Enhanced dental abutment surfaces through metal ion integration

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INTRODUCTION: Peri-implantitis, characterised by inflammation of the gingiva and subsequent bone loss, has a prevalence that may exceed 50% according to some studies and has become a serious oral health problem [1]. Titanium (Ti) and its alloys, which are widely used in the manufacture of dental implants and their abutments, are characterised by superior mechanical properties and high biocompatibility. Ti is biologically inactive and can generate low adhesion between the connective tissue formed by human gingival fibroblasts (HGFs) and implant abutments resulting in a weak gingival seal and facilitating the entry of bacteria. The establishment of a weak gingival seal compared to natural teeth leads to an increasing number of cases of infections by peri-implantitis [2]. By incorporating metal ions (Mg^{2+}, Mn^{2+}, Zn^{2+}, Zr^{4+}, Sr^{2+} and Cu^{2+}), being these biologically active agents involved in many biochemical reactions, it is intended to functionalise the surface of anodised titanium abutments with the aim of improving the affinity with cells representative of the connective tissue, the HGFs [3]. At the same time, bacterial studies are aimed at observing the ability of the new surfaces to reduce bacterial adhesion.

METHODS: Yellow anodised Ti Grade V (12 mm diameter; Ti6Al4V) discs activated by HNO_3 30% were treated by incubation in selected metal chloride solution ($MnCl_2$, $MgCl_2$, $ZnCl_2$, $ZrCl_4$, $SrCl_2$ and $CuCl_2$), followed by heat treatment at 100°C and washing with distilled water. The surface morphology and composition of the discs were studied by SEM/EDX. The surface hydrophilic capacity was characterised using contact angle measurements. ICP measurements were used to calculate the presence and release of metal ions in aqueous medium. Affinity for connective tissue cells was assessed by *in vitro* culture with HGFs, evaluating cell adhesion, migration, proliferation and the effect of surfaces on the ability to promote collagen secretion. Immunoassays were performed to assess the ability of the surfaces to affect gene expression of immune markers. To determine the proteins attached to the surface, the samples were incubated in human serum, eluted and then analysed by proteomic. The antimicrobial potential of the developed treatments was studied using a bacterial strain related to peri-implantitis (Streptococcus Gordoni).

RESULTS: Surfaces treated with the different ions were obtained. Except the Cu^{2+}, all cations resulted in a general improvement of HGFs adhesion on the new surfaces, being remarkable the increased cell projection for Mn^{2+}, Zr^{4+} and Sr^{2+}. Zr^{4+}, Mn^{2+}, Zn^{2+} and Cu^{2+} increased HGFs proliferation at 7 days, as well as generating an increase in cell migration after scratch. Zr^{4+} and Sr^{2+} significantly increased collagen secretion with respect to the non-treated surface. On the other hand, THP-1 *in vitro* results revealed that the ion-treated surfaces showed an immunomodulatory role. Additionally, the presence of the cations modified the protein adsorption profiles on the samples. Bacterial analysis showed no reduction in bacterial colonisation.

DISCUSSION & CONCLUSIONS: In this study, surfaces modified with Cu^{2+}, Mg^{2+}, Zr^{4+}, Mn^{2+}, Zn^{2+}, and Sr^{2+} were obtained and biologically characterized under the same conditions in order to evaluate the potential role of these cations to improve the gingival sealing around dental abutments. In this sense, the surfaces treated with Sr^{2+}, Zr^{4+} and Mn^{2+} displayed the promising *in vitro* results, showing their potential to improve the dental abutment performance.

REFERENCES: [1]V, Astolfi, Int J Environ Res Public Health. 2022;**19(7)**:4147. [2]J. Mouhyi, D.M. Dohan Ehrenfest. Clin Implant Dent Relat Res. 2012;**14(2)**:170-183. [3]N. Wu, H. Gao. ACS Biomater Sci Eng. 2023;**9(6)**:2970-2990.

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Does the formulation of platelet-derived extracellular vesicles with pressure ulcer care treatments enhance their permeability for improved healing?

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INTRODUCTION: Pressure ulcers (PU) are slow-healing wounds that represent a significant public health challenge, underscoring the urgent need for innovative clinical management strategies[1]. One promising approach involves the use of platelet-derived extracellular vesicles (pEV). Despite their potential, the absorption of pEV by the skin can vary depending on individual characteristics[2]. Therefore, this study aims to demonstrate that pEV can be successfully incorporated into commercial pressure ulcer care formulations to enhance the homogeneity of permeability and effectiveness in managing PU.

METHODS: pEV were isolated from platelet lysates (PL) using size exclusion chromatography (SEC) and characterized by the ONI Application Kit™: EV Profiler 2. Four commercial pressure ulcer (PU) care formulations (Linovera™, Mepentol™, Intrasite™, and Purilon™) were tested, comparing their rheological properties using an MCR 302e rheometer, both with and without pEV. An ex vivo PU model was developed using rat skin explants maintained in DMEM/F12 medium. Wounds were induced using 10 mm diameter neodymium magnets pressure overnight. The model was further characterized using MTT viability assays and H&E. For permeability assays, pEV labeled with the PKH26 dye were incorporated into the commercial treatments and applied to 6 mm skin explants for 60 minutes. The explants were fixed and then analyzed via PKH26 fluorescence using confocal microscopy.

RESULTS: The characterization confirmed a typical pEV profile. Incorporating Plasmalyte at a 1:1 ratio significantly altered the rheological properties of the original formulations of the tested biomaterials, including both oils and hydrogels. In the ex vivo PU model, pressure ulcers were developed successfully and an increased metabolic viability was observed in the ulcerated skin areas. pEV permeability after 60 minutes varied depending on the biomaterial applied, highlighting differences in their ability to facilitate pEV penetration in the wounded skin.

DISCUSSION & CONCLUSIONS: We demonstrated that an ex vivo PU model can be generated using magnets to induce localized PU lesions. Finally, the results of the rheological studies and fluorescence analysis in the permeability assays indicate that while it is possible to incorporate pEV into preexisting commercial treatments. The development of new strategies is necessary to enhance the therapeutic delivery, bioactivity, and retention of pEV within wound environments, ultimately improving outcomes in PU manegements.

REFERENCES: [1]Kottner J. et al (2020) *J. Tissue Viability*, **29**, 197–203. doi: 10.1016/j.jtv.2020.04.003. [2]Zhang Y. et al (2024). *Pharmaceutics*, 16, 1394. doi: 10.3390/pharmaceutics16111394.

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Mn-Doped Ti Coatings for Dental Implants: Impact on Osteogenesis, Immune Response, and Protein Interaction

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INTRODUCTION: Titanium (Ti) is a widely used material for dental implants due to its excellent mechanical properties and biocompatibility. However, its bioinert surface and limited osseointegration remain key challenges. Silica-based bioactive coatings, applied through sol-gel techniques, offer a promising solution by enhancing bone binding, improving implant stability, and enabling the incorporation of bioactive molecules and ions. Manganese (Mn), a vital element for enzymes like superoxide dismutase, plays a critical role in bone matrix formation, mineralization, and maintaining osteoblast-osteoclast balance.[1] Protein adsorption on implant surfaces, influenced by their physicochemical properties, is a key determinant of cellular responses and osseointegration. Proteomic analysis of these protein adsorption patterns provides valuable insights for optimizing implant performance and improving clinical outcomes.

METHODS: Coatings for Ti surfaces were synthesized via sol-gel doped with increasing concentrations of Mn and applied as coatings as observed on *Figure 1*.

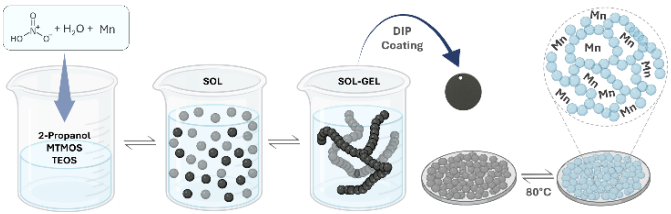

Figure 1: *Synthesis process of Mn-doped coatings*

Biological characterization included cytotoxicity, proliferation, and mineralization assays (ALP activity) in human osteoblasts (HOb), alongside osteogenic gene expression analysis (COL1, BMP2, RANKL). Immune response was assessed in THP-1 macrophages through cytokine quantification (ELISA) and pro- and anti-inflammatory gene expression measurements (RT-PCR). Protein adsorption from human serum was evaluated using nano-LC-MS/MS.

RESULTS: The sol-gel network fully covered the Ti surface, with no Mn accumulations detected. FTIR confirmed Si-O-Si bond formation, while Mn addition slightly increased hydrophobicity without altering surface roughness. Mn-doped materials showed controlled Mn^{2+} release, reducing base degradation over 56 days. *In vitro* studies showed no cytotoxicity, consistent HOb proliferation, and increased expression of osteogenic markers (e.g., COL1, RANKL, BMP2, OCN) in Mn-doped samples, particularly at 14 days. Immune response analysis revealed elevated TNF-α and IFN-γ gene expression in higher Mn compositions, while anti-inflammatory markers (IL-10, TGF-β) increased with lower Mn concentrations. At the same time, proteomic analysis showed that Mn coatings have a higher affinity for proteins related to immune response (e.g., Immunoglobullins, SAA1), cell adhesion, such as CADH1 and CADH5, as well as those involved in osseointegration including FETUA, FETUB among others.

DISCUSSION & CONCLUSIONS: Our findings show that Mn-doped Ti coatings were non-cytotoxic, enhancing surface properties, controlling Mn^{2+} release, and promoting osteogenic gene expression. Proteomic analysis indicated and increased affinity for proteins related to cell adhesion and tissue regeneration on Mn samples, which may contribute to better osseointegration. These results are consistent with existing literature and suggest that Mn-doped coatings can enhance dental implants by improving osseointegration while modulating the immune response.

REFERENCES: [1]Park JW, Kim YJ, Jang JH. Surface characteristics and in vitro biocompatibility of a manganese-containing titanium oxide surface. *Appl Surf Sci*. 2011;258(2):977-985. [2]Romero-Gavilán F, García-Arnáez I, Cerqueira A, et al. Insight into the antibacterial mechanism of Cu-enriched sol-gel coatings employing proteomics. *Biomater Sci*. 2022;11(3):1042-1055.

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Micro-CT evaluation of 3D printed titanium implants with gyroid structure – closed porosity analysis

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INTRODUCTION:

The importance of 3D printing of metal materials is increasing in various areas of biomedical research. The ability to 3D print bone implants offers several advantages, primarily a high degree of customization in shape for use with individual patients. Apart from this key advantage, 3D printing also brings several challenges, such as defects that arise during 3D printing (e.g. porosity), which can lead to stress concentration and mechanical failure of the implant. In an ongoing project focused on the design and fabrication of 3D-printed implants with a gyroid structure for orthopedic or dental applications, we use micro-CT for the characterization of structural parameters (volume, surface etc.). Moreover, closed porosity, which is considered as a material defect, is analyzed. Pilot data from ongoing project will be presented.

METHODS: 3D printed titanium (Ti6Al4V) implants with both gyroid and solid structure were scanned by micro-CT (SkyScan 1272, Bruker micro-CT). Data were processed using Bruker software and 3D analysis of selected structural parameters was performed.

RESULTS: Micro-CT visualizations are presented in figure 1. Example of 3D analysis of selected parameters of gyroid structure in selected specimen is presented in table 1. Comparison of closed porosity in different parts of the specimen is also presented (gyroid, solid and its interface).

Percent object volume	77.94	%
Object surface density	19.07	1/mm
Structure thickness	0.16	mm
Structure separation	0.08	mm
Closed porosity		
Gyroid	0.18	%
Solid	0.07	%
Interface	0.02	%

Tab. 1: Example of micro-CT 3D analysis and closed porosity evaluation in various areas

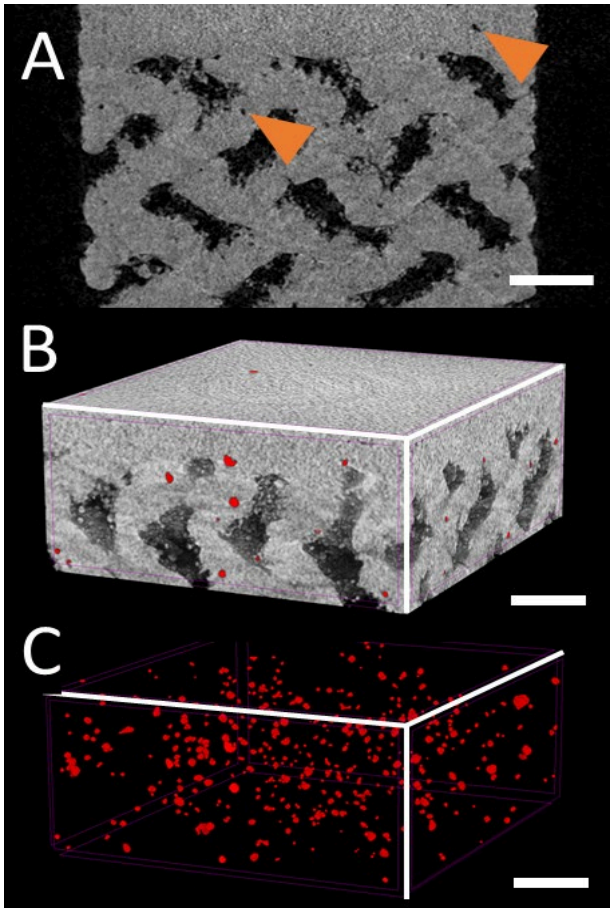

Fig. 1: Micro-CT visualizations: A) cross-section image, orange arrows depict pores; B) 3D visualization with closed pores (red); C) closed pores in the same volume as shows image B; scale bar 500 um

DISCUSSION & CONCLUSIONS: Micro-CT enables quantification of structural parameters of evaluated specimen with the evaluation of closed porosity, which is difficult to obtain using different methods. Volume of closed pores is generally very low (below 1%). Gyroid structure presents higher degree of closed porosity when compared with solid structure. Interestingly, the interface area presents the lowest volume of closed porosity.

