the average diameter of PDNPs resulted to be approximately 200 nm). After a preliminary analysis of PDNP biocompatibility, internalization, and intracellular localization, the effect of the nanoparticles on the steatosis model was analyzed. PDNPs showed the ability to prevent the accumulation of lipids inside HepG2 cells and even stimulate the removal of pre-existent intracellular lipid droplets. Moreover, PDNPs showed the ability to reduce the oxidative stress levels present in steatotic HepG2 cells. Finally, an in-depth proteomic analysis demonstrated the ability of PDNPs to influence key biomolecular pathways involved in hepatic steatosis and lipid metabolism. Altogether out study show the potential of PDNP as a potential tool against hepatic steatosis.

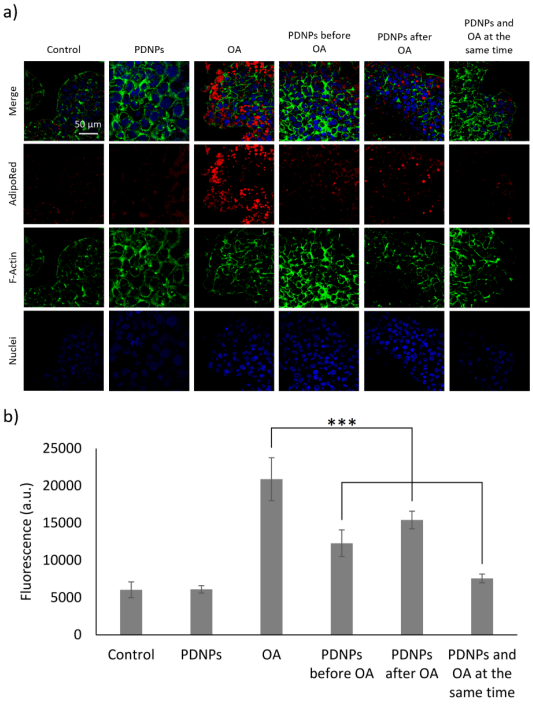

*Fig. 1: Analysis of lipid accumulations. In a) representative images of intracellular lipid droplets. In b) quantitative analysis of lipids content. (*** = p < 0.001,* n *= 3)*

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S7.7-O4

Nanoparticle-mediated silencing of Connexin43 to destabilise scar-forming cells after spinal cord injury

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Abstract

Spinal cord injury (SCI) is devastating traumatic injury characterised by a formation of a growth inhibiting scar around a lesion cavity in the damaged tissue. Consisting of astrocytes and meningeal fibroblasts, this potent barrier impedes the regrowth of injured axons but it also functions to protect undamaged tissue by restricting toxic inflammatory cell ingress. As removal of the scar increases the spread of injury, we hypothesised that reducing Connexin43 (Cx43), the gap junctional protein linking scar cells together, may help to destabilize the scar and controllably reduce, rather than remove its barrier function. To do so, we employed our lab's expertise in scaffold fabrication[1] and gene therapy[2] to develop a biomimetic, gene-activated biomaterial scaffold capable of retaining and releasing nanoparticles complexed with silencing (si)RNA for non-viral delivery to scar forming cells after SCI. First, we determined that treatment with pro-fibrotic TGFβ induces scar-forming behaviours in astrocytes and meningeal cells, as indicated by upregulation of Cx43 and scar-associated Collagen I protein and enhanced cell migration following treatment. Next, soft hyaluronic acid and collagen IV scaffolds that mimic the physico-chemical properties of the native spinal cord were fabricated[3], soak-loaded with peptide nanoparticle-Cx43 siRNA complexes for non-viral gene delivery and then seeded with human astrocytes and meningeal fibroblasts. TGFβ-treated cells grown on gene-activated scaffolds containing Cx43 siRNA nanoparticles demonstrated reduced Cx43 junctional protein expression compared to controls. To test nanoparticle diffusion out of the scaffold to transfect surrounding cells, conditioned media was isolated from the gene-activated scaffold cultures and added to 2D cultures of astrocytes and meningeal fibroblasts. A similar downregulation of Cx43 protein expression was observed in treated cells with increased separation between cells indicating a "loosening" of cell-to-cell connections. In summary, the present work outlines the development of a scaffold-based gene therapy strategy targeting cell-cell connections in the fibroglial scar that forms after cord injury.

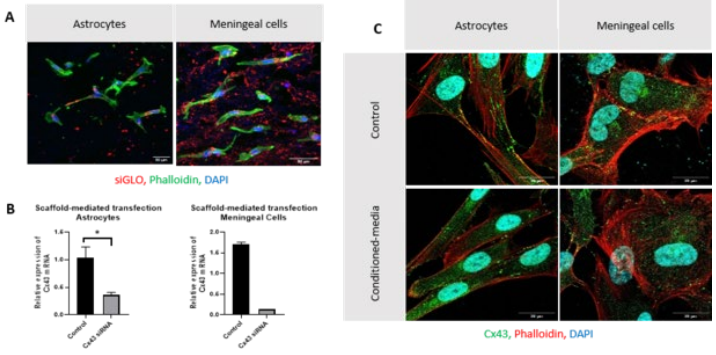

Fig. 1 | Development of a gene-activated scaffold for transfecting scar cells A) Astrocytes and meningeal fibroblasts grown on scaffolds loaded with fluorescently-tagged reporter siRNA (siGLO; red) complexed with GET nanoparticles show cytoplasmic uptake of the nucleic material. B) PCR analysis of Cx43 expression in scaffold-seeded astrocytes and meningeal fibroblasts. C) Astrocytes and meningeal fibroblasts transfected by conditioned media isolated from gene-activated scaffolds showed reduced Cx43 expression and increased cell separation.

References: [1]Ryan et al., 2017; Adv Healthc Mater.; [2]Power et al., 2022; Int. J. Mol. Sci.; [3]Woods et al., 2022; Adv Healthc Mater.

S8.2-O1

Personalized reconstruction of cleft palate deformities in dog patients with innovative 3D-printed organo-mineral cements

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Abstract

Introduction. Developments in the field of additive manufacturing have allowed significant improvements in the design and production of scaffolds with biologically relevant features to treat bone defects. Unfortunately, the clinical and manufacturing workflow to generate personalized scaffolds is source of inaccuracies which lead to a poor fit between the implant and patients' bone defects. In addition, scaffolds are often brittle and fragile, uneasing their handling by surgeons, with significant risks of rupture during their insertion in the defect. Consequently, we developed organo-mineral cementitious scaffolds displaying evolutive mechanical properties which are currently being evaluated to treat cleft lip/palate deformities in veterinary clinics.

Methods. Treatment of dog patients was approved by ethic and welfare committees (CERVO-2022-14-V). To date, 8 puppies with cleft palate/lip deformities received the following treatment. Two weeks prior surgery, CT-scan of patient's skull was performed to allow for surgical planning and scaffold designing. Organo-mineral printable pastes were formulated by mixing an inorganic cement precursor (α-$Ca_3(PO_4)_2$) to a self-reticulating hydrogel (silanized hyaluronic acid) supplemented with a viscosifier (hydroxymethylpropylcellulose). Scaffolds were produced by robocasting of these pastes using 25 or 27G cones (BioX, Cellink). If needed, Pluronic F127 (40% $_{w/w}$) was used to support the printing of the cementitious structure. Surgical interventions included the reconstruction of soft tissues, and the insertion of the scaffold soaked with autologous bone marrow. Bone formation was monitored 3 and 6 months after reconstruction, and a biopsy at 6 months was performed for more detailed analyses. (µCT, histology, SEM).

Results. Scaffolds displayed great handling properties and were inserted within bone defects with a relevant bone edges/scaffold contact without significant issues. Osteointegration of the scaffolds was observed after 3 months, and regeneration of the defect at 6 months seemed promising.

Discussion & Conclusions. Preliminary results have demonstrated an interesting potential of the set-up strategy to treat cleft lip/palate deformities in real spontaneous clinical setting. It also avoided the use of any animal model; patients are going back to their owners after reconstruction.

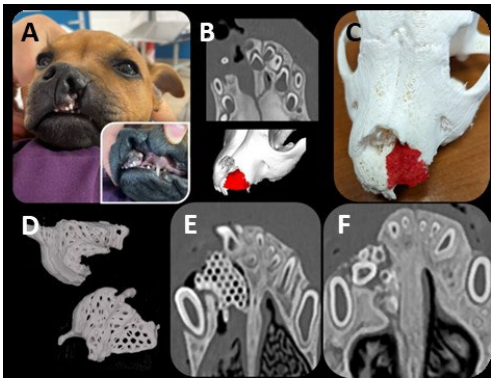

Figure 1. Patient specific treatment of cleft/lip palate deformity (A) the general view of the deformity, (B) the pre-op scanner and implant design, (C) the surgical planning using anatomical models, (D) the printed organo-mineral implants, (E) the post implantation scanner and (F) defect regeneration after 6 months of implantation.

Acknowledgements. French National research agency (ANR-20-CE17-0018). Filière de Santé et Maladie Rares TETECOU (Impulsion research Grant).

S8.2-O2

Fully implantable intraspinal microstimulation device for swine models

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Abstract

Intraspinal microstimulation (ISMS) is a neuromodulation technique that targets the lumbar spinal cord containing control centers for locomotion. ISMS implants were successfully used in cats and were able to restore standing and walking after spinal cord injury, suggesting that this may be a viable approach for restoring mobility in humans. However, there are challenges in translating the ISMS implants to humans. This is due to the higher range of motion in the spinal cord, which could potentially lead to electrode dislodgement and failure of the implant. This study aimed to design, fabricate, and characterize an implantable ISMS device suitable for a clinically relevant large animal model (pigs).

Microwires (50 µm, PtIr, 80%:20% insulated with polyimide; California Fine Wire, USA) were used to fabricate electrodes. The tips of the microwires were de-insulated and sharpened using nanosecond (KrF, 248 nm) and femtosecond (Fiber laser, 343 nm) lasers, respectively. The calculated surface area of the electrode tip was 1.39´105 µm2 on average (n = 8). Microcoils (OD: 170 µm) with different pitch sizes were fabricated from 25 µm microwires. They were dip-coated in poly-dimethyl siloxane (PDMS) at different dip-coating speeds to find the most suitable speed for the ISMS electrodes. The microcoils were then connected to the microelectrodes by laser welding and insulated with PDMS. Sixteen microelectrodes were fabricated, and the lead wires were connected to two Medtronic extension cables (Model 37081). Electrochemical parameters were assessed *in vitro* and *in vivo* using electrochemical impedance spectroscopy (EIS) and voltage transients (VT). A custom wirelessly controlled stimulator was developed and connected to the array using extension cables. Mechanical properties of the microcoils were evaluated using uniaxial tensile testing. Glial cell adhesion to the microcoils and microelectrodes was assessed in mixed glial mouse cell cultures at 7 and 10 days *in vitro*.

The impedances of electrodes measured at 1 kHz were 3.24±0.21 KΩ (*in vitro*) and 20.53±1.99 KΩ (*in vivo*). The microelectrodes could deliver 275 µA (*in vitro*) and 125 µA (*in vivo*) of current while remaining within the safety of the water window as determined from voltage transient measurements. The microcoils with 50 µm pitch and coated at a speed of 5mm/min exhibited adequate elasticity for an ISMS strain-relief system. These microcoils were stretched up to 30% beyond their initial length which is more than the change in length experienced by the pig spinal cord in the thoracolumbar region (~11%).

S8.2-O3

Full Insulin independence after transplantation of 3D bionic pancreas tissue petals – large animals results.

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Abstract

Islets transplantation (ITx) is on the crossroad-in the US is treated as a drug, not a tissue transplantation, in Europe a few countries have a reimbursement for it. Search for a new implantation site didn't bring a breakthrough and still intra-portal ITx with its problems like instant blood-mediated inflammatory reaction(IBMIR) or stripping-off islets from ECM after isolation is a gold standard. Nowadays a few attempts of stem-cell derived β-cells clusters (SCD-Beta) ITx has been shown with inconsistent results. Xeno-derived ITx are also ahead. It seems that ITx into portal vein SCD-Beta or Xeno-derived islets can face exactly the same problems like standard donor derived ITx-IBMIR, lack of ECM and insulin-independence. Additional safety reasons such as clusters in some extend will not be left in the liver make it difficult to monitor. The aim of study was to show results of ITx in large animals of 3Dbioprinted bionic pancreatic tissue models as a further model to be used with SCD-Beta clusters or Xeno-derived islets.

Material&methods: 12 pigs were divided into 4 groups: healthy pigs (n=3); animals after total pancreatectomy, treated with insulin(T1D;n=3); animals after total pancreatectomy and autotransplantation of pancreatic islets into the liver(Liver; n=3); animals after total pancreatectomy, and autotransplantation of 3Dbioprinted bionic pancreatic tissue petals with islets (3D-Petals; n=3). The effectiveness of the ITx was assessed by the daily insulin intake, C-peptide and glucose concentration up to 2 months post-ITx. 3Dbioprinted bionic pancreatic tissue models had 3x3x0.3cm in diameters and were transplanted between rectal muscle and peritoneum.

Results: 3D-Petals group reached full insulin-independence within 4-6 weeks post-ITx. Insulin intake in 2,4,6 weeks after ITx was 42;16;0% of T1D group requirement respectively($P<0.01$). Insulin intake in the Liver group in 2,4,6 weeks after ITx was 24;76;69% of T1D requirement respectively($P<0.01$). None of liver group achieved insulin-independence. Mean fasting C-peptide at 0,2,4,6, weeks in healthy group was :1.7,1.51,1.52,1.21ng/mg vs. 1.5,0,0,0ng/ml in T1D group. C-peptide levels before and post-pancreatectomy and 2,4,6,8 weeks post-ITx in 3D-Petals was 1.9;0.0;0.3;0.16;0.3;0.32ng/ml vs. 2.2;0.0;0.25;0.1;0.1;0.1ng/ml in the Liver respectively ($p<0.05$). The mean insulin intake in the T1D group was 5;5.2;6.4;6.8IU in 2,4,6,8 weeks after pancreatectomy. In the LIVER group was 1.2;3.95;4.4;5IU vs. 2.1;0.85;0.0;0.2IU in the 3D-Petals in 2,4,6,8 weeks after pancreatectomy and ITx.

Conclusion: 3DBioprinted bionic pancreatic tissue petals transplantation achieved FULL insulin-independence in all animals which could be good prognostic before ITx of 3Dbioprinted pancreatic tissue with stem-cell derived islets.

S8.2-O4

Processed inverted human umbilical vessel as nerve regeneration conduit in the treatment of hand nerve section

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Abstract

Statement of the Problem: Treatment of peripheral nerve injury, particularly those with a gap, remains a challenge. Recovery of finger sensitivity is often incomplete and can interfere with personal and occupational activities. The need for better regeneration outcome has given rise to the development of alternative treatments such as nerve conduits. A multicenter, open, and prospective clinical trial was conducted to evaluate the safety and efficacy of a conduit of freeze-dried inverted human umbilical vessel in the regeneration of hand nerve sections. Methodology & Theoretical Orientation: Patients between 18 and 65 years old with hand nerve section of 2 mm to 20 mm with static 2-point discrimination (s2PD) > 15 mm had to be included. The primary objective was the recovery of the sensibility of the hand 12 months after conduit implantation defined by s2DP ≤ 8 mm. Secondary objectives were the evaluation of the clinical tolerance and of the functionality of the hand. Evaluations were performed at 1, 3, 6 and 12 months. Findings: Twenty-four lesions of 23 patients with mean nerve gap of 6.22 mm [2; 30] were included. At 12 months, primary objective, s2PD ≤ 8 mm, was achieved by all but 2 patients (one had chronic alcoholism with peripheral neuropathy, the other had nerve gap of 30 mm) (p-value < 0.001). Complete innervation was recovered by 88% of patients. Pressure sensation and quality of life related to the hand significantly increased while symptoms due to nerve section (pain, cold sensation, hypoesthesia, hyperesthesia) decreased to almost zero. No safety issue related to the nerve conduit were reported. Conclusion & Significance: Processed inverted human umbilical vessel is a safe and effective option as conduit for hand nerve regeneration.

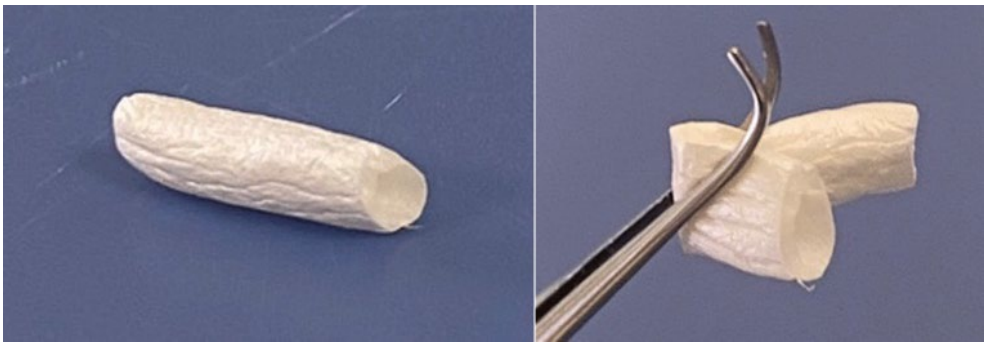

Figure 1: Macroscopical structure of the product

S8.3-K1

New bioinks from natural-based polymers and new bioprinting strategies to develop 3D constructs for tissue engineering

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Abstract

The development of 3D biofabricated constructs requires the use of bioinks that should: (i) exhibit adequate biological properties to elicit desirable interactions with cells and tissues and (ii) present rheological and gel-forming properties to originate strcutures with good definition and reproducibility. These two requirements are many times difficult to be conciliated. In our group we have been developing new bioinks based on proteins (including from human-origin) and polysaccharides with cytocompatible and bioactive properties. Adequate chemical modifications permit to adjust the rheological properties of the materials and their crosslinking ability by the action of adequate stimuli, in order to be used as “universal” bioinks. Alternatively, strategies have been proposed to use adequate supporting baths to freely-bioprint such hydrogels in the volume.

S8.3-O1

FLight biofabrication of anisotropic articular cartilage to provide enhanced mechanical properties

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Abstract

Tissue engineered grafts that recapitulate the biomechanical properties of cartilage are emerging as promising solutions to substitute impaired cartilage. However, despite significant progress, generating engineered cartilage constructs akin to their native counterparts still represents an unmet challenge. In particular, the inability to accurately resemble the zonal architecture of cartilage with varying collagen orientations represents a significant limitation. This extracellular matrix arrangement plays a fundamental role in the mechanical function of the tissue. Here, we show that a novel light-based approach termed Filamented Light (FLight) biofabrication[1] can generate highly porous, cell instructive anisotropic constructs that lead to directional deposition of collagen fibres and promote articular cartilage (AC) maturation.

FLight generates hydrogels within a few seconds composed of aligned microfilaments with diameters ranging from 2-30 µm (Figure 1A). Our photoresin (hyaluronic acid norbornene (HA-NB), 5 M cells/mL, 2 mm path length) enabled the printing of complex designs showing end-to-end microbeam features in the presence of cells. Polydactyly infant chondrocytes were used as an attractive non-immunogenic off-the-shelf cell source.[2] Constructs were generated with a UV box and FLight biofabricated to achieve bulk and aligned hydrogels, respectively (Figure 1B). Constructs were then matured for 8 weeks and evaluated overtime.

Immunohistological stainings were used to compare bulk vs Flight hydrogels. As desired for AC constructs, collagen I was less pronounced than collagen II and limited to pericellular deposition. FLight hydrogels showed that larger microchannels successfully induce the alignment of collagen II fibres while collagen II was found mostly confined to the pericellular space for bulk hydrogels (Figure 1C). We further evaluated tissue maturation by compressive testing. In accordance with histology, stiffness increased over the course of 56 days, eventually reaching native cartilage-like values for FLight hydrogels, both in compressive (~1 MPa) and equilibrium modulus (~ 0.06 MPa) (Figure 1D). Instead, bulk hydrogels only achieved ~0.1 MPa in compressive and ~0.01 MPa in equilibrium modulus. Histologically and mechanically, FLight showed improved tissue maturation compared to homogenous bulk gels. Therefore, FLight is an excellent technology for generating AC with zonal architecture and native-like mechanical properties.

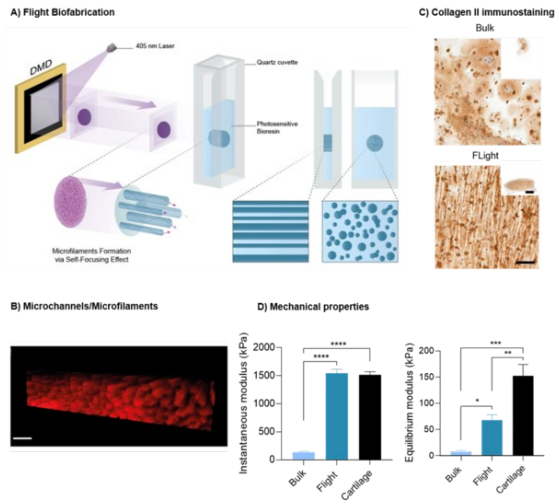

Figure 1. A) Illustration of Flight. B) Microchannels assessment with FITC-Dextran in red (scale bars: 100 µm). C) Collagen II immunostaining (scale bars: 1 mm, 100 µm). D) Compression tests.

References: 1. Liu et al., Adv Mater (2022); 2. Cavalli et al., Sci Rep (2019).

S8.3-O2

Handheld bioprinting of click hydrogels for wound healing

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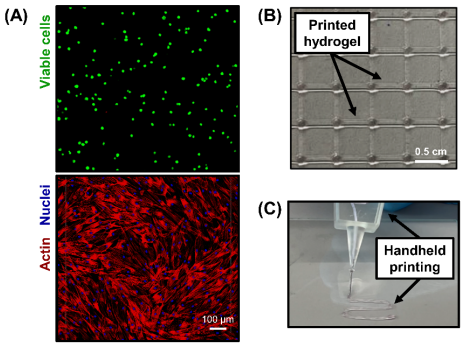
Abstract

Introduction. Chronic wounds don't spontaneously heal and heavily affect patients' quality of life.[1] Current scaffolds are expensive structures that only passively support wound regeneration. Cell-based scaffolds are potential alternatives, but cell-associated risks and costly *ex vivo* fabrication are hindering their translation.[2] We are developing a handheld printing device to biofabricate in situ bioactive cell-free scaffolds to regenerate wounds.

Methods. We functionalised two polymers with bioorthogonal click chemistries,[3] verified the degree of modification via ^{1}H NMR spectroscopy, and mixed them to form hydrogels at different concentrations (2 to 6% w/v). Crosslinking kinetics were investigated via rheometry, mechanical properties via compressive tests. Human adipose-derived stem cells were encapsulated in the hydrogels and cytocompatibility tested via AlamarBlue, live/dead, and nuclei/phalloidin staining. We fabricated a device to print the hydrogels where the precursors are injected, mixed, and extruded as crosslinked bioinks. We optimised the printing shape fidelity by varying concentration, nozzle size, and flow rate. We then assembled a handheld bioprinter to manually print the bioinks and verified the feasibility of manual in situ printing.

Results. The polymers were functionalised with comparable degree of modification (0.1 mmol/g). After mixing the precursors, hydrogels spontaneously formed; faster crosslinking was achieved by increasing polymer concentration (2-15 min). Mechanical properties increased with higher concentration and were suitable for soft tissue regeneration (E=1-6 kPa). Viable cells were encapsulated in the hydrogels, increased metabolic activity was observed over 14 days, and cells showed elongated morphology (Figure_A). We optimised the printing parameters and selected the optimal bioink to print grids with high shape fidelity (>90%, Figure_B). The handheld device was successfully used by different operators to manually print bioinks with defined shape, covering surfaces at 10 cm^2/min (Figure_C). We successfully isolated and encapsulated exosomes in the bioinks, and we are now testing their release and potential for wound regeneration via *in vitro* wound model.



Conclusion. We developed bioorthogonal click bioinks and a handheld device to print them in situ with controlled properties and shape. Our strategy will allow in situ bioprinting, without the need of irradiation or added crosslinkers, to fill wounds and accelerate healing via a cell-free tissue engineering strategy.

Figure. (A) Viable cells encapsulated in bioinks; (B) optimised printing, and (C) handheld bioprinting.

References. [1]Dehkordi et al., 2019; [2]Han et al., 2017; [3]Contessi Negrini et al., 2021

Acknowledgements. Seedcorn Award Rosetrees Trust (N.C.N, S.K., D.W., C.E., A.C.); Dame Julia Higgins Fund 2021 (N.C.N., D.W., M.S.)

