Hybrid Phenol-Functionalized Nanoparticles as Efficient Scavengers for Reactive Oxygen Species with Intrinsic Anti-inflammatory Activity

Andrea C. del Valle[1, 2], Vasiliki Tsikourkitoudi[2], Qiaolin Deng[1], and Georgios A. Sotiriou[2]

[1]*Department of Physiology and Pharmacology, Karolinska Institutet, 14 186 Solna, Sweden*

[2]*Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 14 186 Solna, Sweden*

INTRODUCTION: Reactive oxidative stress (ROS) plays a significant role in various inflammatory diseases, including arthritis, inflammatory bowel disease, and diabetes. Among the ROS-scavenging therapeutics, natural phenols have demonstrated excellent antioxidant properties. Unfortunately, their low water solubility, rapid clearance from the blood, and poor chemical stability hinder their clinical translation. In this direction, recent progress in the synthesis and functionalization of nanoparticles has led to the development of drug delivery systems that can safely and accurately target inflammation. Here, we present hybrid-phenol nanoparticles (HPNPs) for ROS scavenging. The HPNPs were synthesized by one-step polymerization or functionalizing the organic molecules onto inorganic amorphous SiO_2 nanoparticles.

METHODS: DLS and SEM images confirmed the successful nanoparticle synthesis, followed by decreased hydrodynamic diameter and polydispersity in HPNPs compared to the free monomers suspended in water. Dynamic Fourier-transform infrared spectroscopy (FTIR) and Raman were used to identify the antioxidant OH groups on the surface of the HPNPs. *In vitro*, staining with DCFH-DA assay shows the successful ROS scavenging activity of HPNPs.

RESULTS: After synthesizing, the HPNPs improved natural phenols' aqueous solubility and long-term stability. Moreover, FTIR and Raman analysis proved the presence of the antioxidant OH-groups on the surface of the NPs. The radical scavenging activity of HPNPs is evaluated by quenching the free radical DPPH, significantly improving antioxidation activity compared to free molecules. Next, overall toxicity was analyzed using Alamar blue assay in human embryonic kidney (HEK) cells, showing minimal toxicity toward normal cells. Finally, the HPNPs antioxidant effect was further validated *in vitro* after rescuing HELA cells from H_2O_2 oxidation and hypoxia conditions.

DISCUSSION & CONCLUSIONS: Oxidative stress results from an imbalance between ROS production and their elimination by protective mechanisms, leading to chronic inflammation. Natural phenols have a potential anti-inflammatory effect, associated with antioxidant activity and inhibiting enzymes in producing eicosanoids. However, their limited solubility in water and poor chemical stability hinders clinical translation. Hence, developing nanomaterials with efficient ROS scavenging ability, stability, and biocompatibility could be a potential strategy to counteract inflammation. Here, we present a synthesis of polyphenol-containing nanoparticles for depleting ROS. We selected representatives of the most common natural-derived polyphenols, such as stilbenoids (curcumin and resveratrol) and flavonoids (quercetin and gallic acid), to synthesize HPNPs. These findings lay the groundwork for using natural-derived polyphenol nanoparticles for ROS scavenging and demonstrate their potential use in clinical applications.

REFERENCES:

1. ACS Nano. 11(12):12210-12218 (2017).
2. Nat. Commun. 13, 4142 (2022).

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of the KI Research Incubator (KIRI) fellowship.

Interactions of antimicrobial peptide with antibiotics and the use of amphiphilic hydrogel microparticles for enhanced antibacterial activity

A. Stepulane[1], M. Andersson[1]

[1]*Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Sweden*

INTRODUCTION:

The rise of antimicrobial resistance (AMR) and the spread of multidrug-resistant infections have led to combinatory therapies gaining significant interest. Combination of conventional antibiotics (AB) with antimicrobial peptides (AMP) has been deemed a promising approach for prevention and reversal of antibiotic resistance, despite the inherently low stability of AMPs [1]. To improve AMP stability with retained antibacterial activity, covalent attachment onto biomaterials has been extensively reported in literature [2, 3].

Here a novel strategy to enhance and synergize the activity of conventional AB by combining them with AMP functionalized hydrogel microparticles has been explored. Oxacillin (OXA) and vancomycin (VCM) ABs have been combined with plain AMP and AMP functionalized hydrogel microparticles against *Staph. aureus* and methicillin-resistant *Staph. aureus* (MRSA). By synergistic interaction between AMP particles and AB the bacterial antibiotic sensitivity and efficacy has been improved with a potential for repurposing antibiotics and minimizing AMR.

METHODS: AMP (RRP9W4N) functionalized hydrogel microparticles were produced from diacrylated Pluronic F127 based on a previously reported method [3]. Minimal inhibitory conc. (MIC) of AMP, AMP particles, VCM and OXA alone were determined against *Staph. aureus* and MRSA. Checkerboard assays were used to evaluate the potential synergism between plain AMP with OXA and VCM, respectively. Fractional inhibitory conc. (FIC) index was used to categorize the AMP-AB interaction type. Modified checkerboard assay and time-kill studies were employed to evaluate AMP particle interaction with OXA against MRSA.

RESULTS: By combination of plain AMP with OXA the MIC of OXA was reduced from 0.25 µg/ml to 0.13 µg/ml against *Staph. aureus* and from 32 µg/ml to 0.06 µg/ml against MRSA, respectively. MIC of VCM was reduced from 1.00 µg/ml to 0.03 µg/ml against *Staph. aureus* and from 2 µg/ml to 0.06 µg/ml against MRSA, respectively, upon combination with the AMP.

The checkerboard assays of plain AMP with OXA against MRSA displayed most promising results with mean FIC index of 0.39 indicative of synergism. Upon combination of OXA with AMP functionalized hydrogel particles, OXA MIC was reduced by 64-folds from 32 µg/ml to 0.5 µg/ml against MRSA (Fig.1).

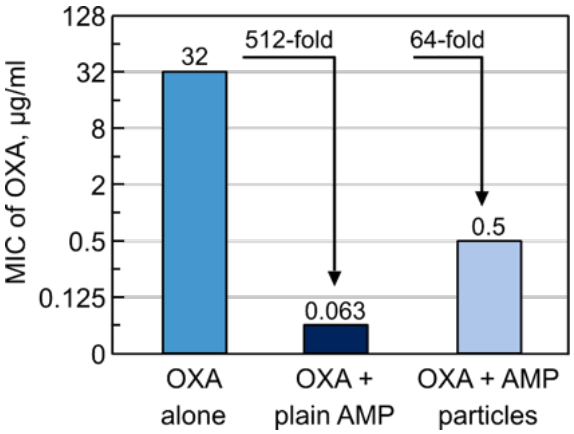

Fig. 1: MIC of oxacillin antibiotic alone and in combination with plain AMP or AMP functionalized hydrogel microparticles against MRSA.

DISCUSSION & CONCLUSIONS: Plain AMPs have been known to exhibit synergism with conventional AB, however no AMP functionalized biomaterials are known to demonstrated synergistic interactions with AB. In this work synergistic interaction with AMP and OXA antibiotic has been demonstrated *in vitro* against OXA-resistant MRSA, both in free AMP state and with AMP functionalized hydrogel microparticles. AMP hydrogel particles show potential applicability in AMP-antibiotic combinatory therapy for repurposing of AB against drug-resistant infections.

REFERENCES: [1]L. Grassi et al (2017) *Front. Microbiol.*, **8**: 314440. [2]S. Atefyekta et al (2021) *ACS Biomater. Sci. Eng.*, **7**: 1693–1702. [3]E. Blomstrand et al (2022) *Int. J. Pharm*, **627**: 122215.

ACKNOWLEDGEMENTS: The authors would like to thank the Wallenberg Foundation and the Chalmers Area of Advance "Materials Science" for funding.

Silk-Based Hydrogels: Versatile Matrices for Biomedical Applications

K.Egle[1,2], A.Grava[1,2], E.Kelle[1,2], A.Dubnika[1,2]

[1]*Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, LV*

[2]*Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, LV*

INTRODUCTION: Silk, a natural protein fibre renowned for its durability and biocompatibility, has shown promising results for biomedical applications [1]. Recent studies have focused on integrating silk fibroin (SF) into hydrogels - three-dimensional polymer networks known for their biomedical versatility [2]. Mixing chitosan (CS), gelatine (G), hyaluronic acid (HA) and fucoidan (FU) with SF during hydrogel preparation process presents a promising strategy in biomaterial research, enhancing mechanical strength, biocompatibility, and bioactivity [3]. On the other hand, calcium phosphates (CaP) and SF hydrogels show potential in mimicking natural tissues, fostering cellular interactions, and advancing innovative biomedical materials [4]. This study examines the development, characterization, and possible use of silk fibroin - based hydrogels, emphasizing their potential in tissue engineering.

METHODS:

A. Preparation of solutions. SF solution was prepared by boiling *Bombyx mori* cocoons according to the method described before [5]. CS solution was prepared by dissolving CS in 1% acetic acid. FU, G and HA solutions were prepared by dissolving them in water in various concentrations.

B. CaP in situ synthesis in SF. CaO was added in a 10% SF solution with stirring, 2M H_3PO_4 was added dropwise and stirred at 300 rpm to reach pH 6, 8, 10, and 11, obtained slurry was used for further hydrogel preparation.

C. Preparation of hydrogels. Hydrogels were prepared using 3 different methods with various rations between the polymers: 1. Physical crosslinking by freezing (-20 °C / -86 °C) or heating (37 °C / 60 °C) [5]. 2. Enzymatical crosslinking with horseradish peroxidase (HRP) and H_2O_2 [6]. 3. Chemical crosslinking with glutaraldehyde (GTA) [3]. See table below for hydrogel compositions.

Method	Composition
1	CS/FU, CS/SF, FU/SF, SF
2	SF/G, SF/G/CaP
3	SF/HA, SF

Table 1. Compositions of prepared hydrogels

All synthesized samples were characterized with Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), swelling, gel fraction, degradation, phase composition with X-Ray diffraction analysis (XRD) and *in vitro* cell viability on mouse fibroblast 3T3 cells.

RESULTS: SF solution treated at higher temperature (60°C) cross-linked faster than solution kept at 37 °C. CS/FU, CS/SF and FU/SF hydrogels frozen at -86°C has smaller pores than hydrogels frozen at -20°C. Physically cross-linked (method 1) SF/FU hydrogels dissolve immediately at all polymer ratios, however SF/CS hydrogels are stable for more than 24h. Enzymatically cross-linked hydrogels containing CaP have a better swelling degree and gel fraction. Chemical crosslinking of SF with HA using GTA proved to be successful and ensured hydrogel stability for more than week. The obtained results show that developed SF based hydrogel samples are not cytotoxic.

DISCUSSION & CONCLUSIONS: Freezing temperature of hydrogels affects their porous structure, heating accelerates the cross-linking time of hydrogels. Addition of CaP to enzymatically cross-linked hydrogels, stabilizes the hydrogel network at pH values above pH 6. Chemical cross-linking requires a minimum concentration of 8% HA and 3.23% GTA.

REFERENCES: [1]Y. Wang et al (2008) *Biomaterials*, 29:3415-3428. [2]Y. Li et al (2012) *Chem Soc Rev*, **41**(6):2193-221. [3] X.-Y. Zhang et al (2019) *Int. J. Biol. Macromol* **136**:1247–1257 [4]R Nemoto et al (2001) *J. Sol. Gel Sci. Technol* **21**:7-12. [5]K. Egle et al (2020) *Key Engineering Materials* **850**:236-241. [6]A. Grava et al (2021) *Materials* **14**:7191.

ACKNOWLEDGEMENTS: The authors acknowledge financial support from the M-era.Net 2 project INJECT-BIO agreement No. ES RTD/2020/14, Latvian Council of Science research project No. lzp-2020/1-0054 MATRI-X and European Union's Horizon 2020 research and innovation programme under the grant agreement No. 857287 BBCE.

Sintering Temperature and Liquid Phase Molarity Influence on Doxorubicin-Loaded Calcium Phosphate Cements: A Comprehensive Study for Prolonged Drug Release in Bone Cancer Therapy

A. Pylostomou[1,2], D. Loca[1,2]

[1]*Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia*

[2]*Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia*

INTRODUCTION: Osteosarcoma, a widespread metastatic affliction, affects people of all ages. The therapeutic interventions that bone cancer patients undergo are traditionally either surgery to remove the tumor, potentially followed by implant fixation (reconstructive surgery), or chemotherapy, and radiotherapy[1,2]. These conventional methods, however, entail challenges such as cancer recurrence, possible implant failure and inflammation, as well as systemic toxicity, particularly with oral or intravenous chemotherapeutics[3]. Additionally, even if the implant leads to a successful bone regeneration, a second surgery is required for implant removal with further potential risks. Calcium phosphate bone cements (CPCs) are promising biomaterials for bone tissue regeneration and cancer treatment, as they are biocompatible, moldable, injectable, and self-setting. Simultaneously, they are biodegradable and porous, eliminating the need for a secondary surgery and facilitating their loading with chemotherapeutic drugs[4]. In this work, we focus on α-tricalcium phosphate (α-TCP) cements laden with doxorubicin (DOX) drug, exploring the influence of the cement powder sintering temperature and of the liquid phase molarity on the DOX-CPC properties.

METHODS: CPC powder underwent BET analysis for specific surface area, utilizing the Quadrasorb SI gas sorption system after 24h degassing with the Autosorb Degasser Model AD-9. Setting time was determined through the Vicat needle method, and XRD elucidated crystallinity and phase composition. Compressive strength was gauged using an INSTRON 10 kN apparatus, and SEM delineated CPC morphology. DOX release kinetics were examined in a 3.5 ml PBS solution for 9 months, with quantification via UV-VIS spectroscopy ($\lambda = 480$ nm).

RESULTS: Higher sintering temperatures reduced CaP particle surface area, decreasing reactivity and prolonging the transformation from α-TCP to HAp. DOX entrapment decreased setting time at 650°C and 700°C, while higher sintering temperatures extended it by up to 61% for a 100°C increase. Reduced liquid phase molarity delayed setting time, with increases of 73.75%, 58.62%, and 137.73% in time at 650°C, 700°C, and 750°C, respectively. Compressive strength results displayed a 550% increase to doubled molarity. Both liquid phase molarity and powder phase sintering temperature significantly impacted drug release profiles.

DISCUSSION & CONCLUSIONS: Our results underscore the intricate interplay of powder phase sintering temperature and liquid phase molarity, influencing properties such as powder surface area, DOX-CPC setting time, mechanical properties, and drug release, as well as the transformation kinetics to HAp. While DOX-CPC combinations display prolonged drug release over 9 months, a comprehensive in vitro biocompatibility assessment is also required and planned for future experimental studies.

REFERENCES: [1]Dickens, E., et al (2018). Surgery (Oxford), 36(3), 134–138, [2] T. Vandeputte et al (2020) J. Cranio-Maxillo-Facial Surg. Off. Publ. Eur. Assoc. Cranio-Maxillo-Facial Surg, 48:38-42, [3]Eguia A, et al. (2020). Med Oral Patol Oral Cir Bucal. 25(1):e71–83, [4]Yousefi AM. (2019). J Appl Biomater Funct Mater. 17(4).