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Lactic acid biofunctionalized cellulose reinforced multiphasic hydrogel for guided bone regeneration

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INTRODUCTION: Biomaterials with multilayer or multiphasic composition are considered exciting for guided tissue regeneration (GTR)[1]. A variety of reported GTR membranes compromise important structural properties like stiffness, and osteogenic property. Natural polymer like chitosan, cellulose based biomaterials has considered as exciting for GTR[2]. The present work focuses the development of lactic acid (Lac) biofunctionalized micro-crystalline cellulose (MCC) reinforced multiphasic hydrogel biomembrane. The multiphasic hydrogel will contain a nonmineralized and a $CaCO_3$-mineralized phase.

METHODS: At first, MCC was biofunctionalized through Lac grafting (MCC-Lac = 1:20, 1:23, 1:25, 1:30 w/w). Thereafter, the various MCC-Lac were characterized using gel permeation chromatography (GPC), Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Then nonmineralized hydrogel biomembranes were prepared by utilizing MCC-Lac-1:20 and 2% w/v chitosan in the following ratios (w/w): 0.0:1.0, 0.1:1.0, 0.5:1.0, 1.0:1.0. Nonmineralized hydrogel without MCC:Lac is kept as control. In this polymer solution, 1% (w/v) polyethylene glycol (PEG) and 1.6% (w/v) agar was also added. The polymer solutions were then stirred for 15-20 min. Then the polymeric solution was subjected to moist heat treatment (120°C for 20 min). Thereafter, genipin (3.75% w/w of chitosan) was added in cooled down polymer solution as a crosslinking agent. The polymer solutions were then solvent casted and kept at 37°C for gel formation. These gels were freeze dried and characterized by compression test and cell viability assay. Thereafter, the nonmineralized hydrogels were used to prepare multiphasic hydrogel through template-mediated biomineralization. To achieve this, one of sides of the hydrogels was sequentially immersed in aqueous $CaCl_2$ solution and aqueous Na_2CO_3 solution for 90 mins and then characterized by FTIR, SEM and energy-dispersive X-ray spectroscopy (EDX).

RESULTS: The degree of functionalization of the MCC-Lac was MCC-Lac: 1:20 >MCC-Lac: 1:25 > MCC-Lac: 1:23 > MCC-Lac: 1:30. Thus, all hydrogels were prepared with MCC:Lac-1:20. The stiffness of the nonmineralized hydrogels was found highest for the MCC:Lac-0.5 sample. On the other hand, increased cytotoxicity (%) was found for the hydrogel sample with MCC:Lac-0.1. Hence, nonmineralized samples with MCC:Lac-0.5 and MCC:Lac-1.0 were selected for the preparation of mineralized multiphasic hydrogels. FT-IR and EDX studies indicated the deposition of calcium carbonate on the mineralized surface. SEM showed increased surface roughness on the mineralized side of the of the multiphasic hydrogel.

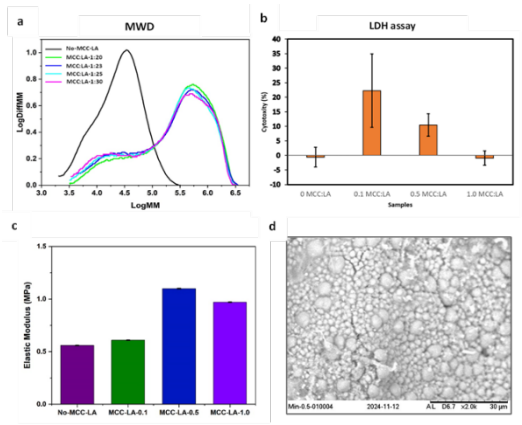

Fig.1. MWD of MCC-Lac and (a) LDH assay (b) and Young Modulus of nonmineralized hydrogel; (c) SEM of mineralize hydrogel.

DISCUSSION & CONCLUSIONS: The multiphasic hydrogels with MCC:LA (1:20)-0.5 and 1.0 demonstrated less cytotoxic behavior compared to the multiphasic gel with MCC:LA (1:20)-0.1. Moreover, the elastic modulus is found highest for MCC:LA (1:20)-0.5 samples. On the other hand, the significant surface roughness can be found with mineralized MCC:LA (1:20)-0.5 samples. Based on these results, MCC:LA (1:20)-0.5 hydrogel is considered to have the highest potential for GTR applications.

REFERENCES: [1]Liao et al.,2005, Biomaterials, 26(36):7564-71, [2]Namanloo et al., 2022, *Materials Science and Engineering*, 2489399, 14.

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Biomineralization Mediated by Cellular Interactions in a Biomimetic Hydrogel

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NTRODUCTION: Biomineralization, the cellular process behind mineralized tissue formation, is critical for addressing serious bone defects. Governed by the interplay of cellular behavior, ion transport, and matrix properties, this process is explored using a custom-built model, BioMingel, which combines agent-based modeling (ABM) and partial differential equation (PDE) solvers with the FiPy[1] framework. This computational-experimental approach tracks individual cell behaviors, assigns unique properties, and replicates biomaterial characteristics as a digital twin. The combined model provides insights into mineral deposition, matrix degradation, and stiffness evolution, bridging in silico and in vitro techniques.

METHODS: The digital twin integrates:

• ABM: Models cell-specific responses.

• PDEs: Capture ion diffusion, enzymatic matrix degradation, and stiffness changes.

Simulations explore the effects of enzymatic activity, mineral transformation kinetics, and gel stiffness on biomineralization.

Experimentally, PEG-Norbornene (PEG-NB) is crosslinked with matrix metalloproteinase (MMP)-sensitive peptide linkers and cRGD attachment motifs using LAP photoinitiation to form a hydrogel mesh[2]. Encapsulation of MC3T3-E1 cells within the matrix assessed using Alizarin Red Staining (ARS), Alkaline Phosphatase (ALP) assays, and a viability assay.

RESULTS: The initial results reveal biomineralization is influenced by:

- Simulations demonstrate that regions of high MMP activity correlate with localized stiffness increases due to mineral transformation.
- Calcium and phosphate ions are dynamically redistributed through cell migration and crosslinking changes, driving heterogeneous mineralization patterns dictated by initial matrix properties.
- A threshold in MMP-driven remodeling triggers a rapid ACP-to-HA transition, accelerating stiffening beyond biphasic patterns, acting as tunable hydrogel switch.

These results underscore the importance of interplay among matrix properties, ion dynamics, and cellular behavior, showing the necessity of modeling individual cell interactions and customized biomaterial properties.

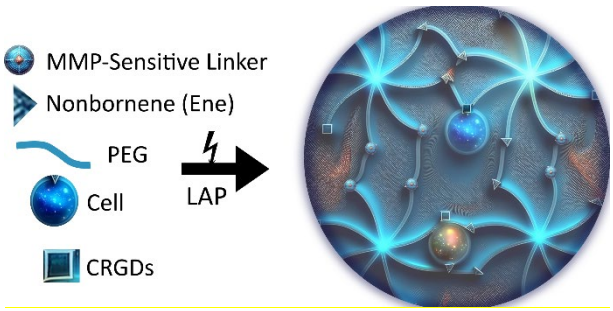

Fig. 1: Schematic of the hydrogel mesh: PEG-Norbornene (PEG-NB) is functionalized with MMP-sensitive linkers plus cRGD cell-attachment motifs. UV activation using the photoinitiator LAP enables cell encapsulation within the matrix.

DISCUSSION & CONCLUSIONS: BioMingel is introduced to examine biomineralization in biomimetic hydrogels through the digital twin principle. The capacity to track individual cell responses, assign cell-specific properties, and adapt the gel structure to mimic varied biomaterials broadens its potential applications. This strategy offers insights for refining tissue engineering materials, with uses in regenerative medicine.

REFERENCES: [1] J.E. Guyer et al. (2009) Comput. Sci. Eng. 11(3), 6–15., [2]B.D. Fairbanks et al. (2009) Adv. Mater. 21(48), 5005–5010.

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Traceless Photopolymerization with Red Light: A Novel Approach for High-Stiffness, Colorless Polymers Suitable for bio 3D Printing

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Photopolymerization with visible light, especially red light, offers substantial advantages over traditional ultraviolet initiated methods, such as improved biocompatibility and deeper tissue penetration without harmful side effects.[1-2] Using red light irradiation to produce colorless crosslinked hydrogels offers numerous benefits, especially in biomedical applications where transparency facilitates deeper and more even curing and allowing for precise monitoring.[3] In this study, we utilized methylene blue (MB^+), an FDA-approved, water-soluble dye, as a sacrificial photosensitizer and triethanolamine (TEA) as a one-electron/one-proton ($1e^-/1H^+$) donor for traceless photopolymerization using red light. In the presence of TEA, methylene blue excited triplet state ($3MB^{*+}$) reduced to coloreless leuco form (Leuco-MB) and result in TEA radicals which initiate radical polymerization. Under ambient conditions, reactive oxygen species (ROS) are produced from the triplet excited state of methylene blue (MB^+). Continuous irradiation leads to the degradation of ground state MB^+, resulting in a permanently transparent, crosslinked hydrogel. The polymerization kinetics, studied by in situ photorheology and H-NMR, demonstrated that the storage modulus reached 1 kPa within 5 minutes, plateaued at 5 kPa within 10 minutes, and achieved >80% monomer conversion within 30 minutes. Furthermore, this photocuring system was successfully applied in an extrusion-based 3D printing system to produce stable, colourless 3D structures. Finally, this photocuring system demonstrated good biocompatibility during polymerization in the presence of cells, expanding its potential applications.

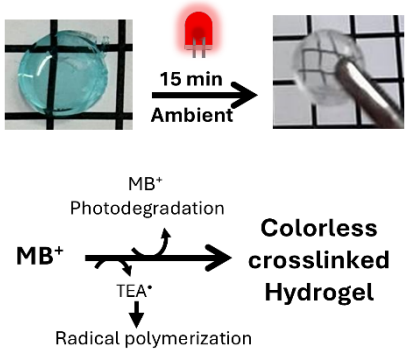

Fig. 1: Photocrosslinking of gelatin methacrylate under ambient condition upon red light irradiation which result in colourless crosslinked hydrogel.

CONCLUSIONS: We demonstrated traceless polymerization using red light, methylene blue, and triethanolamine in water under ambient conditions. This method rapidly produces transparent, crosslinked hydrogels (e.g., 4% GelMA reaches >1 kPa in 5 minutes and >80% conversion in 30 minutes) and is compatible with extrusion-based 3D printing. The resulting colorless hydrogels support high cell viability and allow unhindered light transmission, making them ideal for medical diagnostics, phototherapy, and tissue engineering.

REFERENCES: [1]Balzani Balzani V. et al. (2015) Angew. Chem. Int. Ed., 54: 11320–11337. [2]Yagci Y. et al. (2010) Macromolecules, 43: 6245–6260. [3]Dumur F. (2023) Eur. Polym. J., 186: 111874.

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Tannic acid and arginine nanocoatings for soft tissue integration of titanium dental implants

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INTRODUCTION: Titanium implants are the preferred replacements of missing teeth [1]. However, integration of the transmucosal region of implants to surrounding soft tissues remains challenging. Tight mucosal seal is important to protect the implant from bacterial invasion to avoid peri-mucositis, peri-implantitis and implant failure [2]. Bioactive coatings on implant surfaces that are in direct contact with soft tissues have the possibility to locally suppress inflammation and support mucosal repair to prevent these clinical complications. Tannic acid (TA) exhibits antimicrobial and antioxidative properties and can be continuously deposited with silicic acid to surfaces to form nanocoatings [3]. In turn, arginine-based biomaterials are reported to promote wound healing, decrease inflammation and be antimicrobial [4]. The aim of this research was therefore to study various combinations of these biomolecules to create multifunctional and non-cytotoxic nanocoatings for soft tissue integration of smooth titanium surfaces.

METHODS: Polished titanium coins were coated with TA, L-arginine (LR) and poly-L-arginine (PLR) in mono- or multilayers at pH 6.8. Monolayer coatings consisted of either TA, LR or PLR alone, or a mix of TA and LR (TA+LR). Multilayers were formed by sequential deposition of TA and PLR layers and varying the topmost layer: $(TA\text{-}PLR)_4TA$ or $(TA\text{-}PLR)_5$. PLR with molecular weight of 5-15 kDa (PLR_{low}) and 15-70 kDa (PLR_{high}) were tested. Cellular experiments were performed by culturing human gingival fibroblasts (HGFs) on precoated coins. Possible cytotoxicity of coatings was tested in cell culture medium after 1 and 7 days by measurement of lactate dehydrogenase (LDH) activity and cell viability was assessed with alamarBlue assay. Morphological changes were studied with confocal laser scanning microscopy (CLSM) by immunostaining of focal adhesions with vinculin and cytoskeleton with phalloidin. To evaluate the potential of the coatings to attenuate inflammation, cells cultured on top of the coatings were exposed to 10 µg/ml lipopolysaccharide (LPS) from *E. coli*. Cell culture medium was collected for ELISA analysis of the inflammatory cytokine IL-6 after 6, 24 and 48 hours.

RESULTS: Measurement of LDH release did not reveal any acute cytotoxicity from the coatings except for PLR monolayers. Cell viability analysis corresponded to these findings by showing high overall cell viability from all coatings apart from PLR monolayers. CLSM further confirmed these findings demonstrating cell attachment and elongated fibroblast morphology on all coatings but PLR monolayers (Fig.1.). However, cells tended to aggregate more when cultured on TA coatings compared to uncoated titanium coins or LR monolayers. The TA+LR, $(TA\text{-}PLR)_4TA$ and $(TA\text{-}PLR)_5$ coatings reduced IL-6 secretion by HGFs exposed to LPS compared to TA coating alone. Interestingly, coatings with PLR on top showed the greatest effect on IL-6 reduction.