S8.3-O3

Enzyme-living hydrogel: a novel approach to create custom-sculptured channels in tissue mimetic hydrogels

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Abstract

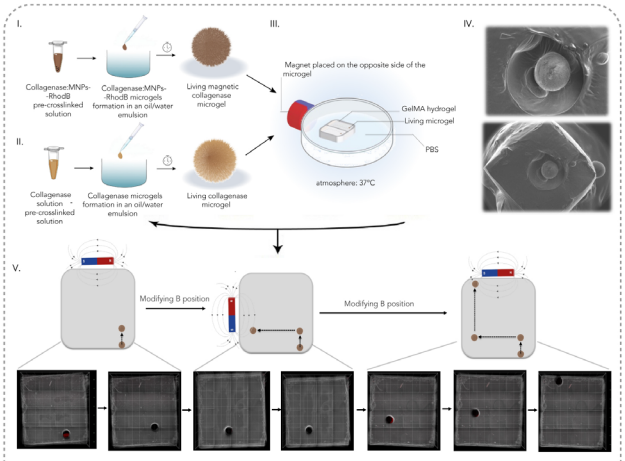

Fig. Illustration of the set-up followed to sculpt channels in tissue mimetic hydrogels. *I) Production of magnetic collagenase microgels and II) collagenase microgels by oil/water emulsion. III) Subsequent encapsulation within GelMA hydrogels with the magnetic field positioned on the opposite side of the microgel. IV) SEM images of the sculptured channel-structure after the hydrogel engravement. V) Set-up followed to create different topographies by changing the position of the magnetic field.*

Tissue Engineering aims to create relevant tissue constructs with appropriate vasculature able to facilitate the distribution of oxygen and crucial nutrients within the 3D architecture, while simultaneously removing metabolic waste. To create 3D perfused materials that can mimic human tissues, researchers have been exploring the use of i) cell-adhesive peptides, growth factors, metalloproteinases and/or crosslinking density to create physicochemical cues that encourage cells to adhere and spread; and ii) sacrificial molding or printing techniques to design channel-like structures into hydrogels that controls cells deposition and growth.[1][2] Despite of the potential of these techniques, reproducing vascular structures using these approaches can be laborious and costly.[2] Therefore, herein we propose a novel strategy to produce a custom-sculptured pattern in a methacrylated gelatin (GelMA) hydrogel using one single enzyme-living microgel. We crosslinked collagenase with different amounts of magnetic nanoparticles labelled with Rhodamine B, into spherical enzymatic microgels and encapsulated them at one of the sides of GelMA hydrogel before its crosslinking. After crosslinking, the resulting constructs were subjected to a magnetic field that was positioned on the opposite side of the spherical enzymatic microgel, forcing the engravement. In a single step, we were able to produce different living microgels with high shape- and size- fidelity without compromising the secondary structure of the enzyme. By simply changing the magnetic field intensity or the concentration of MNPs-RhodB within the microgels, we could adjust the velocity of the sculpturing process. Moreover, by varying the magnetic field position or the size of the microgel, we were able to create different topographies and sculptured patterns with different widths. With this, we demonstrate the ability of creating a simple and innovative strategy to create sculptured channels within 3D hydrogels, that relies in the highly specific protein:enzyme pairing. Given the living and remotely-controlled nature of microgels, such cutting-edge technology may be applied for delivery of cells, biomolecules, or therapeutic agents in various biomedical platforms, overcoming the diffusion problems of conventional 3D hydrogels.

References: [1] A. Malheiro, et. al, ACS Biomater. Sci. Eng. 2016, 2, 1694. [2] C. F. V. Sousa, et. al, Biomolecules 2021, 11, 863.

Aknowledgments. 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).” The authors also acknowledge the FCT project “PROMENADE”-PTDC/BTM-MAT/29830/2017, the doctoral grant SFRH/BD/146740/2019 and the individual contract 2021.02196.CEECIND (A. Sofia Silva).

S8.3-O4

3D bioprinting of a biomimetic leaflet scaffold for heart valve repair

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Abstract

Heart valve disease is a prevalent and increasing clinical burden with limited resort to surgical repair or valvular replacement. Existing treatment alternatives, such as biological and mechanical valve replacements, are inadequate for pediatric patients due to the inability of the valves to grow or respond biologically to their microenvironment, which often leads to multiple valve-refitting surgeries and lifelong coagulation status follow-up. Tissue-engineered heart valves (TEHVs) have emerged as an attractive therapeutic solution to address these challenges, enabling the development of patient-specific models for self-repair and remodeling (see Figure 1).

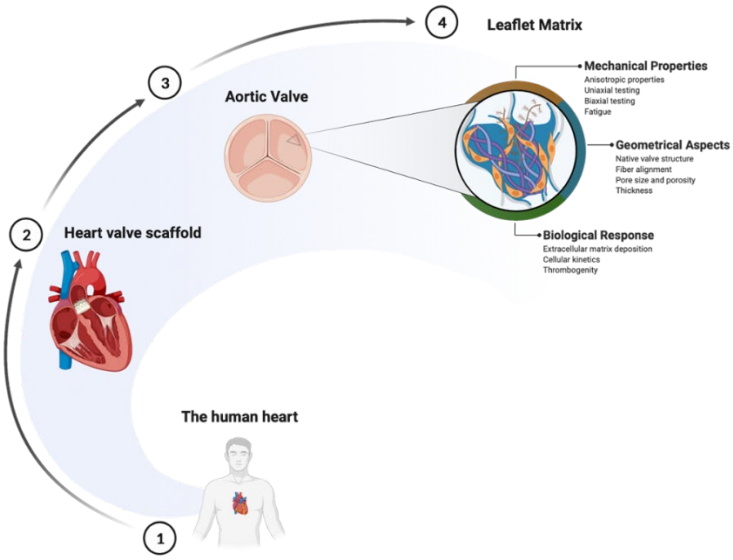

Figure 1. Tissue-engineered approach for heart valve replacement.

To this extent, a 3D bioprinted TEHV prototype was developed to recreate the three-layer leaflet structure of an aortic valve and incorporate valvular interstitial-like cells (see Figure 2). A tunable cell-laden hydrogel scaffold, made from gelatin-methacrylate and polyethylene glycol diacrylate (GelMA/PEGDA), was developed to provide cell encapsulation and transplantation while a layer of polycaprolactone (PCL) was used to recapitulate the mechanics of a native valve structure. A combination of 7.5% GelMA and 5% PEGDA scaffold was made with a white-light crosslinkable method after extrusion printing. As a result, swelling volume decreased and the mechanical modulus and structural stability of the hydrogel increased compared to pure GelMA for seven days. Cells remained over 75% viable, and surface treatment for the PCL with methacrylate acid enabled a more robust integration of the material layer with the hydrogel scaffold for improved cell attachment. The TEHV was tested in a left heart bioreactor system and demonstrated proper opening/closing function under

physiological conditions. Overall, the valve scaffold can become cellularized and mature inside a bioreactor under cyclic conditions for the increased presence of extracellular matrix proteins (e.g., collagen type I) to exhibit remodeling capacity. The assembled TEHV mimics the physiological properties and structure of a native aortic valve, which leads to proper function and stability. Further studies will assess the long-term functionality of the TEHV for appropriate mechanics and biological integration through ECM production.

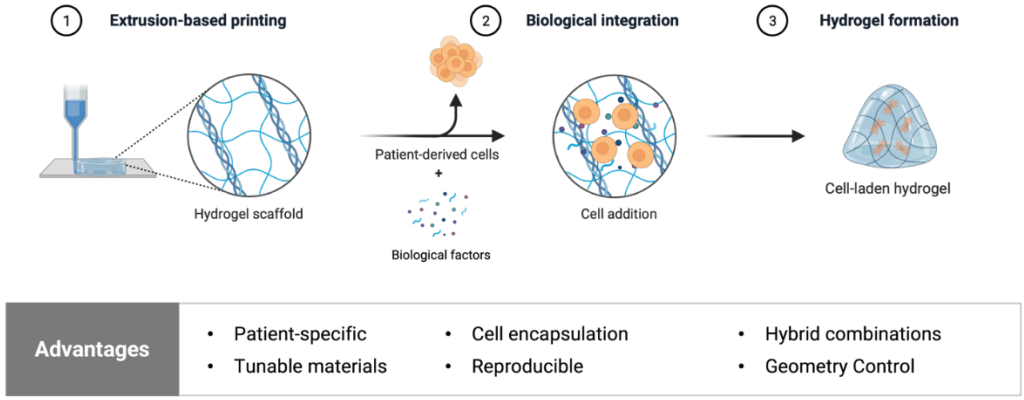

Figure 2. Advantages of 3D bioprinting for patient-specific hydrogel scaffolds.

S8.4-K1

What Can biofabrication do for space and what can space do for biofabrication?

Lorenzo Moroni

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Abstract

Biofabrication in space, and in particular bioprinting, is one of the novel promising and perspective research directions in the rapidly emerging field of space biomedical sciences. There are several advantages of bioprinting in space. First, under the conditions of microgravity (µg), it is possible to bioprint constructs employing more fluidic channels and, thus, more biocompatible bioinks. Second, µg conditions enable 3D bioprinting of tissue and organ constructs of more complex geometries with voids, cavities, and tunnels. Third, a novel scaffold-free, label-free, and nozzle-free technology based on multi-levitation principles can be implemented under the condition of µg. The ideal space bioprinters must be safe, automated, compact, and user friendly. Thus, there are no doubts that systematic exploration of 3D bioprinting in space will advance biofabrication and bioprinting technology per se. Vice versa 3D bioprinted tissues could be used to study pathophysiological biological phenomena, when exposed to µg and cosmic radiation that will be useful on Earth to understand ageing conditioning of tissues, and in space for the crew of deep space manned missions.

Here, we provide some leading concepts on what mutual benefit can be drawn by the application of biofabrication technologies in space, and sketch a future scenario where such marriage could enable advancements in space biological programs and of our ageing society.

S8.4-K2

ESA's initiative on culturing 3D systems on the ISS

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Abstract

Bioprinting is enabling technology for space exploration missions such as medical applications, Bioreactors & closed loop life-support systems, food production, biomining, and bioconstruction. ESA is in the process of developing a 3D Biosystem for the International Space Station, including the development of a 3D bioprinter and 3D cell culture system, as well as in-situ capabilities for incubating, maturing, stimulating, and analysing 3D bioprinted cell constructs. The objectives of ESA's 3D Bioprinting and 3D cell culture system in space is the development of biomaterials for biology research and biomaterials for clinical cases in different space mission scenarios in order to provide innovative and impactful research for science and medicine. To this end, ESA has installed a consortium of 3D Bioprinting facilities across Europe, and will make use of these to perform proof-of-concept studies for the development of an in-flight 3D Bioprinting facility

S8.4-O1

Storability of cell-laden bioinks for applications in space

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Abstract

Introduction. Bioprinting has reached space as a first bioprinting system has been installed at the International Space Station (ISS). Currently, the European Space Agency has commissioned the construction of another bioprinter together with a 3D cellculture system for the ISS. However, delivery and handling of live cells at the ISS are complicated and processes are needed that require a minimum of crew-time. Therefore, it would be advantageous if bioinks could be prepared on ground and brought to the ISS ready-to-use.
Materials & Methods. Different established bioinks based on alginate and methylcellulose and water, PBS or human blood plasma as solvents were combined with the microalgae strain *Chlorella vulgaris*, an immortalised human mesenchymal stem cell line (hTERT-MSC), the osteosarcoma cell line SAOS-2, the hepatocellular carcinoma cell line HepG2 and primary dental pulp stem cells of two donors and stored for up to 28 days at +4°C. At different time points, the rheology, printability (GeSiM BioScaffolder) and cell viability after bioprinting was investigated. For this, bioprinted constructs were cultivated for up to 28 days to investigate possible recovery of the cells.
Results. All bioink compositions showed good printability, even after storage for 28 days. Viability of the microalgae was only decreased marginally, even in case of storage for 4 weeks prior to bioprinting. In contrast, viability of the tested human cell types was reduced by the storage time of the bioinks, compared to freshly prepared ones. However, astonishingly in many experiments no statistically significant differences could be observed between the groups that were stored for 1, 2, 3 or 4 weeks. While cell viability was strongly reduced directly after bioprinting in case of cold-stored bioinks, those cells that survived the process recovered over time and often similar viability values could be reached at day 28 of cultivation. However, the number of live cells was significantly reduced for all cell types. The cell line HepG2 appeared to be the most sensitive cell type concerning cold storage.
Conclusions. While the microalgae strain Chlorella vulgaris showed no significant loss in viability, the investigated human cell types tolerated such conditions less. However, even after 4 weeks of cold storage, viable cells were found that partly recovered after bioprinting and during further cultivation. Launching of premixed (cell-laden) bioinks, stored at 4°C for bioprinting experiments at the ISS therefore might be possible, however, detailed investigations are necessary to find an optimal bioink composition for each cell type.

S8.4-O2

3D collagen hydrogel system to study the effects of microgravity on immune cells

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Abstract

Microgravity has deleterious effects on various physiological systems, and it is well acknowledged that astronauts have increased susceptibility to infections and poor response to vaccination. Immunologically, dendritic cells (DCs) are the key players in linking the innate and adaptive immune responses. Their distinct and optimized differentiation and maturation phases play a critical role in presenting antigens and mounting effective lymphocyte responses for long-term immunity. Despite their importance, no studies to date have effectively investigated the effects of microgravity on DCs in their native microenvironment, which is primarily located within tissues. Here, we address a significantly outstanding research gap by examining the effects of simulated microgravity via a random positioning machine on both immature and mature DCs cultured in biomimetic collagen hydrogels, a surrogate for tissue matrices. Furthermore, we explored the effects of loose and dense tissues via differences in collagen concentration. Under these various environmental conditions, the DC phenotype was characterized using surface markers, cytokines, function, and transcriptomic profiles. Our data indicate that aged or loose tissue and exposure to RPM-induced simulated microgravity both independently alter the immunogenicity of immature and mature DCs. Interestingly, cells cultured in denser matrices experience fewer effects of simulated microgravity at the transcriptome level. Our findings are a step forward to better facilitate healthier future space travel and enhance our understanding of the aging immune system on Earth.

S8.5-K1

Engineering with agential materials: endogenous bioelectric circuits as the interface to reprogramming the software of life

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Abstract

In this talk, I will describe the concept of agential materials, and the strategies of engineering appropriate for exploiting the built-on competencies of living cells and tissues. I will show how engineers can use the native bioelectric interface to drive advances in the control of embryogenesis, regeneration, and synthetic bioengineering. By merging techniques in biophysics, computer science, and behavioral science to reprogram the collective intelligence of cells, new capabilities in bioengineering can be achieved.

S8.5-O1

Engineered Living Materials for light-induced synthesis of β-carotene

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Abstract

Retinoids and their precursors are a family of molecules that comprises lycopene, β-carotene, retinal, retinol (vitamin A), and retinoic acid (Fig.), and are widely employed in biomedicine, and in the food and cosmetic industries, due to their antioxidant, anti-inflammatory and antiaging activity. These compounds are traditionally obtained from fruit and vegetables sources, which requires long and expensive procedures. Furthermore, most retinoids are easily oxidizable and thermally unstable, so other routes to obtain them in a controlled fashion and with a greener approach are under investigation. Among them, the use of synthetic biology to genetic engineering cells is emerging as a valuable tool to regulate its metabolism, resulting in living and responsive "cellular factories". Such living entities can be classified as Engineered Living Materials (ELMs), both alone and in combination with a matrix. In this regard, *Lactococcus lactis* emerges as a suitable choice to develop ELMs since it is a probiotic that confers health benefits, is Generally Recognised as Safe, and has been genetically modified within the last 35 years for therapeutic purposes. In this regard, our group has a wide experience in modifying *L. lactis* for cell/tissue engineering applications [1].

Although different apocarotenoids have been produced under IPTG-induction in *Escherichia coli*, only up to lycopene in the synthesis process (Fig.) has been produced in a nisin-controlled system in *L. lactis* [2]. In this work, we introduce a radical different approach: the opto-genetical engineering (OGE) of *L. lactis* to obtain lycopene and β-carotene by implementing the blue light-inducible T7 RNA polymerase (Opto-T7RNAPs) [4]. The use of light, an orthogonal stimulus, enables a non-invasive and precise spatiotemporal control of the synthesis and overcomes challenges not addressed by the traditional small-molecule inducible systems. Moreover, this OGE probiotic can be incorporated in a skin-suitable matrix to obtain an ELM for the topical delivery of these compounds, showing a cytoprotective effect against oxidative stress when cocultured with epithelial cells.

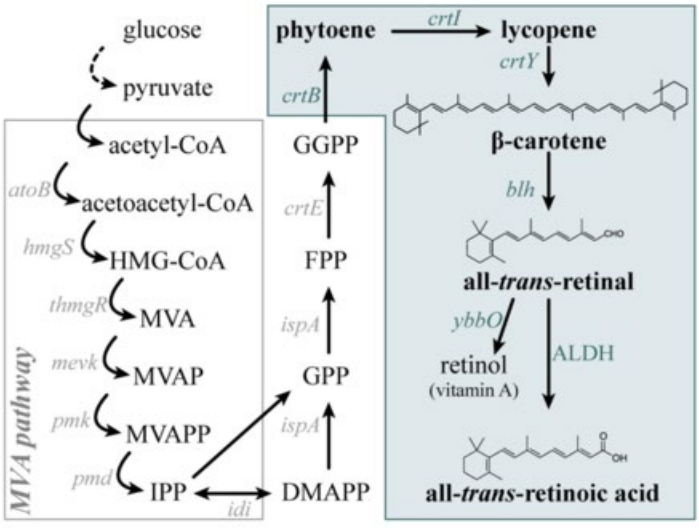

Figure. Natural (white background) and engineered (blue background) pathways needed to synthesise retinoids in L. lactis. Phytoene synthase (crtB), phytoene desaturase (crtI) and lycopene β-cyclase (crtY) must be incorporated into the metabolic machinery to obtain β-carotene from geranyl-geranyl pyrophosphate (GGP).

Acknowledgements: HORIZON-MSCA-2022-PF

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S8.5-O2

AI designed modular recombinant self-assembly protein system as viral entry blocker for pandemic prevention

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Abstract

Since the outbreak of COVID-19 in Wuhan, China in late 2019, it has ravaged the world and caused a large number of infections and deaths. With the rapid and vigorous evolution of AI, it becomes possible to directly design proteins. Here we demonstrate a system based on AI protein simulation to generate blocking peptides that can specifically bind to viruses against the gene sequences of specific viruses (Figure 1). We show a proof-of-concept air suspension SARS-CoV-2 inhibiting particle whitch is constructed by fusion of Qβ phage coat protein to ACE2 binding domain using the system in Figure 1. The particle can both inhibit the binding of spike protein to human ACE2 and stay in the air for enough time until encounter a virus. The simulation results in Figure 2A and Figure 2B show that Qβ phage capsid protein (QβCP) with ACE2 mimic domain (AMD) fusion can bind to the SARS-CoV-2 spike RBD for both delta and omicron variants. In addition, we have also demonstrated the industrial production possibility of this system and have completed the mass production of 500L grade. The produced AMD fused Qβ virus-like particles (AMD-VLPs) can be observed in diameter of around 40nm (Figure 2C) and cantaining two types of coat protien, include ACE2 fusion protein (Figure 2D). The AMD VLPs can inhibit above 60% of high concentration pseudovirus *in vitro* (Figure 2E) and can accelerated the settlement of aerosol form pseudovirus to avoid the contact of virus to human body (Figure 2F). The AMD-VLPs also inhibit the infection of pseudovirus *in vivo* (Figure 2G) and show no significant damages in lung and tracheal tissues (2H). In summary, the results indicates that this system can be used in design a particle that can inhibit the infection of SARS-CoV-2. This system has the potential to respond quickly and stop the spread of emerging viruses in the future.

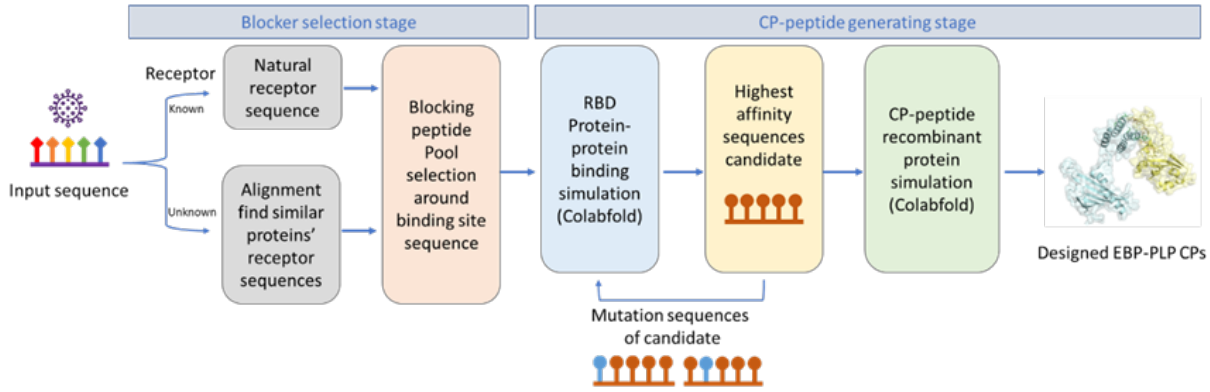

Figure 1. The AI driven design of blocking peptide for this study.

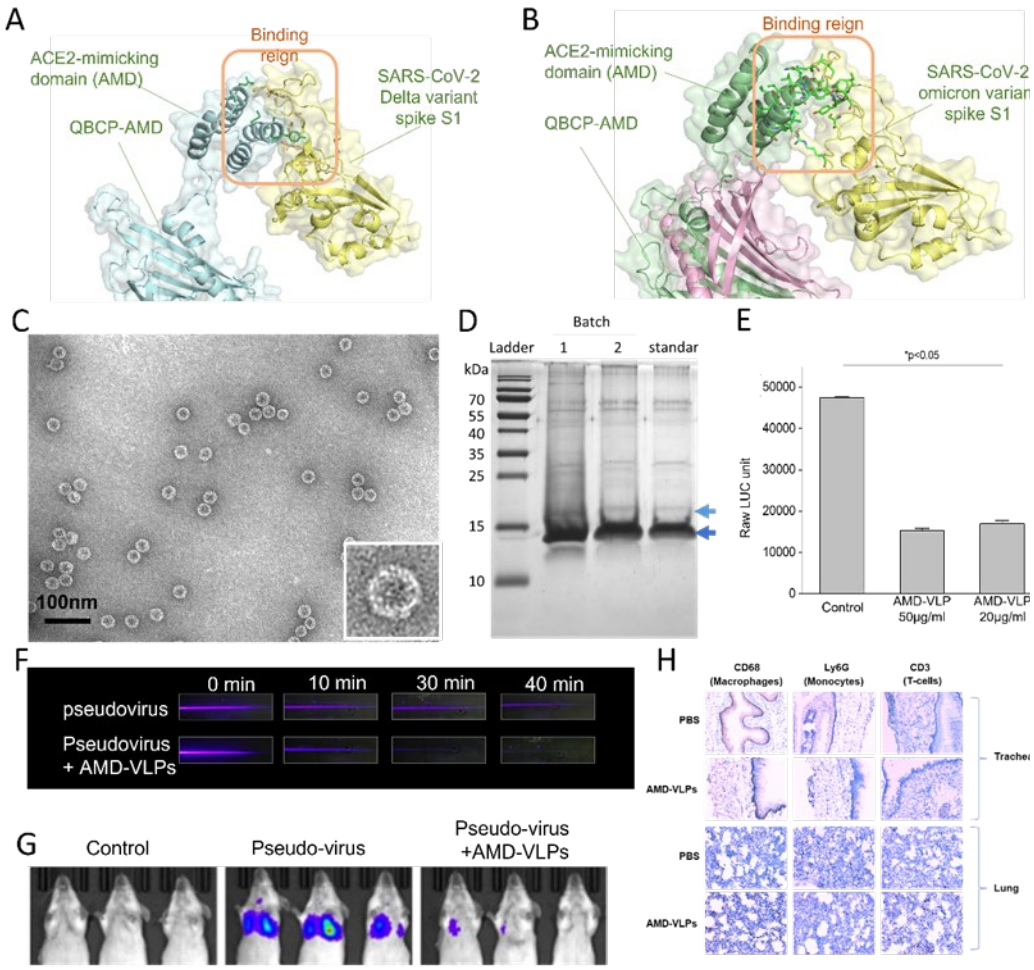

Figure 2. (A) and (B) are simulation of binding between ACE2 mimic domain fused Qβ phage capsid (QβCP) and SARS-Co' Delta S1 (A) and Omicron S1 (B). (C) TEM image of AMD-VLPs. (D) SDS page of AMD-VLPs in different mass product batches and lab-made standard. Blue arrow: origin QβCP. Green: AMD-QβCP. (E) *In vitro* luciferase pseudovirus inhibition different concentration of AMD-VLPs. (F) Spray capture in the air of pseudovirus by AMD VLPs. (G) *In vivo* lung inhalation SARS-CoV-2 pseudovirus infection inhibition. (H) Lung and Tracheal biopsy of mice after inhalation of AMD VLPs.