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

Metallic Nanoparticles and Their Impact on Dopamine Electro-Sensing

B Mostafiz[1], E.D.J Cabrera[1], V Sharma[1], E Peltola[1]

[1]*Department of Mechanical and Materials Engineering, University of Turku, Turku, Finland*

INTRODUCTION: Alzheimer's and Parkinson's diseases, as progressive neurological conditions, present a pressing need for fast, accurate, and reliable detection methods [1-3]. This project focuses on developing an innovative electrochemical (EC) sensor, by modifying carbon-based materials, for the sensitive and selective detection of dopamine (DA) - a key neurotransmitter implicated in these diseases. Despite the growing use of EC methods for dopamine detection, challenges such as low analyte concentration, fouling (both EC and biological), and host response limit their efficacy. This research aims to overcome these issues by integrating novel metallic nanoparticles (gold and platinum) with carbon-based materials, enhancing the sensor's EC sensitivity and selectivity. The study also explores the impact of nanoparticles' aspect ratios, shapes, and sizes on EC responses to optimize sensing conditions.

METHODS: This research's key method components include A. Chemical Fabrication and Integration of Nanoparticles: A 4-step chemical process is used to fabricate metallic core-shell nanoparticles. These are then integrated onto the surface of carbon-based materials to enhance the electrodes' electrochemical response to DA. B. Chemical and electrochemical Characterization: Using Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), the nanoparticles are characterized to confirm their structure and geometry, which is crucial for their function in the sensor. Techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) are employed to characterize and determine DA behavior at low physiological concentrations. C. Addressing Interferences: The study also aims to resolve issues related to interferences (like ascorbic acid and uric acid).

RESULTS: Initial findings are encouraging, indicating that the use of the fabricated nanoparticles enhances the sensitivity for dopamine on a carbon substrate, as shown in Figure 1.a. Furthermore, interference studies conducted with the modified electrode have yielded positive results. Traditionally, the electrochemical detection of DA in the brain is complicated by the overlapping oxidation potentials of uric acid and ascorbic acid, which are prevalent in the brain, with that of DA. This overlap often leads to merged peak currents and inaccurate measurements. However, the recent modifications to our carbon substrates, incorporating fabricated nanoparticles (NPs), have resulted in a platform that demonstrates negligible sensitivity to these interfering substances and a significantly increased affinity for DA (Figure 1.b).

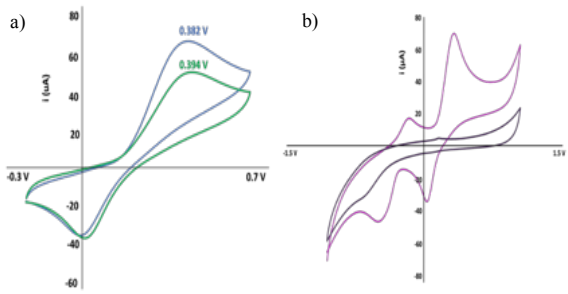

Figure.1 (a) Electrochemical performance of unmodified (green) and modified carbon-based material with Aquept NPs (blue) in a 1mM DA solution. (b) selectivity of Aquept nanoparticles modification. 1mM of ascorbic acid (purple) versus the response for 1mM of ascorbic acid + 1mM DA (pink) in PBS buffer vs. Ag/AgCl reference electrode.

DISCUSSION & CONCLUSIONS: Although these preliminary results are promising, our research will continue by testing different morphologies of Au@Pt core-shells and even move on to test several other types of novel nanoparticles, such as bone-shaped silver nanoparticles. We hope we can provide a comprehensive insight to incorporating these modifiers and what they do to impact the biological selectivity, fouling, and the temporal resolution.

REFERENCES: [1]J.-M. Beaulieu et al (2011) *Pharmacol. Rev.*, **63**, 182–217. [2]S. Verma et al (2020) *Biosens. Bioelectron.*, **165**, 112347, [3]K. Min et al (2009) *Talanta.*, **80**, 1007–1011.

ACKNOWLEDGEMENTS: This project is funded by The Research Counsel of Finland (Project No. 352421, 352422, and 352899)

Biopolymers from Riboflavin for CO_2 capture and O_2 reduction

Corina Schimanofsky, Nadine Kleinbruckner, Elisabeth Leeb, Dominik Wielend, Mihai Irimia-Vladu, and Niyazi Serdar Sariciftci

Linz Institute for Organic Solar Cells (LIOS), Institute of Physical Chemistry, Johannes Kepler University, Linz

INTRODUCTION: Biomaterials are an increasingly important field of research in times of global warming. Due to this omnipresent topic of climate change, efforts are being made to make industrial processes more eco-climate-friendly and to reduce the greenhouse gas emissions. As a result, biomaterials are gaining more and more attention as an environmentally friendly alternative, even if they are not yet completely climate neutral [1]. Examples for promising organic molecules for the reduction of greenhouse gases and the dependency of carbon-based fuels are the naturally occurring molecule classes of anthraquinones and riboflavin (vitamin B2), which are known as efficient, organic photo-electrocatalysts [2]. Both the monomers as well as the respective polymers are reported here for O_2 reduction to H_2O_2, and CO_2 capture and conversion. Furthermore, the monomers can be used in medical applications and in organic batteries, while the polymers can additionally also be used in sensors or electrodes for various devices [3,4]. In this contribution, we report on the successful polymerization and immobilization of two aminoanthraquinones and riboflavin on different carbon-based electrodes using an oxidative electropolymerization process [5].

METHODS: The polymerization of the mentioned biomaterials was performed on different carbon-based electrodes as well as on an optically transparent electrode with an oxidative polymerization in acidic media. The resulting polymers were further characterized using infrared (IR) spectroscopy, cyclic voltammetry (CV) in aqueous media, and scanning electron microscopy (SEM) images, providing insights into the structure-property relations.

RESULTS: In this work we report the electropolymerization and the capability of electrochemical carbon dioxide capture as well as oxygen reduction of these materials. The CV provided further information about the redox properties of the resulting polymers and about the stability as well as the interaction with O_2 and CO_2. The performed IR spectroscopy gave information about the structure of the polymers even though the polymerization of riboflavin onto an optically transparent electrode was not straightforward. SEM images provide a suitable proof of the successful polymerization of these biomaterials.

DISCUSSION & CONCLUSIONS: In conclusion, the polymerization was successfully optimized, and the resulting biopolymers exhibit a homogeneous film and good CV stability on the electrode. The behavior of the polymers under O_2 and CO_2 can be compared with the previous studies of the corresponding monomers [6,7,8]. The IR spectra confirm the successful polymerization and provide information on where the polymerization takes place. In addition, the broadening of the peaks indicates that a conducting/semiconducting polymer is obtained with extended π-electron delocalization.

REFERENCES: [1]E.A.R. Zuiderveen et al. (2023) Nature Communications, **14**, 8521. [2] A. Kormanyos et al. (2016) Chemistry – A European Journal, **22**(27), 9209-9217. [3] M.R. Gerhardt, L. et al. (2017) *Adva. Energy Mater.*, **7**(8), 1601488. [4] W. A. Badawy et al. (2013) *Zeitschrift für Physikalische Chemie*, **227**(12), 1741-1757. [5] N. Kleinbruckner et al. (2023) *Adv. Sustainable Syst*, 2300352. [6] E. Leeb et al. (2022) Electrochem. Sci. Adv., **2**(6), e2100211. [7] A. Kerschbaumer et al. (2022) *Catal Sci Technol*, **13**(3), 834-843. [8] C. Schimanofsky et al. (2022) *J. Phys. Chem. C*, **126**(33), 14138- 14154.

ACKNOWLEDGEMENTS: The author wants to thank all the co-workers for their help. Furthermore, all the authors thank the financial support of the Austrian Agency for International Cooperation in Education and Research (OeAD-GmBH, WTZ, CZ01/2020, 8J20AT025). In addition, we gratefully acknowledge financial support from the Austrian Science Foundation (FWF) within the Wittgenstein Prize from Prof. Sariciftci (Z222-N19), and from the European Research Executive Agency within the Project EINSTEIN (Project No. 101136377, HORIZEN-WIDERE-2023-ACCESS-03).

QCM-D-based analysis of cell adhesion: a relationship between QCM-D signal change and focal adhesion kinase activation

A Rogala, H Tiainen, D Zaytseva-Zotova

Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo, Norway

INTRODUCTION: Cell interaction with a surface of biomaterials is one of the key factors determining the fate of a biomaterial within a body. An acoustic quartz crystal microbalance with dissipation monitoring (QCM-D) technique allows analyzing the kinetics of interactions between cells and an underlying substrate non-invasively and in real time. QCM-D signals have been proposed to quantitively reflect the dynamics of receptor-mediated cell attachments or establishment of focal adhesion complexes (FAs) through which cells bind to extracellular matrix [1]. The association of QCM-D signals with integrin-RGD binding and their correlation with integrin expression levels have been later proven using photo-activatable RGD peptides [2]. It has been also demonstrated that the areal density of FAs, measured from fluorescence images, correlates with a magnitude of the ΔD response [3]. Here, we aimed to further investigate the relationship between QCM-D signals and FAs establishment. For this, we measured the adhesion of human gingival fibroblasts (hGF) using QCM-D and correlated the obtained responses with activation of focal adhesion kinase (FAK) determined via ELISA as FAK activation has been shown to be an important regulator of assembly and turnover of FAs [4].

METHODS: Cell adhesion process was studied on two substrates: polystyrene oxidized by UV-ozone treatment for 15 min (PS_{ox}) and tannic acid nanocoatings deposited on polystyrene (PS-TA) at pH 7.8 during 1 hour [5]. Cell attachment was monitored in a QCM-D QSense® window module (QWM 401) using a QCM-D QSense® E4 (QSX 310, Biolin Scientific). The sensors (QSX 305) were prepared according to the manufacturer's protocol, oxidized or coated with tannic acid nanolayer, equilibrated at 37ºC in serum-free medium for 1 hour and after that in a respective test medium (without FBS or with 10% FBS) at 50 µl/min for approximately 1 hour. HGFs were then injected into a QCM module at 300 µl/min (30 sec) at a concentration of 0.5×10^6 cells/ml. Measurements were performed at 37ºC under 10 µl/min flow of test medium overnight. Level of FAK activation was determined by using human FAK[pY397] and FAK total ELISA kits (Invitrogen) and confirmed by a immunocytochemistry analysis at 1, 2, 4, 6 and 24 hours of hGFs cultivation in static conditions.

RESULTS: The QCM-D results and microscopic analysis of cells revealed that cell adhesion to the substrates was rapid in serum-free medium but significantly delayed in 10% FBS. Cells adhered to a higher degree to PS_{ox} than PS-TA in serum-free medium. In presence of 10% FBS, hGFs showed slow adhesion to PS_{ox}, whereas cell attachment to PS-TA was almost completely hindered. A strong positive correlation was found between activated FAK and ΔD, but not Δf, for all tested conditions (Fig.1).

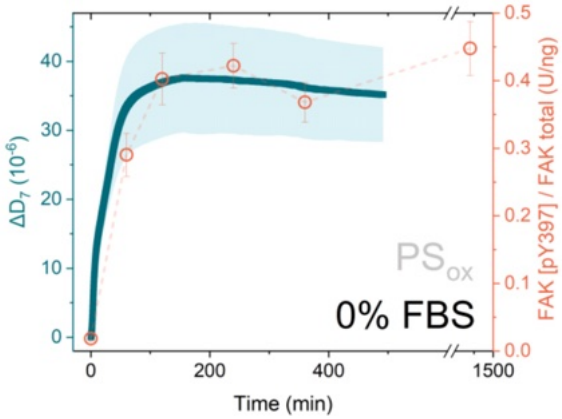

Fig. 1: Fibroblasts adhesion to PS_{ox} surface in serum-free medium. Left axis (blue): change in dissipation (n=7) monitored with a QCM-D. Right axis (red): a degree of FAK activation measured with ELISA.

DISCUSSION & CONCLUSIONS: This study shows a linear correlation between the activation of FAK and QCM-D responses. This result is in agreement with previous studies and confirms that QCM-D is a valuable technique for analysis of early cell adhesion process and establishment of FAs.

REFERENCES: [1]C. Fredriksson et al (1998) *J. Mater Sci: Mater Med*, **9**:785−8. [2]J. Iturri et al (2015) *Sci Rep*, **5**:9533. [3]J.Y. Chen et al (2019) *Exp Cell Res*, **2**:105-13. [4]S.K. Mitra et al (2005) *Nat Rev Mol Cell Biol*, **6**:56-68. [5]F. Weber er al (2019) *Langmuir* **35**:3327-36.

ACKNOWLEDGEMENTS: This work has received funding from The Research Council of Norway (# 302590).

Nanozyme-Mediated Antioxidant Protection of Hemoglobin-Based Oxygen Carriers

D. Douka[1], X. Cun[1], L. Hosta-Rigau[1]

[1]Department of Health Technology, Technical University of Denmark, Denmark

INTRODUCTION: The constant supply of oxygen (O_2) into the tissues of the body is a crucial function for survival. Blood facilitates the delivery of O_2 and in severe accidents or blood diseases, transfusion is the sole method for substitution. Even though transfusion of donor blood is a well-established procedure, it is characterized by some limitations, because of its short shelf-life and the need for blood typing and matching [1]. The development of hemoglobin (Hb)-based O_2 carriers (HBOCs), as blood substitutes is considered a promising alternative with several advantages over conventional blood transfusions.
However, a crucial limitation of current HBOCs is the autoxidation of Hb into metHb, which lacks O_2-carrying capacity [2]. Native red blood cells possess a complex antioxidant system, including catalase (CAT) or superoxide dismutase (SOD) enzymes, able to prevent and revert the conversion of Hb into metHb.
However, the direct incorporation of HBOCs with natural enzymes is limited due to the high production cost, the low stability, and the short catalytic half-lives [3]. A new approach, which includes the use of nanoparticles (NPs) with enzymatic-like activity, referred to as nanozymes (NZs), has emerged as a novel antioxidant system, protecting the Hb from autoxidation into metHb [4].

METHODS: Metal-organic framework (MOF)-based NPs, encapsulating Hb (Hb@MOF NPs) were prepared and coated with different NZs. Next, the resulting NZs-loaded Hb@MOF NPs (Hb@MOF/NZs NPs) were tested for their superoxide radical- and peroxide-scavenging abilities, with Amplex Red and WST-1 assays, respectively. Finally, the O_2 binding capacity of Hb@MOF/NZs NPs was measured and compared to that of free Hb with a needle-type O_2 microsensor.

RESULTS: The results for the enzymatic activity indicated that the Hb@MOF/NZs NPs displayed SOD- and CAT-like activity, with the former activity being superior.

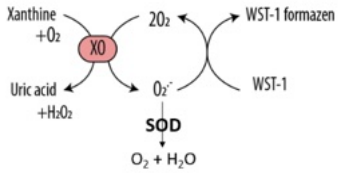

Figure 1: SOD-like activity of Hb@MOF/NZs NPs. O_2 is converted into $O_2^{\bullet-}$ by the xanthine/XO system. $O_2^{\bullet-}$ oxidizes the WST-1 reagent into formazan, which is detected by UV-vis. In presence of SOD-like activity, $O_2^{\bullet-}$ is consumed, decreasing the amount of oxidized formazan.

The O_2 release and binding studies showed that the Hb@MOF/NZs NPs assembled with lower concentrations of NZs present higher O_2 binding capacity.