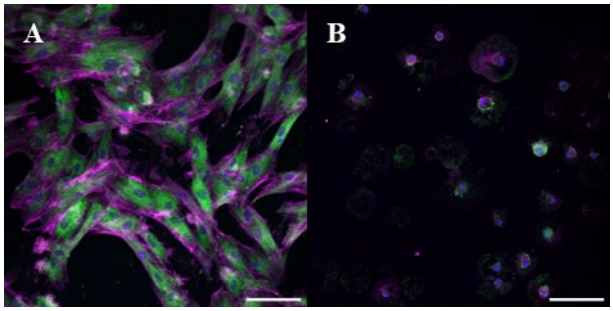

Fig. 1: CLSM of HGFs cultured on titanium coins coated with $(TA\text{-}PLR_{low})_4TA$ (A) or (PLR_{low}) (B) at day 1. Green – vinculin, magenta – phalloidin, blue – DAPI. Scale bar: 100µm.

DISCUSSION & CONCLUSIONS: Our experiments are in agreement with previous results showing HGF adhesion to TA coated smooth titanium surfaces [5]. In addition, our findings indicate that TA coatings combined with LR or PLR can attenuate inflammation without being cytotoxic to HGFs.

REFERENCES: [1]J. Hasan et al. (2022) *ACS Biomater Sci Eng*, **8**: 4697-4737. [2]S. Jin et al. (2024) *Bioact Mater*, **42**: 404-432. [3]F. Weber et al. (2019) *Chemistry*, **25**:9870-9874. [4]Y. Zhou et al. (2021) *J Mater Chem B*, **9**:6738-6750. [5]A. Rogala et al. (2024) *Biomater Sci*, **12**: 3345-3359.

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In vitro testing of biodegradable zinc alloys: main challenges

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INTRODUCTION: Biodegradable zinc alloys have emerged as promising materials for biomedical applications, particularly in orthopaedics. Their ability to degrade in the body environment while maintaining biocompatibility addresses limitations of traditional metallic implants, such as the need for removal surgeries. These alloys also do not exhibit negative corrosion phenomena such as the formation of massive corrosion products in the case of iron and the evolution of hydrogen in the case of magnesium. Zinc's physiological compatibility, combined with the controlled addition of alloying elements like magnesium and strontium, enhances mechanical properties and corrosion resistance. However, replicating *in vivo* conditions during in vitro experiments poses significant challenges. Key factors influencing the degradation behaviour, such as electrolyte composition, medium volume, and ion interactions, must be carefully optimized to ensure that in vitro results accurately predict *in vivo* performance.

METHODS: The study focused on evaluating the corrosion behaviour of the Zn-0.8Mg-0.2Sr alloy in various test environments. Different electrolyte volumes (small vs. large) and compositions (e.g., physiological solution, L-15 medium) were assessed to simulate *in vivo* conditions more accurately. Corrosion rates and polarization resistance were key metrics analysed. Additionally, the study explored optimal *in vitro* testing environments, including the use of cell cultivation media such as RPMI, L-15, and DMEM/Ham's, to identify conditions minimizing zinc ion interference and maximizing cell viability.

RESULTS: Corrosion behaviour was influenced by electrolyte volume and composition. Small volumes led to localized corrosion, while larger volumes promoted uniform degradation. Physiological solutions caused more localized corrosion compared to L-15 medium. RPMI medium exhibited the lowest corrosion rates due to high phosphate content, which passivated the alloy surface. L-15 showed high corrosion rates likely due to amino acid interactions with Zn^{2+} ions. Biological tests using hFOB 1.19 cells indicated higher viability in RPMI and L-15 compared to DMEM/Ham's. Preliminary contact tests demonstrated cell adhesion on the alloy surface in RPMI. Interference from released Zn^{2+} ions affected assay outcomes, necessitating careful selection and validation of evaluation methods, such as fluorescence-based assays.

DISCUSSION & CONCLUSIONS: The findings highlight the importance of carefully tailoring *in vitro* testing conditions to reflect the complexity *in vivo* environment. Variations in electrolyte composition and volume significantly impact the corrosion behaviour of the Zn-0.8Mg-0.2Sr alloy. RPMI medium, with its high phosphate content, emerged as the most suitable medium due to its ability to passivate the alloy surface and support cell viability. In contrast, the high amino acid content in L-15 medium increased the corrosion rate but demonstrated potential for specific applications requiring faster degradation. The study underscores the need to account for zinc ion interference in viability assays, which can compromise the accuracy of biological assessments. Fluorescence-based methods and alternative assays may mitigate these issues, ensuring reliable evaluation of cell-material interactions. Overall, the Zn-0.8Mg-0.2Sr alloy exhibits promising potential for orthopaedic applications, provided that experimental conditions are optimized. Future research should focus on refining in vitro methods to bridge the gap between laboratory and clinical environments, further enhancing the predictive power of these tests for *in vivo* outcomes.

ACKNOWLEDGEMENTS: The research was supported by Czech Science Foundation Grant No. 23-05592S.

The Healing Cargo: pEVs versus cEVs in Osteoarthritis Therapy

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INTRODUCTION: Extracellular vesicles (EVs) are promising candidates in regenerative medicine due to their unique properties and potential as cell-free therapy. Their versatility extends from direct injection to integration with biomaterials, such as hyaluronic acid hydrogels, or coatings for biomaterials such as titanium implants, enhancing their therapeutic applicability [1]. In fact, preclinical in vivo studies, such as osteoarthritis (OA) rat model, highlight their potential for clinical translation [2].

While mesenchymal stem cells (MSCs) are a widely used EV source, platelet-derived EVs (pEVs) have demonstrated superior regenerative capacity in our osteoarthritis *in vivo* model [2]. Despite similar physical properties and targeting abilities, pEVs consistently outperform cEVs. This difference may stem from variations in their cargo, such as miRNA, metabolites and/or proteins.

Here, we aim to uncover the molecular factors driving the superior regenerative effects of pEVs through an omic approach, paving the way for their use either as standalone therapy or in combination with biomaterials for therapeutic applications.

METHODS: EVs were isolated by Size Exclusion Chromatography (SEC) either from platelet lysate pools or human umbilical cord MSCs conditioned media (passage 7 to 13 from 3 different donors), obtained from the IdISBa Biobank (1955/12BIO). EVs were characterized in terms of size and particle concentration by Nanoparticle Tracking Analysis (NTA), and total RNA content. Subsequently, samples were prepared for multi-omics analyses. Transcriptomic profiling was performed using a GeneChip miRNA 4.0 array and analysed with the Transcriptome Analysis Console (TAC). Additional omics analyses, including metabolomics and proteomics, were considered to explore the comprehensive molecular landscape of the EVs, and samples were prepared accordingly.

RESULTS: cEVs and pEVs presented statistically significant differences in their characterization in terms of particle concentration and total RNA content. Regarding to their specific miRNA cargo, both groups included miRNA associated with the regulation of an osteoarthritic process. In cEVs, miRNAs such as has-miR-4508, has-miR-1228, and has-miR-4454 were identified, all of which are implicated in inflammatory pathways associated with joint diseases like rheumatoid arthritis. Additionally, has-miR-663a has been shown to promote apoptosis and inhibit the proliferation of osteoarthritic chondrocytes.

In contrast, has-miR-106-5p, has-miR-16-5p or has-miR-320a were identified in pEVs. These miRNAs are known to upregulate extracellular matrix (ECM) components, downregulate degradative proteins, and alleviate pathological changes in cartilage associated with an osteoarthritic process.

DISCUSSION & CONCLUSIONS: These findings are consistent with previous studies that have demonstrated differential effects of EVs derived from various sources in *in vivo* OA models. The data underscore the complexity of EV mechanisms and the critical role of their cargo in driving therapeutic outcomes. Overall, these results provide valuable insight into the molecular content of EV functionality and support the use of pEVs as promising cell-free therapy for OA and other diseases.

REFERENCES: [1]M. Antich-Rosselló et al. (2021) *International Journal of Molecular Sciences*, **22**: 8580. [2]M.A. Forteza-Genestra et al. (2024) *Journal of Orthopaedic Translation*, **45**: 1-9.

ACKNOWLEDGEMENTS: This research was funded by ISCIII, Ministerio de Economía y Competitividad, co-funded by the ESF European Social Fund and the ERDF European Regional Development Fund (CP16/00124; PI20/00127), PROGRAMA JUNIOR del project TALENT PLUS, construyendo SALUD, generando VALOR (JUNIOR01/18), financed by the sustainable tourism tax of the Balearic Islands, and the Direcció General d'Investigació and Conselleria d'Investigació, Govern Balear (FPI/2046/2017; FPI/87/2022); and the Ministerio de Ciencia, Innovación y Universidades (FPU22/04432).

Assessment of Embedded Bioprinting for the Fabrication of Smooth Muscle Tissue Models

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INTRODUCTION: Phenotypic modulation of vascular smooth muscle cells (SMCs) is a key factor in vascular pathophysiology and influences processes such as vascular remodelling, atherosclerosis and hypertension. Recent studies using 3D bioprinted constructs have shown that microstructural and mechanical factors are critical for the contractile phenotype of SMCs[1,2]. However, the structural integrity and long-term stability of the 3D constructs remains a challenge. Embedded bioprinting techniques, in which hydrogel constructs are printed in a supporting medium, have shown promise for improving the shape fidelity and stability of printed structures[3]. Our long-term goal is developing a robust approach for the fabrication of engineered vascular tissue models. This study investigates the effect of embedded bioprinting in an agarose slurry support bath on the printability, stability and *in vitro* biocompatibility of hydrogel constructs loaded with human adipose-derived stem cells (hADSCs).

METHODS: For the formulation of the bioink, a hydrogel was prepared from 6 % GelMA and 2.4 % alginate (w/v). To prepare the agarose slurry support bath, a 1% agarose gel was prepared by cooling, stirring and centrifugation. Extrusion-based bioprinting (BioX, Cellink) was used to fabricate ring-shaped constructs with hADSCs. The constructs printed into the support bath were referred to as Group 1, while those printed directly onto a plate were referred to as Group 2. Cellink XPLORE ink was used as a control. The printability of the cell-loaded constructs was assessed by digital image processing using ImageJ. The viability of the cells was assessed using live/dead staining.

RESULTS: Immediately after bioprinting, the constructs of Group 1 retained a continuous and uniform ring shape, while Group 2 showed deformation of the filaments (Fig. 1A). The normalized cross-sectional area of Group 1 closely matched the control hydrogel, indicating a high degree of shape fidelity. After three days, the constructs of Group 2 showed significant shrinkage, while Group 1 retained their original dimensions (Fig. 1B). Cell viability remained above 80% in both groups, with no significant differences over time (Fig. 1C).

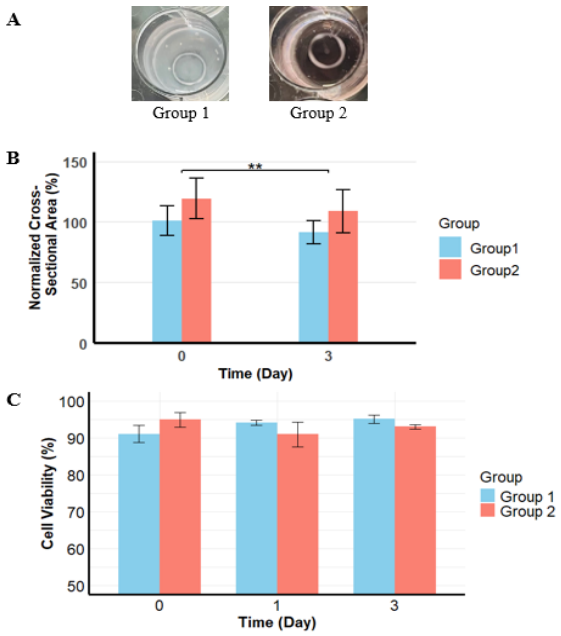

*Fig. 1: Bioprinting performance of different groups. (A) Representative images of newly printed constructs. (B) Quantitative analysis of cross-sectional area of the constructs (**p<0.01, n = 6). (C) Quantitative analysis of live/dead staining of encapsulated cells (n = 3).*

DISCUSSION & CONCLUSIONS: Our study is consistent with existing findings and shows that printing GelMA/alginate hydrogel in an agarose bath ensures high structural fidelity and stability[3]. The bath supports the constructs and counteracts gravity and surface tension to minimise deformation. At the same time, it is possible to print constructs that remain suspended in the gel bath and contain viable cells, so it will be possible to monitor cell contraction and relaxation without restriction. This bioprinted model is expected to provide a reliable platform for studying the contractile function of VSMCs constructs *in vitro*.

REFERENCES: [1]Xuan, Z., et al. (2023) *Tissue Eng. Regen. Med.*, **20:** 199-212. [2]Xuan, Z., et al. (2023) *Biotechnol. Bioeng.* **120**(11):3396-3408. [3]Mirdamadi, E., et al. (2019) *3D Print. Addit. Manuf.*, **6:** 158-164.

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Analysis of the hydrogel injectability for the pain-minimized administration

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INTRODUCTION: Application of advanced hydrogels for tissue reconstruction as a medical device or a therapeutic agent requires a careful administration with a controlled injection rate to avoid excess pain and reach the intended outcomes. For non-Newtonian fluids at manual injection it is challenging as in general there is limited knowledge of the rate at specific needle, depth and angle, as well as opposing underlying tissues. A "safe" level of internal pressures for soft tissues is considered to be below 5~10 kPa (preferably <4 kPa), and for a short-term 13~20 kPa as blood flow may temporarily cease, leading to local anoxia and ischemia [1,2].

In this work a new method has been developed to uniquely characterize hydrogels injectability without a need of the particular rheological model or assumptions, allowing predictive estimation of the programmed injection requiring no explicit "smart needle" rate control.