S8.5-O3

Assessing biomaterial-induced stem cell lineage fate by machine learning-based artificial intelligence

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Abstract

Statement of the Problem: Current functional assessment of biomaterial-induced stem cell lineage fate *in vitro* mainly relies on biomarker-dependent methods with limited accuracy and efficiency. This study aims to evaluate the function of biomaterials in inducing stem cell differentiation with the transcriptome of hMSCs (human mesenchymal stem cells) as a quantitative basis. A gene expression reference and an intelligent assessment model would be established for hMSCs differentiation based on big data and machine learning.

Methodology & Theoretical Orientation: A framework named “Mesenchymal stem cell Differentiation Prediction (MeD-P)” was reported for biomaterial-induced cell lineage fate prediction. MeD-P contains a cell-type-specific gene expression profile as a reference by integrating public RNA-seq data related to tri-lineage differentiation (osteogenesis, chondrogenesis, and adipogenesis) of human mesenchymal stem cells (hMSCs) and a predictive model for classifying hMSCs differentiation lineages using the k-nearest neighbors (kNN) strategy.

Findings: MeD-P exhibits an overall accuracy of 90.63% on testing datasets, which is significantly higher than the model constructed based on canonical marker genes (80.21%). Moreover, evaluations of multiple biomaterials show that MeD-P provides accurate prediction of lineage fate on different types of biomaterials as early as the first week of hMSCs culture.

Conclusion & Significance: MeD-P is an efficient and accurate strategy for stem cell lineage fate prediction. This study demonstrated that machine learning-based artificial intelligence strategies can be widely used for standardized functional biomaterial evaluation, which could hasten the progress of regenerative medicine research.

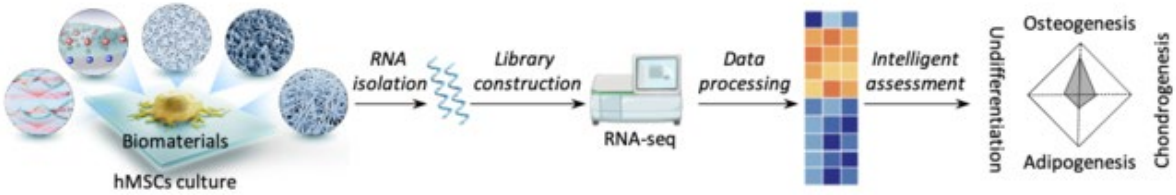

Figure 1. Overview of the MeD-P workflow. Briefly, hMSCs were cultured on biomaterials and harvested for RNA-seq. Processed RNA-seq data were loaded into the MeD-P and generated a report containing three-lineage differentiation probabilities.

S8.5-O4

Machine learning based prediction of immunomodulatory properties of polymer: towards a faster and easier development of anti-inflammatory

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Abstract

In biomaterials development, creating materials with desirable properties can be a time-consuming and resource-intensive process, often relying on serendipitous discoveries. A potential route to accelerate this process is to employ artificial intelligence (AI) methodologies such as machine learning (ML). In this study, we propose a new machine learning approach for screening anti-inflammatory polymers, which could serve as components for designing medical devices with anti-inflammatory properties. We conducted cellular assays with 50 different polymers using the murine macrophage cell line RAW 264.7 as a model. These experiments generated a dataset which we used to develop a machine learning models based on Bayesian logistic regression. After conducting a Bayesian logistic regression analysis, we employed two machine learning models, K-Nearest Neighbors (KNN) and Naive Bayes, to predict anti-inflammatory polymers properties. The study found that the probability of a polymer having anti-inflammatory properties is multiplied by three if it is a polycation, suggesting that incorporating polycations in material design could potentially improve their anti-inflammatory properties. Additionally, the analysis showed that the stimulation of NO secretion is a potential indicator in determining the anti-inflammatory properties of a polymer. These findings highlight the importance of using in silico models for comprehensively considering the biological mechanisms underlying anti-inflammatory responses when developing materials with these properties.

Furthermore, the study compared the performance of two algorithms, KNN and Naive Bayes, in distinguishing between anti-inflammatory and pro-inflammatory polymers. Overall, the study suggests that KNN is a slightly better model for this particular task, but both models perform similarly well. These findings have important implications for the development of more accurate and reliable methods for distinguishing between anti-inflammatory and pro-inflammatory polymers. The study suggests that with appropriate dataset design, ML techniques can provide valuable information on functional polymer properties, enabling faster and more efficient biomaterial development. This has the potential to accelerate multifunctional biomaterial design, ultimately contributing to patient health and well-being. Overall, the results demonstrate the potential of ML techniques in rapidly developing biomaterials.

Keywords: polymers, inflammation, machine learning, Bayesian logistic regression, predictive models, in silico

S8.6-K1

From bench to chairside: Mesenchymal stem cells and biomedical engineering in bone regeneration

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Abstract

Bone defects due to ablative surgery, injuries and pathological and physiological bone resorption still represent a major challenge. The use of advanced therapy, in the form of cell therapy and tissue engineered products, has been recently increased and considered as a promising new approach in regenerative medicine. Based on evidence from preclinical and comprehensive clinical studies, the safety and regenerative potential of mesenchymal stem cells (MSC) in combination with biomaterials have been demonstrated. A substantial amount of work and effort have been placed aiming to develop a novel synthetic bone substitute that triggers bone healing in patients and used as a carrier for MSC. The data generated from recent clinical trials demonstrated that bone marrow stem cells expanded successfully in the laboratory and, combined with synthetic bone substitute biomaterial in patients to augment mandibular bones, induced significant new bone formation. The regenerated bone volume was adequate for dental implant installation. Healing was uneventful. The patients were satisfied with the aesthetic and functional outcomes. No side effects were observed. This novel therapeutic approach can successfully promote bone regeneration, with no unexpected adverse events. Furthermore, preclinical data underscore the advantage of using 3D-printed scaffolds for the successful outcome in bone regeneration.

S8.6-O1

Investigating the regenerative potential of 3D-printed PLLGA/Alginate composite scaffolds for the treatment of articular cartilage defects

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Abstract

Cartilage repair through bioresorbable scaffolds offers a promising alternative to current treatments, by providing cartilage regeneration and avoiding late-stage complications[1]. Commonly employed cell-encapsulated hydrogels offer limited success due to their insufficient biomechanical properties. To address this, 3D-printed PLLGA/Alginate composite scaffolds have been fabricated and chondrogenic potential investigated with three cell types – goat mesenchymal stem cells(GBMSc), chondrocytes(GCh) and a co-culture (GBMSc:GCh) in a 42-day static culture. The composite scaffolds could respond to current unmet needs in a biomechanically comparable scaffold; increasing strength with a PLLGA matrix and promoting chondrogenesis using cell-encapsulated alginate. Zone-wise comparisons in scaffold (upper, middle, lower) were conducted to understand the effect of culture conditions, and dynamic compression stimulation was applied to investigate the effects of dynamic culture on cartilage.

The 3D-printed PLLGA matrix containing crosslinked alginate in its pores(Fig.1e) displayed interconnected pores(Fig.1a&b) with minimal filament defects(Fig.1c&d). The compressive, equilibrium, dynamic moduli;1.05MPa,1.74MPa, and 4.56MPa respectively were biomechanically comparable to the native cartilage[4]. GBMSc, GCh and GBMSc:GCh groups showcased high cell viability (>80%) and a strong cell population composed of chondrocyte-like cells, in a fibrous matrix with even distribution across layers(Fig.1f). DNA quantification displayed stability in the GCh and GBMSc:GCh indicating a cartilage-like behavior where cells do not proliferate(Fig.1g), while GBMSc decreased potentially due to the hypoxic conditions in the scaffold core. An increased sulphated-Glucosaminoglycan(sGAG) production in GBMSc:GCh displayed stronger chondrogenic potential (Fig.1h) in the co-culture group. Further studies on histology are currently in progress to assess variance in cartilage components across the various scaffold layers. The PLLGA/Alginate composites demonstrated potential for cartilage tissue engineering applications due to their ability to balance the scaffold requirement with robust mechanical properties and strong chondrogenesis across cell types. Biomechanical stimulation study using a bioreactor is ongoing to investigate the effects of dynamic culture on cartilage regeneration, which could provide valuable insights into its future translation as a medical device. It is hypothesized that the co-culture would enhance cartilage regeneration over the other cell groups in a dynamic environment.

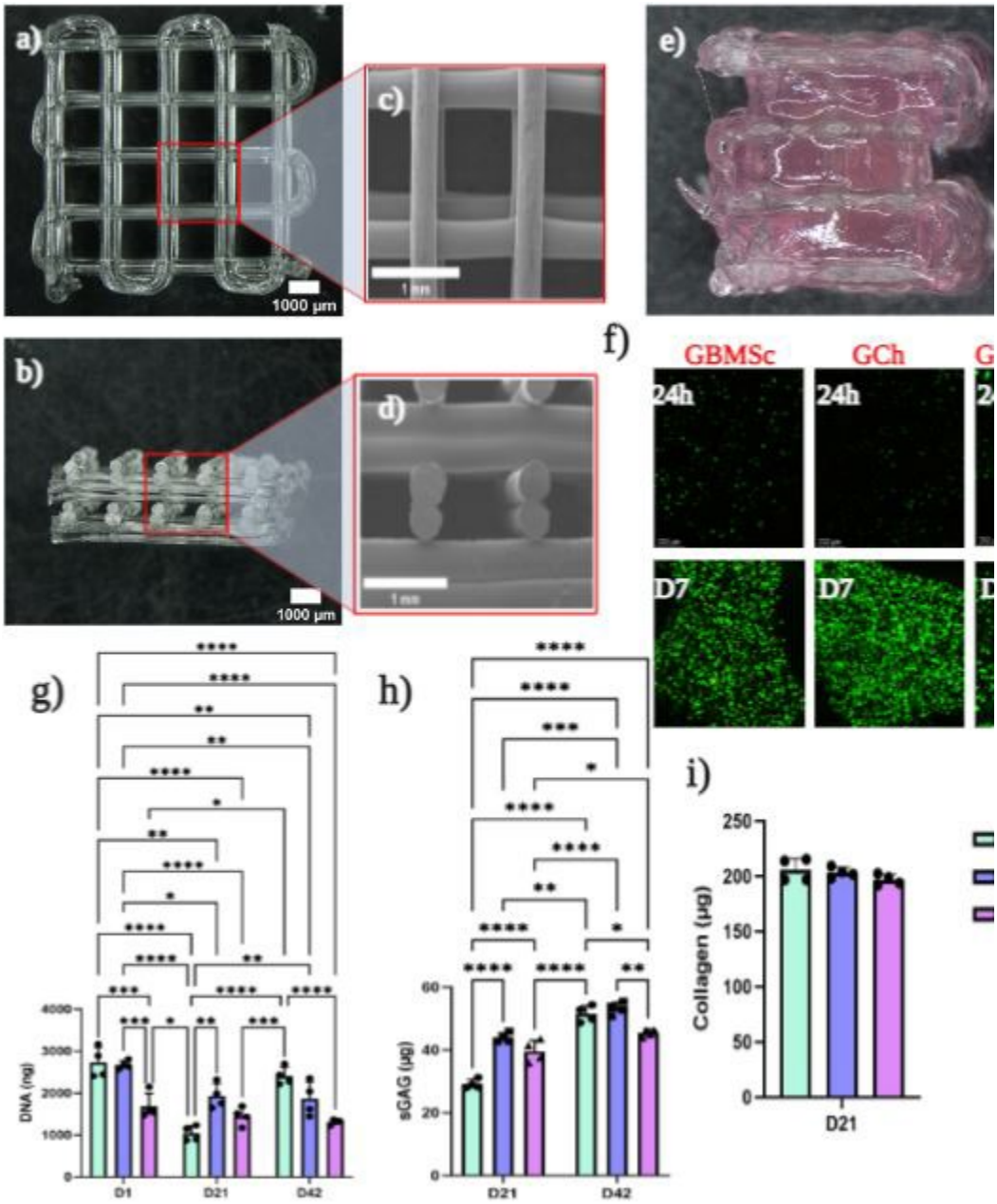

*Fig1(a)3D-printed PLLGA scaffold (b)cross-sectional image (c)SEM of scaffold (d)SEM of cross-section (e)composite scaffold (f)Live/dead (g)DNA (h)sGAG (i)collagen quantification, *p<0.05, **p<0.005, ***p<0.0005*

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S8.6-O2

Pre-vascularized hMSC and hPDC spheroids for bone tissue engineering and their potential as building block units for 3D bioprinting

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Abstract

Spheroids represent an attractive building block unit for bone tissue engineering (TE), especially when pre-vascularized. Spheroid-Bioprinting has been recently investigated to form complex-shaped constructs for tissue and organ regeneration, and provides the means to control how spheroids can be placed in space and time, thus creating more precise bioassembled constructs [1].
In this study, we aim to use pre-endothelialized spheroids for bone TE to evaluate the potential of endothelial cells and their role in osteogenic differentiation. First, we investigated the culture conditions to generate vascularized spheroids using human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs), in order to select the optimal culture conditions that would promote good endothelial cell viability, organization and enhanced bone formation potential *in vitro*. In parallel, the osteogenic potential of human periosteum-derived cells (hPDC) being an important local physiologic osteogenic source was also assessed. For this, spheroids with different cell type ratios were formed and cultured using different osteogenic differentiation media. Spheroid fusion in suspension and encapsulated in photocrosslinkable hydrogels were investigated to access the assembly into larger tissue intermediates to form 3D complex vascularized macrotissues. Gelatin methacryloyl and methacrylated hyaluronic acid were selected for these studies. Lastly, we evaluated the bioprintability of these microtissues and their potential to form a vascular network.
Results showed that the spheroids presented changes in both cellular and ECM components over time. Furthermore, hMSCs and hPDCs presented different osteogenic profiles. HUVECs formed internal clusters, but also peripheral monolayers surrounding the stem cells. hMSC/HUVEC spheroids could fuse together in suspension and inside hydrogels. Initial studies showed that not all ratios allowed the formation of capillary structures. Based on this, we proceeded the bioprinting studies using the 50:50 ratio. Our results showed that it is possible to bioprint a high spheroid density and obtain a good spheroid distribution within the bioprinted construct. Spheroid-laden constructs, confirmed 1 week post-printing the wide distribution of viable spheroids, cellular outgrowth and spheroid fusion. After 7 days, several capillary-like microvessels were already formed within the hydrogel.
This demonstrated that the biomaterial components used to form the bioink and the bioprinting process was mild preserving spheroid viability and phenotype.

References. [1] D. N. Heo et al., Biofabrication, Oct. 2020.
Acknowledgments. Research funded by the European Union's Horizon 2020 framework program, call SC1-BHC-07-2019 - Regenerative medicine: from new insights to new applications, JointPromise - Precision manufacturing of microengineered complex joint implants, under grant agreement 874837.

S8.6-O3

Advanced Gelatin-π-Methacryloyl (GPM) hydrogels: A breakthrough in biocompatibility and strength for biomedical applications

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[1]1Department of Chemistry - Ångström, Macromolecular Chemistry, Uppsala, Sweden. [2]Department of Surgical Sciences,, Uppsala, Sweden. [3]Department of Chemistry - Ångström, Macromolecular Chemistr, Uppsala, Sweden. [4]Department of Materials Science and Engineering, Applied Mechanics, Uppsala, Sweden

Abstract

Introduction. Gelatin-based hydrogels, such as methacryloyl gelatin (GelMa), are widely utilized in biomedical applications due to their biocompatibility, tunable properties, and ease of use. However, their poor mechanical strength, brittle nature, and high swelling can limit their effectiveness in certain applications, particularly those requiring toughness, such as synthetic cornea. This study describes the synthesis of a novel gelatin molecule, gelatin-π-methacryloyl (GPM), and its use for preparing hydrogels with improved toughness, ductility, and reduced swelling. The added π-π interactions were designed to provide ductility and energy dissipation in the hydrogel, whereas the methacryloyl functional groups were designed to achieve covalent crosslinking.

Experimental methods. Gelatin was dual functionalized to install aromatic moieties, providing π-π interactions, and methacryloyl groups for photo-initiated covalent crosslinking. A series of experiments were conducted to evaluate the behavior of the hydrogels in an aqueous environment, measuring changes in G' (storage modulus), swelling, and tensile properties over time. GelMa hydrogels were used as control.

Results**:** Rheology was performed to analyze the viscoelastic behavior of the hydrogels. The initial G' values for 5% GPM, 5% GelMa, and 10% GPM were found to be 200 Pa, 1487 Pa, and 2043 Pa, respectively. These results indicate higher stiffness for the GelMa hydrogels.

The tensile test revealed that the novel material exhibited a remarkable 250% higher strain compared to GelMa's 55% strain, signifying a dramatic enhancement in the ductility of the innovative material in contrast to GelMa . This indicated that the introduction of π-π interactions between the chains resulted in an increased ductility of the hydrogel. The toughness of GPM (179 kJ·m^-3) was found to be significantly higher than that of GelMa (84 kJ·m^-3), indicating its improved ability to absorb energy and resist fracture under mechanical stress (Figure 1).

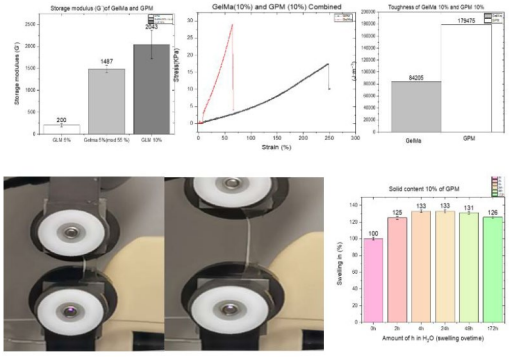

Figure 1. Properties of the GPM hydrogel, including G' values for 5% GPM, 5% GelMa, and 10% GPM; stress-strain curves for 10% GelMa and 10% GPM; toughness values for GelMa and GPM; images of the gels before and after elongation; and swelling data.

Discussion. The novel GPM hydrogel outperformed traditional GelMa hydrogels in terms of mechanical properties, ductility, and swelling behavior. The introduction of π-π interactions between the chains successfully increased the ductility and toughness of the hydrogel. The reduced swelling behavior suggests that the GPM hydrogel could be more suitable for applications requiring implantation in a wet environment, such as in synthetic cornea.

Conclusion. This study successfully synthesized a novel gelatin-π-methacryloyl (GPM) hydrogel that exhibits improved mechanical properties, enhanced ductility, and reduced swelling behavior compared to traditional GelMa hydrogels. The GPM hydrogel holds potential for various biomedical applications, and further experiments are needed to determine its suitability for different applications.

S8.6-O4

An osteochondral bio-engineered model mimicking osteoarthritis *in vitro*

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Abstract

Osteoarthritis (OA) is a joint degenerative pathology characterized by an intricate interaction of inflammatory processes affecting synovium, articular cartilage (AC), and subchondral bone (SB). The OA pathophysiology is problematic to be understood due to a not recognized early phase of the disease, and the lack of reliable and consistent *in vivo* and *in vitro* models able to reproduce the entire pathology. This work aims to develop an engineered *in vitro* model of osteochondral human tissues mimicking the main features of OA as a tool for investigating the disease physiology, biology, and progression. A polylactic acid (PLA) porous trabecular-like construct was 3D printed via Fused Deposition Modelling (Rokit INVIVO Bioprinter) and functionalized with gelatin (Gel) and hydroxyapatite (nHA) to obtain the SB layer. To mimic the AC deep zone, a photocurable Gellan Gum (GG; 3%w/v) multi-channeled hydrogel structure was obtained via soft-lithography, and let adhere to the PLA scaffold.Chondrocytes (differentiated from Y2O1 bone marrow mesenchymal stromal cells) were encapsulated within GG channel embedded in a chondroitin sulfate-based hydrogel, while Y2O1 cells were seeded on the PLA scaffold (Fig. 1). OA environment was simulated by adding IL-6, IL-1β, and TNF-α. GG hydrogel displayed high water uptake capability (initial rapid uptake of 1433±57% at 3 h then achieving plateau), high porosity (pore range size of 100-150µm 30%), and suitable mechanical properties (48±5kPa as compressive Young's modulus). All the materials were capable to sustain cell viability and metabolic activity for up to 7 days (Fig. 2A). The presence of cytokines: (i) decreased the construct mechanical properties (Fig. 2B); (ii) increased the DL calcification, as indicated by higher collagen X levels and decreased the glycosaminoglycans production (0.36±0.01µg in the Healthy model compared to 0.22±0.02µg in the OA samples) at day 21 (Fig. 2D); (iii) led to a higher expression of pro-angiogenic factors (vegf) and a decrease in osteogenic markers (coll1, spp1, runx2) in the SB.The developed OA *in vitro* model was highly reproducible endowed with quickly manufacturing, easy manipulation, and large availability. The selected "cytokines cocktail" was capable to imitate *in vitro* an OA milieu. This innovative approach offers a new way to investigate the development and progression of OA as well as it could be applied to test new therapeutics and to study cell crosstalk interactions.

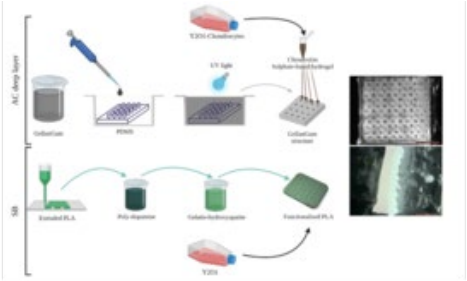

Figure 1: Scheme of model preparation

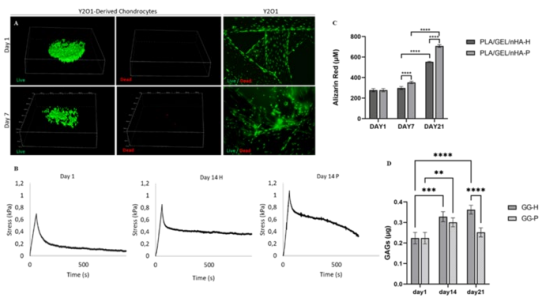

Figure 2: (A) Live and Dead; (B) Stress relaxation; (C) Alizarin red; (D) GAGs

S8.7-K1

Real-scale models of the brain cancer microenvironment

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Abstract

The physiological complexity of cellular interactions is often neglected using *in vitro* models: these interactions can affect the behavior of the cells and diversify the outcome of nanomedicine treatments. *In vitro* investigations on complex biomimetic systems allows for obtaining more predictive results before moving towards pre-clinical testing. For this reason, in recent years, *in vitro* research focused its attention on more complex experimental setups with respect to the standard static 2D cultures.
In this talk, we will show the design, the production, and the characterization of 3D modular co-culture systems, specifically designed to recapitulate the physio-pathological microenvironment of brain tumor. Magnetic microscaffolds have been fabricated through two-photon polymerization (2pp), a disruptive mesoscale manufacturing technique that enables low-cost obtainment of microstructures with nanometric resolution, characterized by unprecedented levels of accuracy and reproducibility [1]. A microtubular structure scaffolding endothelial cells and connected to a fluidic system is exploited to mimic the blood-brain barrier [2]: this biohybrid device is the base for the assembly of magnetic "microcages" hosting glioblastoma cells, and will be provided with docking systems for further magnetic "microcages" carrying endothelial cells and neurons.
This approach represents a disruptive innovation with respect to other 3D models available in the literature, as it will allow a faithful recapitulation of the complex glioblastoma microenvironment through a platform that can be very easily handled in any laboratory.

References. [1] Tricinci O., […], Ciofani G. Advanced Materials Technologies, 5(10): 2000540 (2020); [2] Marino A., […], Ciofani G. Small, 14(6): 1702959 (2018)
Acknowledgments. This work was funded by the European Research Council (ERC) under the European Union's Horizon Europe research and innovation program (grant agreement 101081539, MagDock).