DISCUSSION & CONCLUSIONS: The Hb@MOF/NZs NPs were successfully synthesized and coated with the different NZs. Their ability to deplete superoxide anions ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) was demonstrated prior and following incorporation into the Hb@MOF/NZs NPs. An O_2 electrode was employed to assess the O_2 binding and releasing properties of the encapsulated Hb, and the results showed no significant difference compared to the free Hb. As a further step, the surface modification of the NPs is proposed, to improve their stability and then continue further with biological evaluation in-vitro. In summary, these data demonstrate the potential of the as-prepared HBOCs as O_2 delivery systems with built-in antioxidant protection against reactive O_2 species.

REFERENCES: [1]A. S. Gupta (2019) *Shock (Augusta, Ga.)*, **52**:70. [2]Y. Jia (2016) *Advanced Materials*, **28**:1312-18. [3]R. Ragg (2016) *European Journal of Inorganic Chemistry,* **2016**:1906-15. [4]X. Liu (2023) *Biomaterials Science,* **11**:2551-65.

PLL coated CaP NPs as nanocarriers for DNA delivery

E. Chronopoulou[1], A. Maheshwari[1], A. C. del Valle[1, 2], B. Henriques-Normark[1,3], G. A. Sotiriou[1]

[1]*Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm*
[2]*Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm*
[3]*Clinical Microbiology, Karolinska University Hospital, Stockholm*

INTRODUCTION: Nucleic acid vaccines have gained significant attention, especially after the development of COVID-19 mRNA vaccine. DNA vaccines are safe, inexpensive and can be rapidly designed and produced. However, challenges like the inefficient in vivo delivery and cell uptake limit their application [1]. The development of a more precise and potent vaccine platform is therefore needed.

Nanocarriers display unique features in protecting biomolecules from degradation, targeting the delivery, and controlling their release [2]. Calcium phosphate nanoparticles (CaP NPs) are one of the most promising DNA delivery platforms because of their biocompatibility and biodegradability [3]. In addition, CaP has a high affinity for DNA and has been widely used for DNA transfection studies.

To enhance the transfection efficiency and protect DNA from early degradation, polymers are often used in nano-vaccine formulations. Due to its cationic nature, polymerized lysine (PLL) can interact electrostatically with cells, facilitating efficient internalization for intracellular delivery[4]. Here we present a vaccine formulation, using CaP NPs for the loading of DNA which further is modified with polylysine as uptake enhancer.

METHODS: CaP nanoparticles were generated through a scalable single-step process employing flame spray pyrolysis (FSP). FSP is a commonly used industrial technique due to its high reproducibility and scalability. The CaP NPs were characterized using the Brunauer-Emmett-Teller (BET) method to evaluate the specific surface area (SSA) and X-ray diffraction (XRD) for crystallinity. The lysine polycondensation was conducted at 240°C; as at higher temperatures the amino acid degradation takes place. Transfection experiments were conducted in human embryonic kidney cells (HEK-293T) by delivering the linear vector of EGFP (enhanced green fluorescent protein), which was later analyzed via flow cytometry and confocal microscopy.

RESULTS: We synthesized amorphous CaP NPs confirmed with XRD, with a specific surface area of 230 m^2/g. We achieved more than 80% DNA loading efficiency on bare CaP NPs. DNA loaded CaP nanocarriers were formulated with and without polylysine for transfection studies. The PLL coated CaP NPs demonstrated enhanced DNA uptake by cells indicated by increased GFP production.

DISCUSSION & CONCLUSIONS: The utilization of calcium phosphate nanoparticles coated with lysine polymers showcased improved DNA uptake by cells, followed by the corresponding protein production. This presents a promising foundation for developing an effective DNA delivery platform.

REFERENCES: [1]M. Shafaati et al (2022), *Future Virology*, ***17***(1), pp.49-66. [2]Z. Sun et al (2021), *Frontiers in Materials*, ***8***, p.788373. [3]W. Habraken et al (2016), *Materials Today*, ***19***(2), pp.69-87. [4]S. Manouchehri et al (2022), *Molecular Pharmaceutics*, ***18***(10), pp.3652-3670.

ACKNOWLEDGEMENTS: This work received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (ERC Grant Agreement No. 758705). Funding from the Karolinska Institutet, the Swedish Foundation for Strategic Research (SSF) (FFL18-0043, RMX18-0043) and the Swedish Research Council (No. 2021-05494, 2021-02059) is kindly acknowledged.

Porcine skin-derived hydrogels as bioink

E Palierse[1], AM Mihailescu[1], C Persson[1], M Aramesh[1]

[1] *Division of Biomedical Engineering, Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden*

INTRODUCTION: The extracellular matrix (ECM) provides biochemical and structural support to the surrounding cells in living tissues. Comprising proteins and polysaccharides arranged in an intricate porous network, ECM incorporates cells, enzymes and growth factors [1]. Recreating its complexity in vitro, in terms of composition, structure, and mechanical properties, is a challenging task. The use of 3D bioprinting to fabricate 3D constructs with a hierarchical architecture similar to the native tissue could meet some of these challenges. Bioinks hence need to be formulated to give the biochemical cues for cell adhesion and proliferation [2], which may be missing in commonly used polymers. Decellularized ECM (dECM), obtained from the decellularization of ECM, is a promising candidate to that respect. dECM gels preserve the composition of ECM and thereby contain protein domains essential for cell stimulation [3]. However, 3D bioprinting using dECM gels is often unsuccessful due to their slow gelation kinetics and low mechanical properties. The concentration of dECM hydrogels could be a key parameter to formulate printable dECM bioinks. Here, we investigate how the formulation of skin-derived dECM hydrogels affects their gelation and viscoelastic properties, and therefore their printability and further use as bioinks.

METHODS: The dECM material was obtained from the decellularization of porcine skin tissue. Briefly, the tissue was first delipidated in acetone before several washing steps with surfactants, oxidant solution, buffers and ultrapure water. The quality of decellularization was assessed through biochemical assays. The dECM was then digested with pepsin at acidic pH for 24 h before adjusting the pH at 7.4 to obtain gels with several dECM concentrations. Gelation kinetics at physiological pH and shear viscosity were assessed with rheology measurements. The printability of dECM hydrogels was observed visually and with optical microscopy.

RESULTS: The decellularization removed the DNA while maintaining the levels of collagen and glycosaminoglycans in the dECM. dECM hydrogels with a storage modulus ranging from ca 100 Pa to 5 kPa were obtained after 10 min at 37 °C for concentrations of dECM up to 10 mg.mL^{-1} (Fig. 1a,b). The more concentrated bioinks can be extruded with a 30 G needle at speed up to 1000 mm.min^{-1}, and the prints presented a good shape fidelity (Fig. 1c).

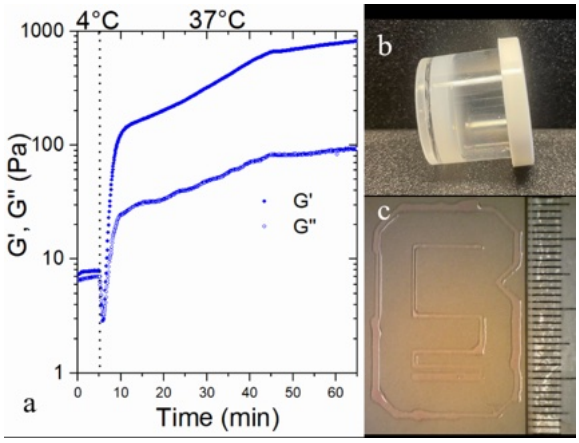

Fig. 1: a) Gelation kinetics of dECM hydrogel at 10 mg.mL^{-1}, b) picture of dECM hydrogel after gelation, c) printability test using a 10 mg.mL^{-1}dECM bioink (needle 30 G, 600 mm.min^{-1})

DISCUSSION & CONCLUSIONS: In this study, we prepared porcine skin-derived hydrogels. The gelation kinetics and moduli were comparable with other studies for gels obtained from various decellularized tissues. The gels at 10 mg.mL^{-1} may even compete with pure collagen hydrogels. These gels were also extrudable, which suggests the possibility to further process them by extrusion-based 3D printing. Thanks to the similarity of dECM with native tissues, dECM hydrogels have great chances to promote cell adhesion and proliferation. Further studies to fabricate 3D printed constructs are ongoing, together with biocompatibility studies. These constructs may be used for tissue engineering or in vitro tissue modelling.

REFERENCES: [1]C. Frantz et al (2010) *J Cell Sci*, **15**:4195-4200. [2]K. Da Silva et al (2020) *J Biomed Mater Res*, **108**:2324-50. [3]J. Kort-Mascort et al (2023) *Biomater Sci*, **11**:400-31.

ACKNOWLEDGEMENTS: We thank Carl Tryggers Stiftelse (CTS 22:2367) for funding.

Optimization of an air-liquid interface system using electrospun membranes to mimic human airway epithelium for development of next-generation high-throughput airway model.

Indra Putra Wendi[1, 2, 3], Hani Alsafadi[1, 2, 3], Emil Rehnberg[1, 2, 3], Iran Augusto Neves da Silva[1, 2, 3], Sinem Tas[1, 2, 3], Maximilian Ottosson[4] , Albin Jakobsson[4] , Darcy Wagner[1, 2, 3]

[1]Lung Bioengineering and Regeneration, Dept of Experimental Medical Sciences, Lund University, Lund, Sweden. [2]Stem Cell Centre, Lund University, Lund, Sweden. [3]Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden. [4]Cellevate AB, Lund, Sweden.

INTRODUCTION: History of pandemics within the last century has been dominated by respiratory diseases, with COVID-19 being the most recent, making them remain a long-term public healthcare concern. As such, establishment of a new airway model is imperative to facilitate advancement in airway disease drug development. Air-liquid interface (ALI) is an established culturing technique which has been shown to have higher *in vivo* relevance compared to regular 2D cell culture, owing to its higher degree of biological and structural complexity [1] and electrospinning-based scaffolds [2] has been shown to have beneficial effects to cellular growth and maintenance due to its closer to *in vivo* topography. Here, we established the feasibility of combining both approach as a potential model amenable to high or medium-throughput screening (HTS) for lead compound identification purposes in primary human airway epithelial cells.

METHODS: We first characterized the optical transmittance of the PCL membranes in the visible light range using high-content plate reader and subsequently established proof-of-principle using a live-cell, far red fluorescent dye to monitor monolayer formation on the membrane. Different surface modification methods and ECM protein coating were utilized and the surface energy were tested using water contact angle (WCA) measurement. Next, we tested different methods to minimize the evaporation due to the "edge-effect" in 96- electrospun transwell plates.

RESULTS: On the visible light range, the lowest optical density was recorded at 630 nm wavelength. The used live-red dye were able to be used to visually track cell distribution on the membrane and treatment with 0.1% atelocollagen I were shown to be able to reduce the water contact angle (mean±SD = 75.125±1.536) Combination of breathable tape, liquid reservoir, and custom-made humidification chamber were able to reduce edge-effects by 71.50% (CI 95%=64.13 – 78.87).

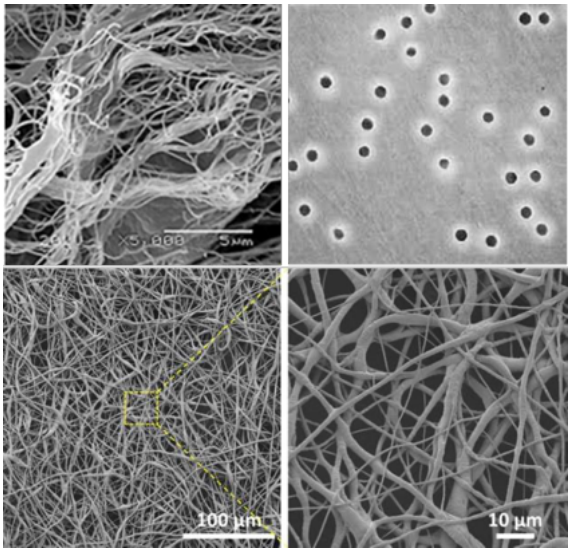

Fig. 1: Comparison between de celled airway basal membrane (Bridge et al 2015 top left) with commercial culture insert membrane (top right) and an electrospun PCL membrane (Tas et al 2021 bottom)

DISCUSSION & CONCLUSIONS: These early proofs of principles give hints on how to work with the electrospun PCL membrane for designing a high throughput drug screening model. Utilization of physiologically and clinically more relevant cells such as human airway cells may be implemented in the future to evaluate the proper biological response towards the material.

REFERENCES: [1] X. Cao et al (2021) *In Vitro Cell.Dev.Biol-Animal*, **57**:7265-68. [2] J. Xie et al (2021) *Mater Sci Eng C Mater Biol Appl.***120**: 111787..

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) and Sweden's Innovation Agency (Vinnova). All electrospun membrane were manufactured by Cellevate AB.

Simultaneous delivery of cannabidiol and insulin-like growth factor 1 for tissue regeneration

I Jurgelane[1,2], D Galkina[1,2], A Grava[1,2], G Salms[2,3], A Dubnika[1,2]

[1]*Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Department of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Latvia*

[2]*Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia*

[3]*Rīga Stradiņš University, Institute of Stomatology, Latvia*

INTRODUCTION: Oral soft tissue regeneration after ablative surgery, trauma or oral cancer is still a challenging goal. Oral cancer is primarily treated with surgery, combined with chemotherapy and radiation, causing a significant loss of soft tissue, particularly gum [1]. The main problems for soft tissue regeneration are cell ingrowth, inflammatory and related pain issues. The wound-healing process includes cell migration, proliferation and differentiation, and the key signaling molecules that regulate these responses are growth factors. Insulin-like growth factor 1 (IGF-1) has an essential role in inducing cell proliferation and collagen synthesis in the fibro-proliferative process [2]. Furthermore, in recent years, medical cannabis has gained significant attention for its potential therapeutic benefits. Phytocannabinoids, such as *cannabidiol* (CBD), is known to speed healing of the wounds [3] and to relieve pain in chemotherapy [4]. The novel combination of IGF-1 and CBD could increase tissue regeneration and decrease chemotherapy-induced side effects. The delivery of both IGF-1 and CBD will be achieved through liposomal systems – by encapsulating them into liposomes. The aim of this work is to encapsulate both bioactive molecules into phospholipid-based liposomes and to evaluate their potential for simultaneously providing anti-inflammatory and antioxidant effect (by CBD) and inducing cell proliferation and collagen synthesis (by IGF-1).

METHODS: Liposomes were synthesized using cholesterol and various phospholipids (DSPE-PEG-Amine (MW3400), DSPC and DPPC) with thin-film hydration method. CBD was incorporated during synthesis process but IGF-1 – during hydration process of the liposomes. The chemical composition of liposomes was characterized by Fourier transmission infrared spectroscopy and the particle size and zeta potential was determined by dynamic light scattering analysis. The release of CBD and IGF-1 was determined using ultra performance liquid chromatography [5] and ELISA method, respectively. The *in vitro* cytotoxicity tests were conducted with gingiva-derived mesenchymal stem cells (GMSC) isolated from human patients according to the decision No. 6-1/12/47 (26.11.2020) of Riga Stradiņš University Research Ethics Committee. CCK8 assay was used for cell viability determination.

RESULTS: The particle size of liposomes varies between 3-9 µm and depends on the phospholipids used in the synthesis. The encapsulation of CBD and IGF-1 alter the particles size as the presence of CBD reduces the particle size. Based on the FTIR results, the synthesis process does not affect the chemical structure of the bioactive molecules. The cell viability for all samples (pure liposomes, with CBD, with IGF-1 and with CBD and IGF-1) is not lower than 70%.