METHODS: In the demonstrated example, the method is shown for own experimental data of the of the amount of hydrogel (μL) injected via a defined nozzle (needle gauge), per applied local pressure (kPa), which is complemented of testing the same hydrogel and needle setup into the skin phantom. All tests were done at 22°C and RH 25% in a under laminar flow cabinet of ISO Class 5 (ESCO, Singapore). Syringes of 1 mL capacity were used with sterile needles 29G x 1/2". Acrylic hydrophilic gel was used as a simulation material.

In the creep mode the constant force was applied stepwise for the for the location of the needle as free, immersed into a skin phantom vertically at 90° and inclined by 45° down to ~2 mm depth. After the tests, the data were first checked with the Proteus 6.1 software (Netzsch Gerätebau GmbH, Germany). Time-invariant values such as viscous stiffness of the gel in injection conditions and its memory value have been extracted with a patented method [3].

RESULTS: Experimental data clearly revealed a non-linearity of the flow kinetics with time and applied pressure, Fig. 1. It shows that numeric values are changing with applied pressure in a non-monotonic way, disclosing that there are might be different limiting phenomena affecting the flow.

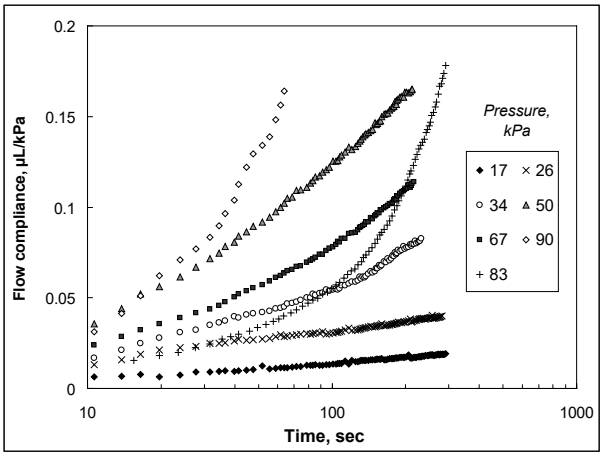

Fig. 1. Flow compliance in time as function of applied pressures (kPa).

The curves of viscous stiffness and memory are almost linear for all applied pressures (data not shown here). The hydrogel injectability map has been constructed showing the gel in the syringe facing friction, flow resistance and no-slip effects at low pressures when memory values are much less than unity.

DISCUSSION & CONCLUSIONS: The method developed can assess the injectability properties vs. specific injection system conditions which usually are very different from standard rheology setups, giving inconsistent outcomes due to uneven contact, inertia and elastic instability effects, fitting to assumed models, lack of proper loading history assessment, etc.

New method allows bypassing these limitations and can be directly deployed in optimized injection systems design compliant to MDR 2017/745 and smart hydrogels deployment [4,5].

REFERENCES: [1] *Materials* **14** (2021), 2845; [2] *Tissue Eng.* **A28** (2022), S141; [3] US Patent 10809171 B2 (2019); [4] *Int. J. Mol. Sci.* **23** (2022), 7, 3665; [5] *Biomed. Mater.* **12** (2017), 025004.

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Biophotonic composite scaffolds for controlled nitric oxide release upon NIR excitation

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INTRODUCTION: Bone grafting is a common procedure in orthopaedic, trauma, and maxillofacial surgery, aiding nearly half a million patients annually in the United States and Europe. Despite its benefits, it poses significant infection risks, often associated with biofilm formation on implants, which are resistant to antibiotics and immune responses. Bioactive glass (BAG) materials promote bone regeneration and angiogenesis due to their exceptional bioactivity and versatility. Recently, a borosilicate glass composition has shown promise with enhanced bioresorbability and thermal stability, enabling the fabrication of porous scaffolds suitable for bone regeneration. While bioactive glass particles have been associated with antimicrobial properties, the smaller surface area of scaffolds, compared to granules is unlikely to prevent biofilm formation. Therefore, it is of outmost importance to develop a biomaterial, loaded with an antimicrobial drug, that can be actuated, in-vivo, for spatial-temporal control. [1, 2]

METHODS: The $26.93SiO_2$–$26.93B_2O_3$–$22.66Na_2O$–$1.72P_2O_5$–21.77CaO (mol%) bioactive glass was synthesized using the melt-quenching technique, crushed to <38 µm, and mixed with $CaWO_4$ crystals codoped with Yb^{3+} (15 at%) and Er^{3+} (0.75 at%), prepared via solid-state reaction. Scaffolds were fabricated using the porogen burn-off method, incorporating 10 wt% crystals, and sintered at 555 °C for 1 h. S-Nitroso-N-Acetylpenicillamine (SNAP), a NO-donor, was coated on the scaffold. Scaffolds were evaluated for upconversion properties, bioactivity in simulated body fluid (SBF), mechanical strength, and cytocompatibility with human adipose stem cells (hADSCs) using Live/Dead assays and fluorescence microscopy. NO release, under 980mnm irradiation was quantified.

RESULTS: The prepared scaffolds exhibited interconnected porosity, mechanical properties similar to the cancellous bone, and bioactivity. Incorporation of $CaWO_4$ crystals codoped with Yb^{3+} and Er^{3+} enabled NIR-to-green upconversion under 980 nm excitation, with sufficient brightness (~2.23 cd/m²) to release nitric oxide (NO) from S-Nitroso-N-Acetylpenicillamine (SNAP). NO release was precisely controlled by NIR light, with no release observed in scaffolds without upconversion crystals. Despite the slow degradation of the crystals no cytotoxicity was reported, during culture of human adipose stem cells (hADSCs), in direct contact. [3]

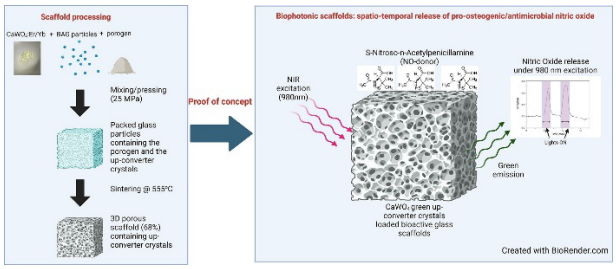

Fig. 1: Schematic image of scaffold processing and release of Nitric oxide

DISCUSSION & CONCLUSIONS: The biophotonic scaffolds demonstrate great potential for controlled drug delivery and bone regeneration. Incorporating upconversion crystals allowed efficient NO release while maintaining scaffold integrity, bioactivity, and cytocompatibility. Consistent upconversion emissions after two weeks in SBF suggest sustained functionality, paving the way for light-triggered therapeutic systems.

REFERENCES: [1] Erasmus, E., P. Sule, R. Johnson, O. T. Massera, J. Sigalas, I. (2018) Sci Rep., 8(1) : 3699. [2] Ghanavati. S, Petit. L, Massera. J. (2023) J. Non-Cryst. Solids, 616 : 122446. [3]Ghanavati, S., et al. (2024) Mater. Des., 247 : 113369.

Investigating the Impact of Extracellular Vesicles on the Cytocompatibility of Low-Modulus PMMA Bone Cement

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INTRODUCTION: Polymethyl methacrylate (PMMA) bone cement is widely used in vertebroplasty to treat osteoporosis-induced vertebral compression fractures.[1] However, PMMA has some drawbacks. The high stiffness of the cement may facilitate adjacent vertebral fractures, and as a bioinert material, it has poor bone integration.[2] Previous studies showed that linoleic acid (LA) is a promising additive to reduce mechanically-related complications.[1] Also, LA-loaded cement (LA-PMMA) showed a much lower maximum polymerization temperature,[1] which increases the possibility of adding bioactive substances to the cement, such as extracellular vesicles (EVs). EVs, known for their roles in intercellular communication, immune modulation, and tissue regeneration, present the potential for mitigating cytotoxic effects and enhancing bone regeneration.[3] This study incorporated EVs in LA-loaded bone cement and investigated the effect of EVs on the cytocompatibility of the cement.

METHODS: EVs isolated from tomato plants were used in this study. The EVs were mixed with LA and MMA monomer to create an EVs-loaded liquid blend, which was further mixed with the powder component of the cement and the resulting paste was injected into moulds (EV-LA-PMMA). Cement discs (diameter: 12.95±0.1 mm, height: 2.00±0.1 mm) were obtained and incubated in 1.2 mL cell culture media for 72 hours. Cell culture media was replaced every 24 hours and saved for evaluating the cytotoxicity of the extracts. The cytotoxicity assessment was done by exposing the murine preosteoblastic cell line MC3T3-E1, subclone 14 (CRL-2594, ATCC) to undiluted, 4-fold diluted, and 10-fold diluted cement extracts. Cell metabolic activity was evaluated by the Alamar blue assay after 24 h of cell exposure to the extracts. LA-PMMA discs without EVs were used as a reference. The mechanical and handling properties of the EVs-loaded cement were evaluated following ASTM-F451 standards.

RESULTS: The obtained results suggest that the addition of EVs to the low-modulus PMMA bone cement reduces its cytotoxic effects to some extent. In particular, the marked cytotoxic effect of the 48h-extract of LA-PMMA was abolished when EVs were incorporated into the cement (Fig 1). In terms of mechanical properties, the addition of EVs maintained the characteristic low modulus, 916 ± 71 MPa, of the LA-PMMA cement.

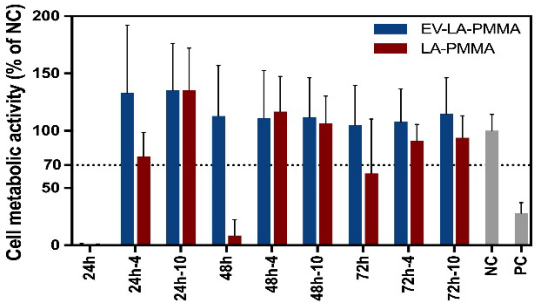

Fig. 1: Cell metabolic activity of preosteoblasts exposed to cement extracts for 24 h. n = 15, from 3 independent experiments with 5 replicates each. Mean and standard deviation are presented. LA-PMMA: low-modulus cement; EV-LA-PMMA: tomato plant EVs-loaded low-modulus cement; Times (24h, 48h, 72h) indicate extract collection time points; -4 and -10 indicate dilution factors. NC indicates negative control (only media). PC indicates positive control (5 vol% dimethyl sulfoxide in media). Cell metabolic activity greater than 70% indicates no cytotoxic effect.

DISCUSSION & CONCLUSIONS: The incorporation of EVs into low-modulus LA-PMMA bone cement shows promise in improving its cytocompatibility without compromising its mechanical properties. Future studies will investigate the effect of EVs-loaded low-modulus cement on the mineralization of preosteoblasts and the response of inflammatory cells.

REFERENCES: [1]P. Heini et al. (2000) *E. Spine J.*, **9**:445-450. [2]Zhao et al. (2023) *Chem. Eng. J.*, **474**: 145609. [3]F. Fang et al. (2024) *Bone Res.*, **12**: 4.

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Novel Adhesive and 3D-Printable Biomaterials for Corneal Tissue Engineering

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INTRODUCTION: Due to the global shortage of donor corneas, only 1 in 70 visually impaired patients can be treated with a corneal transplant to restore their sight[1]. Creating a cornea-mimicking structures by 3D-bioprinting has potential to answer this unmet need for alternative solutions. 3D-bioprinting allows precise control of the fine structure, controlling dispersion of cells and adjustment of mechanical and chemical properties within the structure, and thus offers the best potential to this date[2]. The rise of this technology has generated a demand for innovative and customizable 3D-printable bioinks. Many synthetic and natural materials have been investigated for their suitability as bioink[3]. The bioink supports cell growth and must be selected with care. Current options face significant challenges, including non-biocompatibility, weak wet adhesion, poor mechanical strength, and the requirement for UV light or harmful crosslinkers.
Here, we have modified the previously reported hyaluronan-based adhesive bioink[4] with dynamic and cell-friendly crosslinking system. To explore whether we can increase the adhesion, we grafted multiple catechol derivatives to hyaluronic acid backbone and provide a systematic comparison of these catechol-based components.

METHODS: We modified hyaluronic acid with catechols using well-established EDC/NHS chemistry and our previously reported method[4]. Degree of modification was determined with both NMR and UV-Vis spectroscopy. Mechanical characterization included rheological measurements to assess viscosity, shear thinning, and storage (G') and loss (G'') moduli of hydrogels, along with compression testing for self-healing capability and tensile testing for adhesion of synthesized materials. Finally, we printed grids through a 32G needle to characterise printability using high speed and low pressure for minimal flow of bioink. We also printed with low speed and high pressure to enhance the bioink flow.

RESULTS: NMR proved successful modification of hyaluronic acid with different catechols without compromising the cell-friendly crosslinking method. Crosslinked bioink remains soft but could be handled and showed shear thinning behaviour critical for extrusion printing and good shape fidelity. All but one catechol derivative enhanced the printability and stiffness of the bioink. We used bioink without catechol modification as the reference. Catechol bioinks had better shape fidelity, as shown in Fig. 1., which was especially prominent with high flow of biomaterial.
Additionally, tissue glued together with the ink could carry loads and could be stretched multiple times of its own length. Catechol modified hydrogels also showed self-healing properties.

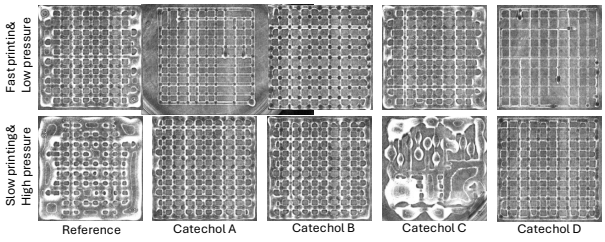

Fig. 1: Images of 4 different printed catechol-hydrogels with two different printing conditions.