S8.7-O1

Towards a dynamic *in vitro* model of the intestine using smart hydrogels

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Abstract

Intestinal peristalsis plays a crucial role in digestion, nutrient absorption and overall health. Mechanically active *in vitro* intestinal models capable of undergoing peristalsis are very attractive because they reduce the dependence on testing in animal models by mimicking intestinal physiology and dynamics [1]. However, peristaltic contractions are difficult to recreate *in vitro* due to practical difficulties in achieving force control and periodicity during compression of soft substrates. In this work, we explore possibilities of achieving gut motility *in vitro* in a non-contact manner using light as a trigger.

To mimic intestinal muscles that cause a peristaltic contraction, we generate light-responsive micro-particles, which exhibit photo-induced shrinkage, using two different techniques – microfluidics and electrospinning. The first proof-of-concept is a trans-well setup, containing the micro-particles in a hydrogel representing the basement membrane of the intestine, where the intestinal epithelium and mucus-producing cells are represented by Caco-2 cells and HT-29 MTX, respectively. We co-culture different ratios of these cells to mimic different parts of the intestine, such as the colon and the small intestine, and analyze cell differentiation and micro-villi formation by electron microscopy. Monolayer barrier properties are assessed through trans-epithelial electrical resistance (TEER) measurements and permeability response to different-sized molecules.

We show successful fabrication of light responsive nanofibers via electrospinning and confirm our observations with electron microscopy. Culturing cells on basement membrane mimicking hydrogel, we observe altered micro-villi formation as well as mucus secretion *in vitro*, when compared to a stiff substrate such as poly(ethylene terephthalate) that is often used in intestinal models. We also demonstrate physiologically relevant barrier integrity of cellular monolayers after differentiation on such substrates.

Thus, we aim at designing a modular platform where user-defined mechanical cues can be transferred to cells, resembling peristaltic stimulations in a physiologically relevant manner, to gain insights into the contribution of gut motility to human health.

References. [1] Jalali-Firoozinezhad et al, Nat Biomed Eng, 2019

S8.7-O2

Development of 3D cell-printed tissue-specific type 2 diabetes multiple-organ on-a-chip model applicable to diabetes complications

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Abstract

Type 2 diabetes (T2D) is a metabolic disease that affects several organs and causes a variety of complications; it is associated with over 1.6 million deaths each year. Accumulation of visceral adipose tissue (vAT) and its acquisition of an inflammatory phenotype may be an important predictor of T2D. Compared to subcutaneous adipose tissue (sAT), vAT exhibits increased macrophage activity during a diabetic pathology. Despite the increasing demand for *in vitro* T2D models that replicate the complex tissue microenvironment *in vivo*, reconstructing the tissue-specific microenvironment and complications of T2D remains challenging. Therefore, in this study, we developed a dynamic multiple-organ on-a-chip that mimics T2D in a hyperglycemic environment and vAT using three-dimensional (3D) cell printing with decellularized extracellular matrix (dECM) bioinks. This chip consisted of separate compartments for the pancreas, adipose tissue, and liver, closely related to T2D, and a retinal compartment was added to identify T2D complications. In addition, the pathological features of T2D were clearly revealed in the chip using vAT-derived dECM bioink in a hyperglycemic environment. These pathological features have resulted in cellular dysfunctions of the retinal compartment. Moreover, the T2D medications treated on the chip showed the same clinically efficacy. Our chip is suitable for recapitulating the key features of T2D and is a promising platform for drug testing and the study of T2D complications.

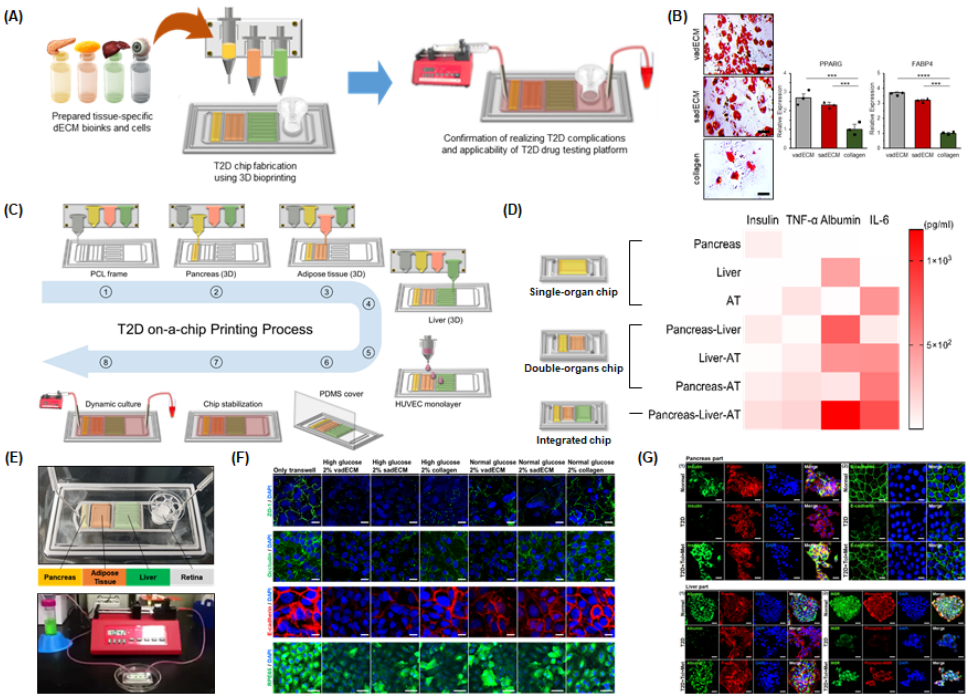

Figure 1. (A) Schematic of T2D on-a-chip fabrication and application. (B) Effects of sadECM and vadECM bioinks on adipogenic differentiation (C) Schematic of the fabrication of a T2D on-a-chip using a 3D cell printer. (D) Multiple-organ interaction effects using the quantitative measurement of metabolite productions. (E) Images of a T2D complication model (top) and dynamic culture using a syringe pump system (bottom). Immunofluorescence staining for confirming (F) hRPE tight junction breakdown and dysfunction, and (G) reaction level of commercial drugs for T2D on the T2D on-a-chip

S8.7-O3

Advancing tumor modeling and drug screening with a dynamic system integrating a 3D human-based hydrogel to support spheroid invasion

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Abstract

To date, advances in biomimetic materials have prompted great progresses in recapitulating the complexity of tumor invasion. However, the synthetic and animal origin of the biomaterials' majority has opening up some concerns regarding the need for a close recapitulation of the tissues' biochemical and biomechanical properties. Human methacryloyl platelet lysates (PLMA, Metatissue®) hydrogels were recently proposed as humanized 3D platforms for tumor invasiveness modeling.1,2 The recapitulation of tumor architecture and synergistic interaction of stromal cells with invading osteosarcoma (OS) tumor highlighted the suitability of PLMA hydrogels to develop accurate cell invasiveness models.2

Given the recognized potential of microphysiological systems to improve tumor physiopathology recapitulation, a tumor-on-a-chip incorporating PLMA hydrogels was developed 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 hydrogel, and co-cultured with human mesenchymal stem cells. Live imaging analysis revealed the recapitulation of the tumor-stromal cell and cell-extracellular matrix interaction in an invading tumor.(Fig.1)

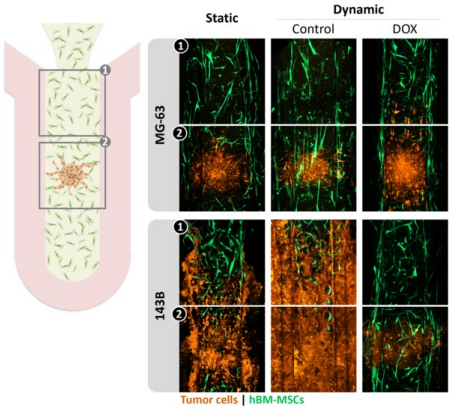

Figure 1.Confocal images of tumor-on-a-chip models, at 7 days.

Moreover, the distinct metastatic ability of the tumor cell lines was mimicked, revealing that metastatic cells showed more aggressive invasive behavior, enhanced in the dynamic environment. Doxorubicin treatment showed an improved drug resistance of the metastatic model, highlighting the suitability of these models for therapy testing.(Fig.2) To further understand how dynamic environment influences tumor metastatic ability, protein and gene expression was performed. Specifically, VEGF was over-produced by 143B cells and, contrarily, MG-63 cells showed an increased expression of collagen, associated with a non-metastatic phenotype.(Fig.2) Exometabolomics profiling further identified metabolic features correlated with tumor progression, demonstrating the glycolytic metabolism preference of metastatic cells in the dynamic system.

Overall, this study clearly validates the feasibility of PLMA hydrogels integration into microphysiological systems to develop accurate cell invasiveness models, following the animal-free tendency.

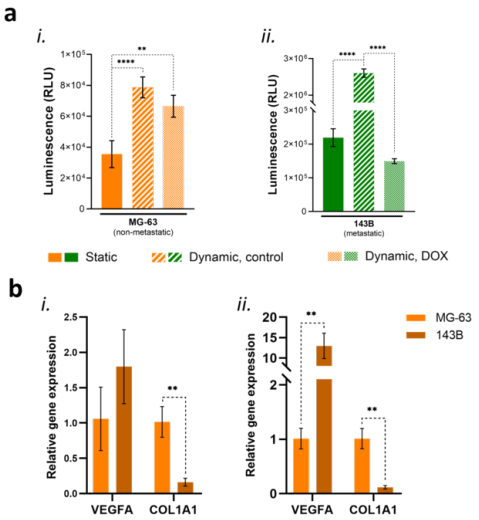

Figure 2.(a) Tumor cell viability of (i)MG-63 and (ii)143B-based tumor-on-a-chip models and (b) their relative gene expression in (i)static and (ii)dynamic conditions, at day 7.

References. 1.Monteiro C.F. et al. Advanced Science (2020); 2.Monteiro C.F. et al. Acta Biomaterialia (2021).
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, and the doctoral grant SFRH/BD/144640/2019 of Cátia Monteiro, financed through the FCT/MCTES (PIDDAC). Catarina Custódio acknowledges FCT for the individual contract 2020.01647.CEECIND. The authors would also like to acknowledge the support from Metatissue®.

S8.7-O4

Vascularized liver tumor model for endovascular embolic agents testing

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Abstract

Chemoembolization is a standard treatment for liver cancer. However, testing of newly developed embolic agents is dependent on animal models, which do not correspond with human vascular anatomy and characterization of vascular response in real-time is challenging. In this study, we developed an *in vitro* microfluidic device with vascularized human liver tumor model for embolic agents testing and chemotherapy release studies. Vascular bed was obtained by seeding fibrin suspension with endothelial cells and fibroblasts. The cells formed perfusable vascular networks within 5 days surrounding an empty hole where liver tumor spheroid was embedded in collagen (3mg/ml) and fibrin (10mg/ml) mixture. Chemoembolization was then conducted by injection of drug-eluting shear thinning material (DESTH) with 5Fr catheter into one media microchannel while the other lining microchannel was left unblocked (Figure 1a). The perfusability of the vascular bed was proved prior to DESTH application by injecting dextran (blue channel) to one of the media channels resulting in fast distribution of dextran through microvessels to the opposite channel (Figure 1b). Application of doxorubicin (DOX)-loaded DESTH into a channel blocked the diffusion of dextran into vasculature bed. The results from fluorescence microscopy after 24 and 48 hours revealed the collapse of vascular bed. Live and dead staining of the vascularized tumor spheroids after 48 hours showed that tumor cells treated with DOX-loaded DESTHs significantly increased the number of dead cells.

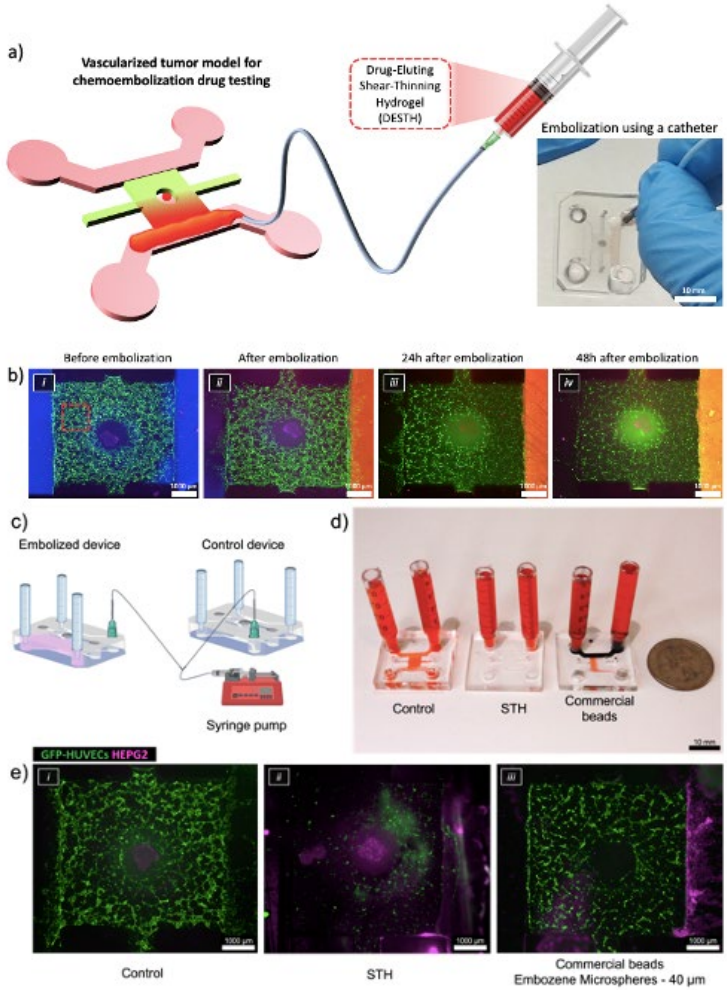

Fig.1: Chemoembolization-on-a-chip experimental setup.

In order to analyze the effect of the embolization itself and to compare the efficacy of the used embolizing material, we compared shear thinning material used in the previous experiment to commercially available Embozene™ microspheres (Figure 1c). Microspheres or shear-thinning hydrogel (STH) blocked water flow across fibrin gel within device when a hydrostatic pressure was applied (Figure 1d). After embolization, we observed vascular bed regression when the devices were treated with STH or Embozene™ treatment 4 days in a flow setup compared to the corresponding control devices. The commercial beads only showed a modest result while STH blocked the vasculature more efficiently **(Figure 1e)**. These results demonstrate the feasibility of developed vascularized liver tumor spheroid model to evaluate different mechanisms of hydrogels and drug-loaded hydrogels for embolization efficiency and chemotherapy release testing.

Acknowledgments. This work was supported by the National Institutes of Health (R01AR074234, R01AR073135, UH3TR003148, R34NS126032, R01GM126571 and NIH R01 CA257558-01A1) and by the Slovak Research and Development Agency under the Contract no. APVV-20-0073 and VEGA 1/0413/23.

S9.1-K1

From nanoscale precision towards processing at the speed of light: A versatile photo-crosslinkable polymer platform

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Abstract

In an ideal world, damaged tissue would be restored back to its initial state, without the lingering presence of body foreign material, in a patient-specific manner. A generally accepted method by which this could be achieved is by means of a temporary scaffold. This scaffold would temporarily take over the necessary functions of the defective tissue. Over time, newly formed tissue would then replace the scaffold, re-take its function and restore the defective area to its initial state. To progress towards the outlined ideal scenario, additive manufacturing of biodegradable polymers plays a key role. Herein, the synthesis and characterization of thiol-ene crosslinkable poly-ε-caprolactone (PCL) and gelatin will be discussed as well as their processing through digital light processing, computed axial lithography and two-photon polymerization. Furthermore, the materials' biocompatibility and capability to serve various tissue engineering applications as evidenced through *in vitro* and *in vivo* assays will be presented. In a final part, polymers chemically tailored to enable monitoring their *in vivo* fate in a non-destructive fashion will be addressed.

References: Thijssen et al. Advanced Materials 2023, 10.1002/adma.202210136; Thijssen et al. Advanced Functional Materials 2022, 32, 2108869; Thijssen et al. Biomacromolecules 2023, 24, 1638-1647; Gréant et al. Advanced Functional Materials 2023, 10.1002/adfm.202212641; Kolouchova et al. Chemistry of Materials 2022, 34, 10902-10916

S9.1-O1

A synthetic dynamic photoresin for fast volumetric bioprinting of functional hydrogel constructs

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Institute for Biomechanics, Zurich, Switzerland

Abstract

Volumetric bioprinting (VBP) has emerged as a powerful tool for fast 3D bioprinting of living cellular constructs in one step. However, existing bioresins that meet the physicochemical requirements of VBP are limited to thermo-responsive photosensitive gelatin derivatives of high polymer concentrations. For this project, a series of synthetic bioresins were formulated with varying amounts of norbornene-functionalized PVA (nPVA), sacrificial gelatin, PEG dithiol crosslinker, LAP photoinitiator and embedded human mesenchymal stem cells (hMSC). Photo-rheology and unconfined compression tests were applied to assess printability and gel mechanics.

Using 5% gelatin as a temporary network enabled the printability of nPVA resins with low polymer concentrations down to 1.5%. Unconfined compression testing shows that the presence of gelatin renders the printed matrices viscoelastic (Figure 1A). After 24 h incubation at 37 °C, ~85% gelatin was released out of the constructs. Resins containing 1.5% and 3% nPVA were used to print soft and stiff hydrogel constructs with a compressive modulus of 2.7 ± 0.1 kPa and 25.1 ± 1.7 kPa, respectively. After bioprinting, cells were highly viable (>90%), indicating good cell-compatibility of the printing process (Figure 1B). However, the morphology of embedded cells showed remarkable differences in soft and stiff matrices. After 7 days, cells embedded in a soft matrix showed longer protrusions compared to cells in a stiff matrix (Figure 1C). We reasoned that the presence of the temporary gelatin network in the matrix and the resulting viscoelasticity facilitated fast cell spreading. Additionally, complex cell-laden constructs such as trabecular bone were printed using nPVA resins within 7-15 s. Interestingly, single hMSCs self-organized into multicellular aggregates in alignment with the orientation of the printed channels. In conclusion, we developed a new synthetic bioresin by introducing a thermo-releasable gelatin network into a photo-clickable PVA hydrogel for fast VBP of functional hydrogel constructs.

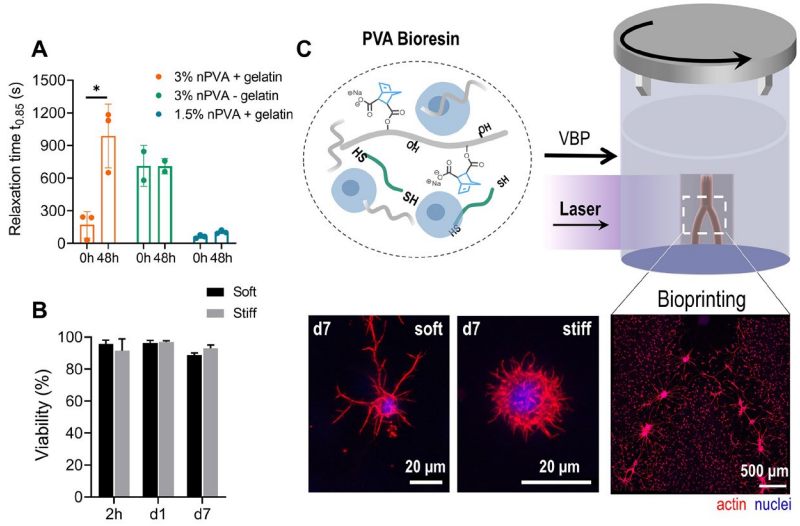

*Figure 1. (A) The stress relaxation time (t0.85) of different hydrogel samples before (0 h) and after incubation in PBS at 37 °C for 48 h (48 h). Data presented as mean ± SD. Statistical analysis was evaluated with a multiple paired t-test. *p = 0.0435 (n ≥ 2). (B) Quantification of cell viability of hMSCs at different time points. (C) Illustration of volumetric bioprinting and cell morphology in 3D-printed hydrogel constructs.*

S9.1-O2

Photoclickable and isotonic collagen-based matrices for rapid volumetric additive manufacturing

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Abstract

Volumetric additive manufacturing/printing (VP; **Fig. 1A**) combined with recent advances in photoclick chemistry has brought forth a paradigm shift in the field of tissue biofabrication, by enabling tissue printing at high resolution within seconds. Importantly, one of the most widely used materials in biofabrication and regenerative medicine is collagen, but VP of collagen-based matrices has not yet been explored. The temperature and pH sensitivity of the collagen formulations, where the collagen undergoes fibril formation at neutral pH, can complicate procedures involving the mixing of cells, resin handling, printing and post processing. In this work, we first demonstrate a photoclickable collagen system which is compatible with VP (**Fig. 1B**) and allows prints at a high resolution (up to 150 µm, **Fig. 1C**) and high speed (capable of printing a cm-scale construct in < 20 s, see example in **Fig. 1D**). We then demonstrate how the fibrillogenic potential of the collagen molecules at neutral pH can be temporarily inhibited (see thermorheology in **Fig. 1E**) using a customized collagen fibrillogenesis inhibition (CFI) buffer system. The CFI buffer allows easy mixing of the cells at high densities (up to 10 M cells/ml) and post-processing within the collagen formulations without affecting the print fidelity and cell viability. The buffer also does not affect the molecular structure (via circular dichroism measurements, **Fig. 1F**) or the photocrosslinkability (via light-dose tests) of the formulations. Finally, we also demonstrate multimaterial volumetric printing of the formulations, where we create tissue constructs featuring muscle and connective tissue interfaces and bifurcating vessels with concentric layers of endothelial cells and connective tissue. The photoclickable, isotonic and neutral formulations demonstrated in this work can potentially transform the field of light-based biofabrication involving collagen matrices.

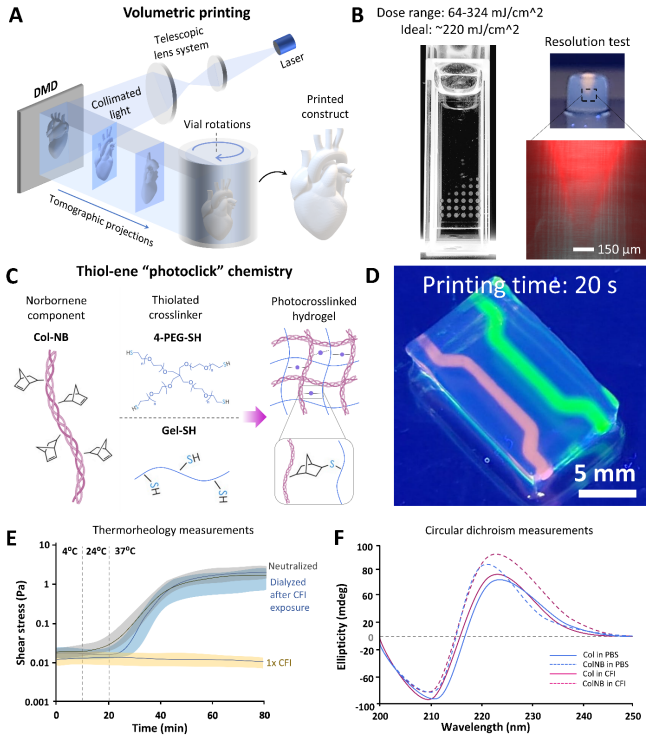

Figure 1. A. Scheme of volumetric printing (VP) using tomographic light projections for the biofabrication of tissues. B. Scheme of thiol-ene crosslinking for matrices containing collagen norbornene (Col-NB) and a crosslinker based on thiolated 4-arm polyethylene glycol (4-PEG-SH) or thiolated gelatin (GelSH). C. Dose tests and resolution tests for the volumetric prints. D. Example perfusable construct printed with the collagen-based resin. E. The CFI buffer temporary inhibits fibrillogenic potential of Col-NB (i.e., there is no evident crosslinking at 37°C), while the fibrillogenesis is restored once the CFI is removed through dialysis. F. Circular dichriosm measurements demonstrate retention of triple helical conformations (negative peak at 225 nm) and constitutive α-helix chains (negative peak at 210 nm) in collagen.