DISCUSSION & CONCLUSIONS: Based on the cell viability results, all investigated samples are considered as non-cytotoxic, according to the ISO 10993-5:2009. The highest cell viability was observed for liposomes containing DSPE-PEG-Amine (MW 3400). Liposomes with IGF-1 show increased cell proliferation due to the mitogenic effect of IGF-1 [6].

REFERENCES: [1]American Cancer Society, Treatment option for oral cavity cancer by stage. Online [01.12.2023] – https://www.cancer.org/content/dam/CRC/PDF/Public/8766.00.pdf. [2]C.-H. Tsai et al (2005) *Oral Oncol*, **41**:940-6. [3]M. Klein et al (2018) *Phytoter Res*, **32**:2275-81. [4]T. Fisher et al (2016) *Curr Oncol*, **23**:S15-22. [5]Australian Goverment, Department of Health. Online [05.12.2023] – https://www.tga.gov.au/sites/default/files/test-method-uplcc01-cannabinoids.pdf. [6]N.-Z. Bashir (2021) *Arch Oral Biol*, **122**:104993.

ACKNOWLEDGEMENTS: This research has received funding from the M-era.Net 2 project INJECT-BIO under agreement No. ES RTD/2020/14 and European Union's Horizon 2020 research and innovation programme under the grant agreement No. 857287 (BBCE).

Rapid Manufacture of Engineered Muscle Platforms via In-Air Printing of Elastomer Composites

I Papiano[1], A Dogan[1,2], J Chan[1], C Radeke[1], J U Lind[1]

[1]*Department of Health Technology, Technical University of Denmark, Denmark*

[2] *Novo Nordisk Foundation*

INTRODUCTION: Conventional muscle microphysiological systems (MPSs) designed for drug screening and disease modeling rely on measurements of electrophysiological parameters and contractile forces, as muscle tissues exhibit both electrical and mechanical activity [1]. However, the current manufacturing process for muscle MPSs involves multiple steps[1] and limits the design flexibility. Direct Ink Writing (DIW) is a valuable 3D printing technique that has been proven to enable the simple production of a variety of complex geometries using appropriately engineered materials [2, 3]. This study proposes to explore the use of DIW as a rapid manufacturing modality for muscle MPSs. We have formulated an elastomeric composite conductive ink utilizing styrene-ethylene-butylene-styrene (SEBS) polymer and graphite microparticles in butyl acetate (ButAc) solvent. We have demonstrated that this material can be 3D printed in air, forming suspended filaments over several centimeters while retaining elasticity and conductive properties. As a proof of concept, we have used embedded additive manufacturing to bioprint C2C12 cells around two consecutive filaments in a sacrificial matrix. The resulting engineered muscle system holds promise for monitoring cell contractility.

METHODS: The DIW 3D printing method was used to print both the conductive ink and the muscle tissue. The graphite-based ink was characterized mechanically through tensile tests, electrically through multimeter measurements, and printability-wise through experimental prints and brightfield images. Tissue constructs were analyzed using brightfield images as well as actin-stained fluorescent images on a confocal microscope.

RESULTS: We developed an in-air 3D-printable material that shows both elasticity and conductivity properties to be used in muscle contractility tracking. In our previous work, we demonstrated the possibility of printing SEBS/ButAc-based materials in-air over centimeters in length (Figure 1a). In this paper, we explored a composite with graphite, which was characterized both mechanically and electrically. Embedded bioprinting of C2C12 cells was proven to be a valuable method for the creation of connected tissue constructs in precise placements around the support structures (Figure 1b).

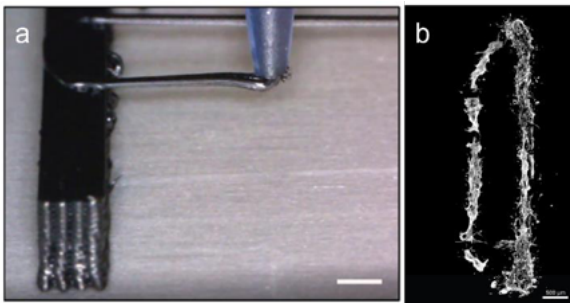

Fig. 1: (a) Free-standing in-air printing of the SEBS/graphite composite. Scale bar: 1 mm. Reproduced from[2]. (b) Confocal image of an actin-stained C2C12 tissue at 8 days in culture. Scale bar: 500 μm.

DISCUSSION & CONCLUSIONS: The SEBS/ButAc inks doped with graphite microparticles hold great promise in their ability to be free-suspended in addition to having both elastic as well as conductive properties. We believe that the combination of optimal solvent evaporation rate, particle gelation, and particle-polymer interactions allows the material to be printed in air without relying on support substrates. The embedded bioprinting allowed good localization of the tissue around the ribbons, paving the way for future electrical tracking of cell contraction.

REFERENCES: [1]Q. Wu et al (2023) *Biofabrication*, **15**:035023. [2]C.J. Pless et al (2023) *Adv Electron Mater*, 2201173. [3]J.L. Lind et al (2017) *Nature Mater,* **16**:303-308.

ACKNOWLEDGEMENTS: I.P., J.C., A.D., C.R., and J.U.L. would like to gratefully acknowledge The European Commission MSCA-IF (798820), the Lundbeck Foundation (R250-2017-1425), The Independent Research Fund Denmark, Sapere Aude Starting Grant (8048-00050), and the Novo Nordisk Foundation (NNF21OC0066994).

Microglial physiology changes in a stiffness-dependent manner in different 3D environments

Irene Tomé-Velasco[1,2], Ane García-García[3,4], Leyre Pérez-Álvarez[3,4], Federico N. Soria[1]

[1]*Achucarro Basque Center for Neuroscience, UPV/EHU Science Park, 48940 Leioa, Spain.*

[2]*Neuroscience Department, University of Basque Country (UPV/EHU), 48940 Leioa, Spain.*

[3]*Physics Chemistry Department, University of Basque Country (UPV/EHU), 48940 Leioa, Spain.*

[4]*BCMaterials, Basque Center for Materials, UPV/EHU Science Park, 48940 Leioa, Spain.*

INTRODUCTION: Biomaterials have been used in research for decades as a tridimensional microenvironment for cell culture, being the acrylamide-bisacrylamide-based hydrogels the most widely used material. Many of these biomaterials do not have a biologically mimetic composition for the cell grow, in particular when modelling the brain parenchyma.

Microglia are resident macrophages of the brain that play an essential role in maintaining the homeostasis of the central nervous system (CNS) by sensing the environment.

Here we explore several biomaterials to achieve a more brain mimetic-like 3D-environment and studying whether matrix stiffness affect brain cell physiology in general and microglia in particular.

METHODS: We created 3D scaffolds with different methacrylated polymers, such as alginate, gelatine, and hyaluronan or chitosan, and using Matrigel as control biomaterial. We evaluated cell viability, phagocytosis, motility and morphology in SV40 microglia cells.

RESULTS: SV40 microglia are more viable in methacrylate alginate, gelatine and Matrigel. Alginate-based scaffold allowed microglia to adopt the more brain-like morphology, while softer Matrigel scaffolds allowed for increased motility.

DISCUSSION & CONCLUSIONS: Tridimensionality clearly affects microglial physiology and function, and it is in a 3D environment where brain cells can better mimic their in vivo counterparts. Whereas stiffness has a clear effect on movement and morphology, it is still not clear whether composition has a major effect on these parameters. However, composition and assembly protocol has a clear effect on cell viability. 3D scaffolds might prove a clear advantage over 2D cultures to better mimic the mechanical and chemical cues that cells find in the real brain, which might be useful for in vitro testing and screening

ACKNOWLEDGEMENTS: We thank the Microscopy Facilities at Achucarro Basque Center for Neuroscience and the UPV/EHU (Sgiker) for providing infrastructural support.

Save Your Tears for the Assay: Carbon Nanotubes Still Fooling Scientists

Johanna Suni[1], Salli Valkama [1], Emilia Peltola [1]

[1] *Department of Mechanical and Materials Engineering, University of Turku, Finland*

INTRODUCTION: Carbon nanotubes (CNTs) are widely used material in different fields of industry and medicine due their specific properties. Because of their large-scale use, possible toxic effects on humans and the environment have been extensively studied. A common method for assessing the toxicity of CNTs are different cell viability assays based on tetrazolium salts, such as MTT assay. The international standard ISO 10993-5 also describes MTT assay and related tests for *in vitro* cytotoxicity testing. However, it has been observed that some of the assay dyes may interact with CNTs, leading to potential false results [1,2]. The issue of interactions between cell viability assay molecules and carbon nanomaterials remains to be investigated.

METHODS: We observed spectrophotometrically interactions between multi-walled carbon nanotubes (MWCNTs) and six different coloured tetrazolium salts: MTT, MTS, INT, XTT, WST-1, and WST-8. In metabolically active cells, tetrazolium salts reduce to formazan crystals by NAD(P)H-dependent oxidoreductase enzymes. Therefore, we utilized the formazan forms of dye molecules and cell-free systems to exclusively observe interactions between assay dyes and MWCNTs.

RESULTS: We found that all tested tetrazolium salt dyes interacted with MWCNTs, as evidenced by coloured dye molecules being absorbed into them. At a high concentration of MWCNTs, the absorbance intensity immediately starts decreasing at the beginning of each assay, and the intensity continues to decrease over time.

DISCUSSION & CONCLUSIONS: Our results demonstrate that cell viability assays are not reliable methods to study toxicity of CNTs. It is necessary to carefully investigate which *in vitro* methods are truly suitable for CNTs. For this purpose, it is important to study the interactions between carbon nanomaterials and cell viability assay molecules.

REFERENCES:. [1] J.M. Wörle-Kirsch (2006) Oops They Did It Again! Carbon Nanotubes Hoax Scientists in Viability Assays. *Nano Lett*, **6**:1261–1268. [2] L. Belyanskaya et al (2007) The reliability and limits of the MTT reduction assay for carbon nanotubes–cell interaction. *Carbon*, **45**:2643–2648.

ACKNOWLEDGEMENTS: This research has been supported by the Research Council of Finland (grants #347021, #352421, #352422, #352899).

Drug to polymer conjugates – a novel use of polyanhydrides in drug delivery systems

Konrad Kwiecień[1], Karolina Knap[1], Katarzyna Reczyńska-Kolman[1], Przemysław Mielczarek[2], Dorota Ochońska[3], Daria Niewolik[4], Katarzyna Jaszcz[4], Monika Brzychczy-Włoch[3], Elżbieta Pamuła[1]

[1]*Department of Biomaterials and Composites, Faculty of Materials Science and Ceramics, AGH University of Krakow, Poland*

[2]*Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics, AGH University of Krakow, Poland*

[3]*Department of Molecular Medical Microbiology, Chair of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland*

[4]*Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland*

INTRODUCTION: Polyanhydrides, e.g. poly(sebacic anhydride) (PSA), are considered drug carrier materials and have been used in clinical practice [1]. Recently, PSA was found to be promising in the delivery of azithromycin to the lungs in the form of dry powders for inhalation [2]. The success of the study was based on almost complete encapsulation efficiency. The phenomenon was explained by the creation of ester bonds between the polymer and the drug. Herein, we present a detailed mass spectrometry study on the establishment of drug-polymer conjugates with hydrophobic drugs with hydroxyl or phenol groups, i.e. azithromycin (AZM) and curcumin (CU), respectively.

METHODS: PSA was obtained by melt polycondensation. Microparticles (MPs) were manufactured by oil-in-water (O/W) emulsification, where O is a drug (either AZM or CU) and PSA dissolved in dichloromethane in various ratios, and W is a poly(vinyl alcohol) solution in MilliQ water. For CU-loaded MPs, the W-phase was additionally acidified with acetic acid, hydrochloric acid, or sulfuric acid. The morphology and size distribution of the MPs was assessed by optical and fluorescent microscopic observations and laser diffraction-based size measurements. The encapsulation efficiency (EE) and drug loading (DL) of AZM-loaded MPs were assessed by mass spectrometry (MS) of the supernatants, while for CU-loaded MPs, it was evaluated by fluorometric measurement after decomposing MPs in dimethyl sulfoxide. For analysis of the conjugates, the MPs were dissolved in chloroform, diluted 100 times, and injected directly into the mass spectrometer to detect the ions of conjoined drugs and monomers. The analysed ions were separated and fragmented to ensure their conjugate origin. To check the potential disturbance of the process on the bioactive properties, AZM-loaded MPs were tested with *Staphylococcus aureus* and the release of the pure drug in biological environment was tested by incubation with human microsomes.

RESULTS: The MPs obtained were spherical with most diameters in the range of 1-3 µm however, they showed a tendency to agglomerate resulting in median sizes between 10-30 µm in the form of dry powder. CU-loaded MPs were yellow due to the presence of the drug and exhibited a green glow when observed under the fluorescent microscope. Encapsulation evaluation showed that EE of AZM was nearly 100% while for CU-loaded it was up to 70% which was increased compared to around 10% for non-acidified conditions. MS analysis discovered conjoined drug molecules with up to several monomers at a time. Isolation and fragmentation confirmed their origin. Incubation with human microsomes showed gradual detachment of the conjugates in the biological environment, and AZM-loaded MPs effectively inhibited the growth of *S. aureaus*.

DISCUSSION & CONCLUSIONS: Hydrophobic drugs with hydroxyl or phenol groups can be encapsulated into poly(sebacic anhydride) with high efficiency due to the creation of drug-polymer conjugates; however, phenols require a low pH of water phase. The drugs do not lose their properties during the manufacturing process and can be released as pure substances in the human body. Furthermore, the issue of excessive agglomeration should be addressed in future studies.

REFERENCES: [1]J. P. Jain et al (2008) *Expert Opnion on Drug Delivery*, **5**(8):889-907. [2] K. Knap et al (2023) *Biomaterials Advances*, **153**:213540

ACKNOWLEDGEMENTS: This study was supported by National Science Centre, Poland (project No 2019/35/B/ST5/01103)

In vivo applications of Poly(glycerol sebacate urethane) Scaffolds

Andreas Samourides[1]*, Zacharoula Xenou[1], Despoina Kokkinidou[1], Kyriakos Spanoudes[2], Konstantinos Kapnisis[1], Andreas Anayiotos[1]

[1] *1Department of Mechanical Engineering and Materials Science and Engineering, Cyprus University of Technology, Limassol 3036, Cyprus*

[2] *VET EX MACHINA LTD, Cyprus*

INTRODUCTION: Soft tissue engineering (TE) is a multidisciplinary field with the aim to regenerate or replace a dysfunctional or damaged tissue. Usually, a three-dimensional porous scaffold is produced to provide a temporary structure and mechanical support while the tissue grows and subsequently replaces it. For this reason the scaffold should be highly tunable, with interconnected porous structure, be biocompatible, biodegradable and ideally to degrade linearly at the same rate as the tissue develops In this study, three PGSU scaffolds, with different mechanical and microstructure properties were fabricated and investigated for in vivo for their microstructure, biocompatibility and their ability to degrade at the same rate as tissue develops into the scaffold.