DISCUSSION & CONCLUSIONS: Developed materials fill demands of next generation bioinks with excellent printability. They are elastic and adhere to surfaces and tissue. The hydrazone crosslinking method is dynamic; the structure heals itself after subjected to deforming stress. These adhesive bioinks show promise for soft tissue engineering applications and have unique adhesive properties.

REFERENCES: [1]P.Gain et al. (2016) *JAMA Ophthalmology* **134**: 167–173. [2]A. Schwab et al. (2020) *Chem. Rev.* **120**: 11028–11055. [3]D.Gogoi, M. Kumar. & J. Singh. (2024) *Annals of 3D Printed Medicine* **15**: 100159 [4]A.Mörö et al. (2022) *Biofabrication* **15**, 015020

ACKNOWLEDGEMENTS: We are thankful for Business Finland, Vilho, Yrjö and Kalle Väisälä Foundation, Eye and Tissue Bank Foundation, Foundation of Finnish Chemistry Congress and Jane and Aatos Erkko Foundation for funding. The authors wish to thank Alexander Efimov for helping to perform NMR measurements, Maija Kauppila for her help with rheological characterization and Hanna Kaiser for preparing UV-Vis standards.

Exploring osteoimmunomodulatory ion combinations to stimulate cellular processes for bone growth

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INTRODUCTION: The healing of critical-sized bone defects is a significant clinical challenge, primarily due to the necessity of coordinated interactions between the various cell types in a complex biological environment. Osteoimmunomodulation is an interdisciplinary approach that integrates the principles of immunology and bone biology and uses biomaterials such as ions to adjust the balance between bone formation and resorption, thereby promoting more effective bone forming and repair [1]. Macrophages are crucial for bone healing by mediating interactions not only between biomaterials and bone cells but also between the cells and biomaterials. Pro-inflammatory (M1) and anti-inflammatory (M2) macrophages contribute to the different stages of healing process by first mediating the pro-inflammatory responses and stem cell recruitment and later contributing to anabolic responses. All this occurs through the secretion of a variety of signalling molecules, growth factors, and cytokines [2].

In this study, we will investigate the direction of macrophage polarisation, regulation of inflammatory signalling, and promotion of osteogenic differentiation when the immune cells are treated with different combinations and concentrations of therapeutic ions. By optimising ionic compositions, we aim to create a pro-regenerative immune microenvironment that accelerates bone healing processes and improves bone tissue integration.

METHODS: Effects of biologically active ions on bone formation will be investigated by polarising macrophages to the M1 phenotype and treating them with various combinations and concentrations of osteoimmunomodulatory ions and assess their polarisation into the M2 phenotype. Polarisation will be assessed using flow cytometry, RT-qPCR, and ELISA to analyse specific marker expression and cytokine secretion. Moreover, conditioned media from ion-treated macrophages will be used to culture pre-osteoblasts and mesenchymal stem cells and the osteogenic differentiation will be evaluated by ALP activity, Alizarin Red staining, and RT-qPCR for osteogenic markers.

RESULTS: We aim to find optimised ion combinations and concentrations to modulate macrophage polarisation that enhance M2-driven regeneration while regulating M1-mediated inflammation. This immune modulation has potential to promote osteoblast differentiation and mineralisation, which could improve bone formation in critical-sized defects.

DISCUSSION & CONCLUSIONS: Our findings are expected to demonstrate how specific ion combinations modulate macrophage polarisation, potentially enhancing M2-driven tissue regeneration while balancing M1-mediated inflammation. This aligns with emerging research highlighting the role of immune regulation in bone healing. Our findings will contribute to the development of novel ion-based biomaterials with tailored osteoimmunomodulatory properties, offering new therapeutic strategies for enhanced large bone defect healing.

REFERENCES: [1]C. Schlundt et al. (2015) *J. Exp. Orthop.*, **2**: 1-0. [2]D. Zhang et al. (2023) *J. Tissue Eng. Regen. Med.*, **1**:1512966.

ACKNOWLEDGEMENTS: The authors would like to acknowledge the Finnish Ministry of Education and Culture, Makarna Agneta och Carl-Erik Olins fond, and Ellida och Tor Mauritz Ljungbergs stipendiefond for financial support. This study is part of the activities of the Åbo Akademi University Foundation (SÅA)-funded Centre of Excellence in Research "Materials-driven Solutions for Combatting Antimicrobial Resistance (MADNESS)".

Maximizing Bovine Embryo Survival and Development in vitro through Novel Biomimetic Hydrogel Cultivation

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INTRODUCTION: Assisted reproduction technology (ART), including in vitro fertilisation (IVF), is widely used for animal breeding, particularly for the conservation of endangered species and cattle breeding [1]. Transvaginal ovum pick-up (OPU) with *in vitro* embryo production is a cost-effective and reliable method for cattle [2]. However, *in vitro* embryo culture has generally low success rates, with only 20–40% blastocyst maturation, due to factors such as epigenetic changes [3]. Employing a three-dimensional (3D) culture medium can provide sufficient mechanical support, leading to increased embryo yield and improved survival rates.

In this study, a non-toxic, photocrosslinkable 3D cultivation hydrogel system suitable for bovine embryo cultivation was developed. The main component of the hydrogel, the copolymer of lactic and glycolic acid, and poly(ethylene glycol), PLGA-PEG-PLGA, provides thermosensitivity, photocrosslinking provides additional mechanical stability, and stabilised fibroblast growth factor-2 (FGF2-STAB®) supports blastocyst development.

METHODS: The PLGA-PEG-PLGA copolymer was synthesised via ring-opening polymerisation on the Shlenk's line. The hydrogels were formed by mixing a PLGA-PEG-PLGA hydrogel with a photo-crosslinker, ethylene glycol dimethacrylate (EGDMA), or poly(ethylene glycol dimethacrylate) (PEGDMA). The polymer was characterised by NMR, and the mechanical properties of the hydrogels were evaluated by dynamic rheological analysis. The release of FGF2-STAB® was determined using ELISA and cytotoxicity was evaluated using a life-dead assay on mouse fibroblast cells.

RESULTS: By combining PLGA-PEG-PLGA with photocrosslinkers, a transparent and hydrolytically stable hydrogel system was achieved. Importantly, these hydrogels exhibited no release of acidic compounds even after six days of exposure to embryo cultivation media, maintaining a stable pH level. They were found to be non-toxic and supportive of the viability of mouse fibroblasts (Fig.1).

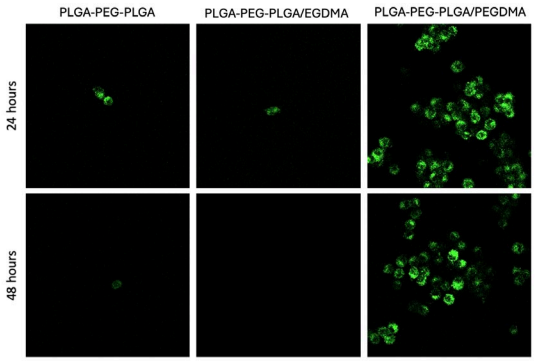

Fig. 1: Viability of mouse fibroblasts on hydrogel surface after 24 and 48 h

Rheological analysis demonstrated that brief irradiation effectively crosslinked photocrosslinkers within the hydrogels. The release profile of FGF2-STAB® followed a zero-order mechanism: hydrogels formed from PLGA-PEG-PLGA combined with PEGDMA exhibited a slower release, with approximately 45% released over 14 days.

DISCUSSION & CONCLUSIONS: The photocrosslinked hydrogel system offers a stable, non-toxic, and promising 3D cultivation medium for cattle oocytes with potential applications in human-assisted reproduction.

REFERENCES: [1]R. Santymire et al. (2014) *Adv. Exp. Med. Biol.*, **753**: 119-134. [2]C. Galli et al. (2014) *Theriogenology*, **81** (1): 138-151. [3]L. Ferré et al. (2020) *Animal*, **14** (5): 991-1004.

ACKNOWLEDGEMENTS: This project was supported with the state support of the Technology Agency of the Czech Republic as part of the National Center of Competence Program no. TN02000017 and also by the AD622302001 Jaroslav Koča Bridge Fund of CEITEC. CzechNanoLab project LM2023051 funded by MEYS CR is gratefully acknowledged for the financial support of the measurements/sample fabrication at CEITEC Nano Research Infrastructure.

Bioactive Glass S53P4 Suppresses Inflammatory Phenotype in Macrophages While Promoting Oxidative Phosphorylation

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INTRODUCTION: Macrophage metabolism is closely linked to their phenotype and function[1], which is why there is growing interest in studying the metabolic reprogramming of macrophages. Bioactive glass (BG) S53P4 is a bioactive material used especially in bone applications. Additionally, BG S53P4 has been shown to affect macrophages[2,3], but the mechanisms through which the possible immunomodulatory effects are conveyed remain unclear. The aim of this study was to provide a more comprehensive understanding of the effects of bioactive glasses on macrophage phenotype and function.

METHODS: The effects of BG S53P4 on macrophages were studied in vitro using mouse macrophage cell lines (RAW 264.7 and J774-Dual) and an LPS-induced inflammation model. The cells were grown both in direct contact with the BG as well as in BG conditioned medium. Macrophage growth rate and confluence was analyzed using the IncuCyte Basic Analysis Software.

The potential metabolic rewiring of macrophages was studied by assessing mitochondrial respiration and glycolytic rate with the Seahorse analyzer (Agilent) that allows the measurement of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).

The inflammatory cell signaling cascades were studied with the J774-Dual macrophage cell line, which allows a simultaneous study on the effects of BG S53P4 on both NF-κB and interferon regulatory factor (IRF) pathways.

Nitric oxide production was measured with the Griess reaction, and the mRNA expression of inflammation related genes was quantified with qPCR.

RESULTS: An overall increase in cell proliferation was observed after LPS activation. Cell confluence at 48 hours was lower when macrophages were cultured in direct contact with BG S53P4, but when the cells were cultured in biomaterial conditioned medium, no such effect was observed.

Our results show that BG S53P4 increases both oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of macrophages. Additionally, BG S53P4 dampened the activation of NF-κB and IRF pathways, which were triggered by LPS stimulus, as well as decreased the mRNA levels of inflammatory genes IL-1β and IL-6. LPS also induced nitric oxide secretion from cells after 24 hours, but in the presence of BG S53P4, this effect was slightly reduced.

DISCUSSION & CONCLUSIONS: BG S53P4 reverses the suppression of oxidative phosphorylation in LPS-activated macrophages and guides the cells towards an anti-inflammatory phenotype. These changes did not depend on macrophages being in direct contact with BG S53P4 indicating an important role for indirect effects, such as the dissolution products or the pH increase associated with BG S53P4. Taken together, BG S53P4 demonstrates properties of an applicable immunomodulatory material for future use.

REFERENCES: [1]Viola et al. (2019). *Front Immunol*, **10**: 1462. [2]Björkenheim et al. (2021). *Eur Cell Mater*, **41**: 15-30. [3]Barrak et al. (2022). *J Dent*, **127**: 104296.

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MSc Vilma Tupala and BScs Martina Pérez Jiménez and Kaisla Kangas are kindly acknowledged for technical assistance and conducting part of the experiments.

Exploring cancer cell homing using a transwell-based alginate hydrogel platform

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INTRODUCTION: Cancer metastasis, driven by the ability of malignant cells to home to distant organs, is the leading cause of cancer-related mortality. The process of homing is critical for cancer spread, and understanding its mechanisms is essential for developing effective treatments. Accordingly, this study applied a transwell-based alginate hydrogel (TAH) platform as a cancer microenvironment to demonstrate the homing behavior and metastatic potential of gastric cancer stem cells (CSCs). Based on the findings of Chu et al. (2020) [1] and Busuttil et al. (2018) [2], we aim to evaluate the effects of the TAH niche on the behavior of CSCs.

METHODS: The gastric cancer cell lines MKN45 or AGS was co-cultured with endothelial cells (HMEC-1) or mesothelial cells (MeT-5A) using the TAH model. This TAH platform, which mimicked the tumor microenvironment, facilitated cell-to-cell interactions across distinct chambers. The upper chamber contained endothelial or mesothelial cells, while the lower chamber cultured cancer cells. The cancer cells that migrated to the alginate hydrogel were characterized and recognized as the CSC population. Homing indices were determined based on the migration and adhesion of the CSCs.

RESULTS: By using the TAH model, MKN45 showed significantly higher migration and adhesion of CSCs than those of AGS under co-culture fashion (either HMEC-1 or MeT-5A cells). Their homing index was 0.63 for HMEC-1 and 0.75 for MeT-5A, respectively. In contrast, AGS exhibited negative indices (-0.45 for HMEC-1 and -0.43 for MeT-5A, respectively), suggesting a stronger homing ability in MKN45.

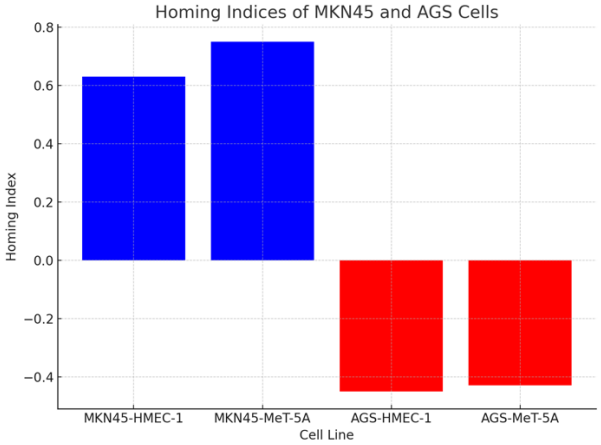

Fig. 1: Homing indices of MKN45 and AGS CSCs co-cultured with HMEC-1 and MeT-5A. MKN45 showed positive homing indices, while AGS displayed negative indices.