S9.1-O3

A flexible allyl-modified gelatin photoclick bioresin designed for volumetric 3D printing of soft matrices for Adipose-derived Stromal Cells (ASC) and pre-differentiated adipocytes culture

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Abstract

Volumetric bioprinting (VBP) is a layerless light-based 3D printing technique which recently favored a paradigm shift for additive manufacturing (AM) platforms since it enables the fabrication of convoluted cell-laden structures in tens of seconds with high spatiotemporal control. A flexible allyl-modified gelatin (gelAGE)-based photoresin was developed to produce exceptionally soft polymeric matrices (0.2 - 1.0 kPa), although maintaining high shape fidelity and stability *in vitro.* The gelAGE-based formulations were tailored to exploit the fast thiol-ene click chemistry with a multi-arm thiolated polyethylene glycol (PEG4SH), in the presence of 0.1% of lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as photoinitiator. The flexibility of the gelAGE biomaterial platform enabled to vary its concentration ranging from 2.75% to 6% and to change the allyl to thiol molar ratio without interfering with the resin photocrosslinking efficiency. The thiol-ene crosslinking strategy allowed to fabricate viable cell-laden structures with a high throughput in tens of seconds. The suitability of the gelAGE-based photoresins was highlighted by the adipogenic differentiation of adipose-derived stromal cells (ASC) after VBP, and by volumetric 3D printing of mechanically fragile pre-differentiated adipocytes-laden constructs as a proof-of-concept. Altogether, this study introduced a soft photoclick resin to enlarge the library of suitable biomaterials for VBP toward soft tissue engineering applications.

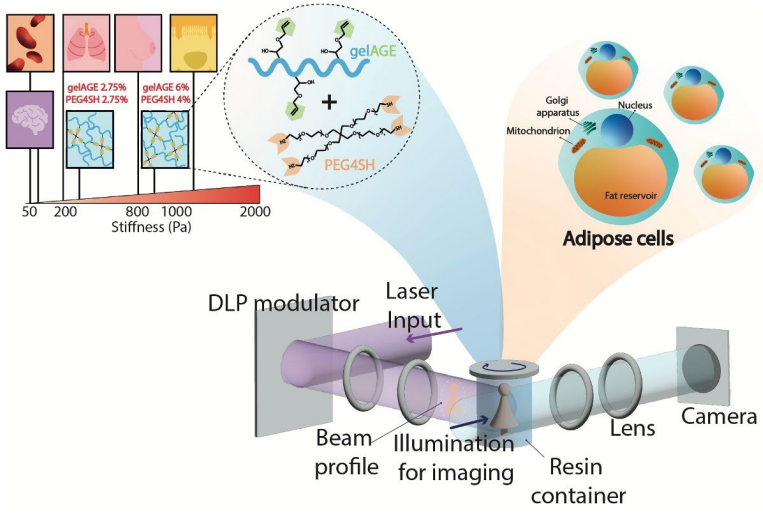

Figure 1. Schematic of volumetric bioprinting of gelAGE-based photoclick resins for soft tissue engineering applications. The matrix stiffness of both bioresins with low and high gelAGE concentration, respectively, was contextualized with other relevant tissues (e.g., brain, lung, adipose (breast) and endothelial).[1]

References: [1] T. R. Cox, J. T. Erler, Dis Model Mech 2011, 4, 165.

S9.1-O4

4D printing of temperature-triggered shrinking hydrogels to achieve physiological dimensions for kidney tissue engineering via volumetric printing

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Abstract

To develop advanced *in vitro* models, it is well-known that scaffold geometrical cues play an important role in cell behavior. However, a major challenge in kidney tissue engineering is fabricating complex tubule-mimicking constructs with physiologically relevant dimensions to promote cell function (inner tubule diameter of 10-70µm). Recently, interest has been growing in on-demand shrinking technologies to further reduce the minimal achievable dimensions of 3D-printed hydrogels. This post-printing process is driven by an increase in hydrophobicity of the crosslinked polymers upon application of an external trigger, which leads to partial dehydration and, consequently, to a volume reduction of the printed construct. Taking advantage of thermo-responsive polymers, mild heat can be used as trigger to induce shrinkage. This project aimed to develop a new hydrogel able to shrink upon a cytocompatible temperature increase (T<=37°C). Poly(N-isopropyl acrylamide) (PNIPAM) was selected because of its lower critical solution temperature (LCST) transition at ~32°C. The new hydrogels are composed of a fully synthetic polymer obtained by polymerizing NIPAM and hydroxyethyl acrylate (HEA) via controlled radical polymerization from a bifunctional polyethylene glycol (PEG) macroinitiator. Subsequently, methacrylate groups (MA) were incorporated, yielding the hydrogel precursor here abbreviated as PNH-MA (Fig.1A). Mechanically robust gels were obtained by photo-crosslinking at 4°C (T<LCST) (Fig.1B). Upon incubation in aqueous solution at 37°C, the desired shrinking behavior was observed showing 85% of the maximum shrinking capacity achieved after 6 hours (Fig.1C). By tuning the degree of methacrylation and the length of the thermo-responsive domains, the extent of shrinkage could be controlled, with a 5-fold reduction in volume as maximum shrinkage capacity. 3D-printed hydrogels with perfusable channels were successfully printed via volumetric printing, and subsequently shrunk by temperature increase, while retaining scaffold shape and open channels (Fig.1D). Conditionally immortalized proximal tubule epithelial cells (ciPTECs) were seeded into the swollen channels, previously coated with L-DOPA to enable cell adhesion. Then, the cell-laden channels were shrunk and cell viability was assessed (Fig.1E). These results indicate that both the material and the shrinking process are cytocompatible and provide a promising platform for *in vitro* models with relevant dimensions.

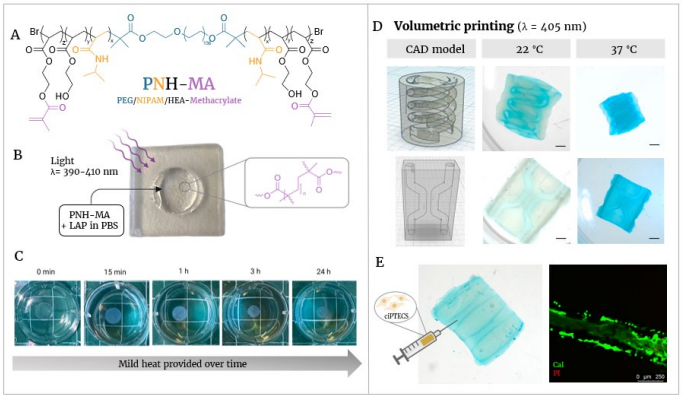

Fig.1 Graphical abstract. A) Chemical structure of the thermo-responsive hydrogel precursors. B) Mechanically robust hydrogels obtained by photo-crosslinking reaction. C) Temperature-triggered shrinking behavior upon incubation at 37°C in PBS. D) 3D-printed hydrogels obtained via volumetric printing, subsequently shrunk to reduce scaffold dimensions (scale bar=1mm). E) Shrunken ciPTECs-laden channels (Live/Dead staining).

S9.2-K1

Cell instructive polymer biomaterials

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Abstract

In regenerative medicine and medical devices, decoration of materials with biomolecules or coating with complex mixtures such as Martigel® are common strategies to control cell attachment and phenotype for both *in vitro* and for *in vivo* use.[1] It is desirable to use polymers that can control cells without biomolecule addition for regulatory and reproducibility reasons. Unfortunately, while there is a very developed literature on cell-biomolecule interactions, our understanding of the response of cells to most manmade materials is not well established. Only in isolated cases is there a good description of cell-material surface interactions and fewer still where material-tissue interactions are well characterised and understood.

This paucity of information on the mechanism of biomaterial interactions with cells and the body acts as a roadblock to rational design. Consequently, we have taken a high throughput screening approach across a number of cell types to discover new bio-instructive polymers from large chemical libraries of synthetic monomers presented as micro arrays.[2] This approach, akin to engineering serendipitous discovery of new materials, has resulted in novel materials which we have taken all the way from the lab to the clinic.

More recently we have extended our approach from polymer identity, to explore the opportunities offered by micro topography and 3D shape manipulation to provide bio-instructive topographic cues to immune cells, stromal cells and pathogenic bacterial cells. To do this we have developed and adopted a range of high throughput screening platforms, including the TopoChip[3,4], ChemoTopoChip[5] and used 3D printing to produce the ChemoArchiChip[6].

The talk will focus on introducing these topographic platforms and highlighting our findings in the field of topographic and material chemical control of both *in vitro* macrophage and *in vivo* host immune response to topography.[3-6]

References: 1. Celiz (2014) Materials for stem cell factories of the future -Nature Materials; 2. Yang (2021) High-throughput methods in the discovery and study of biomaterials and materiobiology -Chemical Reviews; 3. Nasir (2023) Polymer functionalised micro-topographies for maturation of human pluripotent stem cell derived cardiomyocytes -unpublished; 4. Vassey (2020) Immune modulation by design: using topography to control human monocyte attachment and macrophage differentiation -Advanced Science; 5. Burroughs (2021) Discovery of synergistic material-topography combinations to achieve immunomodulatory osteoinductive biomaterials using a novel *in vitro* screening method: The ChemoTopoChip -Biomaterials; 6. Vassey (2023) Innate immune cell instruction using micron-scale 3D objects of varied architecture and polymer chemistry: The ChemoArchiChip -Matter (Cell Press)

S9.2-O1

Alginate-Norbornene as a versatile hydrogel platform for kidney *in vitro* modeling

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Abstract

Currently 10% of the worldwide population is estimated to be affected by kidney disease. Unfortunately, available *in vitro* models are not sufficiently biologically relevant to provide full understanding of progression stages of kidney diseases. When developing *in vitro* models, the first step to increase the biomimicry is to engineer a 3D microenvironment for cells that is able to mimic the native ECM. Our aim was to engineer alginate-based hydrogels as a versatile ECM mimicking material. To achieve this, two chemical modifications were introduced in this polysaccharide backbone (as schematized in Figure 1): sulfation and norbornene functionalization. Sulfated polysaccharides mimic heparan-sulphate and aid with the retention of endogenous growth factors (heparin-binding proteins). Furthermore, norbornene groups enable the use of thiol-ene click reaction, which has three main functions:

1. Covalent crosslinking and mechanical properties tuning
2. Allowing hydrogels remodeling by cells (thiol-pending cleavable crosslinker)
3. Biological functionalization (cysteine-pending peptides)

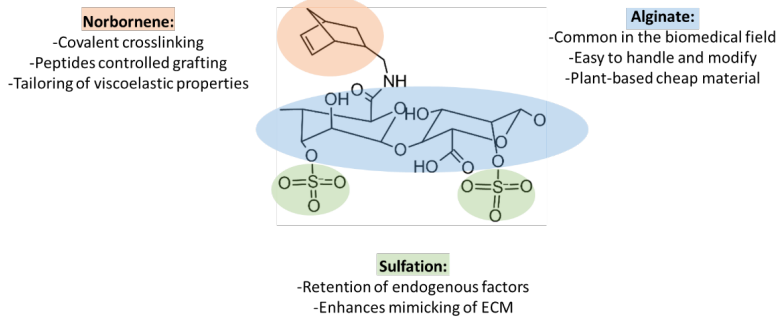

Figure 1 Scheme representing the modified alginate and the function of the various components.

These features make this platform highly tailorable from the material science point of view, as the viscoelastic properties, the swelling and the degradation can be tuned by selection of the crosslinker. Photo-triggering of the thiol-ene coupling was selected to ease the use of these hydrogels for bioprinting. The tunability in the viscoelastic properties, degradation, and crosslinking kinetics of the platform were tested by photo-rheology and nanoindentation. Results showed that the most effective parameters to control the mechanical properties of the hydrogels were the concentration of alginate and of dithiol crosslinker, whereas the crosslinking kinetics were mainly affected by the amount of photocrosslinker and the intensity of the used UV light. These results allowed tuning of the modified alginate properties for extrusion bioprinting (BioX), and for microfluidic bioprinting (Aspect Biosystems), both with Ca^{2+} crosslinking and via a novel Ca^{2+}-free approach. The degree of substitution of norbornene of 25±5% guaranteed free sites for further attachment of peptides to introduce biological cues in the hydrogels (RGD and nephronectin). Biological characterization is currently ongoing on single cell models (cell lines and iPSCs derived kidney progenitors) and on iPSCs-derived kidney organoids. The modified alginate was shown to be a highly tunable material and adaptable to different bioprinting technologies. Moreover, on the basis of the preliminary biological *in vitro* evaluations, it can be suitable for the design of novel *in vitro* kidney models.

S9.2-O2

Versatile click alginate hydrogels with protease-sensitive domains as cell-responsive/instructive 3D microenvironments

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Abstract

Alginate (ALG) is a natural polymer widely used to create artificial extracellular matrices (ECM) for tissue engineering applications. Since it does not degrade in the human body, imparting proteolytic sensitivity to ALG hydrogels leverages their properties as ECM-mimics. Still, this requires effective chemical strategies [1,2]. Herein, we explored the biocompatible/bioorthogonal strain-promoted azide-alkyne cycloaddition (SPAAC) to graft bi-functional matrix metalloproteinase (MMP)-sensitive peptides to cyclooctyne-modified alginate (ALG-K) for partial crosslinking, followed by clicking integrin-binding peptides. Multifunctional hydrogels were formed via a secondary ionic-crosslinking step. Hydrogel properties were fine-tuned to provide cell-responsive/instructive microenvironments for 3D cell-culture. ALG was first modified by carbodiimide chemistry with BCN-amine (K) to incorporate SPAAC-clickable cyclooctyne moieties (ALG-K) [3]. Modification degree (MD≈3%) was assessed by ^{1}H-NMR and cyclooctyne incorporation (new peaks:2.18-2.35/3.34ppm) was confirmed. MMP-sensitive GGYGPVGLIGGK (PVGLIG) and GGGGRGDSP (RGD) peptides were successfully modified with azide groups (N_3), as proved by LC-MS/MS. Bi-functional N_3-PVGLIG-N_3 was SPAAC-clicked to ALG-K at different concentrations (0-250µM). PVGLIG-ALG-K grafting was confirmed by appearance of characteristic FTIR-peaks and higher molecular-weight species on Triple-SEC analysis, suggesting occurrence of peptide-mediated crosslinking. SPAAC-clicking potential of ALG-K derivatives was assessed with fluorescent azido-tags. PVGLIG-ALG-K susceptibility to enzymatic cleavage by collagenase was confirmed. PVGLIG-ALG-K was combined with RGD-ALG-K to produce multifunctional PVGLIG/RGD-ALG-K hydrogels laden with fibroblasts (hDNF) by ionic crosslinking with Ca^{2+}. 3D-cultured hDNF were analyzed for viability (live-dead assay), gene expression (qPCR), MMP production (immunostaining, zymography), morphology, cellular interconnectivity quantification, ECM production (CLSM). Hydrogels with intermediate PVGLIG concentration (125µM) presented slightly higher stiffness while promoting extensive cell-spreading and higher degree of cell-cell interconnections, which was likely favored by cell-driven proteolytic remodeling of the network. 3D-cultured hDNF not only produced their own pericellular ECM, but also expressed ECM-cleaving proteases (MMP-2 and MMP-14), and produced a secretome with proven enzymatic activity towards PVGLIG moieties. Overall, we report the successful production of MMP-sensitive alginate hydrogels by bioconjugation with cell-instructive/responsive peptides via SPAAC click-chemistry followed by ionic crosslinking. Double-end PVGLIG grafting not only provided partial crosslinking of the network but also rendered hydrogels sensitive to cell-driven enzymatic cleavage and proteolytic remodeling. By recapitulating key ECM-like features, these multifunctional hydrogels provide biologically-relevant 3D matrices for soft tissue regeneration.

Acknowledgments. FCT-Portuguese Foundation for Science and Technology: project PTDC/BTM-ORG/5154/2020, fellowships SFRH/BD/129855/2017 and 2022.10184.BD.

References. 1.Fonseca KB et al., Acta Biomater2011:7(4);1674; 2.Fonseca KB et al., Soft Matter2013;9:3283; 3.Jain E et al., ACS Appl Bio Mater (2011):4(2);1229

S9.2-O3

Self-assembling peptide-hyaluronan hydrogels as biomimetic cell microenvironments

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Abstract

There is a great interest in hydrogels for the 3D culture of cells that offer a biomimetic environment for cells to grow and proliferate and/or direct specific cell behaviors. Here, we are presenting a new class of hyaluronan (HA) hydrogels that are formed by supramolecular assembly of unmodified HA with cationic beta-sheet peptides [$(KI)_nK$, lysine (K), isoleucine (I), n = 2–6] (1). The hydrogels exhibit tunable structural and mechanical properties and trigger the formation mesenchymal stem cell spheroids (Figure 1), facilitating and expanding their applicability for 3D stem cell and organoid cultures. In addition, HA is a major constituent of connective tissues and present in the tumor microenvironment of many cancers. Its presence in the hydrogels in the native form is biologically relevant, providing a more realistic environment to probe its role in fundamental *in vitro* studies.

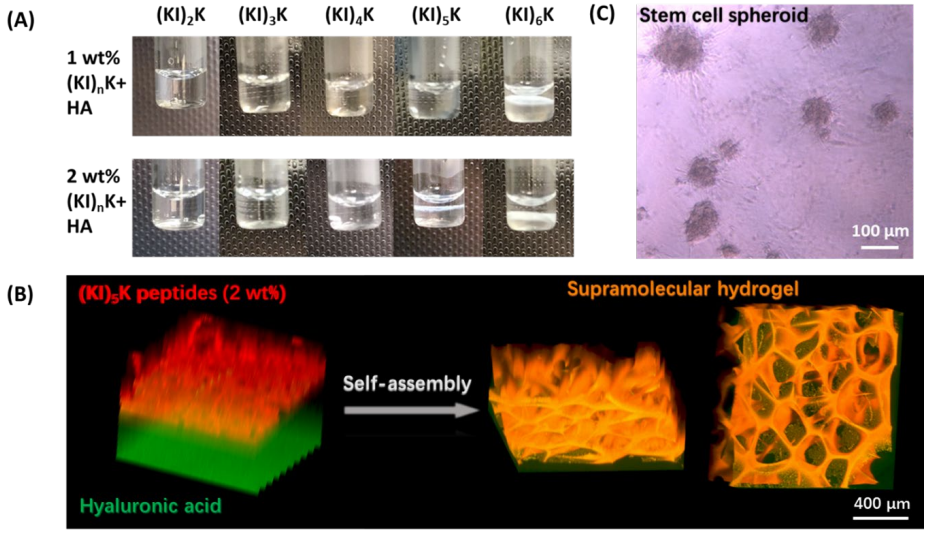

Figure 1: Combining HA with $(KI)_nK$ peptides of various sequence lengths leads to homogeneous complexes or hydrogels with phase separation (A) where a microporous structure is observed in some cases (B). MSCs cultured on the $(KI)_nK$-HA hydrogels form spheroids (C).

Acknowledgements**:** H.S.A. thanks the MOBILIsE project funded by the European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 951723. Y.Y. thanks China Scholarship Council for providing her PhD scholarship (Grant No. 201706630005). J.B. acknowledges Marie Sklodowska-Curie Individual Fellowship granted by the European Commission (MSCA-IF-2014-658351). Y.S. thanks the Shanghai Pujiang Program (21PJ1404100). The authors thank the Diamond Light Source (DLS) for the access to beamline I22 (Proposal No SM25037) and the technical support by DLS personnel, Dr Nicholas Terrill and Dr Andy Smith.
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S9.2-O4

Guiding nasal chondrocytes through 3D bioprinted design to generate an osteochondral tissue

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Abstract

Introduction: Osteochondral defects due to trauma or osteoarthritis affect the cartilage layer and the underlying subchondral bone. Cartilage tissue is divided into the articular and hypertrophic zones. Recent work has shown that the phenotype of chondrocytes within the different zones can be controlled by regulating oxygen concentration. This study investigates the role of hypoxia in cartilage formation, using human nasal chondrocytes (hNCs) embedded in a collagen/tyramine hyaluronic-acid-based (Col/THA) hydrogel, by controlling oxygen gradient using a 3D-bioprinting approach.

Methods: Primary hNCs were isolated from the nasal septum cartilage of patients undergoing septoplasty and were cultured in 2D and 3D aggregates (i.e., pellets) under normoxic conditions (21%-O2), hypoxic conditions (2%-O2), or supplemented with hypoxia-inducing compound (21%-O2 + 1mM-DMOG). hNCs were embedded into the Col/THA (2.5%/15%) hydrogel with ruthenium/sodium persulfate (0.2 mM/2 mM) as a photoinitiator. Constructs were printed using a pneumatic bioprinter (REGENHU, Villaz-St-Pierre, Switzerland) and crosslinked using visible light for 10 min. Printed constructs were analyzed for viability using calcein-AM/ethidium-homodimer-1 and assessed for quality using Alcian Blue (AB) and Safranin-O. Gene expression on COL2A1/ACAN/SOX9/COL1A1/RUNX2/ALPL/SMADs was performed on days 7/14.

Results: 2D cultured hNCs in hypoxia and with DMOG maintained the native spheroidal morphology, showing positive AB staining (Figure 1A). Conversely, the normoxic conditions showed an elongated fibroblastic morphology and a limited amount of AB-positivity. Hypoxia-conditioned hNCs exhibited statistically significant upregulation of the chondrogenic markers SOX9/ACAN/COL2A1 and a lower expression of the hypertrophic cartilage markers RUNX2/ALPL/COL1A1 (Figure 1B). Similar results were obtained in 3D pellet cultures. The viability study at 28 days in the bioconstruct showed high survival of hNCs in the Col/THA-based hydrogel. On the histological level, safranin-O staining showed new extracellular matrix formation which was further confirmed by positive immunofluorescence staining for collagen type I/II.

Conclusion: Hypoxia condition positively supports the generation of articular-like cartilage tissue using hNCs. Furthermore, topological studies on the role of hydrogel architecture and oxygen gradients diffusion are undergoing using 3D-bioprinting in the direction of stable osteochrondral construct formation. The integration of the heterogenous cartilage layer with a subchondral bone scaffold for the cartilage-bone interface will be the next challenge addressed in this project.

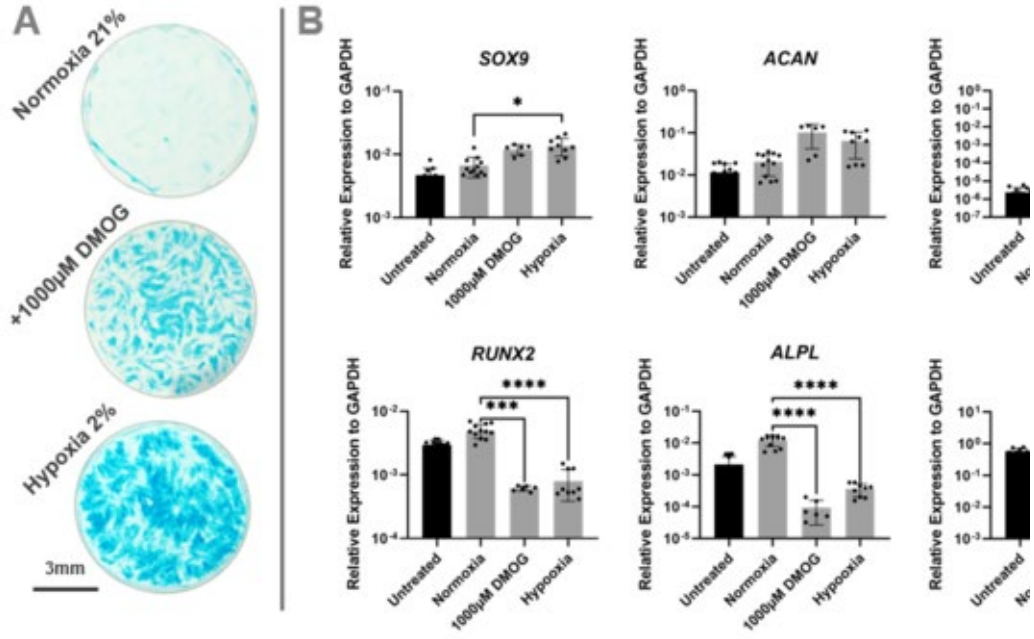

Figure 1: A) Representative images of AB-stained hNCs monolayer at day 14. Scale bar=3mm. B) Analysis of chondrogenic (SOX9/ACAN/COL2A1) and hypertrophic (RUNX2/ ALAP/COL1A1) gene expression markers at day 14. Expression of each gene were normalized to untreated samples. GAPDH was used as a housekeeping gene.

S9.3-K1

Promoting neurovascular regeneration in musculoskeletal repair

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Abstract

Tissue engineering provides a viable means of regenerating bone and skeletal muscle tissues following injuries that lead to large volumetric defects. Our lab has developed advanced biomaterial and stem cell-based approaches to promote functional recovery following volumetric muscle loss (VML) and critical-sized craniofacial bone injuries. In this presentation, I will summarize several of these studies.