METHODS: PGSU scaffolds with hexamethylene diisocyanate (HDI) ratios of 0.8 and 1.0 and polymer concentrations (w/v%) equal to 10% and 15%. Briefly, the PGS pre-polymer was dissolved in 1,4-dioxane at the required w/v concentration and HDI was added at 0.8 or 1.0 ratio to glycerol. The solution was left to react for five hours at 55°C. The solution was then frozen in an in-house customized mold (see 2) and freeze dried for 16 hours. In vivo biocompatibility of the scaffold was investigated by implanting scaffolds subcutaneously in CD1 albino mice for six weeks. Specifically, in total 16 mice had scaffolds implanted subcutaneously, 12 of which were implanted with four scaffolds of each sample group and the rest of them with Ethilon® Nylon suture 4-0 spheres which acted as the positive control. The scaffolds were also characterized for their microstructure using SEM. Finally, to assess any inflammatory response, whole body imaging (WBI) was used after

RESULTS: Figure 1A and B show the cross-sectional microstructure of the PGSU scaffolds before and after in vivo implantation. It was found that the previously open pore microstructure was filled with new tissue, demonstrating uniform tissue ingrowth. Subsequently, as the density increases the porosity decreases, in combination with the decrease in volume and no change in mass it can be said that the scaffolds degraded by surface erosion and the tissue ingrowth into the scaffolds is similar to their rate of biodegradation. Finally, no inflammatory response was observed during the in vivo imaging (see Figure 1C and D), which shows that the scaffolds are biocompatible and their degradation by-products do not cause any additional immune response.

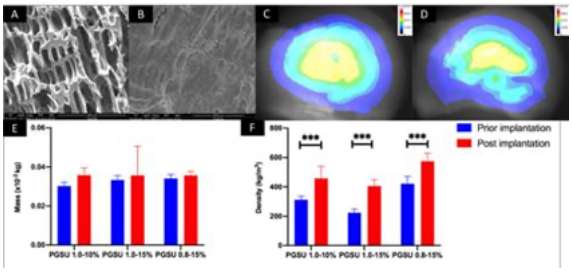

Fig. 1: (A) Representative cross section microstructure of a PGSU scaffold prior implantation, (B) and 42 days post implantation; (C) whole body imaging of the mouse with the positive control, (D) and of the mouse with PGSU scaffold 42 days post implantation; (E) mass and (F) density of the PGSU scaffolds prior and post implantation. *** *when* $p < 0.001$.

DISCUSSION & CONCLUSIONS: Large PGSU scaffolds were synthesized, fabricated and implanted subcutaneously into a mouse model for six weeks. The scaffolds demonstrated excellent integration with the surrounding tissue, and it promoted tissue ingrowth. At the same time, the scaffolds began degrading at a similar rate to the tissue development which is the ideal characteristic of a scaffold for the purpose to replace a damaged tissue. PGSU was shown in vivo to be biocompatible, biodegradable and promote tissue ingrowth at a rate that synchronizes with its degradation.

REFERENCES: [1]Sang Y. et al., ACS Appl. Mat. and Inter. 10(11):9290-9300, 2018 [2]Samourides A. et al., Mat. Sci. and Eng.: C, 108, 110384, 2020 [3]Samourides A. et al., Appl. Sci., 11(11), 5004, 2021

ACKNOWLEDGEMENTS: This work was co-funded by the European Regional Development Fund and the Republic of Cyprus through the Research and Innovation Foundation (Grant number: OPPORTUNITY/0916/MSCA/0017).

Assessing Mechanical Degradation of Porcine Tissue

M Ruding[1, 2], M Fossum[2], J U Lind[1]

[1]*Department of Health Technology, Technical University of Denmark, Denmark*
[2]*Labratory of Tissue Engineering, Rigshospitalet, Denmark*

INTRODUCTION: Autologous micrografting, a theory proposed by Meek in 1958, describes the concept of increasing the surface area (i.e. wound edges) of graft tissue to increase the rate of wound expansion [1]. Previous work has examined the expansion potential of micrografts fragmented from centimeters to millimeters [2]. Little is known, however, about how fragmentation on a micrometer scale can promote proliferation. This study aims to evaluate the proliferative potential of porcine endothelial micrografts when mechanically degraded to the micrometer scale.

METHODS: Porcine bladder harvesting: Tissue samples were collected from adult, female pigs used for a butchering course at ZBC Roskilde. After euthanization, bladder samples were collected through a midline incision and immediately washed in PBS (phosphate buffered saline, Sigma-Aldrich, St. Louis, US). Samples were transported on ice and suspended in 1×DMEM (Dulbecco's Modified Eagle Medium, Sigma-Aldrich) and antibiotics (penicillin 50 U/mL, streptomycin 50 µg/mL, and amphotericin B 2 µg/mL, Invitrogen, Thermo Fisher Scientific, Waltham, US). The bladder walls were pinned to sterile cutting boards to isolate the epithelium, which was cut into small fragments of approximately 2 mm^2 size and used in experiments or plated as control groups.

Mechanical degradation: Tissue dissociation was achieved using the FFX TissueGrinder (TG, Fast Forward Discoveries GmbH, Frankfurt, Germany). Pre-washed and cut tissue was loaded with 500 µL 1×DMEM into the inner circle of the TG's rotor. The stator and 40 µm cell strainer were placed on the rotor and enclosed in a 50 ml Falcon tube. The grinding process parameters were modified from the manufacturer's protocols (Table 1).

Table 1: Steps of the TG protocol used to process porcine tissue.

Process	Speed (rpm)	Duration (sec)
Cutting	+ 8	30
Grinding	- 8	30
Cutting	+ 15	40
Grinding	- 15	40
Cutting	+ 10	30
Grinding	- 10	30

After the protocol, the Falcon tube was inverted, the rotor was removed from the stator and sieve, and 100-200 µL of remnants were collected from the filter. The sample was resuspended and pipetted into 12-well plates for analysis and culture.

RESULTS: The imaged tissue, taken directly after degradation by the TG, show a heterogeneous fragment population (Fig. 1).

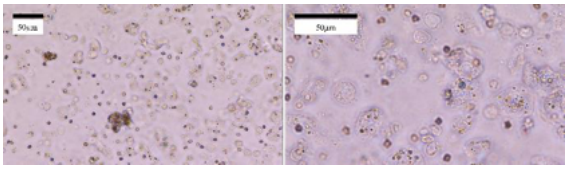

Fig. 1: Microscope images of processed tissue taken with 20X (left) and 40X (right) objectives on Day 0.

The tissue fragment sizes were estimated in ImageJ [3], assuming they were roughly spherical, to calculate the size distribution (Fig. 2).

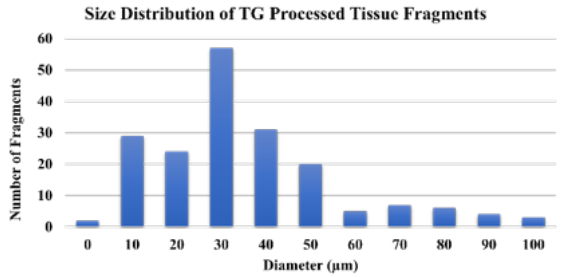

Fig. 2: Distribution of the diameter of processed tissue fragments up to 100 µm.

After nine days, endothelial cells and fibroblasts were observed in wells of TG samples (Figure 3) and little to none were observed in control groups.

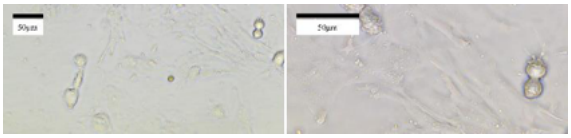

Fig. 3: Microscope images of processed tissue taken with 20X (left) and 40X (right) objectives on Day 15.

DISCUSSION & CONCLUSIONS: Porcine bladder epithelium mechanically processed by the TG showed rapid dissociation and proliferation in culture. Further investigation is needed to assess the reproducibility of this method with porcine bladder epithelium and additional tissues.

REFERENCES: [1]C.P. Meek (1958) *Am. J. Surg.* **96**, 557-558. [2]N. Juul et al (2023) *Sci Rep* **13**, 13589. [3]C. A. Schneider et al (2012) *Nature Methods*, 9(7), 671–675.

ACKNOWLEDGEMENTS: CAG Regenerative Medicine for Urogenital Surgery and Fertility (SURF). Special thanks to ZBC Roskilde and Rie Jensen.

Comparative Study of Coating Textile Knitted Scaffolds with Human Bone Powder vs. Hydroxyapatite Powder for Bone Regeneration

M Persson[1], J Holopainen[2], M Skrifvars[3], J Tuukkanen[4], PP Lehenkari[4]

[1] *Department of Textile Technology, Faculty of Textiles, Engineering and Business, University of Borås, Sweden*

[2]*Department of Chemistry, University of Helsinki, Finland*

[3]*Swedish Centre for Resource Recovery, Faculty of Textiles, Engineering and Business, University of Borås, Sweden*

[4]*Research Unit of Translational Medicine, Anatomy and Cell Biology, Faculty of Medicine, University of Oulu, Finland*

INTRODUCTION: Hydroxyapatite (HA), which closely mimics the structure of natural bone mineral, has been extensively researched for its potential in bone regeneration [1,2]. The mineral has shown promise in enhancing the osteoconductive properties of implant surfaces, making it a focal point in the field of orthopaedic and dental implants [1]. The present study aims to contribute to this body of research by conducting a comparative investigation into the coating of textile scaffolds with human-derived bone powder and commercially available HA powder. The primary objective is to explore potential differences in the source of the mineral and its implication for bone regeneration.

METHODS: Knitted spacer fabric made of Poly(lactic acid) (PLA) monofilament were coated with powder derived from human femoral heads or commercially available hydroxyapatite (< 200 nm, Sigma-Aldrich Co) in an ethanol (EtOH) solution using an ultrasonic bath. Prior to the coating, the knitted PLA scaffold underwent surface activation using 1M NH_3. The distribution and characteristics of the coating were analysed using scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR). Subsequently, the cellular attachment was assessed through *in vitro* screening of bone marrow-derived human mesenchymal stem cells (hMSCs) at various time points using fluorescence and confocal microscopy. The differentiation of the hMSCs into osteoblast was evaluated by measuring the alkaline phosphate activity (ALP).

RESULTS: The findings of this study demonstrate the uniform distribution of the coating on the knitted PLA scaffold, as confirmed by SEM and FTIR analysis. Furthermore, the knitted PLA scaffold when coated with human-derived bone powder or HA, supported the attachment and the proliferation of hMSCs (Fig. 1). Additionally, the comparable osteoconductive properties of HA and human-derived bone powder were substantiated through ALP activity assessment.

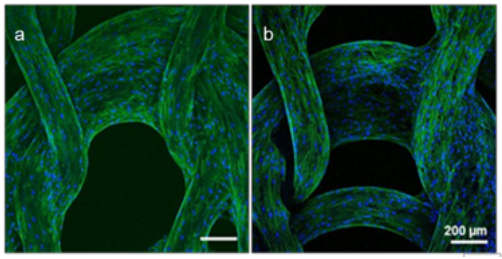

Fig. 1: Morphology of hMSCs cultured in osteogenic induction medium after 21 days as visualized by fluorescent staining of actin filaments (green) and nuclei (blue, a) Human derived bone powder and b) HA coated knitted PLA scaffold. Bar = 200 µm

DISCUSSION & CONCLUSIONS: The results from this study revealed that both human-derived bone powder and commercially available HA effectively enhanced the osteoconductive properties of the knitted PLA scaffold. Notably, no significant difference was observed between the two apatite powders in terms of their osteoconductive effects. However, a notable distinction was identified in the particle morphology of the two types of powder. Specifically, the commercially available HA exhibited a spherical shape, whereas the human-derived bone powder displayed a more irregular shape. Given the substantial impact of particle morphology and topography on cell attachment and proliferation [1], it is advisable to further optimize the shape and size of the particles for a more comparable study in the future.

REFERENCES: [1]M Persson et al (2014) *Colloids Surf. B*, **121**:409-416. [2]M Persson et al (2018) *Sci Rep* **8**:10457.

ACKNOWLEDGEMENTS: This work has been performed in the project Scaffolds tissue Engineering which belongs to MATERA Era-Net program and is financed by national agencies TEKES and MIUR

Antimicrobial activity of Ag/SiO_2 flame-made nanoparticles

M. Samara[1], V. Tsikourkitoudi[1] and G.A. Sotiriou[1]

[1]*Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, SE-1 71 65 Stockholm, Sweden*

INTRODUCTION: The global rise in antibiotic-resistant bacteria, primarily fueled by the overuse and misuse of antibiotics, presents a pressing public health concern, necessitating innovative approaches to combat infections. Nanoparticles, offer a promising approach against antibiotic-resistant infections. Silver nanoparticles (Ag NPs), due to their small size, are extensively studied for their ability to increase the bacterial outer membrane permeability, interact with intracellular components, and release Ag^+ ions, leading to destabilization and cell death [1]. Additionally, the incorporation of silica (SiO_2) into composite Ag/SiO_2 nanoparticles enhances their stability, prevents their agglomeration, and augments their antibacterial properties [2].

METHODS: In this study, Ag/SiO_2 nanoparticles were produced by flame spray pyrolysis (FSP), a scalable and reproducible aerosol-based method. Varied Ag content (0, 20, 40, and 60 %wt) was achieved while maintaining a constant total metal concentration. Thorough analysis involved specific surface area (SSA) measurement via N_2 adsorption, crystallite size determination using X-ray diffraction patterns, and structural analysis through Transmission electron microscopy. The release of Ag^+ ions at different incubation timepoints was quantified. Moreover, the *in vitro* assessment of antibacterial effectiveness against *Methicillin-resistant Staphylococcus aureus* (MRSA) involved colony-forming units (CFUs) enumeration method (Fig. 1), covering nanoparticle concentrations ranging from 12.5 to 100 µg/ml. To evaluate practical application in physiologically relevant context, NPs *ex vivo* testing was performed on porcine skin wounds infected with MRSA. As final formulation, the nanoparticles were incorporated into 4% w/w methylcellulose solutions. The antimicrobial activity and zone of inhibition against MRSA were assessed using the disk diffusion assay.

RESULTS: Ag/SiO_2 nanoparticles demonstrated a notable reduction in CFUs/ml compared to SiO_2, positioning them as promising nanoantibiotic agents. The study reports a dose-dependent antibacterial efficiency of Ag/SiO_2 nanoparticles, with enhanced antibacterial efficacy observed in nanoparticles with higher Ag content. Remarkably, Ag/SiO_2 nanoparticles displayed antibiotic effects comparable to, and in some cases, surpassing those of *vancomycin*—an established MRSA antibiotic.

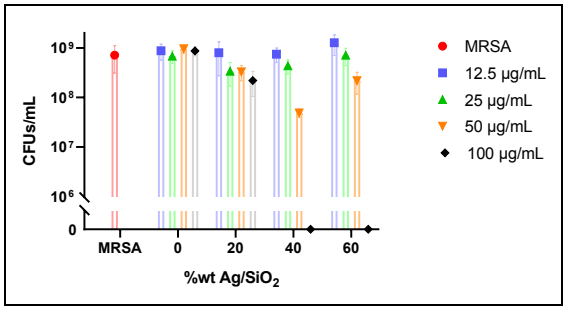

Fig. 1:Mean values and standard deviations of the number of MRSA CFU/mL (log 10), obtained by the CFU enumeration method of xAg/SiO2 NPs treatment, in four different concentrations.

DISCUSSION & CONCLUSIONS: Overall, this study underscores the heightened potential of nanoparticle-mediated therapeutic strategies utilizing inorganic nanoparticles in the fight against antibiotic-resistance infections. These findings hold promise for addressing the critical issue of antibiotic resistance, both in controlled laboratory settings and within physiologically relevant conditions.