DISCUSSION & CONCLUSIONS: The results revealed that MKN45 has higher homing indices than AGS, correlating with their more significant metastatic potential. The TAH model provides valuable insights into CSC dynamics and highlights the role of microenvironmental cues in homing behavior. This platform offers a promise for metastasis research and the development of anti-metastatic therapies, serving as a robust tool for studying cancer metastasis and testing potential treatments.

REFERENCES: [1]H.Y. Chu et al. (2020) *Cancers*, **12**: 2176. [2]R.A. Busuttil et al. (2018) *Scientific Reports*, **8**: 825.

ACKNOWLEDGEMENTS: This study was supported by the Ministry of Science and Technology, Taiwan.

Evaluating bone-implant contact in metal-based implants: Comparison of 2D histology and 3D micro-CT analysis

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INTRODUCTION: Successful orthopaedic implants require intimate bone–implant contact (BIC) for optimal function and long-term success. BIC, defined as the percentage of the implant surface in direct contact with bone tissue, is a critical parameter influencing implant stability and long-term success. Traditional 2D histological methods assess bone-to-implant contact (**2D-BIC**) by measuring the length of the bone-implant interface along one or more planes of section and dividing it by the total implant perimeter within those respective planes. In contrast, micro-CT analysis enables the three-dimensional evaluation of BIC (**3D-BIC**), potentially offering a more comprehensive assessment of the bone-implant interface by quantifying the contact area across the entire implant surface.

METHODS: This study compared the *in vivo* performance of a commercially available Mg-Y-RE-Zr alloy (MAGNEZIX®) with three experimental biodegradable Mg-Y-(Li)-based alloys processed by different plastic deformation techniques. The alloys were implanted in the form of screws/pins into the femurs of rats for 6 months. 3D-BIC was evaluated using micro-CT with a voxel size of ~ 6 µm, providing detailed 3D information about the interface between the implant and the surrounding bone. To assess the accuracy of 2D histological methods, 2D-BIC was also evaluated from histological sections.

RESULTS: A key focus of the results will be to quantitatively assess the variability of 2D-BIC values across different histological planes and to determine whether 2D-BIC values from a single plane accurately represent the overall 3D-BIC.

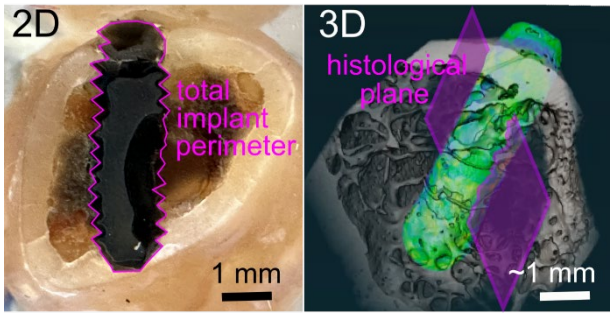

Fig. 1: (Left) Image of a bone-implant specimen embedded in poly(methyl methacrylate) for histological analysis. The magenta line indicates the total implant perimeter for 2D-BIC evaluation. (Right) 3D micro-CT reconstruction. Implant is shown in green–blue, bone in grey, and a magenta plane represents an example of a histological section for 2D-BIC evaluation.

DISCUSSION & CONCLUSIONS: This study aims to critically evaluate the accuracy and limitations of traditional 2D histological methods for assessing BIC in biodegradable metal-based implants. The findings of this study will contribute to a better understanding of the factors influencing peri-implant bone remodelling and guide the development of more effective and predictable metal-based bone implants.

ACKNOWLEDGEMENTS: Supported by the project Biodegradable (OP JAC; reg. no. CZ.02.01.01/00/23_020/0008512) – co-funded by the European Union and by the State Budget of the Czech Republic.

Interaction of primary fibroblasts with 100% nanofibrous yarn for surgical suture materials

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INTRODUCTION: Nanofibers are a popular material in tissue engineering application because of their excellent properties, such as fiber diameter similar to extracellular matrix protein, huge porosity and large number of contact points for cell adhesion. Our project focuses on the preparation of nanofibrous yarns using alternating current (AC) electrospinning [1] and their use as surgical sutures. Sutures play a critical role in wound healing by providing support and facilitating tissue repair after surgery or trauma. The basic function of sutures is to hold layers of tissue together, promoting proper alignment for healing. In addition, by closing the wound, sutures help reduce the exposure of internal tissues to bacteria, thereby reducing the risk of infection. As the wound heals, sutures promote the formation of collagen, which strengthens the tissue over time. We expect that the nanofibrous surface of suture will support cell adhesion and protein synthesis and enhance its incorporation into the surrounding tissue.

In the current study, we tested two nanofibrous yarns with different diameters and compared them with a planar nanofibrous sample. All samples were prepared from polycaprolactone (PCL) by AC electrospinning. We tested the samples for cell adhesion, proliferation and synthesis of ECM proteins.

METHODS: Planar nanofibrous material and nanofibrous filaments were seeded with neonatal human dermal fibroblasts HDFn (ATCC) and cultured for 14 days. Cell adhesion and further proliferation and viability were determined by MTS assay, dsDNA quantification and confocal microscopy. Synthesis of proteins typical for cell adhesion (vinculin) and extracellular matrix (type I collagen, fibronectin) was detected by gene expression and protein detection using immunofluorescence staining.

RESULTS: MTT assay for cell metabolic activity and dsDNA quantification showed good cell adhesion and proliferation on nanofibrous yarns. The values measured in both assays were higher compared to the planar sample. Immunofluorescence staining showed comparable positivity for vinculin on all samples. We also detected type I collagen and fibronectin, proteins typical of extracellular matrix.

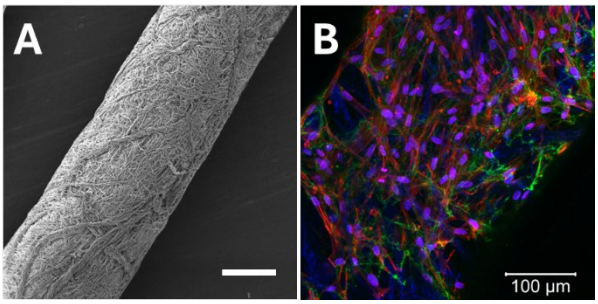

Fig. 1: 100% nanofibrous yarn visualized using SEM (A), scale 500 µm. Immunofluorescence staining of fibronectin produced by fibroblasts adhered on nanofibrous yarn (green color) (B). Cell nuclei were stained using Hoechst (blue color) and actin fibers using phalloidin (red color). Visualized using confocal microscope, scale bar 50 µm

DISCUSSION & CONCLUSIONS: The study showed that the nanofibrous surface of the yarns promoted cell adhesion and proliferation, and the measured values were even higher than for a planar sample of the same material. HDFn synthesized proteins typical of the ECM, collagen I and fibronectin. Thus, we confirmed our prediction that the nanofibrous surface of surgical yarns can promote cellular activity and integration of the material into the surrounding tissue.

REFERENCES: [1]J. Beran et al., "A method of the production of nanofiber yarn by alternating electrical spinning of the polymer solution or melt and an equipment to perform the method," PV2022-370, (2024). Available: https://isdv.upv.gov.cz/webapp/!resdb.pta.frm

ACKNOWLEDGEMENTS: This research was supported by Ministry of Health of the Czech Republic, grant nr. NW24-08-00133.

Beyond the Critical Micelle Concentration: Formulation of the Bilayer Vesicles in PLGA–PEG–PLGA Copolymers Targeting for Drug Delivery

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INTRODUCTION: Poly(D,L-lactic acid-co-glycolic acid)-b-poly(ethylene glycol)-b-poly(D,L-lactic acid-co-glycolic acid) (PLGA–PEG–PLGA) is amphiphilic triblock copolymer able to form an organized nanostructures when exceeding the critical micelle concentration (CMC). We hypothesize that PLGA–PEG–PLGA can form polymer bilayers (i.e., polymersomes) even below the CMC, challenging the traditional paradigm. The possibility of these bilayer vesicles bears similarities to lipid-based membranes. Understanding whether these nanoscale assemblies under sub-CMC conditions is crucial for drug delivery and biomedical applications at low polymer concentrations.

METHODS: PLGA–PEG–PLGA was synthesized via ring-opening polymerization. Size exclusion chromatography confirmed molecular weight and narrow dispersity (Mn ~ 5.3 kDa; PDI ~1.14). The UV-Vis was employed to determine the CMC. Dynamic light scattering provided complementary measures of size distribution, concentration, and diffusion coefficients. Fluorescence anisotropy assessed the rigidity of bilayer structures. The cryo-TEM offered visualization of nanoscale assemblies. A polymer model was built in GROMACS and compared to a phospholipid bilayer; the outputs provided insight into immobilized water at the bilayer interface.

RESULTS: Below the measured CMC (0.04 w/v%), fluorescence results indicated significant hydrophobic region suggesting that the copolymer does not exist as free chains. Dynamic light scattering confirmed stable nanoscale assemblies of ~ 90 nm even at sub-CMC concentrations, while high fluorescence anisotropy values (from 0.320 to 0.382) supported the presence of a rigid, bilayer-like environment rather than a simple micellar core. Cryo-TEM images revealed vesicular structures. Using molecular dynamics simulations, we found water molecules strongly bound to the polymer interface. An AI model (simplified X-ception) classified a fraction as “frozen” indicating restricted mobility that likely stabilizes the bilayer (Fig. 1).

Fig. 1: Schematic polymer assembly with a bilayer (hydrophilic heads outward, hydrophobic tails inward)

DISCUSSION & CONCLUSIONS: Other studies showed that the PLGA–PEG–PLGA have CMC of approximately 0.03% w/v. Below this range, these systems are typically assumed to exist as free polymer chains rather than organized assemblies [1]. However, our fluorescence anisotropy measurements suggest a highly ordered hydrophobic domain. Notably, these anisotropy values are comparable to those observed in phospholipid bilayers (e.g., DPSC (1,2-distearoyl-sn-glycero-3-phosphocholine) ~ 0.3) [2]. This contrast underscores the organized, bilayer-like character of the PLGA–PEG–PLGA domains, even under conditions where the copolymer is assumed to be largely unimeric. The intrinsic flexibility of the PEG backbone allows the PLGA blocks sufficient freedom to form robust supramolecular structures [3], supporting our hypothesis that polymersome-like assemblies may exist below the conventional CMC. Such bilayer organization provides a distinct environment for hydrophobic molecules highlighting the potential of sub-CMC polymer assemblies in biomedical applications.

REFERENCES: [1]L. Yu et al. (2011), *Biomacromolecules*, **12**:1290-1297. [2] J. Kotoucek et al. (2020), *Sci Rep*, **10**:5595. [3]K. Abe et al. (2015), *Colloids Surf A Physicochem Eng Asp*, **474**:81-88.

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Shifted laser surface texturing of titanium: micro-nanopattering and enrichment with silica-phosphate glass-derived products

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INTRODUCTION: Surface modifications of titanium and titanium alloys, including texturing, roughening, and biological activation, have been extensively studied to improve osseointegration. Various techniques, such as advanced physicochemical methods, laser-induced patterning, and biomimetic modifications, have been successfully tested for stable implant integration. All these surface modification techniques for titanium implants have demonstrated enhanced bone formation and improved osseointegration compared to untreated surfaces [1]. It is widely recognized that laser surface texturing is an efficient technique for producing precise structures with specific geometries. A novel method known as shifted laser surface texturing (sLST) has the potential to enhance productivity and enable the creation of complex-shaped objects [2]. Moreover, the use of multiple chemical agents has been shown to promote bone formation, while the biological rationale behind bioglass coatings lies in their ability to form a well-defined porous structure and release specific ions that influence the tissue-implant interface [3]. Here, titanium scaffolds were subjected to shifted laser surface texturing (sLST) to create an open-pore macrotopography with micro-nanopatterning. Additional surface treatment using sol-gel products derived from bioglass has been explored.

METHODS: Three-dimensional cavity patterns with depths and diameters in the range of hundreds of micrometers, featuring various geometries, were created by laser ablation of titanium using a nanosecond SPI G3 series laser and the sLST technique. Impregnation and surface modification were subsequently carried out using silica-phosphate glass and its laser ablation products.
Pristine and modified surfaces were analyzed using chemical, thermal, and structural techniques. Additionally, the human SCP-1 cell line was used for the following experiments. A resazurin assay was conducted to evaluate cell proliferative activity. Live/dead staining was performed to assess cell survival on the scaffolds, and Alizarin Red staining was used to analyze osteogenic differentiation capacity.

RESULTS: The porous micro-/nano-structured titanium surface features well-defined macro circular cavities with an irregular morphology, covered by a moss-like structure at the nanoscale. Sol-gel silica-phosphate glasses modified with TTIP were easily pipetted and successfully filled the circular pores. These glasses exhibited tunable porosity, reaching up to 70 vol% in the meso-/macro-region, while maintaining excellent mechanical integrity during the calcination process

DISCUSSION & CONCLUSIONS: The presence of 7.5 mol% TiO_2 promotes the formation of a fully interconnected framework at a macroscale of 10–30 µm. The osteogenic differentiation capacity of hMSCs was significantly enhanced on sLST-Ti compared to sol-gel-modified surfaces. Despite this, TTIP-modified surfaces demonstrated safety and good biocompatibility.

REFERENCES: [1]T. Stich et al. (2022) *Bioeng. Trans. Med*, **7**:e10239. [2]J. Martan et al. (2019) *J. Laser Appl.* **31**, 022507. [3]S. Naseri et al. (2017) *J. Mater. Chem. B* **5**, 6167-6174.