For the treatment of VML, we designed a 3D human cell-derived muscle construct with neuromuscular regenerative potential and applied it in combination with a rehabilitative exercise regime in mice to enhance recovery from VML injury. We hypothesized that there may be a synergistic effect between the mechanical stimulation produced by rehabilitative exercise and the acetylcholine receptor (AChR) cluster-promoting ability of agrin. We found that our engineered muscle graft expressed pro-regenerative markers and induced robust growth of de novo host myofibers that were vascularized, displayed AChR clusters, and showed some nerve ingrowth into the defect area. All factors were enhanced by functionalization of the scaffolds and the inclusion of rehabilitative exercise.

To regenerate critical-sized bone defects in murine calvaria, our lab has long investigated the potential for using 3D-printed scaffolds infused with adipose-derived stem/stromal cells. In this presentation, I will report the design and fabrication of 3D printed oxygen-generating calcium peroxide-polycaprolactone bone tissue engineering scaffold that once hydrolytically activated, provides long-term generation of oxygen at a controlled, concentration-dependent manner, without triggering burst release at early time points. We employed a quantitative 3D imaging platform to demonstrate the positive effect of oxygen release on vascular phenotype and volume (i.e. enhanced CD31hiEmcnhivessel volume) along with the increased number of Osterix+ skeletal progenitor cells in post-implantation defect healing and bone regenerating microenvironment.

Finally, in craniofacial bones, such as the calvaria, few studies have focused on developing a quantitative understanding of nerve interactions with the vasculature and osteoprogenitors during healing. Through our quantitative lightsheet microscopy platform, which can image the entire skull at single-cell resolution, we have mapped the nerves and their associations with vasculature during homeostasis, aging, and bone healing in the murine calvaria. These studies reveal insights int the kinetics and distribution of nerves in non-healing injuries that may provide insights for the design of biomaterials used to treat these injuries.

S9.3-O1

Peptide-hydroxyapatite nanoparticles loaded into a thermoresponsive hydrogel for enhanced bone regeneration

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Abstract

Introduction. In situ forming hydrogels present a minimally invasive technique for sustained local drug delivery. We have synthesised a thermoresponsive Chitosan (Cs)-grafted N-isopropylacrylamide (NIPAAm) hydrogel designed to degrade over 8 weeks. This hydrogel has been loaded with cell penetrating peptide (RALA)/hydroxyapatite (HA) nanoparticles (NPs). The aim of this study was to synthesise Cs-g-PNIPAAm hydrogel loaded with RALA/HA NPs for bone regeneration *in vitro* and *in vivo*.

Materials & Methods. Cs-grafted-PNIPAAm was synthesised by free radical polymerisation. NPs were formulated via electrostatic attraction by incubating HA with the RALA peptide for 30 min at RT. Hydrogel morphology was analysed by SEM. *In vitro* degradation was measured in 0 and 3 mg/mL lysozyme-containing PBS at 37°C. *In vivo* degradation was measured in C57BL/6J mice up to 8 weeks following subcutaneous injection of 200 µL hydrogel. Viability of NCTC-929 cells following NP treatment was determined via MTS assay. This treatment was assessed using a condyle model in rats with a critical size unilateral defect.

Results/Discussion. The hydrogel formed a complex structure with a highly connected and meshed network (Fig.1A). The copolymer exhibited a sol-gel transition at 35.2 ± 1.13°C, which was unaffected by NP incorporation (Fig.1B). Eight weeks post-injection 82.7% ± 4.4% of the hydrogel degraded *in vivo*, matching degradation in 3 mg/mL lysozyme *in vitro* (Fig.1B/C). No cytotoxic effects from the hydrogel were observed in NCTC-929 cells (Fig.1D). In the rat condyle defect model, RALA/HA NPs (4.5 µg) delivered via a thermoresponsive hydrogel increased bone volume by 81.3% at 8 weeks compared to 60% in empty defect (Fig.2).

Conclusion. This study shows successful synthesis of a thermoresponsive and biodegradable Cs-g-PNIPAAm hydrogel through cost-effective free-radical polymerisation. RALA/HA NPs were successfully loaded onto the hydrogel and released over time, maintaining functionality. Treatment with the RALA/HA loaded hydrogel led to improved bone regeneration in an *in vivo* rat condyle defect model.

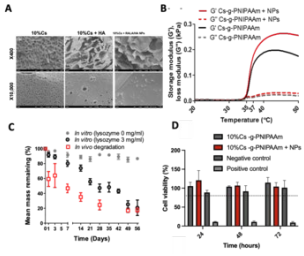

Figure 1: (A) SEM of hydrogel at X400 (above) and X10,000 (below). (B) Storage/loss modulus of Cs-g-PNIPAAM ± HA NPs. (C) In vitro and in vivo correlation studies for degradation of 10 wt% Cs-g-PNIPAAm. (D) Cell viability of NCTC-929 fibroblasts co-cultured for 24 h with the hydrogel degradation products indicated.

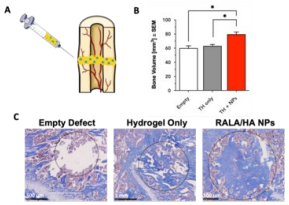

Figure 2: (A) Representative images of rat condyle defect showing region of interest 8 weeks after surgery +/- treatment. (B) Bone volume within region of interest at 8 weeks. Data shows average of 6 defects +/- SEM.

S9.3-O2

A composite elastin derivative-based hydrogel designed for promoting bone formation, vascularization, and innervation: *In vivo* evaluation in ectopic and heterotopic models

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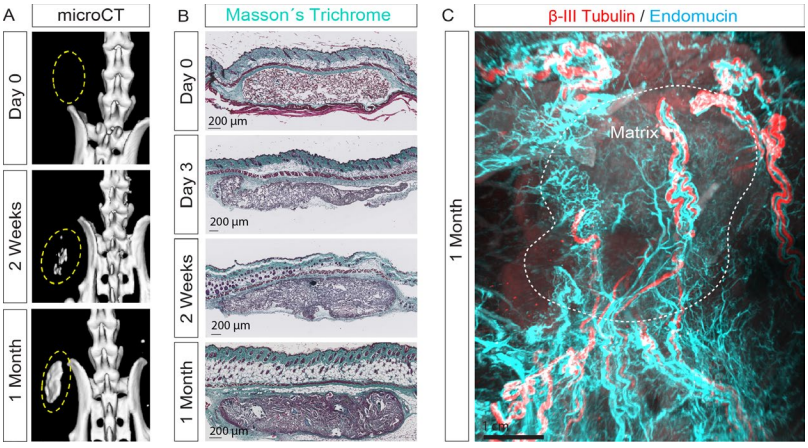
Abstract

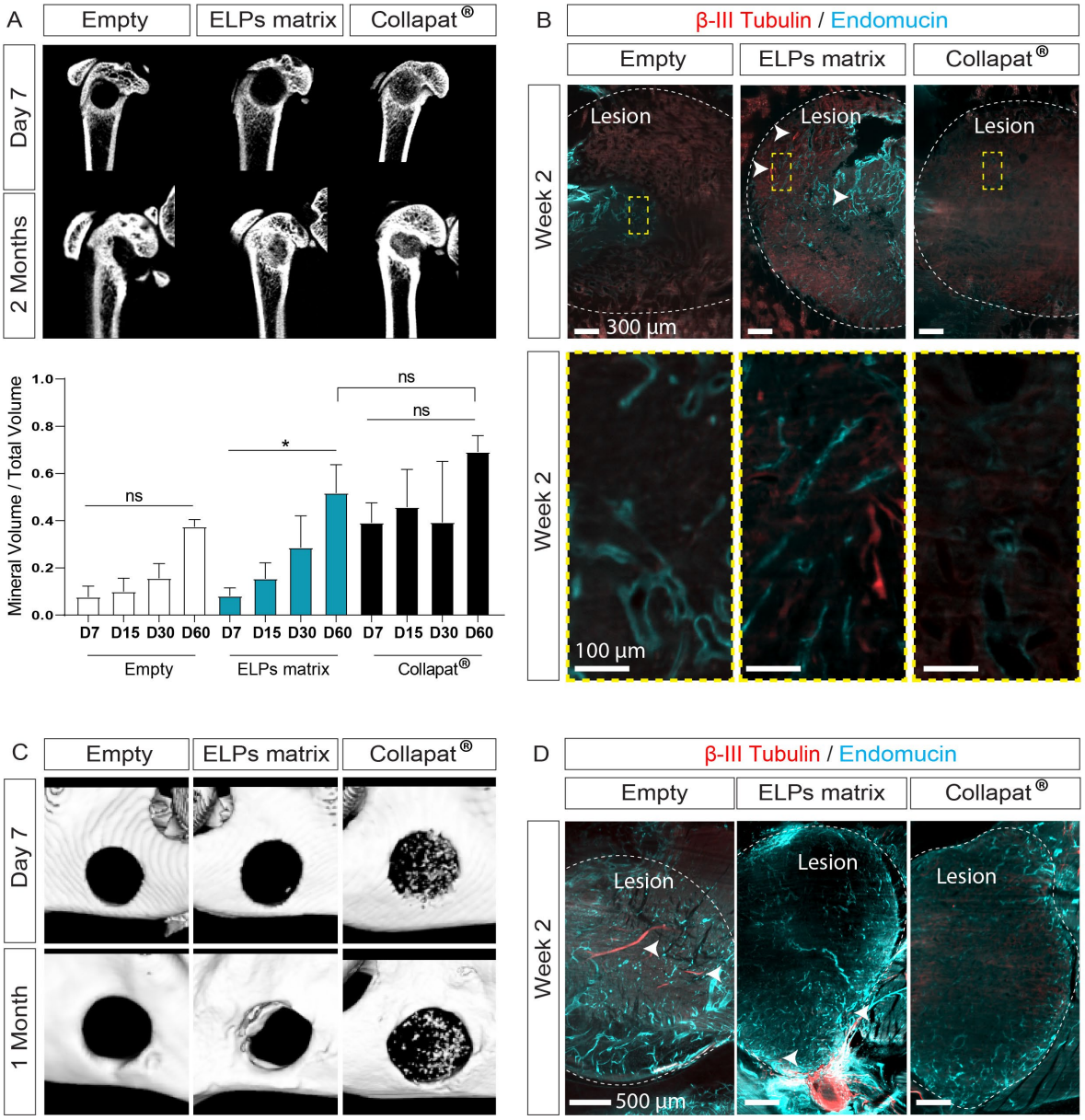
Despite continuous progress in bone tissue engineering, the successful reconstruction of large bone losses remains a major unsolved problem in the clinical sector. This project focuses on developing an innovative biomaterial that allows the recruitment of bone cells, endothelial cells and neuronal fibers within the same matrix, allowing the regeneration of bone tissue. This bioactive hydrogel is based on a matrix of recombinant Elastin-Like Polypeptides (ELPs), chemically modified to allow the graft of laminin-derived peptides intended to stimulate angiogenesis and innervation, combined with calcium phosphate particles to promote bone repair. After its physicochemical characterization, the objective of this work was to evaluate the effectiveness of this functionalized ELPs-based hydrogel *in vivo* in murine preclinical studies.

The functionalized ELPs-based hydrogel was first implanted ectopically in a mice subcutaneous model. Micro-CT analysis confirmed the formation of ectopic mineralized tissue within the implants, which increased over time (Fig.1A). Histological sections of the hydrogel showed cell colonization, formation of osteoid tissue, no significant fibrosis, and normal recruitment of immune cells (Fig.1B). Nerves were always present in the periphery of the material, and vascularization started 1 week after implantation, moreover, 1 month after, the gel was fully vascularized (Fig.1C).



The ELPs-based hydrogel was therefore tested in a femoral condyle defect model in rat. This hydrogel, a negative control (empty defect) and a positive control (Collapat®) were followed by longitudinal micro-CT, showing that the volume of mineralized tissue formed after 2 months was not significantly higher in empty defects nor in Collapat®, but there was a significant increase in hydrogels (Fig.2A). For lesions filled with the hydrogels, a network of blood vessels was formed after 2 weeks, and nerve fibers could be seen inside the lesions (Fig.2B). To evaluate the use of this hydrogel for craniofacial bone

reconstruction, we created a critical mandibular bone defect model in rat. The same conditions were tested. Interestingly, a partial reconstruction was observed in the periphery of the defect after 1 month only for the ELPs-based hydrogel (Fig.2C). Moreover, after 2 weeks the lesions filled with ELP-based hydrogels were vascularized and innervated (Fig.2D).



The cell-free and growth factor-free hydrogel is biocompatible, capable of inducing mineralized tissue, angiogenesis and nerve sprouting. Such a holistic approach represents a technological breakthrough contrasting with current strategies based solely on bone and endothelial cells. These results are very encouraging, and we are currently pursuing further *in vivo* testing in mini-pigs for pre-clinical evaluation.

S9.3-O3

Multi-layered mechanically reinforced collagen-based scaffold promotes osteochondral repair of critical sized medial condyle defects in both young and elderly goats

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Abstract

Articular cartilage in joints has a low regenerative capacity which decreases with age[1]. Our lab has previously developed collagen based matrices designed with a 3D printed polycaprolactone (PCL) framework mimicking the mechanical properties of the native tissue which have shown significant potential for osteochondral tissue regeneration[2,3]. Following their in successful *in vitro* evaluation[3], this study aimed to evaluate the regenerative capacity of these mechanically reinforced multi-layered collagen-based scaffold in osteochondral defects in a well-stablished *in vivo* goat stifle/knee model in both a young and elderly goat population. To this end, the mechanically reinforced multi-layered collagen-based scaffold were prepared according to previously reported methods[2,3]. Subsequently, these scaffolds were implanted in critically sized defects (Ø6mm x 6 mm height) in the goat medial femoral condyle. Animals were divided in two groups, elderly (7-8y) and young skeletally mature adults (2-3y), with 8 and 12 goats respectively. After six months animals were sacrificed and assessed macroscopically, histologically and by µCT to assess integration in the subchondral bone. Overall, the scaffold promoted the regeneration of the osteochondral unit in all animals regardless of their age even at this relatively early time point of only six months post-implantation. Macroscopic and histological evaluation of the articular cartilage at the defect site revealed the formation of new cartilage covering the full defect area in 5 out of 8 animals in the elderly group and 6 out 12 in the adult group (Fig. 1).

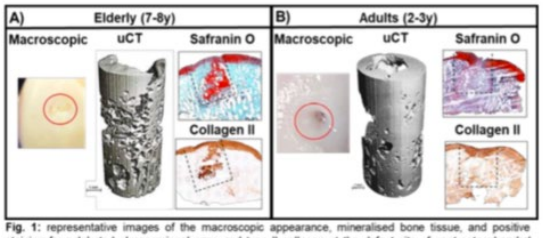

Fig. 1: representative images of the macroscopic appearance, mineralised bone tissue, and positive staining for sulphated glycosaminoglycans and type II collagen at the defect site of goat osteochondral medial femoral condyle defects in the elderly (A) and adults (B) group. Red circle and dotted line squares denote the defect area.

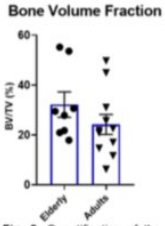

Fig. 2. Quantification of the bone mineral fraction in the defect site after 6 months of implantation. No significant differences were observed.

Interestingly, the scaffold was able to promote the regeneration of the subchondral bone in both groups, with the elderly group having a mean BV/TV of 32.12% and the adults a mean BV/TV of 24.16% (Fig. 2). Taken together, this study has demonstrated the *in vivo* osteochondral regenerative capacity, directed by host cells, of a novel mechanically reinforced tri-layer scaffold implanted cell free in the goat medial femoral condyle after only 6 months of treatment. Interestingly, the regenerative capacity of our reinforced tri-layer scaffold is not age-dependent, thus demonstrating the promise of our scaffold for promoting osteochondral repair in elderly patients with lower intrinsic regenerative potential than young adults.

References. [1]Rando TA; Nature 2006 441:1080-6. [2]Levingstone T.J.; et al, Acta Biomater, 2016 32:149-160. [3]LeMoine M.: et al. ORS 2022 Annual Meeting Paper No. 32.

Acknowledgments. Funding from the European Research Council (ERC), (ReCaP, Grant# 788753) and from Advanced Materials and BioEngineering Research (AMBER) Research Center funded by Science Foundation Ireland, (Grant# SFI/12/RC/2278).

S9.3-O4

Piezoelectric scaffolds enhance the osteogenic differentiation of pre-osteoblastic cells by applying uniaxial compression

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Abstract

Bone is a highly dynamic tissue that undergoes continuous mechanical forces through lifetime. Mechanical stimuli applied on scaffolds resembling a part of the human bone tissue could have major effect on osteogenesis. Poly(3,4-ethylenedioxythiophene) (PEDOT) is a piezoelectric material that responds to mechanical stimulation producing an electrical signal that promotes the osteogenic differentiation of pre-osteoblastic cells by opening voltage-gated calcium channels. The aim of this study was to examine the biological behavior of pre-osteoblastic cells when seeded onto lyophilized piezoelectric PEDOT-containing scaffolds applying uniaxial compression.

Two different concentrations of PEDOT (0.15% w/v and 0.10% w/v) were combined with a 5% w/v poly(vinyl alcohol) (PVA) and 5% w/v gelatin, casted into wells, freeze dried and crosslinked with 2% v/v (3-glycidyloxypropyl)trimethoxysilane and 0.025% w/v glutaraldehyde.

The scaffolds were physicochemically characterized by FTIR, measurement of elastic modulus, swelling ratio, degradation rate. They were subjected to uniaxial compression with a frequency of 1 Hz and a strain of 10% for 1 h every second day for 21 days. The loading parameters were selected to resemble the *in vivo* loading situation. Cell viability and morphology on the PEDOT/PVA/gelatin scaffolds was determined. The alkaline phosphatase (ALP) activity, the collagen and calcium production were assessed to validate the effect of the piezoelectric scaffolds and the uniaxial compression on the osteogenesis.

PEDOT/PVA/gelatin scaffolds presented favorable mechanical properties for bone tissue engineering. Their elastic modulus ranged between 1 and 5 MPa. The degradation rates indicate a mass loss of 15% after 21 days. The cell viability indicates an increase of cell number over time, while SEM images display well-spread cells. The ALP activity at days 3 and 7 is higher in the dynamic culture compared to the static one. The cells clearly evidenced an osteogenic differentiation as confirmed by the high ALP activity levels and the collagen secretion. Moreover, energy dispersive spectroscopy analysis revealed the presence of calcium phosphate in the extracellular matrix after 21 days.

Our results indicate that the PEDOT/PVA/gelatin scaffolds support the adhesion, proliferation, and osteogenic differentiation of the pre-osteoblastic cells under mechanical stimulation, thus favoring bone and other load-bearing tissue engineering with amplified matrix production.

S9.4-K1

Unleashing the versatility of calcium phosphate nanoparticles: extending their impact from bone repair to diverse applications

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Abstract

Calcium phosphate glasses (CPg) have gained extreme importance in the field of bone tissue engineering due to their remarkable ability to stimulate bone formation and angiogenesis. While their osteoinductive properties have been extensively studied, recent research has unveiled their potential in other crucial areas, such as wound healing and antimicrobial effects.

The controlled release of essential ions can accelerate tissue repair and promote a favorable cell proliferation and migration environment. These ions actively participate in signaling pathways, fostering the formation of new blood vessels and facilitating the regeneration of damaged tissues.

As research progresses, the versatility of calcium phosphate glasses in leveraging ions for bone repair, wound healing, and antimicrobial effects continues to unfold. These multifunctional materials promise to revolutionize tissue engineering and regenerative medicine, paving the way for innovative treatments that address diverse medical challenges.

S9.4-O1

Magnesium-based platforms as therapy for cardiovascular diseases: material optimization, biocompatibility, and immune response

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Abstract

Magnesium (Mg) is an interesting material for clinical applications such as non-permanent implants due to its low weight, ductility, and good mechanical properties. Mg is an essential element for the human body, so in principle, it is considered biodegradable, and its degradation products are not harmful or toxic. However, at the same time, Mg is highly chemically reactive. As part of its degradation, hydrogen gas and production of Mg(OH)2 develop at implant sites and may cause unwanted tissue dysfunction such as inflammation or necrosis. Fortunately, the degradation rate can be altered by physicochemical modification of the material to alleviate adverse biological responses. A successful procedure is the plasma electrolytic oxidation (PEO) technique, which generates a surface layer of $MgO/Mg(OH)_2$ in a controlled way. Thus, the degradation rate of the Mg can be carefully tuned and reduced. An additional advantage of PEO is that adequately designed topographical surfaces can be produced that improve the adhesion and function of, e.g., therapeutic stem cells. This work aimed to use PEO to modify the surface of c.p Mg (chemically pure Mg) to improve its degradation, considering using this support that allows the healing of vascular lesions. Then, a complete study was carried out on modified Mg to understand the biological effect of this new material on different cell types and hemocompatibility. Cells related to blood vessels (arteries) were used to study the cellular response to Mg, including endothelial cells, smooth muscle cells, macrophages, and fibroblasts. The technique implemented covered diverse *in vitro* and *ex-vivo* techniques such as cytotoxicity, proliferation, differentiation, cell-material interaction, immunofluorescence staining, molecular test (PCR and western-blot), and flow cytometry (Fig.1). Finally, the immune response to Mg degradation products was studied using TEM and molecular techniques. After analyzing all the results from the degradation of the material and the biological characterization, this work yielded a prototype coating that reduces the degradation rate of the material while improving biocompatibility. We conclude that modification of c.p Mg implants by the PEO technique is promising for cardiovascular devices that support the healing of the vascular lesion.

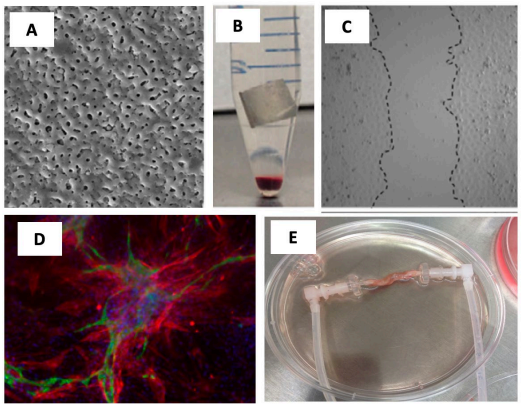

Figure 1. A) Mg modified by PEO B) Hemocompatibility test on Mg samples C) Wound healing process after the exposition of cells with Mg products D) Angiogenesis text in the presence of Mg products E) studies ex vivo in animal arteries.

S9.4-O2

Balancing bone health: regulating osteoclasts function with bioactive glass ions in health and disease

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Abstract

Introduction. Bone health relies on the anabolic and catabolic balance of osteoblasts and osteoclast, which is affected by age, physical activity, medication, and disease. Diabetes disrupts bone homeostasis, increasing fracture risk and prolonging bone fracture repair[1,2]. Hyperglycaemia increases the Reactive Oxygen Species (ROS)[3] and iron bioavailability[3] and decreases Hypoxia Inducible Factor-1α (HIF-1α) stabilisation[4]. Here, we investigate the role of individual ions (*e.g.* Si, Co, P, Ca) and bioactive glass (BG) dissolution products on osteoclasts function in different glucose concentrations. These ions could have different roles in regulating osteoclasts; for example, Co^{2+} has been reported to stabilise HIF-1α[4], whilst Si can decrease ROS[5]. Controlled ion release of Co and Si from BGs, may therefore control osteoclast activity and offer a more personalised approach to regulating bone health.

Methods. Osteoclasts were derived from primary macrophages (6-10-week-old MF1-mice) and an osteoclastic subclone of RAW264.7 (with high TRAP-5b activity) and cultured in MEM (+10%FBS, 1%P/S, 0.25µg/ml amphotericin B, 2mM L-glutamine, and 200ng/ml M-CSF) and DMEM (+10%FBS and 1%P/S), respectively. Cells were seeded on dentine slices at $6x10^5$ and $1.5x10^4$ cells/cm^2 (3ng/mL RANKL) and allowed to attach for 48 hours. Na_2SiO_3 (0.4-2mM) or $CoCl_2$ (12.5-50µM) treatments were applied for 7 days, and resorption was assessed via TRAP-5b activity/staining, Scanning Electron Microscopy (SEM,Jeol-7401F) and quantitative surface measurements (Nexview™-NX2-3D Optical-Interferometer, Zygo) after lowering pH to 7 or 6.8 on day 5.

Results and discussion. Si (2mM) inhibited osteoclastogenesis in low glucose (Fig.1 a,b), while Co (12.5-50µM) restored hyperglycemia-inhibited osteoclasts to low glucose levels (Fig.1c). Other preliminary results (not shown) suggest Ca upregulates osteoclasts in hyperglycemia. The difference between ions and species released from Si and P-based BGs will be discussed.