REFERENCES: [1]T. Bruna et al (2021) *Int. J. Mol. Sci.* 22 (13):7202. [2]D. Mosselhy et al (2017) *Nanomaterials*, 7 (9):261

ACKNOWLEDGEMENTS: Fundings from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (ERC Grant agreement n° 758705), from Karolinska Institutet Faculty Board, Swedish Research Council (nrs 2018-05798, 2021-02059 and 2021-05494), Torsten Söderberg Foundation (M87/18) and Swedish Foundation for Strategic Research (FFL18-0043 and RMX18-0041) are kindly acknowledged.

Enhancing Hemoglobin-Based Oxygen Carriers: Insights from Tannic Acid Interaction and Metal Ion Influence

M Nadimifar[1,2], L Hosta-Rigau[1]

[1]*Institute of Biochemistry and Biophysics, University of Tehran, Iran*

[2]*Department of Health Technology, Technical University of Denmark, Denmark*

INTRODUCTION: In addressing challenges associated with the standard transfusion of donor blood, including limited availability, disease transmission risk, and delays due to blood typing requirements, the development of hemoglobin-based oxygen carriers (HBOCs) has emerged. HBOCs, leveraging hemoglobin (Hb), aim to replicate the oxygen-carrying capacity of blood without the constraints of blood typing. Despite attention on encapsulating Hb in micro- and nanosized carriers, achieving high Hb loading remains a challenge [1]. A recent one-step self-assembly method in water, incorporating polyethylene glycol (PEG), phenolic ligands, metal ions, and bioactive macromolecules, presents a promising approach to synthesize functional protein nanoparticles, addressing drawbacks in conventional blood transfusions [2]. Notably, phenolic ligands, beyond their role in assembly, can interact with Hb, potentially altering its structure and function. Controlling these structural changes can offering potential improvements in product efficacy.

METHODS: Utilizing fluorescence spectroscopy, we investigated the interaction between Tannic acid (TA), a key phenolic component in current Hb nanoparticles (Hb-NPs), and Hb. The nature of this interaction was probed using the Stern-Volmer and van't Hoff equations, while computational analysis provided deeper insights. Hb-NPs were synthesized through a one-pot assembly method, incorporating various concentrations of PEG, manganese (Mn^{2+}), and TA into a Hb suspension. The impact of different Mn^{2+} concentrations on Hb functionality was examined using an oxygen meter, revealing potential alterations in Hb functionality due to increased interaction opportunities between TA and Mn^{2+}.

RESULTS: Upon the addition of TA, fluorescence emission spectra exhibited quenching. Analysis using the Stern–Volmer equation unveiled a static quenching process dominated by the formation of a compound between Hb and TA. The apparent binding constant (K_A) increased with temperature, indicating robust and temperature-stable binding, with a probable single binding site near the β-37 Trp residue. Thermodynamic analysis disclosed spontaneous and endothermic binding, primarily propelled by hydrophobic interactions. Docking simulation depicted TA's preference for interacting with hydrophobic residues of Hb, particularly the β-37 Trp residue, emphasizing its capability to trap Hb clusters through hydrophobic interactions. The preservation of Hb's function was observed by increasing Mn^{2+} concentrations.

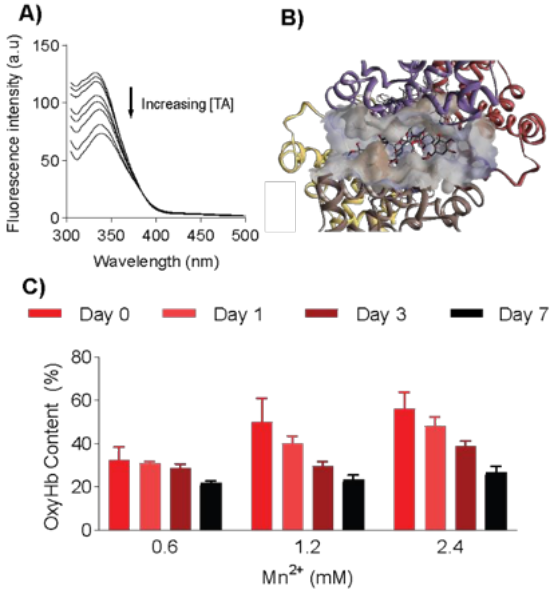

Fig. 1: A) TA quenching effect on intrinsic Hb fluorescence. B) Docking simulation of Hb and TA. C) OxyHb content of Hb-NPs fabricated using three different concentrations of Mn^{2+}.

DISCUSSION & CONCLUSIONS: While Hb-NPs consistently preserved Hb's functionality through multiple oxygenation and deoxygenation cycles, the O_2 loading capacity was influenced by Mn^{2+} concentration. Higher Mn^{2+} concentrations led to an augmented oxyHb content, proposing a potential effect from reduced TA availability to interact with Hb at elevated Mn^{2+} levels.

REFERENCES: [1]M.M.T. Jansman (2018) *Adv Colloid Interface Sci*, **260**:65–84. [2]J. Chen et al (2022) *Adv Mater*, **34**:2108624.

ACKNOWLEDGEMENTS: We express our gratitude to 3B's group members at DTU for their valuable collaboration during this research.

Coaxial nanofibrous wound dressing with antibacterial and photothermal flame-made nanoparticles

Morten Klabunde[1], Reshma Vasantha Ramachandran[1], Georgios Sotiriou[1]

[1] *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Sweden*

INTRODUCTION: Nanofibers have found significant applications in diverse fields such as tissue engineering, drug delivery, wound dressings, energy cells, and textiles. Among the various techniques that exist to produce nanofibers, electrospinning offers a significant advantage of tuneable fiber characteristics such as diameters and morphologies, while being cost-effective. Coaxial electrospinning is a modification of this technique that can be used to fabricate core-sheath nanofibers [1]. They can be used for encapsulating cargo with a controlled release rate. Here we have developed a core-sheath nanofibrous anti-bacterial wound dressing. The sheath consists of silver-silica nanoparticles (Ag/SiO_2 NPs), which possess anti-bacterial characteristics [3] that targets wound infections- a phenomenon that significantly impedes the healing process. On the other hand, the core contains photothermal gold-silica (Au/SiO_2 NPs) that provide localized heating in response to infrared radiation- a process known to aid in wound healing [2].

METHODS: Ag/SiO_2 and Au/SiO_2 NPs are produced using flame spray pyrolysis (FSP) where silver acetate and gold acetate respectively are used as precursor materials alongside a combination of acetonitrile and 2-ethylhexanoic acid [4]. These particles are further incorporated into core-sheath fibers using the electrospinning setup Fluidnatek LE-50. The heat response was obtained using a thermal camera (Ti480 Pro, Fluke) under laser irradiation at 808 nm.

RESULTS: The presence of both the antimicrobial Ag/SiO_2 NPs as well as the photothermal Au/SiO_2 NPs renders the core-sheath fibers multifunctional. We demonstrate the anti-bacterial effect against Methicillin-Resistant Staphylococcus Aureus (MRSA)- a pathogen known to cause wound infections. Further, we validate the photothermal effect of the membrane by near-IR laser irradiation.

DISCUSSION & CONCLUSIONS: Wound infections and removal of wound dressings remain critical challenges to overcome in this domain. We present a step towards this by producing a core-sheath nanofibrous membrane using the cost-effective industrial manufacturing processes of flame spray pyrolysis and electrospinning. The membrane initially targets the wound infections using the anti-bacterial particles encapsulated in its sheath while the photothermal particles in the core structure enables localized heating which is known to assist in wound healing.

REFERENCES: [1]P. Rathore et al (2021) *ACS Applied Materials & Interfaces*, **13 (1)**:48-66. [2]J. Ziesmer et al (2022) *Adv Materials Interfaces*, **9 (34)**. [3]F. J. Geissel et al (2021) *Journal of Colloid and Interface Science*, **608 (3)**: 3141-3150. [4]G. A. Sotiriou et al. (2010) *Environ. Sci. Technol*., **44** (14) 5649–5654.

ACKNOWLEDGEMENTS: Fundings from the Karolinska Institutet Board of Research (no. C114766133) and the European Research Council (no. 758705), are kindly acknowledged.

Development of Multifunctional Self-Healing Sensors for Enhanced Wearable Health Monitoring

Morteza Alehosseini[1], Tiberiu Gabriel Zsurzsan[2], Jon Spangenberg[3], Firoz Babu Kadumudi[1] and Alireza Dolatshahi-Pirouz[1]

[1]*Department of Health Technology, Technical University of Denmark, 2800 Kgs, Lyngby, Denmark*
[2]*Department of Electrical Engineering, Technical University of Denmark, 2800 Kgs, Lyngby, Denmark*
[3]*Department of Mechanical Engineering, Technical University of Denmark, 2800 Kgs, Lyngby, Denmark*

INTRODUCTION: Wearable health monitoring devices due to the variety of qualities that they offer have become the prime focus in modern health care. These bioelectronic devices are flexible, skin conforming, portable, durable, highly sensitive, and can provide real-time monitoring.

They have proved to be highly useful in numerous cases such as patients going through the rehabilitation process, in the evaluation of the post-surgery performance of athletes, translation of sign language for individuals with inability to hear, early diagnosis and prognosis of certain illnesses such as Parkinson disease and also in sports performance and human-machine interfaces for robotics [1]. The strain sensors can also be widely applied in sports performance and human-machine interfaces for robotics. Flexible wearable sensors are the key point for monitoring motion and physiological signals and daily activity. The current studies on flexible and stretchable strain sensors have hardly succeeded to cover the important characteristics of the next generation of wearable electronics for rehabilitations [2]. These features involve low cost, high sensitivity, high elasticity, self-healing capability, and multi-functionality. Personalized cheap and accurate wearable electronics for human motion monitoring can help patients and doctors for rehabilitation.

METHODS: Here a sensitive, self-healable, and elastic strain sensor is developed by embedding nanomaterials along with a conductive polymer, PEDOT PSS. The sensors are sensitive to strain, pressure, and temperature and can achieve high stretchability up to 600% and great durability due to the self-healing properties.

RESULTS: The electrical properties, sensitivity, and mechanical properties of the materials were tested at different strains. The conductivity of the materials goes up to 10^{-3} S/cm while showing sensitivity to different temperatures between 15 ºC to 60 ºC based on the mechanical test. It can heal itself more than 95% in less than 3min. Sensitivity to pressure was measured too and it shows significant changes in conductivity when a wide range of pressures from 1kPa to 1MPa was applied to the sensor.

DISCUSSION & CONCLUSIONS: In this study, we developed a low cost and accurate and self-healable sensor for healthcare monitoring. We expanded the functionality of the material to also monitoring temperature of the body. This sensing material was also highly durable because of self-healing properties.

REFERENCES:

[1]Sepehr T. et al., Adv Science. 6: 1801664, 2019

[2]Firoz B.K. et al., Adv Science. 6: 1801241, 2019

Lactic acid grafted microcrystalline cellulose loaded membranes for guided bone regeneration

Probal Basu and Hanna Tiainen
Institute of Clinical Dentistry, Department of Biomaterials,
University of Oslo, Norway

INTRODUCTION: Cellulose based biomaterials exhibit important properties such as porosity, elasticity that makes them exciting for guided tissue regeneration (GTR) application [1]. Biomaterials for guided tissue regeneration need to have a balance between material stiffness, elasticity, and flexibility to maintain their barrier property. On the other hand, they should maintain their structural properties such as porosity, biocompatibility and have antibacterial properties [2]. Most of the reported GTR specific biomaterials/membranes compromise structural properties such as porosity for strength and typically lack antibacterial properties. The current work focuses on the development of lactic acid (Lac) biofunctionalized microcrystalline cellulose (MCC) reinforced membrane for GTR. .

METHODS: The MCC was biofunctionalized through lactic acid (Lac) grafting (MCC: Lac = 1:20, 1:23, 1:25, 1:30 w/w) and they were characterized based on physical and chemical properties using Fourier-transform infrared spectroscopy (FT-IR), zeta potential and scanning electron microscopy (SEM). The membrane was prepared by solvent casting by mixing different ratios of MCC-Lac with chitosan-aqueous acetic acid solution where 1.5% w/v chitosan was present. Other polymers like polyethylene glycol (PEG, 5% w/v) was also mixed and stirred for 10-15 min.In this mixture, genipin (0.07% w/v) is given as a biosafe crosslinking agent and stirred for 5-8 min. The polymer solution was then solvent casted in petri plates and incubated at 24 h at 37 ºC. Finally, MCC-Lac reinforced chitosan membrane was obtained.

RESULTS: Lactic acid biofunctionalized MCC was successfully prepared through grafting. The degree of functionalization and zeta potential of the grafted samples are as follows: MCC-Lac: 1:30 > MCC-Lac: 1:25 > MCC-Lac: 1:23 > MCC-Lac: 1:20. The SEM and FTIR study indicated notable grafting of lactic acid on the surface of MCC. The MCC-Lac= 1:30 samples was utilized to prepare chitosan-based biofunctionalized membrane. The prepared membrane was freeze-died and characterized on the basis of physiochemical properties. The FTIR analysis indicated the successful grafting of Lac on MCC, and presence of chitosan. The membrane also exhibits notable internal porosity. Additionally, SEM study indicated significant porous structures. (Fig. 1).

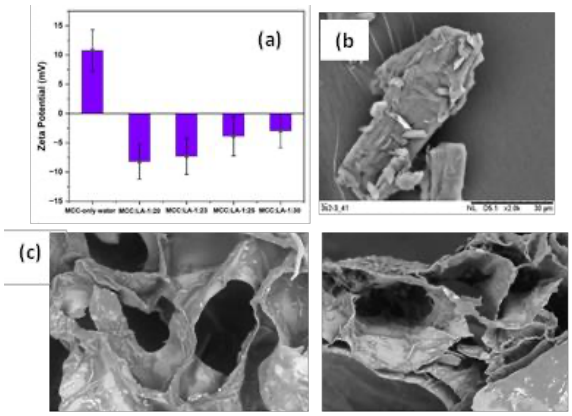

Fig. 1: (a) Zeta potential and (b) SEM of biofunctionalized MCC: Lac=1:30 and (c) MCC-Lac= 1:30 reinforced chitosan based membrane.

DISCUSSION & CONCLUSIONS: MCC is being considered as a significant reinforcement filler material [3]. Lac has antibacterial property. The major objective of biofunctionalization of MCC with Lac is to prepare highly efficient reinforcement filler with the antimicrobial property for the developed membrane. MCC-Lac: 1:30 exhibited high degree of biofunctionalization that is indicated through zeta potential, FTIR analysis. The high degree of biofunctionalization resulted from the higher degree of Lac grafting. Hence, this sample was used to prepare the membrane. The SEM images of the membrane indicated significant macroporous structure. These are important for infiltration and growth of cells which is necessary for the guided tissue regeneration.

REFERENCES: [1]Lee et al., 2015, J Adv Prosthodont., ***7***(6): 484-495. [2]Yang et al., 2022, Front. Bioeng. Biotechnol., ***10***:921576. [3]Ashori A and Nourbakhsh A. 2010, Composites Part B: Engineering**.,** ***41***(7), 578-581.

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 101108847.