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Microfluidic Cell Electrospinning for Neuron Tissue Engineering

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INTRODUCTION: Neurological disorders are estimated to affect 15 % of the worldwide population and are responsible for both physical and cognitive disabilities.[1] Neurodegenerative diseases are generally poorly understood and despite extensive investigation, no effective treatments that reverse pathological processes exist.[2] Typical *in vitro* model systems such as 2D cell culture and organoids are often insufficient to fully mimic the complexity of human brain tissue. Recently, more attention has been brought to the fact that extracellular environmental cues plays a large role for cell response through various mechanotransduction pathways.[3] Bioengineered 3D tissue models have attracted great attention as they can reconstruct diverse features of native tissue such as topographical, mechanical and biochemical cues while preserving simplicity for straight forward conclusions.[2] This study aims on creating a new biomimetic 3D nerve model that can be utilized for *in vitro* studies of diverse neurodegenerative diseases.

METHODS: Cell electrospinning is used to create nano-to-micron sized fibers encapsulating single cells. For characterization of the cell encapsulation efficiency, viability and biological function, live/dead assays, immunofluorescence staining, atomic force microscopy and in-fiber differentiation of are performed. Microfluidics has proven valuable for the creation of core-shell cell-laden microfibers mimicking natural morphologies [4] and is currently investigated for precise control of complex configurations of multiple fluids in combination with cell electrospinning for the creation of more advanced neuronal disease models.

RESULTS: The cell electrospinning process was studied using PC12 cells and key parameters for cell embedding efficiency and post-electrospinning viability were studied. It was found that optimization of solution viscosity and ratio of materials were crucial for obtaining thin, viable cell-embedding fibers that were crosslinkable and stable in cell culture for at least one week. It was found that cells, while compromised directly after electrospinning, could re-gain functionality after days in culture inside the electrospun fibers. Disease relevant primary cells and microfluidic creation of core/shell structures is currently studied for optimized cell encapsulation and functionality as a neuron fiber model.

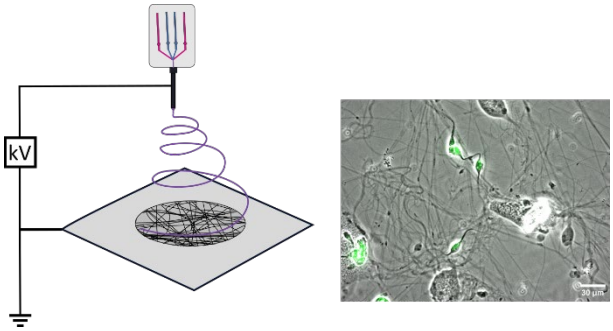

Fig. 1: Electrospinning combined with microfluidics is proposed for the fabrication of a biomimetic nerve model.

DISCUSSION & CONCLUSIONS: This study presents a new biofabrication approach with combined microfluidic system and electrospinning for production of an *in vitro* neural 3D model for studying neurodegenerative disorders and their pathological mechanisms. Single cell embedded fibers were successfully obtained and cell viability, morphologies and function were studied to lay the foundation for more complex artificial neuron models.

REFERENCES: [1]J. Van Schependom and M. D'Haeseleer (2023) Journal of Clinical Medicine, **12**(5): p. 1709. [2]A.S. Mullis and D.L. Kaplan (2023) Biomaterials, **298**: p. 122143. [3]I. Tortorella et al. (2022) Cells, **11**(19): p. 3093. [4]H. Onoe et al. (2013) Nature Materials, **12**(6): p. 584-590.

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Ascorbic Acid 2-Phosphate-Releasing Poly(L-lactide-Co-Epsilon-Caprolactone) Membranes for Pelvic Organ Prolapse: An *In Vivo* Study

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INTRODUCTION: Pelvic organ prolapse (POP) is a common condition affecting up to 40% of post-menopausal women. In POP, pelvic organs, such as bladder or uterus, descend through vagina causing various symptoms affecting the patients' quality of life. [1] Weakening of the pelvic support tissues and disrupted collagen and other extracellular matrix (ECM) protein metabolism is considered a major pathological characteristic of POP. [2] Relapsed POP was previously repaired using transvaginal nonabsorbable polypropylene (PP) meshes. However, in 2019, the FDA ordered manufacturers to stop selling these devices due to safety concerns. Therefore, there is a critical need for better and safer alternative repair materials for treating POP.

The current study investigates holed bioabsorbable ascorbic acid 2-phosphate (A2P) -releasing poly-l-lactide-co-epsilon-caprolactone (PLCL) membranes as a novel alternative for POP repair. Ascorbic acid and its derivatives, such as A2P, have been shown to enhance collagen production and cell proliferation *in vitro* [3-4]. Our aim with the PLCL-A2P membranes is to promote cell proliferation and ECM-protein, especially collagen, synthesis on site, regenerating the natural support of the weakened pelvic floor. We have previously demonstrated PLCL-A2P membranes to enhance proliferation and collagen production *in vitro* [4]. The current study is the first to assess the effect of PLCL-A2P membranes in an *in vivo* rat model to assess the effect on tissue regeneration.

METHODS: Rats were randomly divided into six groups based on the implanted material: 1) d0 ctrl, 2) SHAM, 3) commercially available PP, 4) PLCL, 5) PLCL-4%A2P, 6) PLCL-8%A2P. Materials were implanted subcutaneously on the abdominal fascia. Timepoints for the study were 1 wk, 4wk, and 26 wk, and N = 7/group/timepoint. Uniaxial tensile test was performed to assess the mechanical strength of the tissue. Histological sections were stained with Masson Trichrome stain to assess the tissue around the implant. The area of collagen, dense collagen and fat accumulation was measured using QuPath digital pathology software.

RESULTS: PLCL-A2P membranes supported the normal healing of the tissue with increased total collagen amount compared to other groups with very small amount of dense collagen localized around the biomaterial. No abnormal stiffness was detected.

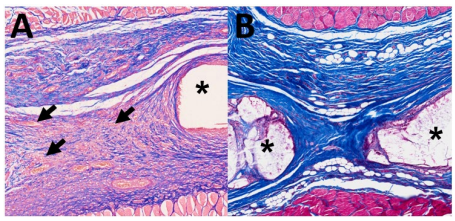

*Fig. 1: Masson trichrome staining at wk4 (A), and wk26 (B) shows connective tissue and new blood vessels (arrows) growing through PLCL-A2P membrane. At wk26, the PLCL-A2P has started to degrade, and tissue grows into the material. * = material*

DISCUSSION & CONCLUSIONS: Similar to previous *in vitro* findings [4], PLCL-A2P increased the total amount of collagen also *in vivo* compared to other material groups. Longer timepoints would provide further insights into the long-term regenerative potential of PLCL-A2P membranes for POP repair, as substantial amount of material was still present at the 26wk timepoint.

REFERENCES: [1]T. Vergeldt et al (2015) *Int. Urogynecol. J.,* **26**:1559-1573. [2]Z. Tian et al (2024) *Int. J. Gynecol. Obstet.*, **131**:1029-1041. [3]R.I. Hata et al (1989) *J. Cell. Physiol.* **138**:8-16. [4]A. Kurki et al (2024) *Regen. Biomater.* **2024**:rbae060.

ACKNOWLEDGEMENTS: We gratefully thank Finnish Centre for Laboratory Animal Pathology (FCLAP), Tampere University's Preclinical Facility, students, Biocenter Finland (BF) and Tampere Imaging Facility (TIF), Academy of Finland, Suomen Kulttuurirahasto, and other collaborators contributing to the study.

Phytate-Coated implants: A promising strategy for improved antibacterial activity in orthopaedic applications.

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INTRODUCTION: A key challenge in implantology is creating antibacterial surfaces that retain osteoconductive properties. Implant-associated infections, responsible for 20 % of implant failures, are often linked to biofilm formation, which complicates treatment. While hydroxyapatite (HA) coatings are widely used for orthopaedic implants, their anti-inflammatory and antibacterial properties are limited [1]. In previous studies, we developed a direct covalent grafted phytate (IP6) surface with osteogenic activity that also reduced bacterial adhesion on machined Ti implants [2]. In this study, we aimed to compare the antibacterial, biocompatibility, and osteogenic properties of rough IP6 and control Al_2O_3-blasted Ti surfaces with HA-coated implants, one of the most important orthopedic coatings, using both in vitro and in vivo models.

METHODS: Al_2O_3-blasted Ti implants were functionalized with IP6 by wet chemistry or with HA by plasma spray. Bacterial adhesion using *S. aureus* was measured. Implant biocompatibility and bioactivity were tested using MC3T3-E1 pre-osteoblasts. Finally, IP6 and HA coated rough implants were studied in an *in vivo* rabbit model. Densitometric, histomorphometric and histopathological studies were performed 8 weeks after implantation to assess osseointegration.

RESULTS: IP6-coated implants significantly reduced *S. aureus* adhesion. Both IP6 and HA-coated implants exhibited good biocompatibility levels, comparable to the control. Furthermore, IP6-coated surfaces demonstrated differentiation markers levels similar to the control but higher than those observed with HA. Additionally, PGE2 release was lower in IP6-coated implants compared to HA. In the *in vivo* study, IP6-coated implants showed improved micro-CT results compared to the HA implants. Moreover, histomorphometric analysis revealed that IP6-coated surfaces exhibited a higher percentage of the area of interest occupied by bone tissue compared to both the control and HA surfaces. Regarding histopathological results, differences were observed only in fibrous tissue score.

DISCUSSION & CONCLUSIONS:

Compared to the gold-standard HA coating, IP6 coating promoted greater bone differentiation in vitro. Additionally, IP6 reduced bacterial adhesion and decreased the production of the inflammatory mediator PGE2 following bacterial challenge in vitro. In an in vivo orthopedic implant model, IP6 demonstrated improved osseointegration compared to HA coatings. These findings underscore the potential of IP6 as an innovative orthopedic implant coating, providing effective antibacterial properties while supporting optimal osseointegration.

REFERENCES: [1]Szcześ, et al. (2017). *Adv. Colloid Interface Sci.* **249**:321–330, [2]Córdoba A, et al (2016). *ACS Appl Mater Interfaces* **8**:11326–35.

ACKNOWLEDGEMENTS: IP6 was obtained from Laboratoris Sanifit. HA and Al_2O_3 coatings were produced by ORCHID Orthopedic Solutions. This work was supported by "Direcció General d'Innovació i Recerca del Govern de les Illes Balears" co-funded ERDF European Regional Development Fund, (Fondos FEDER) (ES01/TCAI/15_2018), the Ministerio de Educación Cultura y Deporte (FPU15/03412).

Long-Term Viability and Adhesion of Human Gingival Fibroblasts in Direct Contact with Dental Restorative Materials

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INTRODUCTION: Various types of materials, such as smart ceramics, composites, resin-modified glass ionomers, amorphous inorganic materials, and shape-memory alloys have been widely applied in dentistry. Over the past few decades, their biological properties have been significantly enhanced [1]. The primary criterion for these materials has been their neutrality in the oral environment, ensuring that they remain passive within the oral cavity, even at the cellular level. However, recent advancements have introduced materials with active properties into modern dentistry. These materials, with improved biocompatibility and smart designs can interact dynamically with the oral environment. They respond to stimuli such as stress, temperature, moisture, or pH, releasing active substances or promoting the healing process [2]. Despite these advancements, the diverse range of restorative dental materials and their varied compositions pose challenges in accurately assessing their behavior *in vitro* and *in vivo*. In this study, we evaluated the long-term viability and adhesion of human gingival fibroblast cell line on four different dental materials (one fiber reinforced composite, one resin-modified glass ionomer and two experimental polymer-based ions releasing materials), providing insight into their biocompatibility and performance.

METHODS: Human gingival fibroblasts (HGF-1) were seeded on the top surface of four different dental materials (6 x 2 mm, Stick Tech Ltd.): Fuji II, EverX Flow, Bioliner 1 (TCBC) and Bioliner 2 (ST). Cell viability was assessed by Alamar Blue assay eight hours after seeding and then every 24 hours for the next 7 days. Along with viability, cell morphology was evaluated by phase-contrast microscopy. On the last day of experiment, samples were visualized via LIVE/DEAD staining or fixed and stained with Hoechst and Phalloidin to evaluate cell morphology and adhesion to the materials. Materials' surface morphology and degradation degree were additionally recorded during the experiment.

RESULTS: Tested materials in direct contact with the HGF-1 cell line showed limited biocompatibility in a long-term Alamar Blue cell viability assay. Surprisingly, cell morphology in the presence of materials was normal and similar to control group, i.e., cells cultured on plastic. LIVE/DEAD staining confirmed the presence of living cells. Finally, the surface of all materials remained similar and no signs of degradation was observed, and HGF-1 cells showed normal morphology and good adhesion to the materials.

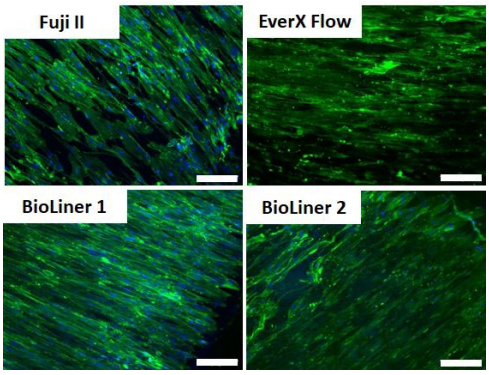

Fig. 1: Attachment of cells to the surface of the tested materials imaged by confocal microscopy. Cells were visualized by staining the cell nuclei (blue) and actin cytoskeleton (green). Scale bar (white) equals 200 µm.

DISCUSSION & CONCLUSIONS: This result indicates that despite of overall suboptimal cell viability (according to ISO 10993-5), Fuji II, EverX Flow, Bioliner 1 and Bioliner 2 can be considered as biocompatible according to the results obtained via microscopic techniques. However, more repetitions of the biocompatibility assay are required before performing more advanced tests.

REFERENCES: [1]L. Parul R et al. (2023) *Cureus*. **15**, [2]V. Badami & B. Ahuja. (2014) *The Scientific World Journal*.