Conclusion. Co and Si ions modulate osteoclast activity (Si inhibits osteoclast formation, while Co restores hyperglycaemia-impaired osteoclast function). Understanding the associated mechanisms can aid in designing the next generation of bioactive glass for restoring bone health in specific disease populations.

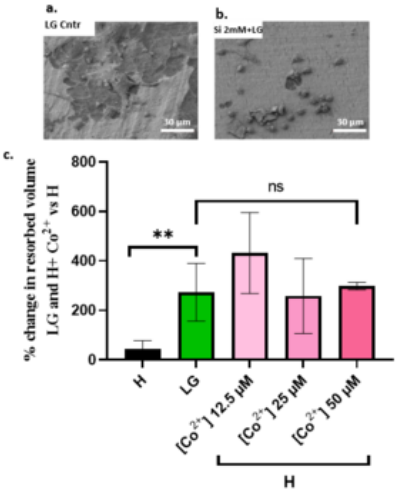

*Figure 1. Si and Co regulation of osteoclasts resorption. (a) SEM images of primary osteoclast and resorption pit in Low Glucose (LG) control, (b) LG+2mMSi, (c) Volume resorbed by subclone-differentiated osteoclasts in LG control, High Glucose (H) control and H+Co^{2+}. [LG]= 5.5mM, [H]= 25mM, values represent mean±SD, n>5, **p<0.01.*

References. [1]Valderrábano R. *et al.*, Clin.Diabetes.Endocrinol., 25:4-9,2018; [2]Loder RT.*et al.* ,Clin.Orthop.Relat.Res., 232:210-216,1988; [3]Li S.*et al.*, Free.Radic.Biol.Med.,162:435-449,2021; [4]Rezaei A.*et al.*, Sci.Rep.,12:13944, 2022; [5]Monte F.*et al.* , Tissue.Eng.Regen.Med., 12(11):2203-2220,2018.

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S9.4-O3

Gallium-containing titanium implant to improve osteointegration in osteoporosis and bone metastasis

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Abstract

Introduction. Titanium (Ti) implants are the most used biomaterials to repair damaged bone. However, diseases such as osteoporosis or bone metastasis can continue to promote bone resorption even when the implant is inserted. Therefore, the risk of fractures is still high and traditional treatments, such as bisphosphonates or denosumab, may not be enough. In this context, a combination of drugs with an implant capable of reducing the abnormal bone resorption in the injured bone would be the ideal approach. In this study, we propose the modification of the titanium surface with Gallium (Ga). In fact, Ga-containing drugs have already been used to treat bone resorption in diseases such as osteoporosis, bone metastasis, and Paget's disease, since it is expected to reduce osteoclast formation by leading to iron-dependent cell death[1].

Experimental methods. Firstly, different concentrations of Ga-doped samples were characterized by RAMAN spectroscopy, and the release of Ga^{3+} was assessed. Secondly, the suitability of the Ga^{3+} was proved by inducing RAW264.7 to be differentiated into osteoclast in a multinuclearity study. To further investigate the involvement of Ga in osteoclastogenesis, ROS specimens were measured and TRAP activity was evaluated. Finally, the behavior of human mesenchymal stem cells (hMSC) was studied in terms of cell adhesion and differentiation.

Results and discussion. The results prove that the Ga-containing calcium titanate layer, formed as a consequence of the treatment, inhibited the formation of the multinucleated cells and the apparition of TRAP-positive cells in a dose-dependent manner (Fig. 1). Even though Ga slightly downregulated the expression of transcription factors involved in osteoclastogenesis, achieved to downregulate by 80% the expression of matrix metalloproteinase 9 (MMP9) which is produced by the mature osteoclast (Fig. 2). Our results suggested that Ga^{3+} may alter the iron metabolism when the energy-demand is enhanced and inhibit the formation of the mature osteoclast by inducing ferroptosis. Furthermore, Ga did not alter the behavior of hMSC in addition to enhancing the differentiation into osteoblast.

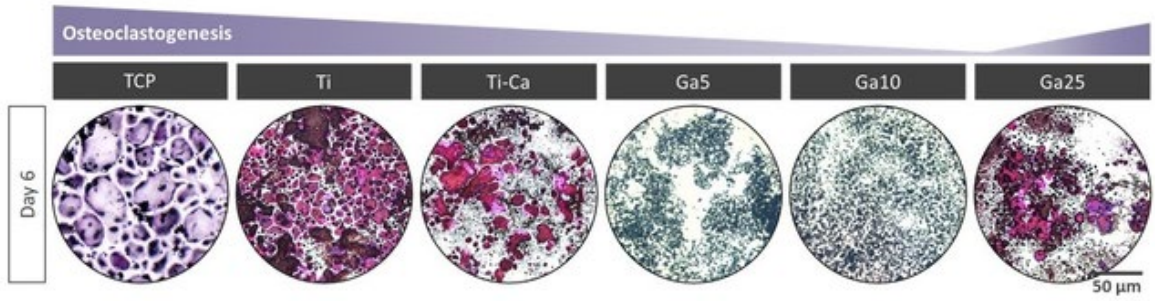

Figure 1. Representative images of RANKL-treated RAW264.7 stained with TRAP

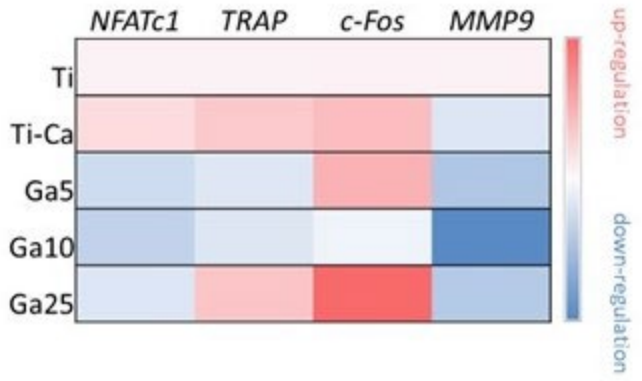

Figure 2. Heat-map of osteoclastic genes expression

Conclusion. This work proved the hypothesis that Ga^{3+} inhibits osteoclastogenesis by inducing ferroptosis. Moreover, Ga-doped titanium displayed osteoinductive properties. Therefore, Ga-containing titanium implants might be advantageous for patients whose fractures have been produced by an excessive bone resorption disease.

References. 1. Z. Gao *et al.* Experimental Gerontology 165 (2022) 111836.

S9.5-K1

Advances in 3D cell culture models for bridging the *in vitro* to *in vivo* gap

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Abstract

Even in research, change is hard. Moving *in vitro* research from 2D to 3D cell culture models is not a simple task, yet, change is needed to be able to predict the *in vivo* behavior of cells. Despite its substantial weakness in completely replicating the physiological environment of cells, *in vitro* research is still the preferred choice due to its advantages over *in vivo* research, and these include the compliance with the principles of the 3Rs (Replace, Reduce and Refine), the tight control of the chemical and physical environments, the reduced cost, and the higher throughput. Transitioning to 3D cell culture models is obligatory not only for drug discovery and development as it extends to all fields of research including regenerative medicine. On the grounds of this, there is a fundamental need for developing and utilizing robust, rapid, reliable, and reproducible research methods implementing 3D cell culture models and providing an output that is predictive of the results that are normally obtained through *in vivo* research. Setting 2D cell culture models side by side with 3D cell culture models reveals that there is a poor correlation or even an absence of correlation between the two. The latter can be explained by the key differences that include cell morphology, cell proliferation, cell migration, cell-cell interactions, cell-extracellular matrix (ECM) interactions, cell differentiation, vasculature, gene expression, protein expression, and mechanical stimulation. All of the aforementioned factors provide a solid foundation for the use of 3D cell culture models rather than 2D ones, even if they are challenging in terms of experimentation, characterization, analysis, and cost. As a result, 3D cell culture models can overcome the inadequacies of the widely used 2D cell culture models, and can be used to develop validated pre-clinical pipelines capable of translating data from *in vitro* to *in vivo* and from preclinical to the clinic. Although having 'gold-standard' 3D cell culture models for regenerative medicine sounds really far-fetched, 3D cell culture models represent a satisfactory compromise between the conventional 2D cell culture models and the extensive use of animals in research.

S9.5-O1

It takes TWO to tango: Roadmap from concept to *in vivo* validation of a brain and tumor DUALLY-targeted nanomedicine for glioblastoma treatment

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Abstract

Minimal therapeutic advances have been achieved over the past two decades for glioblastoma (GBM), which remains an **unmet clinical need**. Here, **stimuli-responsive nanoparticles (NPs)** for anti-cancer **drug delivery to GBM** are reported, with multifunctional features that circumvent insufficient **blood-brain barrier (BBB) trafficking** and lack of **GBM targeting** – two major hurdles for anti-GBM therapies. NPs are dual-surface tailored with a (i) brain-targeted acid-responsive Angiopep-2 moiety that triggers NP structural rearrangement within BBB endosomal vesicles (acidic pH) [1], and (ii) L-Histidine moiety that provides NP preferential accumulation into GBM cells [2] post-BBB crossing. **Docetaxel (DTX)** was loaded into the stimuli-responsive NPs as a model drug (0.04 µM IC50 versus 800 µM for standard temozolomide chemotherapy [3]).

The polymeric matrix of the stimuli-responsive NPs was modified with Angiopep-2 and L-Histidine by carbodiimide, selective carbamate hydrolysis and thiol-maleimide chemical strategies, followed by characterization by NMR, FTIR and MALDI-TOF mass spectrometry. The up-scale manufacture of the NPs was achieved through in-house optimization of a microfluidic technique. Cell studies were performed in GBM invasive margin (GIN) patient primary cells isolated from hospital tumor tissue. Preliminary studies of BBB translocation were conducted in fluid-dynamic hCMEC/D3 Transwell® *in vitro* systems. For *in vivo* studies (biodistribution; efficacy after intratumoral and intravenous administration; safety), an U-87 MG orthotopic mice model of GBM was used, where tumor development was monitored by MRI.

In a panel of GIN patient cells, assembled into 2D and 3D *in vitro* cell models, the stimuli-responsive multifunctional NPs targeted GBM cells, enhanced cell uptake by up to 12-fold, and induced up to 3-times higher cytotoxicity. Moreover, the *in vitro* BBB permeability was increased by 3-fold. A biodistribution trial confirmed a 3-fold enhancement of NP accumulation into the brain in a mice model with an intact BBB. Lastly, the *in vivo* anti-tumor efficacy was validated in GBM orthotopic models following either intratumoral or intravenous administration. Median survival and number of long-term survivors were increased by up to 50%. Histological, hematologic or blood biochemical systemic toxicity was not detected.

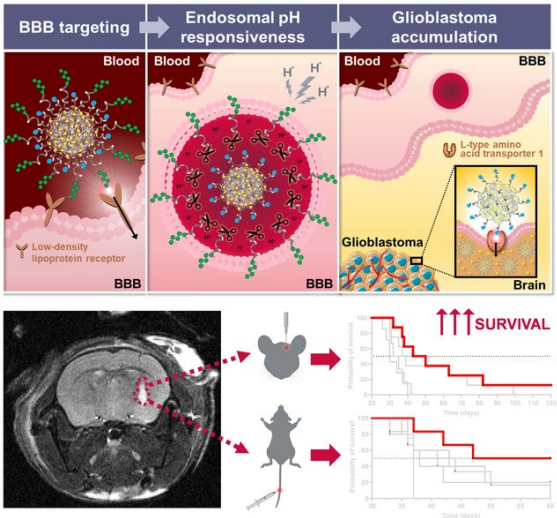

Figure 1 – Rationale and enhancement of GBM mice median survival after treatment.

Altogether, a preclinical proof of concept was provided to support these stimuli-responsive NPs as an effective anti-GBM multistage chemotherapeutic strategy, with ability to respond to multiple fronts of the GBM microenvironment.

References: [1] Clark et al., Proc Natl Acad Sci U S A. 2015,112,12486; [2] Martins et al., J Control Release, 2023,353,77; [3] Yang et al., Nucleic Acids Res,41,D955

S9.5-O2

Hydrogel-based tumour microenvironments as models of vascularized Glioblastoma for validation of nanomedicines

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Abstract

The tumour microenvironment (TME) is the main obstacle limiting the efficacy of treatments against aggressive diseases such as glioblastoma (GBM). The high histological complexity, the infiltration by tumour-supporting cells, and the presence of biological barriers such as the blood-brain barrier (BBB) and the rigid extracellular matrix (ECM) hinder the accumulation of molecules and transporters. Reliable *in vitro* systems that recapitulate the complexity of GBM TME are needed to support the design of innovative drugs and carriers able to overcome these barriers.

This work aims to develop a reliable three-dimensional GBM model to investigate the transport of polymer nanoparticles (NPs)-based drug platforms. The model combines different cell actors involved in human GBM, ECM-like biomaterials, and a microfluidic device to reproduce vascularization, to reliably mimics GBM structure and composition.

GBM spheroids were prepared in low adhesion conditions using a combination of GBM cells and GBM-associated Stem Cells, coupled with other cells of the TME, such as microglia and astrocytes, to replicate tumour histology (Fig. 1A). The spheroids were encapsulated in natural (collagen-based) or synthetic (polysaccharide-based) polymer gels with mechanical properties resembling the GBM ECM.

A vascular network was obtained by inserting the GBM spheroids in a commercial microfluidic platform, containing two lateral perfusion channels coated with human brain endothelial cells, from which angiogenic sprouting was induced to vascularize the spheroid (Fig. 1B). Immunostaining confirmed the homogeneous presence of endothelial cells forming tight junctions (Fig. 1C). Moreover, the microvasculature was able to replicate the barrier effect against NPs, resembling our *in vivo* observations. The model was used to verify the infiltration capacity and viability following treatment with polyurethane NPS loaded with a proteasome inhibitor (Bortezomib, BTZ). The results confirm that the drug can reduce tumour proliferation and infiltration in ECM-like gels, with the effect depending on the cellular composition. NPs-mediated treatment had lower efficacy than the free drug and was able to reduce cytotoxicity on non-tumour cells of TME.

This model represents a promising step in developing a reliable replica of human GBM TME by combining biomaterials and microfluidics. The device could be a valuable tool for the preliminary validation of drugs, nanomedicines, and alternative transporters (e.g., cell-mediated drug delivery).

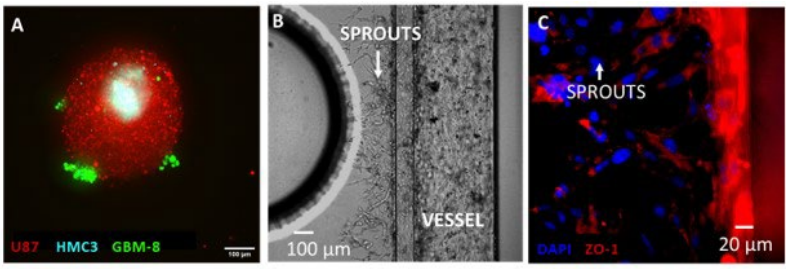

Figure 1 A) Distribution of GBM cells (U87), Cancer Stem Cells (GBM-8) and microglia (HMC3) within the spheroids. B) Brain endothelial vessel sprouting in the microfluidic platform through collagen-based hydrogels. C) Thight junction marker distribution in the artificial vessel.

S9.5-O3

Soft microfluidics: enzymatic-crosslinked silk fibroin hydrogel lab-on-a-chip for colorectal tumor model

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Abstract

Integrating biological material within soft microfluidic systems made of hydrogels offers countless possibilities in biomedical research [1]. To overcome the limitations of traditional microfluidics (PDMS), based on solid, non-biodegradable/biocompatible and non-tunable materials, hydrogel-based microfluidics have been used, with potential to transform *in vitro* cell/tissue culture and modeling [2]. Herein, we describe a innovative methodological approach for developing proprietary soft cell-laden microfluidic device based on enzymatically-crosslinked silk fibroin (eSF) hydrogel [1]. It's unique properties made possible to mimic in a simplistic way the native dynamic 3D microenvironment of colorectal cancer (CRC) and its response to chemotherapeutics. Results show that 14 wt% eSF microfluidic platform has the ability to perfuse fluid while displaying *in vivo*-like biological responses. Tested 14% eSF hydrogels offer stability, flexibility and transparency. SEM images showed the eSF hydrogel maintained the microfluidic features with excellent fidelity after the entire fabrication process. We next investigated the deformation capacity of the soft eSF microfluidic chip. A mean tensile strain of 103.96% was found in 14% eSF. Alamar blue and ATP quantification of cells without any drug perfusion showed that HCT-116 cells are viable overall, and the endothelial cells did form a lumen-like structure. Overall, this work shows that combining of eSF hydrogels and microfluidics can be employed for developing novel soft lab-on-a-chip platforms with superior performance.

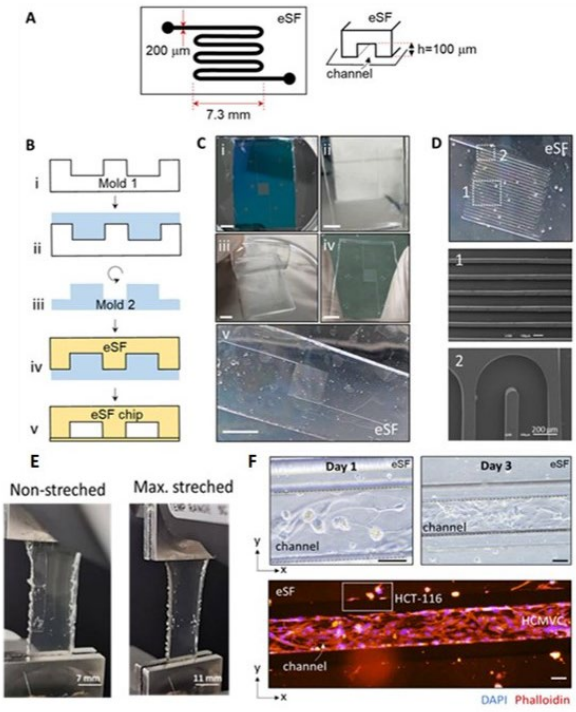

Figure 1 – A) illustration of the eSF microfluidic device. B-C) Scheme and corresponding photographs showing the main steps for fabricating the chip by photolithography and soft lithography. i: SU8 mold 1; ii: PDMS replica; iii-iv: PDMS mold 2; v: eSF chip. D) Photograph of the eSF chip and the corresponding SEM images of two different areas. E) Photograph showcasing the high degree of deformation of the eSF hydrogel. F) Brightfield images of HCoMECs on Day 1/ 3 and Fluorescence microscopy images of HCoMECs after 5 days of culture (DAPI, blue, nuclei; phalloidin, F-actin, red).

Acknowledgments. M.R.C. acknowledges her post-doctoral contract TERM RES Hub – Scientific infrastructure for Tissue Engineering and Regenerative Medicine Norte-01-0145-FEDER-02219015 and Portuguese Foundation for Science and Technology (FCT) for the financial support under the CEEC Individual 2021.00049.CEECIND. This work was partially supported by the IET A. F. Harvey Engineering Research Award 2018 (ENG ThE CANCER)

1. PATENT. M. R. Carvalho, D.C., C. R. Carvalho, J. B. Costa, V. Ribeiro, J. M. Oliveira , Kundu S. C., R. L.. 2019. 2. Shen, C., et al,.Lab on a Chip, 2019. 19(23): p. 3962-3973.

S9.5-O4

Combining tunable biomaterials and flow-based membrane technologies for improved biomanufacturing of T-cell therapies

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Abstract

The manufacturing of adoptive cell therapies, such as the chimeric antigen receptor T-cell (CART), includes isolation, activation, genetic modification, and expansion of a patient's T-cells. These processing steps remain mainly lab-scale procedures, which are time and labor-intensive affording limited opportunities for scale up increasing the cost of treatment. There is an unmet need to develop modular cell therapy production platforms that can recreate elements of the *in vivo* microenvironment to effectively engineer patients' cells with increased efficacy and reduced cost.

Here, we present a modular manufacturing platform that combines soft hydrogels with existing commercial flow-based membrane devices for improved activation, expansion, and transduction of primary human T-cells. Thin poly(ethylene glycol) diacrylate (PEGDA) hydrogel films were formed on microfiltration membranes to prepare hydrogel coated membranes (HCM) with bioinspired mechanical properties and cell-activating anti-CD3 and anti-CD28 through biotin-avidin chemistry. T-cell activation and proliferation in response to HCM in comparison to state-of-the-art controls were extensively characterized by analyzing the changes in expression of T-cell markers. We further introduced HCMs into a tangential flow filtration (TFF) device to efficiently transduce the T-cells with a model lentiviral system under flow. Lastly, we investigated the applicability of the device for selection of specific cell populations by functionalizing the HCM with anti-CD8, allowing for the immobilization of CD8 T-cells on the membrane to yield a CD4 enriched T-cell population.

Culture on antibody-functionalized HCM yielded highly activated and proliferative primary human T-cells that presented a memory phenotype ideal for T-cell therapy applications, especially in comparison to rigid controls. In parallel, T-cell transduction with the HCM under flow in the TFF flow device resulted in a multi-fold increase in transduction efficiency compared to static controls. Finally, the HCM successfully enriched the CD8 target cell population in the TFF device following selection experiments. Together, these results demonstrate that HCM and TFF technologies combine as a modular platform to improve T-cell activation, increase proliferation, enhance transduction, and enrich desired cell populations compared to current industrial biomanufacturing processes. Integrating tunable biomaterials with flow technology presents the potential for improved biomanufacturing of T-cell therapies with relevance to current industrial processes.

S9.6-O1

Physico-chemical and biological characterization of polyelectrolyte coatings with immunoregulatory properties derived from a Bifidobacterium longum exopolysaccharide

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Abstract

Statement of purpose. Interaction of implanted biomaterials with the host is highly influenced by the inflammatory response to the surface. Incorporation of immunomodulatory compounds within the biomaterial offer an attractive approach to control this interaction. In this work we use an exopolysaccharide (EPS) from Bifidobacterium longum (EPS624) which was previously shown to induce immunoregulatory responses in several animal models of allergy. It was also shown to increase interleukin-10 (IL-10) secretion through TLR-2 signaling in human immune cells and inhibit osteoclast differentiation in normal and inflammatory conditions. Here, we incorporate the EPS624 as a structural and biologically active component within polyelectrolyte coatings and characterize their physicochemical properties and immunomodulatory effect *in vitro*.

Methods. EPS624 was produced through the collection, resuspension and centrifugation of bacterial pellets, the supernatant was then filtered and precipitated by ethanol, and the precipitate was dialyzed through a 12-14kDa cellulose membrane, purified through reverse phase column chromatography and finally freeze dried. Polyelectrolyte coatings were produced as seen in Figure 1, using the negatively charged EPS624 (or alginate as control), and a positively charged chitosan. The polyelectrolyte dipping process was repeated as needed to create 1, 5 or 10 double layers.

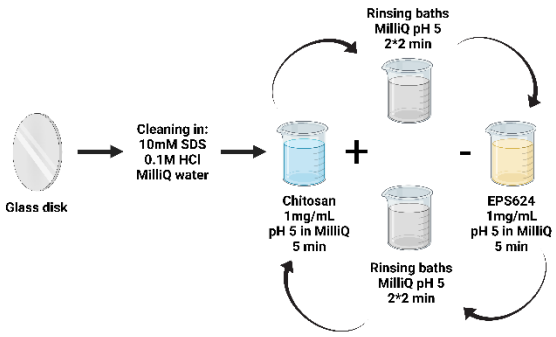

Figure 1: Polyelectrolyte coating of EPS624 and chitosan on glass disks.

EPS624 physico-chemical characterization was performed through dynamic light scattering, zeta potential measurements at 1mg/mL at varying pH values, and size exclusion chromatography (SEC). Coatings were characterized by Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM). Human Peripheral Blood Mononuclear Cells (PBMCs) were exposed to coated surfaces for 24h, as well as EPS624 solutions. Immunoregulatory activity upon exposure to EPS624 was quantified through secreted IL-10 measured by ELISA. Coating cytotoxicity on human fibroblasts was measured by cell titer blue up to 10 days.

Results. An exponential thickness growth model, a linear rugosity growth model as well as evolving topography of the coatings were revealed by AFM characterization. SEC showed 2 sub-components to the EPS624 and a Mw of 639.9kg/mol. Figure 2 shows an increased IL-10 secretion from 1 to 5 layers

plateauing for 10 layers and the specificity of EPS624 action, as replacing EPS624 with Alginate (10A) caused minimal IL-10 induction.