Textile scaffolds of P(3HB)/P(3HB-co-4HB) blend filaments

S Kopf[1], D Åkesson[1], M Skrifvars[1]

[1]*Swedish Centre for Resource Recovery, Faculty of Textiles, Engineering and Business, University of Borås, Sweden*

INTRODUCTION: Tissue engineering is a multidisciplinary field which aims to replace damaged or diseased tissue by artificial implants. Textile technology with its precise control of yarn placement and control of pore size can be one option to produce tissue engineering scaffolds [1]. Besides structural integrity through controlled yarn placement, textile technologies result in controlled anisotropy, mimicking the natural tissue [2]. The success of a textile-based scaffold does not only depend on its design but also the filaments and its properties that built up the final scaffold [3].

Poly(3-hydroxybutyrate) (P(3HB)) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) are biopolymers with excellent biocompatibility and degradation behaviour making them interesting candidates for biomedical applications [4].

This poster investigates the melt spinnability of P(3HB)/P(3HB-co-4HB) blend filaments and their degradation in isotonic media comparable to the human body. Additionally, the filament's potential for textile-based scaffold formation is investigated.

METHODS: The filaments were produced by melt spinning of a P(3HB)/P(3HB-co-4HB) blend (57 mol%/ 43 mol% with a 4HB content of 30 mol% in the copolymer). Tensile testing and differential scanning calorimetry (DSC) was conducted on undrawn filaments before and after seven weeks immersion in phosphate buffered saline solution (PBS) at 37°C. The PBS had a pH value of 7.4 and was changed weekly. To investigate the processability of the filaments a tube was knitted on a small circular knitting machine.

RESULTS: The filaments possessed a degree of crystallinity of around 53% as well as a tensile strength of 21.5 ± 2.3 MPa at an elongation at break of 341 ± 168% and a Young's modulus of 862 ± 168 MPa before the immersion in PBS. Seven weeks after immersion in PBS, no gravimetric weight loss could be detected. However, especially the filament's elongation at break deteriorated dramatically from around 341% to 6.7% after being exposed to PBS. The tensile strength decreased by around 5.5 MPa whereas the Young's modulus increased by 178 MPa. Additionally, DSC showed a slight increase of crystallinity to 60%. To investigate the processability of the filaments into a textile, a small tubular knit was produced (Fig. 1).

Fig. 1: Tubular knitted structure from P(3HB)/P(3HB-co-4HB) blend filament.

DISCUSSION & CONCLUSIONS: To our knowledge, there are no other reports of P(3HB)/P(3HB-co-4HB) blend filaments, thus the mechanical properties before degradation are compared to pure P(3HB-co-4HB) filaments with a 4HB content of 4 mol% [5]. Our filaments are in average 5 times stiffer while exhibiting a 1.5 times higher elongation at break as compared to the P(3HB-co-4HB) filaments. Only the tensile strength of our filaments is half of the pure P(3HB-co-4HB) filaments which can be explained due to the lack of filament drawing and thus molecular alignment in our case. A filament drawing would of course influence the other mechanical parameters and the degree of crystallinity. The deterioration of mechanical properties during aging is because of the faster degradation of the amorphous segments as the crystalline parts of the polymer, which mainly contribute to tensile strength, have a higher resistance to hydrolysis [6]. This is reflected by the increased tensile strength and degree of crystallinity of our filaments and in alignment with Vodicka et al.'s results during the degradation of P(3HB-co-4HB) in artificial body fluids [6]. The textile processability of the filament is shown by the knitted tube, which is promising for the pending textile-based scaffold.

REFERENCES: [1]C. Jiang et al (2021) *Acta Biomater*, **128**:60-76. [2]M. Akbari et al (2014) *Adv Funct Mater* **24**:4060-7. [3]A. Doersam et al (2022) *Adv Mater Technol*, **7**:2101720. [4]C. Gajjar et al (2014) Resorbable Fiber-Forming Polymers for Biotextile Applications, [5]T. Omura et al (2021) *ACS Appl Polym Mater*, **3**:6479-6487 [6]J. Vodicka et al (2022) *Polymers*, **14**:1990 7

ACKNOWLEDGEMENTS: The authors would like to acknowledge Maria Persson for fruitful discussions and Stefan Gustafsson for knitting the sample.

Automated 3D Printing of Micro-structured Substrates for Rapid Generation of Anisotropic Skeletal and Cardiac Muscle Tissues

S. Butdayev[1], S. Leone[1], S. S. Keller[2], J. U. Lind[1]

[1]*Department of Health Technology, Technical University of Denmark, Denmark*

[2]*National Centre for Nano Fabrication and Characterization, Technical University of Denmark, Denmark*

INTRODUCTION: Anisotropic organization of cells and extracellular matrix is a common hallmark for several types of mammalian tissues, not least striated muscle[1,2]. In the absence of unidirectional cellular alignment skeletal myofibers developed in vitro from myoblasts thus tend to be orders of magnitude shorter than their in vivo counterparts. Similarly, cardiac muscles engineered in vitro that are not anisotropic fail to replicate the end-to-end electrical coupling through gap junctions between cells, which is a crucial aspect of in vivo myocardial electrophysiology[3]. Anisotropy is therefore an essential feature to replicate when producing physiologically relevant models of cardiac as well as skeletal muscle. Several methods have been introduced for producing tissue culture substrates with microscale cues to direct the self-assembly of tissues into anisotropic architectures[4,5]. However, methods such as microcontact printing and micro-molding are time-consuming and laborious due to their processing times and manual steps. Here, we show a rapid and automated 3D printing procedure for fabricating micro-structured tissue culture substrates. The method relies on the fabrication and use of macroscopic printing nozzles containing micro-scale tip features that are transferred to the extruded surface during deposition. The procedure therefore relies on the shear-thinning and thixotropic properties of the ink materials.

METHODS: The conventional plastic nozzles were patterned through a hot embossing process on a silicon wafer featuring defined micro-sized ridges. The resultant patterned nozzles were employed in the 3D printing of extrudes, facilitating the transfer of structures onto the surface of the extrudes during the printing process. Subsequently, both human and murine cells were seeded on the patterned substrates, and the alignment of the cells along the established structures was evaluated through microscopy imaging.

RESULTS: The examination of the nozzle end and extruded surface by using optical profilometer measurements proved the successful replication of patterns from the wafer onto the substrates. Consequently, the cells cultivated on these structured surfaces exhibited the formation of anisotropically aligned monolayers.

DISCUSSION & CONCLUSIONS: We demonstrate that in-situ micro-patterned substrates provide sufficient topographical cues for generating unidirectionally aligned cultures of striated muscle tissue. We also demonstrate how micro-structured nozzles for the procedure can be conveniently produced in a low-tech setup by hot embossing of conventional plastic nozzles. Our method provides a route for automated fabrication of complex culture substrates and devices while decreasing the overall fabrication time.

REFERENCES: [1]R. H. Anderson et al (2019) J. Anat., **235**:697-705. [2]I. J. LeGrice et al (1995) Am. J. Physiol. -Hear. Circ. Physiol., **269**:38-2. [3]S. J. Park (2019) Circulation, **140**:390-404. [4]M. L. McCain et al (2014) Biomaterials, **35**:5462-5471. [5]A. W. Feinberg (2007) Science, **317**:1366-1370

ACKNOWLEDGEMENTS: S.B., S.L., S.S.K., and J.U.L. would like to gratefully acknowledge The European Commission MSCA-IF (798820), the Lundbeck Foundation (R250-2017-1425), The Independent Research Fund Denmark, Sapere Aude Starting Grant (8048-00050), and the Novo Nordisk Foundation (NNF21OC0066994).

Optimizing Alginate Blends for Stable Microbead Formation with Controllable Size

S Parvaneh[1,2,3,4], D Wagner[1,2,3,4,5]

[1]Lung Bioengineering and Regeneration, Department of Experimental Medicine, Faculty of Medicine, Lund University, Lund, Sweden, [2]Wallenberg Center for Molecular Medicine, Lund University [3]Lund Stem Cell Center, Lund University [4]NanoLund, Lund University [5]McGill University, Departments of Medicine and Biomedical Engineering

Lung Bioengineering and Regeneration

INTRODUCTION: Microencapsulation of biologics using biomaterials aims to secure and protect bioactive compounds for controlled release and is being extensively investigated across the pharmaceutical, cosmetics, and food industries. Hydrogel beads, especially those containing encapsulated agents, have gained considerable attention. Alginate, a naturally occurring polymer, has garnered widespread use in these technologies owing to its availability, cost-effectiveness, biodegradability, and biocompatibility. Different methods for microbead formation using alginate have been reported such as electrically driven droplet generators, emulsification, and microfluidics. However, their ultimate utility for certain applications, such as in encapsulating cells and molecules, is affected by the physical and mechanical characteristics of the resulting alginate hydrogel. Alginates used for the fabrication of hydrogels differ based on their composition (i.e. guluronic (G) and mannuronic(M)) and chain length. These parameters impact the stability of the resulting hydrogel based on the crosslinking approach used. However, the use of single types of alginates can be a limiting factor as the availability of alginates with both the chain length and G/M ratio needed for specific applications can be limited or cost-prohibitive. Our research aimed to optimize a blend of two alginate types (low and high molecular weight (LMW and HMW) and low/high G/M to fabricate alginate beads that exhibit mechanical and structural stability when fabricated using a water/oil emulsion fabrication system.

METHODS: The emulsion of water in oil protocol [1] was employed alongside Ca-EDTA internal gelation [2], utilizing a design of experiments approach to determine the optimal ratio of different sodium alginates and crosslinking approach. Acetic acid in oil was used to induce internal gelation via calcium release from Ca-EDTA or Ca-CO3.

RESULTS: A combination of 4% LMW, high G/M and 1% HMW, low G/M with 50 mM Ca-EDTA resulted in significantly more stable microbeads than individual use, even at higher concentrations. Stirring speeds of 1000 rpm produced >80% circular microbeads with a diameter of <500µm. 2% v/v acetic acid in oil for 10 minutes was sufficient for crosslinking and internal gelation.

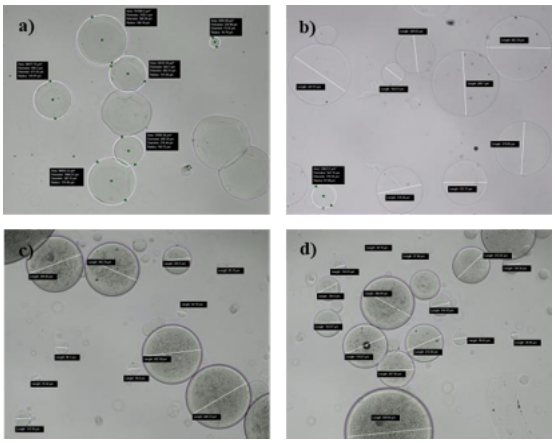

Fig. 1: Sodium alginate microbeads: a) LFR 4%; b) CR 1%; and c and d) LFR 4%+CR 1%.

DISCUSSION & CONCLUSIONS: A combination of alginate characterized by a high G/M ratio and HMW facilitates the creation of stable and flexible microbeads. Employing Ca-EDTA as a calcium source instead of the commonly used CaCO3 enhances solubility without inducing CO2 release in the gelation process. A brief pH change during internal gelation could be an effective strategy for preventing cell death in cell encapsulation but requires further optimization. Next, we will implement this protocol within a microfluidic system for cell encapsulation to illustrate its applicability for cell transplantation.

REFERENCES: [1]Hoesli, C.A et al (2017) *J. Vis. Exp.* (**124**), e55280**.** [2]N Paiboon et al (2023) *j. foodhyd.108712*

ACKNOWLEDGEMENTS: European Research Council (ERC) under the Horizon 2020 research and innovation program (grant agreement No 805361). Lund University and the Strategic Research Area “Emerging Research Topics”

PEGDA-Based PolyMIPE and Hyaluronic Acid Stabilized Gold Nanoparticles Composites for Bone Tissue Engineering

S. Akbari[1,2*], V.S. Parihar[1], J. Hyttinen[2], M. Kellomäki[1]

[1]*Biomaterials and Tissue Engineering Group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland*

[2]*Computational Biophysics and Imaging Group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland*

INTRODUCTION: Polymerized medium internal phase emulsions (PolyMIPEs) are macroporous materials have attracted interest in tissue engineering as highly porous scaffolds to support cell proliferation. The polymerized high internal phase emulsions (PolyHIPEs) developed through water-in-oil (W/O) emulsions are usually hydrophobic in nature [1] and therefore in this work we aimed to use medium internal phase emulsions (MIPEs) to increase the hydrophilicity by using a hydrophilic polymer poly (ethylene glycol) diacrylate (PEGDA) through an oil-in-water (O/W) emulsion. To improve the bioactivity and cell attachment to the scaffolds we loaded the scaffold into the hyaluronic acid (HA) coated gold nanoparticles (HA-AuNPs).

Gold nanoparticles have demonstrated promising applications in tissue engineering due to their ease of synthesis, size controllability, surface plasmon resonance (SPR), and biocompatibility [2]. Recently, it was discovered that AuNPs can regulate cell differentiation and promote the regeneration of bone and cartilage tissue [3].

In this study, we synthesized a PEGDA-based polyMIPE composite incorporating HA-AuNPs. The resulting composite scaffold will be tested for its effectiveness in bone tissue engineering.

METHODS: The polyMIPE was fabricated via thermal polymerization of PEGDA in the internal phase at 70°C for 24 hours followed by washing with ethanol and water and drying. HA-stabilized AuNPs were synthesized by in-situ reduction method, briefly, the gold chloride solution in DI water was refluxed for 2 hours then the temperature was brought down to 40°C followed by the addition of HA- gallic acid (GA) solution with pH 8.5 and stirred at 40°C for 12 hours. Then the porous scaffold was loaded into the HA-AuNPs as shown in Fig. 1.

RESULTS: Hydrophilic porous polyMIPE materials were obtained with improved antioxidant activity after loading with HA-AuNPs, which supports the cell attachment and proliferation. The HA-AuNPs were obtained from the HA stabilized via polymer mediated reduction method and were characterized by dynamic light scattering (DLS). The average particle size was 150 nm and zeta potential were -32. The composites of polyMIPE-HA-Au-NPs will be tested for bone tissue engineering.

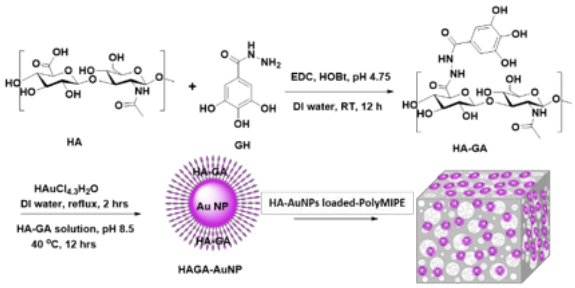

Fig. 1: Synthesis of HA stabilized gold nanoparticles and their composites with polyMIPE.

DISCUSSION & CONCLUSIONS:

The composites obtained from HA-functionalized AuNPs and polyMIPE displayed good antioxidant properties due to the presence of HA-AuNPs. In addition, polyMIPE exhibited good porosity, elasticity and swelling, which can be attributed to the emulsion's volume phase composition and the presence of hydrophilic PEGDA, respectively.

REFERENCES:

[1] B. Aldemir Dikici and F. Claeyssens. (2020) *Front. Bioeng. Biotechnol.*, **8**, 875

[2] S. Mandal *et al (2021) Appl. Radiat. Isot,* **69**, 12:1692–1697

[3] Y. Shi *et al (2021) Front. Chem,* **9**:724188.