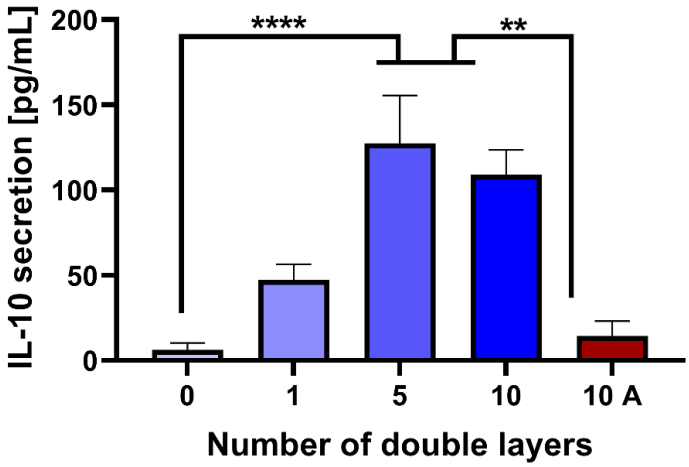

Figure 2: IL-10 induction by polyelectrolyte coatings, as indicator of immunoregulatory activity in hPBMCs.

In addition, the polyelectrolyte coatings supported fibroblasts attachment and proliferation after 10 days. In summary, EPS624/chitosan polyelectrolyte coatings with evolving topographies showed promising *in vitro* immunomodulatory properties and biocompatibility.

S9.6-O2

Evaluating the role of protein coatings in modulating neutrophil activation on 3D printed PCL scaffolds

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Abstract

Introduction. Neutrophils are one of the key players of the immune system that act as a first line of defense at the sites of inflammation. They have a significant impact on the innate immune response when biomaterials are implanted, and their activation can be influenced by the physical and chemical characteristics of the biomaterials due to their sensitivity to environmental changes[1,2]. Therefore, it is important to study and understand the interactions between neutrophils and biomaterials to establish design principles for biomaterials that can promote tissue repair and regeneration by stimulating the immune response. This study aims to determine the effects of protein coating on the neutrophil activation and response.

Experimental Methods. The Polycaprolactone (PCL, MW:45000Da) scaffolds were fabricated by an extrusion-based bioprinter (3D Discovery;RegenHU) and later coated with solutions of 1 and 10 % (v/v) FBS, and collagen I. Human primary neutrophils, isolated from peripheral blood, were seeded on biomaterials and tissue culture plastic (TCP) (3x10^{5}cells/well), then the metabolic activity and percent cytotoxicity were determined (1, 3, 5, 7 and 24 hours) via CellTiter-Blue® (CTB, Promega) and LDH (Roche, Sigma-Aldrich) assays, respectively. A panel of proteins involved in inflammation was measured from the cell culture supernatants using the Olink® immunoassay (OlinkProteomics, Sweden). PMA was used as an inflammatory control.

Results and Discussion. CTB showed that neutrophils were metabolically active throughout 24h. The metabolic activity was lower for the groups containing FBS compared to the FBS-free ones at the initial hours (Figure1A). Similarly, FBS-free groups were less cytotoxic to neutrophils than FBS containing groups from 1st to 7th hour. The presence of FBS resulted in an increased production of inflammatory proteins in the neutrophils compared to the groups without FBS (Figure2). Although protein coating is always present *in vivo*, understanding the factors triggering neutrophil activation *in vitro* is key to dissect complexity and help understanding how these cells interact with biomaterials.

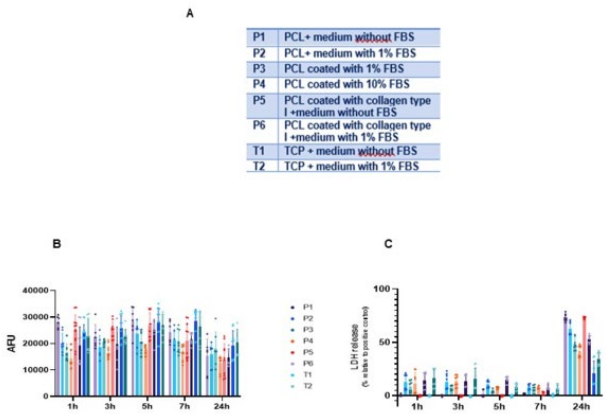

Figure 1. A) Experimental groups used in the study, B) Metabolic activity of neutrophils represented as arbitrary fluorescence units over time, C) LDH release by neutrophils cultured on different materials.

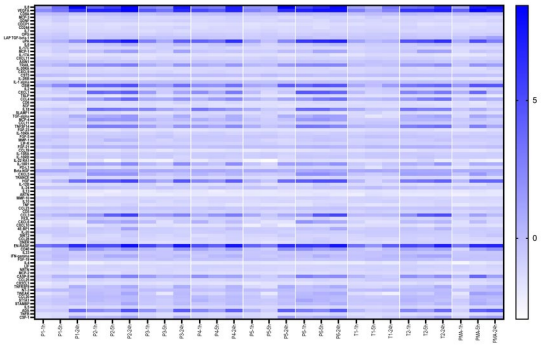

Figure 2: The heatmap analysis of inflammatory proteins via Olink technology.

Conclusion. Evaluating the activation and immune response of neutrophils to various proteins and materials is crucial in establishing guidelines for designing constructs in the field of tissue engineering.

References. 1.Kruger, P. et al. PLoS pathog.11(3):e1004651, 2015. 2.Abaracia, JO. et al., Acta Biomater. 1(133):58-73, 2021

S9.6-O3

Pro-inflammatory characterization of biomaterials using genetically modified THP-1 cells

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Abstract

Investigating biological response to biomaterials is important to understand biocompatibility and cell-material interaction for medical application. Macrophage is one of important cells for early biological response. Previously, macrophage response to material have been investigated through the production of inflammatory and anti-inflammatory cytokines by ELISA and RT-PCR assays, phenotype change by flow cytometry and immune chemical histology. In this presentation, we developed a pro-inflammatory characterization method of biomaterials using the THP-1 cell, whici is a macrophage cell line, tagged with luminescent peptide (HiBiT) genetically. The luminescent gene was inserted into IL-1β gene of THP-1 cell using CRISPR/Cas9 system. When the obtained HiBiT-tagged THP-1 cell was stimulated by LPS, the secretion of IL-1β was detected by luminescent measurement at high-sensitively as well as ELISA and RT-PCR assays. Using the HiBiT-tagged THP-1 cell, it was founded that the production of IL-1β differed between the used materials, nylon, cellulose, PTFE, and so on. Also, the different time course of the IL-1β secretion was observed among them. These results indicated the IL-1β production from the HiBiT-tagged THP-1 cell on the same sample could be measured over time. We believe that this pro-inflammatory characterization method using the genetically engineered macrophage is useful as a complement method of ELISA and RT-PCR assays.

S9.6-O4

Novel immune-instructive materials for tissue regeneration and wound healing

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Abstract

The immune system plays a key role in response to pathogens and foreign materials as well as orchestrating tissue homeostasis following different types of injury. In addition to biochemical signals, immune cells are also highly responsive to physico-chemical cues in their microenvironment. This provides opportunities for modulating immune cells' function through developing novel materials that are embellished with immune-instructive niches.

Wound healing is a complex biological process that is driven by close crosstalk between immune and stromal cells. A close collaboration between macrophages and fibroblasts is known to be crucial for a successful wound healing, by driving regulated transition from acute inflammation to resolution of inflammation, tissue remodelling and healing. Dysregulation in any of these processes, such as in diabetic wounds, results in chronic inflammation and creation of non-healing wounds.

Using a high-throughput screening strategy we screened a library of more than 300 acrylates and methacrylates to identify homo and/or co-polymers that can modify key functional properties of human macrophages and fibroblasts using a mix of cellular and molecular readouts. We further used some of these polymers to develop functional coatings and microparticles which were tested for their bio-instructive properties *in vitro* and *in vivo*.

A selection of polymers were shown to support pro or anti-inflammatory phenotypes in macrophages, and modulate attachment, proliferation and differentiation of human fibroblasts *in vitro*. The ability of these polymers to modulate foreign body response (FBR) against subcutaneous implants, or their impact on chronic wound healing were investigated in relevant *in vivo* models. These experiments revealed that through modulating macrophage and fibroblast functional phenotypes these polymers can significantly suppress FBR and/or promote tissue regeneration and wound healing in non-healing wounds without the need to use any additional exogenous mediators.

The ability to control biomaterials surface attributes provides a powerful tool for modulating the phenotype and function of immune and stromal cells with the aim of reducing detrimental pro-inflammatory responses and promoting beneficial pro-healing responses. We propose that it is possible to use immune-instructive materials to harness the immune system to regulate inflammation and promote pro-healing responses with potential applications in a variety of other inflammatory conditions.

References: 1- Immune-instructive materials as new tools for immunotherapy. Fisher et al. *Curr Opin Biotechnol*. 2022 Apr;74:194-203. doi: 10.1016/j.copbio.2021.11.005. 2- Microparticles Decorated with Cell-Instructive Surface Chemistries Actively Promote Wound Healing. Latif et al. *Adv Mater*. 2023 (online ahead of print) doi: 10.1002/adma.202208364. 2022 Nov 28;e2208364. doi: 10.1002/adma.202208364.

S9.6-O5

The immune response to a 4D-bioprinted cartilage construct carrying an immunomodulatory nanosystem

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Abstract

Injury to articular cartilage is recognized as a cause of significant joint morbidity. Chondral lesions trigger post-traumatic inflammation that when left untreated may develop into osteoarthritis, due to cartilage's inability to overcome the inflammatory response. In tissue engineering, some approaches have been explored to achieve bioprinted cartilage microtissue including different cell sources and biomaterials, however without reconstituting the intrinsic cellular morphologies faithfully, the complex structure of the articular extracellular matrix (ECM), and the functions of articular cartilage.

In this work, we fabricated nanoenabled human cartilage microtissue by bioprinting patients derived human chondrocytes with a hydrogel-containing immunomodulatory system to mitigate local inflammation after implantation in arthritic joint. The capacity of generated nanoenabled microtissues to modulate inflammation was tested *in vivo* using the air pouch mice model, commonly used to study acute and chronic inflammatory responses to biomaterials, and potential therapeutic targets for treating inflammation. Following bioprinting, the human cartilage microtissues (nanoenabled and non-nanoenabled (control)) were maintained in culture till maturation, verified by chondrogenic phenotype and ECM characterization. The secretomes of mature microtissues were then injected in mice air pouches and the inflammatory exudates were analysed after 72h. The immune response was assessed by analysing recruited immune cells (namely natural killer cells, B cells, T cells, neutrophils, macrophages, and dendritic presenting cells) to the air pouch cavity. In addition, the profile of pro and anti-inflammatory cytokines, chemokines, and inflammatory mediators was also assessed by Proteome Profiler Array. The results showed that the secretome of nanoenabled bioprinted human cartilage microtissue displayed a significant decrease, when compared to control, in dendritic cells recruitment which are responsible for presenting antigens to T cells to initiate and regulate the adaptive immune response. Moreover, the levels of pro and anti-inflammatory cytokines (IL1β, TNFα, IL28, IL4, IL5, IL33, and IL27), chemokines (CCL20, CX3CL1, CXCL1, CXCL13, and CXCL16), and inflammatory mediators (B-cell activating factor, Complement Component C5/C5a, Complement Factor D, and C-Reactive Protein) was also reduced compared to the control. These findings suggest that the developed nanoenabled bioprinted human cartilage microtissue carries the immunomodulatory capacity to mitigate local pro-inflammatory responses.

Acknowledgments. This study was supported by European Union's Horizon 2020 research and innovation program under grant agreement number 814558 project RESTORE. The authors acknowledge the Portuguese Foundation for Science and Technology for financially supporting MC with a Ph.D. fellowship (2020.05177.BD), and the support of i3S Scientific Platforms Translational Cytometry, Histology and Electron Microscopy, and Biointerfaces and Nanotechnology.

S9.7-O1

Isolation and characterizations of multidrug-resistant human cancer cells by a biodegradable nano-probe

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Abstract

Multidrug resistance (MDR) remains a significant challenge in cancer therapy, posing a risk of cancer recurrence and metastasis. Tumor drug resistance could be further divided into inherent and acquired drug resistance. In order to comprehend the mechanism behind MDR and evaluate the effectiveness of new treatments for MDR tumors, various MDR cell lines have been established based on a chemo-drug dose selection process. However, these drug-induced MDR cell lines are only able to imitate acquired MDR and may have additional gene mutations that differ from inherent MDR. Up to now, it is still challenging to obtain a cell model with inherent drug resistance. Therefore, we proposed a molecular-beacon-based strategy, DNCP nano-probes, to characterize and isolate the inherent MDR cells without the need of drug selection. It is considered that most tumors are heterogeneous. Some tumor cells express more MDR-related genes and others express less. We hypothesized that our DNCP nano-probes can be utilized as an effective tool to isolate the sub-populations among MCF-7 cells based on their MDR gene expression level. We expected that two groups of MCF-7 cells could have different MDR1 gene expression levels and distinct phenotypes.

DNCP nano-probes were 130 nm in diameter and -6 mV surface potential nanoparticles. After delivering DNCP to the MCF-7/ADR multidrug resistance cell line, good cell viability and high cellular uptake efficiency were confirmed. To verify that DNCP had the ability to isolate MDR1-expressing MCF-7, we delivered DNCP to both MCF-7/ADR and MCF-7 cells. Results showed that DNCP was able to accurately differentiate MDR1 mRNA expression levels between the two cell lines. Subsequently, we used DNCP to detect MCF-7 cells, and then utilized FACS to separate the high fluorescence (Top 10) and low fluorescence (Last 10) groups. qPCR data showed that MDR1 mRNA expression in Top 10 cells was 1.7 times higher than that in Last 10 cells.

We have successfully used DNCP to isolate MCF-7 cells with varying levels of MDR1 expression. This also indicated the heterogeneity of the MCF-7 cell line, as previously hypothesized. Interestingly, we assessed several MDR-related cell phenotypes, finding that cells with higher resistance migrated more quickly and formed tighter spheroids. In summary, we have developed a novel strategy for isolating inherent drug-resistant cells without the need for drug selection. Furthermore, our findings demonstrate that cell phenotype can vary significantly based on the level of MDR gene expression.

S9.7-O2

Transdermal delivery of PD-1 blocking T cells for melanoma treatment by porous microneedles

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Abstract

Immune cell therapy is a treatment method in the middle and late stages of cancer therapy. This means that the immune system plays a crucial role in limiting the development of cancer. Cell therapy is a minimally invasive delivery of autologous cells into bodies and aims to prove the potential for cellular grafting in cancer. As we know, blocking the programmed death-1 (PD-1) pathway on T cells has shown excellent tumor suppressor efficiency for melanoma. However, how to efficiently and painlessly deliver the T cells into skin is urgent demand so far. Herein, we design the porous microneedles (PMNs) with PD-1 blocking T cells encapsulation (TPMNs) allows penetration-mediated transdermal seeding of PD-1 blocking T cells when applied on the melanoma tumor bed to augment T cell infiltration and promote the activity of attacking melanoma cells. The PMNs formed by encapsulating calcium peroxide (CaO_2) microparticles in copolymer (PMVE-co-MA/PEG/PVA)-based MNs. The encapsulated CaO_2 microparticles are then etched with HCl, leaving pores for further PD-1 blocking T cells loading. Our results demonstrated that the cell viability can be maintained above 80% for 24 h after TPMNs production. Furthermore, melanoma cells treated with the TPMNs showed elevated inhibition efficiency of cell proliferation and increased cytokines expression compared to controls. Such microneedle-mediated local cell delivery enhances the immune stimulation of T cells, it also reduces the frequency of injections using syringes. This TPMNs offer an innovative cell delivery platform for scattered seeding of living cells for treating a variety of tumors.

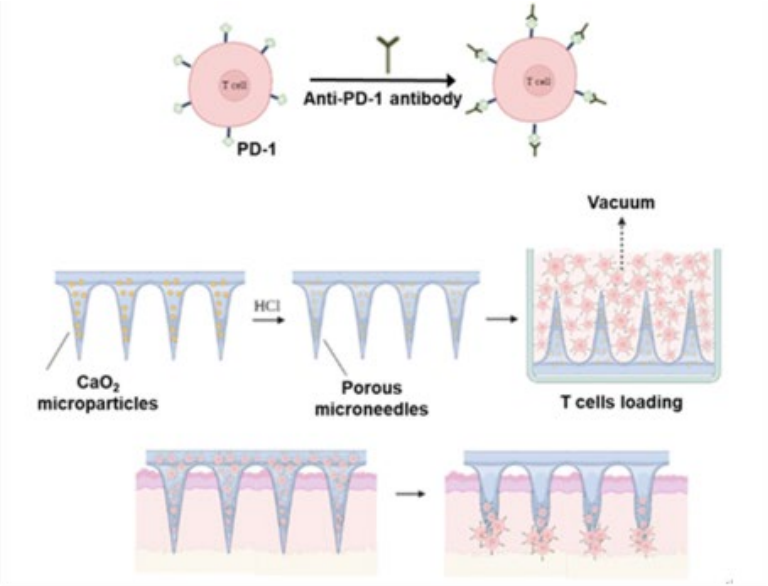

Figure 1. *Schematic of porous microneedles (PMNs) for delivering PD-1 blocking T cells into the skin.*

S9.7-O3

Optimization of post-processing and polymer coating parameters for metallic microneedle arrays as an effective drug delivery system via 3D printing technology

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Abstract

Current transdermal drug delivery systems (DDS) have limitations including poor administration of large drug molecules and skin irritations and a more efficient method is therefore required. This research aims to develop 3D printed stainless-steel microneedle (MN) arrays with optimal mechanical/biological properties as an effective DDS.

Conical-shaped MNs (h=1,000 µm and base Ø:250 µm, 36 MNs per patch) were fabricated using a Direct Metal Laser Melting 3D printing process and subsequently electropolished. Both processes were optimised using Design of Experiment (DoE), varying the printing speed (500–700 mm/s), power (35–70 W), and trace width (0.09–0.12 mm) and time (60–120 s) and current (3–7 A) during the electropolishing. Further experimental investigation was conducted on optimal MNs to assess the penetration force (skin fracture point at force–displacement graph) and depth. The optimal MNs were coated with 10% Poly(vinyl-alcohol) (PVA)–, 10% Polyvinylpyrrolidone (PVP)– and 8% Carboxymethyl-cellulose (CMC)–RhoB solutions using an immersion coating technique and the extent of drug loading and release efficiency were investigated using three different skin models: pig ear, synthetic tissue (SynTissue) and parafilm.

From the DoE studies, optimal MNs with a height of~ 780 µm, tip diameter of~ 25 µm and significantly reduced roughness were fabricated (Fig.1a). The MNs achieved a skin penetration depth of ~ 500 µm without tip fracture at a force of 4 N (Fig.1b), indicating that easy MN application can be achieved without interference with the skin's pain receptors. Furthermore, the immersion coating technique was successfully implemented, with 20 wt.% PVA-RhoB achieving a consistent homogeneous coating thickness of ~ 500 µm (Fig.1c). The coating with polymer-based material and RhoB resulted in a drug loading capacity of approximately 0.4 ± 0.2 µL exclusively on the microneedle surface, without spreading to the surrounding patch area (Fig.1d). The MNs coated with only RhoB demonstrated ~ 80% drug release within 1 min. In contrast, the use of PVA provided a controlled and slow drug release of ~ 95% RhoB over a time–period of 120 min (Fig.1e).

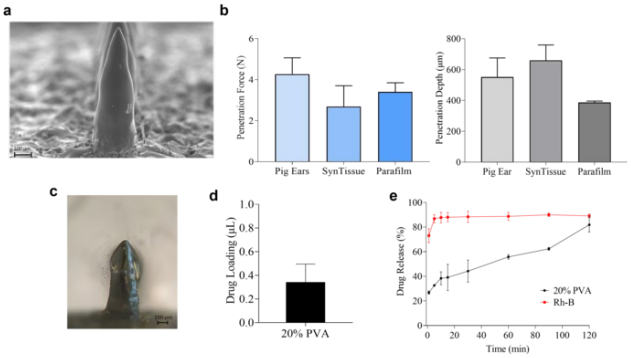

Figure 1: Assessment of optimal MNs determining the a) geometry and printing quality, b) functional properties (i.e., penetration force and depth) and performance of polymer-based coating in terms of c) coating quality, d) drug loading (RhoB), and e) drug release. Bars represent the mean ± standard error of 6 samples (n=6).

In conclusion, the 3D printed metallic-based MNs demonstrated successful skin penetration without fracture or potential painless application. The incorporation of the drug within a polymer coating resulted in a controlled rate of drug release from the MN. Taken together, the proposed coated metallic MN technology provides a platform capable of alleviating the current limitations of conventional DDS with potential painless and controllable drug administration.

S9.7-O4

Microneedle-lateral flow cassette integration for blood-free point-of-care testing of chronic kidney disease during a pandemic

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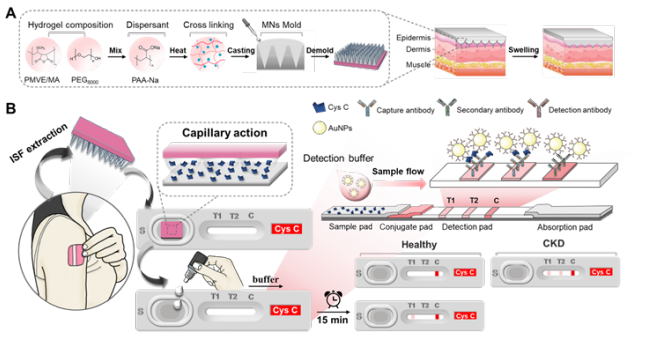
Abstract

Chronic kidney disease (CKD) is the most neglected chronic disease affecting over 750 million persons in the world. Cystatin C (Cys C) has been proposed as a potential glomerular filtration rate (GFR) marker for the early detection of acute kidney injury and CKD. However, most traditional methods for Cys C detection are immunoassays using serum as a sample and are tedious to perform and economically burdensome.

Therefore, there is an urgent need for more reliable biosensing devices to provide timely feedback to patients diagnosed with CKD. In this study, we demonstrated on-site, blood-free, and easy CKD monitoring by integrating MN extraction with a lateral flow cassette (LFC). To collect a larger volume of skin ISF in a shorter period, we added absorbent material, sodium polyacrylate (PAA-Na), into poly(methyl vinyl ether-alt-maleic acid) (PMVE/MA)-based hydrogel MNs (HMNs) to increase the swelling and water holding capabilities. We further designed an LFC containing a lateral flow paper strip and a square sample port for hydrogel microneedle patch (HMNP) assembly to run a Cys C immunoassay using MN-extracted ISF samples (Scheme 1).



Scheme 1. (A) The preparation procedure of HMNPs. (B) Schematic of the proposed blood-free rapid detection for Cys C and the detection mode for the healthy and CKD subjects.

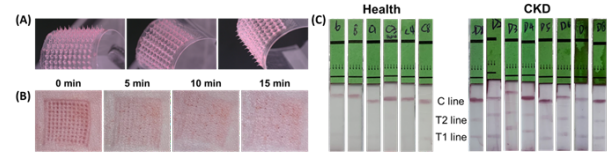


Figure. (A) The photographs of HMNP after bending prove that the HMNP can be bent to conform to the skin. (B) Rat skin puncture marks at a period of 15 min after HMNP removal. (C) Detection of Cys C by an integration of HMNP and LFCCys C in rat skin ISF samples. The visual results of Cys C in the ISF of healthy rats (n = 6) and CKD rats (n = 8) were analysed by LFCCys C (healthy rats: no red line or only one slight red line observed at T1 zone; CKD rats: two red lines were observed at T1 and T2 zones, respectively).

Moreover, to meet our goal, the HMNPs were thumb pressed into the skin of healthy rats and adenine-induced CKD rats for 5 min to ensure sufficient extraction of the skin ISF before removal. The procedure was well tolerated by the rats with no significant erythema, oedema or other effects, and within 15 min, the skin almost returned to a normal appearance (Fig.B). We extracted skin ISF from healthy rats and CKD rats using HMNPs and then directly transferred the HMNPs to LFCCys C for Cys C detection. As shown in Fig.C, no red line or only one slight red line was observed at the T1 zone in the group of healthy rats. Conversely, two red lines were observed at the T1 and T2 zones in the group of CKD rats, indicating that the concentration of Cys C in CKD rats was significantly higher than that in healthy rats and the concentrations in skin ISF for the rats with CKD were all higher than 1 μg/mL. The results confirm that the concept of using an integration of HMNP and LFC for blood-free CKD monitoring is feasible and complete Cys C testing by themselves within 25 min to achieve CKD management anytime for blood-free CKD management by the naked eye and no cross-reaction with other cytokines in ISF.