ACKNOWLEDGEMENTS: S.A is thankful to the Institute of International Education's Scholar Rescue Fund (IIE-SRF) and Finnish National Agency for Education (EDUFI) for supporting this research at Tampere University. The authors are grateful to the Centre of Excellence in Body-on-Chip Research (CoEBoC) for the financial support.

Modification of Fluoride Ion Release Behaviour Dispersed in Dental Poly Methyl Methacrylate Material by Incorporating Polyethylene Oxide

Tianxiao Wang[1], Menghong Li[1], Ziyan Gu[1], Chengjuan Qu[1], Jia Wang[2], Wen Kou[1]

[1]*Department of Odontology, Umeå University, Sweden*
[2]*Department of Physics, Umeå University, Sweden*

INTRODUCTION: Poly Methyl Methacrylate (PMMA) is a commonly used dental material, which is often used to make artificial teeth and splints [1]. Sodium fluoride (NaF) is a commonly used anti-caries drug [2]. Therefore, it is a good idea to add NaF to PMMA, which can prevent dental caries by releasing fluoride ions. However, NaF is water-soluble, while PMMA is hydrophobic. Hence, NaF could not disperse well in PMMA, and it is not conducive to the release of fluoride ions. Therefore, we designed a strategy to add Polyethylene Oxide (PEO) [3], an amphiphilic and biocompatible material, to the mixture of PMMA and NaF to improve the release behaviour of fluoride ions.

METHODS: 10 g of PMMA and 3 g of PEO were mixed in acetone, and then 1 g, 2 g and 3 g of PEO were added to the PMMA/PEO mixture, respectively. The mixture was dried to form thin films. The surface and cross-section of the films were observed by scanning electron microscopy, and their element distribution and functional groups were analysed by energy dispersive spectroscopy and Fourier-transform infrared spectroscopy, respectively. The surface roughness and contact angle of the films were also measured. The fluoride ion release rate of the films was tested by immersion experiments and their *in vitro* biocompatibility was tested by cytotoxicity experiments.

RESULTS: In the images of the film surface morphology, compared with the film without PEO, the NaF clusters on the surface of the film with PEO added were smaller and more dispersed. In the cross-section images, NaF clusters were not only embedded on the surface of the films, but also evenly dispersed inside the films. Similarly, the clusters inside the film without PEO were also larger than those inside the film with PEO added. And there was no phase separation or delamination phenomenon between PMMA and PEO.Fourier transform infrared spectroscopy showed that the spectra of films without PEO added and films with different amounts of PEO added had the same bands, and the intensities of these bands were close.

The average surface roughness test results showed that the average roughness of the films decreased from nearly 0.8 μm to about 0.2 μm after adding PEO. Static contact angle test results show that adding PEO can reduce the film surface contact angle, and the more PEO added, the smaller the contact angle.

The immersion test results showed that adding PEO could significantly increase the release of fluoride ions. After adding 3 g of PEO, the fluoride ion release amount increased nearly 20 times.

Cytotoxicity test results showed that although the cytotoxicity of films containing PEO was greater than that of films without PEO, it was within an acceptable range [4].

DISCUSSION & CONCLUSIONS: It can be proved that PMMA and PEO were uniformly mixed together in this study, while NaF was dispersed in the form of clusters through the scanning electron microscopy images and Fourier transform infrared spectroscopy. Moreover, the addition of PEO could enhance the dispersion of NaF clusters, hence the clusters became smaller and more uniform, which decreased the surface roughness further. Moreover, since PEO is an amphiphilic material, it also decreased the contact angle by adding PEO. The PEO mixed in the films might provide a channel for the internal NaF clusters to release fluoride ions, thus significantly increasing the release of fluoride ions. The released fluoride ions might cause some cytotoxicity, but fortunately the cytotoxicity was within an acceptable range [4].

Above all, this experiment provides a strategy to combine PEO with PMMA, a commonly used dental material, to improve the release behaviour of fluoride ions. It is expected to endow dental appliances with anti-cariogenicity.

REFERENCES: [1]M.S. Zafar et al (2020) *Polymers* **12**:2299 [2]V.R. Nagireddy et al (2019) *Int J Clin Pediatr Dent*, **12**:484-90. [3]R. Emerine et al (2022) *AIMS Bioeng*, **9**:178-96 [4]A. Bruinink et al (2011) *Adv Biochem Eng*, **126**:117-52.

ACKNOWLEDGEMENTS: We would like to thank Sara Henriksson, András Gorzsás and Cheng Choo Lee, the senior research engineers in Umeå University for their help.

Effects of Temperature and pH in the Self-Assembly of Polyphenol Nanoparticles

V. Güsgen[1,2], A. C. del Valle[1,2], Qiaolin Deng[1,2], G. A. Sotiriou[1]

[1] *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm*
[2] *Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm*

INTRODUCTION: Natural polyphenol molecules exhibit several pharmacological properties relevant for preventing and treating diseases: antioxidant, anti-inflammatory, anticoagulant, and anticancer [1]. However, these natural phenolic compounds possess low stability and poor water solubility, essential for their clinical applications [2].

This study aims to optimize polymer-based nanoparticles' *water-in-water* synthesis process and particle growth control. This will enable reproducible synthesis in pharmaceutical product development and ensure the efficacy of the drug-delivery systems.

METHODS: Poly-quercetin nanoparticles (pQu-FA NPs) were synthesized via a one-step polymerization process of quercetin and folic acid in PB solutions at varying pH values. The NP samples were stirred or sonicated at different time lengths. Then, the generated NPs were suspended in ddH_2O for freeze-drying and consequently dissolved in various conditions to test for particle stability.

UV-VIS spectrometry characterized the nanoparticles to identify the characteristic profiles of quercetin and folic acid. Dynamic light scattering (DLS) was performed to assess the size distribution profiles of the pQu-FA NPs. Fourier-transform infrared spectroscopy (FTIR) was also performed to determine surface chemical composition. Scanning electrode microscope (SEM) imaging was used to analyze NP stability. Furthermore, the cytotoxicity and antioxidant activity of pQu-Fa NPs were assessed *in vitro*.

DISCUSSION & CONCLUSIONS: The stability of nanoparticles is highly influenced by external factors such as pH, temperature, stirring speed, and stirring duration.

Preliminary experiments have shown that adding a zwitterionic molecule such as folic acid improves particle encapsulation efficiency and achieves a more homogenous NP solution and consistent size of NPs (**Figure 1**). Additionally, by combining FA with a PB solution of higher pH, the aggregation of NPs decreases, and the permeance of polymeric components is improved (as seen in SEM).

Finally, accomplishing a higher Z-potential heightens the stability of NPs when in suspension [3].

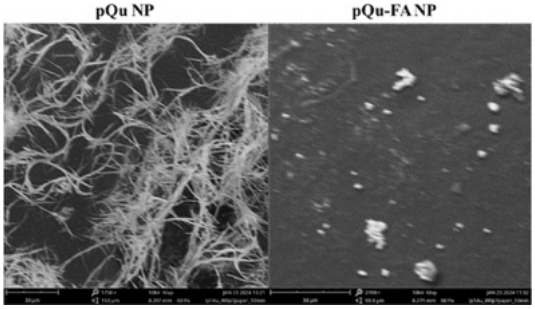

Figure 1: SEM images of polymeric nanoparticles with and without adding folic acid during synthesis.

Our results suggest that the particle size and stability can be controlled by adjusting pH, time, and stirring conditions. Moreover, upon encapsulation, particles show minimal toxicity *in vitro*, suggesting their future applications and research as pharmaceuticals.

REFERENCES: [1] S.Roszkowski (2023), *Molecules*, 28(10). [2] T.Sharma et. al (2022), *OpenNano*, 8. [3] Barrón et. Al (2023), *Biomolecules*, 13(7), 1158.

ACKNOWLEDGEMENTS: We thank the Erasmus Mundus internship support and the KI Research Incubator fellowship.

Hierarchically Porous Metal−Organic Frameworks-Based Hemoglobin Carriers with Surface Click-PEGylation: Boosting Colloidal Stability and Biocompatibility

Weiguang Jin[1], Leticia Hosta-Rigau[1]

[1]*Department of Health Technology, Technical University of Denmark, Denmark*

INTRODUCTION: Timely transfusion of red blood cells (RBCs) is an essential and life-saving procedure for avoiding body hypoxia and subsequent organ failure when patients suffer from acute severe blood loss. However, the limited supply and portability of donor RBCs and the time-consuming blood matching and typing, coupled with their short half-life and special storage requirements, make the opportune RBCs transfusion very difficult, especially during man-made or natural disasters. Incorporating hemoglobin (Hb), the central component of blood for oxygen transport, into nanoparticles to fabricate Hb-based oxygen carriers (HBOCs) can bring promising RBCs substitutes. HBOCs have potential for delivering oxygen and overcoming the abovementioned limitations when donor blood is not available, and incidentally addressing the tissue toxicity of free Hb [1]. Our group previously reported HBOCs by encapsulating Hb within mesoporous metal-organic frameworks (MOFs), PCN-333, and demonstrated their ability for Hb protection and oxygen carrying [2]. Nevertheless, achieving long circulation and biocompatibility are upcoming challenges when developing HBOCs. Meanwhile, expanding the variety of MOFs for macromolecule Hb pore encapsulation is also important since microporous MOFs predominate the reported structural repertoire.

METHODS: As shown as Fig 1A, herein we present a novel HBOCs based on hierarchically porous MOFs, in which the ligand was partially replaced with a sacrificial one with relative weak metal binding affinity, forming structural defective MOFs. After the removal of the sacrificial ligand, the generated mesopore provides appropriate cavities for Hb pore encapsulation. The obtained HBOCs were further functionalized with PEG coating.

RESULTS: The proposed hierarchically porous MOFs successfully encapsulated Hb, overcoming the natural pore size limitation of the pristine micropore MOFs structure (Fig 1B). The resultant HBOCs uniformly appeared as round-shaped particles sized around 190 nm (Fig 1C). Importantly, the surface PEGylation rendered the HBOCs with a significantly improved colloidal stability and stable hydrodynamic diameters compared to their parent counterparts, which is essential for upcoming *in vitro* and *in vivo* studies.

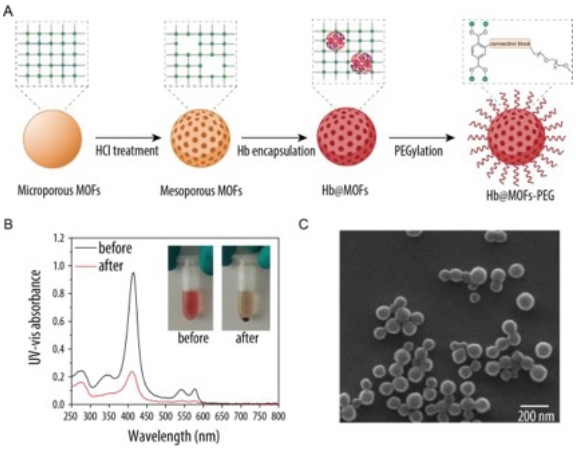

Fig. 1: A) Schematic illustration of the synthesis of PEGylated Hb-encapsulated MOFs; B) UV-vis absorbance of Hb solution revealed the encapsulation; C) SEM of Hb@MOFs-PEG.

DISCUSSION & CONCLUSIONS: These findings highlight the structural engineering of PEGylated hierarchically porous MOFs, providing new insights into the design of MOFs for Hb delivery.

REFERENCES: [1]C.C. Satue et al (2021) Biomater Sci, 9:1135-52. [2]X. Liu et al (2023) Biomater Sci, 11:2551–65.

ACKNOWLEDGEMENTS: This work was supported by the European Research Council under the European Union's Horizon 2020 - Research and Innovation Framework Programme (Grant No. 101002060).

Cell membrane coated biomimetic nanoparticles for tumor targeting

Wujun Xu[1], Lizhi Liu[2], Vesa-Pekka Lehto[1]

[1]*Department of Technical Physics, University of Eastern Finland, Finland*

INTRODUCTION:

Cell membrane (CM)–coated biomimetic nanoparticles (NPs) have attracted a lot of attention in nanomedicines due to their unique biological features such as immune evasion, prolonged blood circulation, and homologous tumor targeting [1]. However, several key questions about the biomimetic NPs, such as CM coating integrity, are still not fully understood, preventing the further advancement of the novel coating technique for cancer therapy. The present study aims to thoroughly investigate the effect of CM coating integrity on tumor targeting [2].

METHODS:

The CMs of CT26 cells were first extracted using the Dounce homogenizer [1]. The CM vesicles and porous silica NPs were mixed with the same mass (protein weight for CM vesicles) and co-extruded through a 200 nm membrane to prepare the CM coated biomimetic NPs. A fluorescence quenching assay was proposed to quantitatively probe the integrity of the CM coating, which is a vital metric to assess the coating quality of the biomimetic NPs. At the end, a novel approach using external phospholipid was designed to improve CM coating efficacy (Fig. 1A). The NPs were characterized with physicochemical methods and evaluated with *in vitro* assays & *in vivo* animal experiments.

RESULTS:

Our results discovered that most of the biomimetic NPs (>90%) were only partially coated with CM [1], which contradicts the common assumption that the NPs would be completely coated. Furthermore, the mechanisms with molecular simulations revealed that the partially coated NPs were internalized into cancer cells through an aggregation-based cooperation mechanism. To address the problem of partial CM coating, the use of external phospholipid increased CM fluidity, promoting the final fusion of CM on the NPs. Consequently, the tumor targeting of the biomimetic NPs was significantly improved by enhancing CM coating efficacy (Fig. 1B) [2].

DISCUSSION & CONCLUSIONS:

NPs coated with CMs using non-optimized techniques have exhibited promising biomedical properties. To enhance the coating techniques for achieving better coverage of NPs, it is crucial to optimize the coating process and study the influencing parameters. This research offers innovative perspectives on cell membrane coating technology, paving the way for the strategic development of biomimetic NPs tailored for targeted cancer therapy.

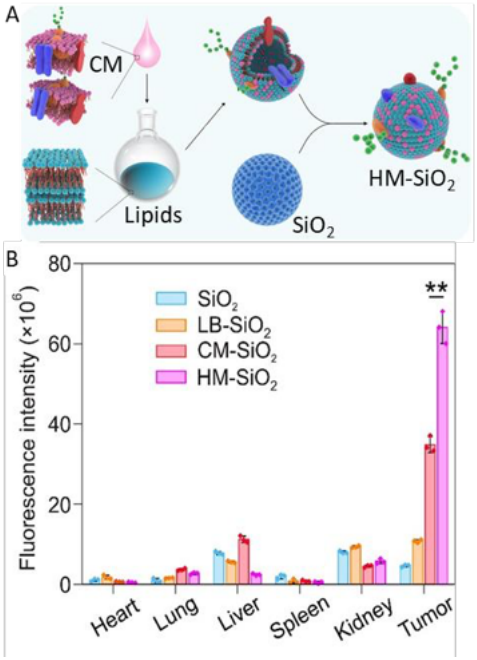

*Fig. 1. A) Schematic showing the process of developing hybrid membrane (HM) coated HM-SiO_2 NPs. B) In vivo tumor targeting by the analysis of the fluorescent signals from the tumor and major organs collected at 96 h after intravenous injection. **$p < 0.01$.*

REFERENCES: [1]L. Liu et al (2021) *Nat. Comm.* **12**: 5726. [2] L. Liu et al (2022) *Nat. Comm.* **13**: 6181

ACKNOWLEDGEMENTS: We acknowledge the support from the Research Council of Finland (projects 314412 and 356056).