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Development of a Novel Coating to Modulate Hypoxic Signaling for Enhanced Implant Tissue Regeneration

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INTRODUCTION: A fast and strong integration of the material with the patient is key for a successful implant performance. However, implants are increasingly being placed in patients with compromised healing capacity due to health conditions such as osteoporosis or chronic inflammatory diseases, often resulting in implant failure [1]. Upregulations of HIF-1α have shown promising results in enhancing wound healing. The 4-prolylhydroxylase inhibitor 1,4-dihydrophenonthrolin-4-one-3 carboxylic acid (DPCA) can effectively stabilize HIF-1α [2]. We aim to functionalize titanium implant surfaces with the sodium salt of DPCA (NaDPCA) to modulate the hypoxic signaling pathways that enhance regeneration of peri-implant tissues.

METHODS: 0,25 mg/ml NaDPCA was dissolved into PBS with NaCl concentration adjusted to 0.6 M at pH 7.4. Real-time interaction of NaDPCA with Ti substrates was studied using a quartz crystal microbalance (QCM-D). Adsorption of NaDPCA on titanium sensors was recorded with and without an intermediate tannic acid (TA) layer deposited on the sensor surface as previously described. Further, co-deposition of 0,25 mg/ml NaDPCA and 1 mg/ml on titanium sensors was assessed. Each layer was deposited for 1 h, and after each deposited layer, a rinsing step with PBS was included to remove loosely bound particles. Release of NaDPCA from Ti discs previously coated with NaDPCA or TA-NaDPCA was studied using high-performance liquid chromatography (HPLC).

RESULTS: NaDPCA adsorbed as a monolayer when deposited on a bare Ti surface, resulting in ~15 Hz decrease in Fig. 1 A, blue. This layer was not removed after PBS rinsing. TA deposition resulted in ΔF ~75 Hz with no further changes in frequency when NaDPCA layer was deposited on top of the TA layer (Fig. 1A, red). However, significant increase in ΔF of ~120 Hz was observed when TA and NaDPCA were co-deposited on Ti Fig. 1A, black).

Immediate release of NaDPCA in PBS was observed irrespective of the co-deposition time. The release continued at a slower rate during the next 24 hours. Additionally, the concentration of released NaDPCA was higher for longer co-deposition times.

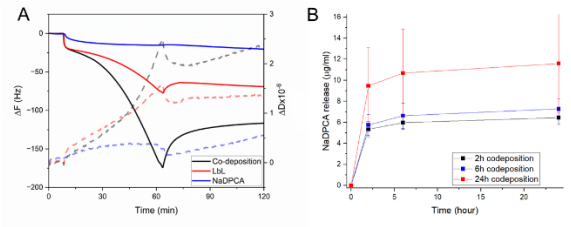

Fig. 1: QCM-D analysis of NaDPCA coatings (A). Release of NaDPCA in PBS (B)

DISCUSSION & CONCLUSIONS: When in direct contact with Ti, NaDPCA formed a monolayer that was stable under physiological conditions, preventing the desired release of the drug. In combination with TA, the formed layer continuously increased in thickness due to TA crosslinking, allowing more drug to be loaded in the coating as observed in a greater ΔF compared to only TA layer deposition. Additionally, NaDPCA could be released from the coatings in physiological conditions, with higher NaDPCA concentrations being detected with HPLC for longer co-deposited times. These coating present a promising approach to deliver DPCA directly to compromised tissues immediately after implant placement. Nevertheless, further optimisation of the loading capacity and release kinetics is required to obtain the most beneficial therapeutic effect.

REFERENCES: 1. Sakka, S. and P. Coulthard, Implant failure: etiology and complications. Med Oral Patol Oral Cir Bucal, 2011. 16(1): p. e42-4. **2**. Drager, J., E.J. Harvey, and J. Barralet, *Hypoxia signalling manipulation for bone regeneration.* Expert reviews in molecular medicine, 2015. **17**: p. e6. **3.** Weber, F., A. Barrantes, and H. Tiainen, *Silicic acid-mediated formation of tannic acid nanocoatings.* Langmuir, 2019. **35**(9): p. 3327-3336.

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Plant-based hydrogels for scalable and sustainable cultivation of adipose tissue

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INTRODUCTION: Cultured meat has recently attracted interest as an innovative approach to the production of meat from cell cultures. In addition to muscle proteins, fat is an important component of meat, providing flavour and moisture retention. Natural scaffolds, such as collagen and gelatine, efficiently support the *in vitro* cultivation of adipose tissue, but their animal origin limits their scalability and sustainability. Recently, plant polysaccharides and proteins have attracted attention as promising scaffold alternatives[1]. Polysaccharide fibres derived from brown algae (alginate) and from the seeds of *Plantago ovata* (psyllium) are considered functional food components with potential as scaffold biomaterials for cultured meat[2,3]. In this work, alginate/psyllium hydrogels are investigated as a platform for cultured adipose tissue.

METHODS: Alginate (Alginic acid sodium salt) was purchased from Sigma-Aldrich. Psyllium polysaccharides were extracted from commercially available powdered husks (FiberHUSK) using a novel chemical-free method. Alginate/psyllium hydrogels were prepared by mixing alginate and psyllium solutions in different ratios. The rheological properties of the hydrogels were analysed using rheometry (TA Instruments). Bovine adipose stromal cells (bASCs) were grown as spheroids in 96-well plates (BIOFLOAT, faCellitate) and cell viability was assessed by live/dead staining. Spheroids and hydrogel mixtures were blend and extruded into 3D constructs in a $CaCl_2$ bath. The cell-loaded constructs were cultured in multiwell plates under dynamic culture conditions on a rocking platform. Adipogenic differentiation was induced with serum-free media and assessed by BODIPY staining.

RESULTS: Our chemical-free extraction method yielded a clear, homogenised psyllium polysaccharide extract (Fig. 1a). Rheological analysis showed that alginate dominated hydrogels had a higher loss than elastic modulus at all frequencies, while 1% (w/v) alginate–1% (w/v) psyllium mixtures exhibited the opposite, with minimal frequency dependence. Psyllium alone did not crosslink in $CaCl_2$ without at least 0.5% (w/v) alginate. Optimal scaffold integrity for cell culture was achieved with 2% (w/v) hydrogels (Fig. 1c). The viability of the bASCs was maintained after spheroid formation (Fig. 1b). After encapsulation and induction of differentiation for 2 weeks, accumulation of lipid droplets confirmed successful differentiation The levels of lipid accumulation appeared similar when comparing spheroids encapsulated in 2% (w/v) alginate vs. spheroids in 1% (w/v) alginate–1% (w/v) psyllium (Fig. 1d).

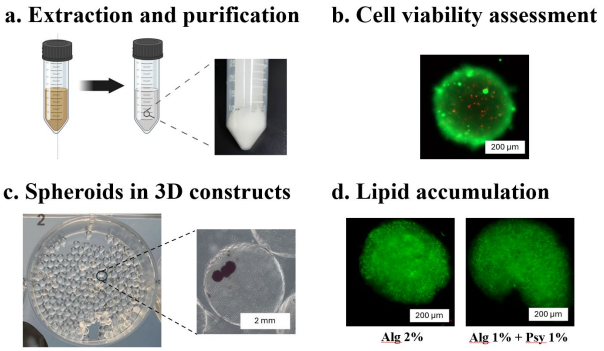

Fig. 1: (a) Clear polysaccharide extract from Psyllium husks (b) Viability of bASC spheroids assessed by calcein-AM and propidium iodide staining. (c) Representative 3D construct with encapsulated spheroids (d) Lipid droplet staining assessed by BODIPY after induction for 2 weeks.

DISCUSSION & CONCLUSIONS: These results show the feasibility of using alginate and psyllium hydrogels for the culture of bASCs. Extraction of psyllium polysaccharides using a chemical-free method was successful and resulted in food-grade materials with a good balance of purity and yield. Rheological tests confirmed favourable mechanical properties that allowed stable extrusion of the droplets into uniform 3D constructs. The successful differentiation of bASCs highlights the potential of these hydrogels for the cultivation of adipose tissue and lays the foundation for future scalable and sustainable production of cultured fat.

REFERENCES: [1]J.O. Wollschlaeger et al. (2022) *Gels,* **8** (2): 94. [2]W.S. Jeong et al. (2023) *Carbohydr. Polym.,* **321**:121287. [3]M. Belorio et al. (2021) *Crit. Rev. Food Sci. Nutr.*, **62**(2): 527-538.

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Decellularized Human Amniotic Membrane Biomaterials: An In Vitro Study

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INTRODUCTION: Human amniotic membrane (hAM) is a thin membrane found in the innermost part of the placenta, delimiting the amniotic cavity. Its regenerative attributes and intrinsic physico-chemical properties make this tissue a promising therapeutic option and an ideal candidate for the generation of engineered tissue biomaterials[1,2]. hAM-derived biomaterials have great potential in the field of wound healing, as they harbour growth factors and cytokines, as well as antifibrotic, immunomodulatory and antibacterial capacities[3]. In addition, hydrogels generated from decellularized hAM can be derived into bioinks, which increase their potential application in regenerative therapies[4]. Therefore, this work presents the generation of decellularized hAM-derived biomaterials (films and hydrogels) and their in vitro characterisation.

METHODS: The hAM-derived biomaterials were generated after chemical decellularization of the tissue (dAM). The assessment of this process was verified by DNA content, analysis of structural components by histology and biochemical determination, and examination of the matrix structure. For hydrogels, freeze-dried dAM was mechanically pulverised and enzymatically digested. The neutralised pre-gel was gelled by temperature. The decellularized films were divided into native and freeze-dried. The resulting biomaterials were characterised by mechanical property analysis, rheometry, swelling and degradation testing and scanning electron microscopy. The effect of the different biomaterials was analysed in vitro by cytotoxicity, metabolic activity and wound healing assays.

RESULTS: DNA content analysis revealed the correct decellularization process of the tissue. In addition, histological staining and scanning electron microscopy images revealed the preservation of the structure after decellularization. The different biomaterials showed good mechanical properties as well as optimal degradation times and good biocompatibility in vitro. Furthermore, wound healing analysis showed a higher percentage of wound closure after treatment with the developed biomaterials (hydrogels and films).

DISCUSSION & CONCLUSIONS: The results obtained reveal that potential of decellularized hAM for the generation of biomaterials with good in vitro biocompatibility, for its application in different regenerative treatments. In a next step, these generated biomaterials could be combined with other biomolecules or drugs as a release scaffold or used for the generation of bio-inks for 3D printing, with a special focus on wound healing. Moreover, further studies are needed to demonstrate their safety and efficacy in vivo.

REFERENCES: [1]A. C. Mamede et al. (2012). *Cell and tissue research*, ***349*** (2): 447–458. [2]M. Fénelon et al. (2021). *Membranes*, ***11***(6): 387. [3]P. Heydari et al. (2024). *International journal of biological macromolecules*, ***281***(Pt 4): 136596. [4]G. Kafili et al. (2024). *Frontiers in bioengineering and biotechnology*, ***12***: 1358977.

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In vitro biocompatibility of differently prepared CMC hydrogels as potential delivery platforms for chronic wound treatment

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INTRODUCTION: Chronic skin wounds represent a significant healthcare challenge, requiring advanced therapeutic approaches that can effectively support tissue regeneration while preventing infection. Carboxymethyl cellulose (CMC)-based hydrogels have emerged as promising scaffold materials due to their biocompatibility [1], easy manipulation, capacity to serve as carriers for therapeutic agents [2], and properties easily adjusted according to desired outcome. The optimization of CMC gel preparation methods is crucial for developing effective delivery systems that can maintain stability while facilitating the controlled release of bioactive compounds [3]. This study explores ways to study the biocompatibility of differently prepared CMC hydrogels towards key skin cell populations, including fibroblasts (3T3, HDFn) and keratinocytes (HaCaT), investigating their potential as delivery platforms for therapeutic agents in chronic skin wound treatment.

METHODS: Three CMC hydrogel formulations were evaluated: room temperature-prepared (CMC/RT), autoclaved (CMC/Aut), and autoclaved with $CaCl_2$ being added right before application (CMC+$CaCl_2$). Initially, testing of CMC/RT was performed on 3T3 fibroblasts via direct application with cells cultured on inserts. Subsequently, all formulations were tested with human cells (HDFn and HaCaT) using three application methods: direct contact, cells seeded onto the gel, and cells cultured in the presence of the gels (indirect exposure). Cell response was assessed through morphology (light microscopy), metabolic activity (MTS), viability (Live/Dead), and proliferation (PicoGreen) assays.

RESULTS: CMC gel prepared at room temperature (CMC/RT) demonstrated biocompatibility with 3T3 cells, maintaining metabolic activity above 70% of untreated cells and showing increasing proliferation over 6 days. However, direct application, as well as cells seeded on top of CMC/RT proved cytotoxic for HDFn and HaCaT cells. When testing how well would HDFn and HaCaT do in indirect contact, HDFn showed initially reduced metabolic activity with CMC/Aut and CMC+$CaCl_2$, recovering to control levels by day 3, while CMC/RT maintained consistent metabolic activity. HaCaT cells demonstrated compatibility with all CMC formulations, showing comparable or enhanced metabolic activity versus controls across three days.

DISCUSSION & CONCLUSIONS: Among the tested 4.5% CMC gel preparations, CMC/RT emerged as the most promising carrier candidate for chronic wound therapeutics, demonstrating an optimal balance of biocompatibility across tested cell lines.

REFERENCES: [1]K.K. Kłosiński et al. (2022) Emadi, A. Mousavian, D.H.J. Alamdari (2024) *Polymers*, **15**:144 [2]C.M. Lai et al. (2024) *Adv.Sci.*, **2412360**. [3]X. Zhang et al. (2024) *Small*, **2400516**.

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Strategy for long-term carbon nanotube alignment in GelMA via magnetic field

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INTRODUCTION: Multi-walled carbon nanotubes (MWCNTs) have emerged as a promising material for use in three-dimensional (3D) biomaterials due to their exceptional mechanical, electrical, optical, and structural properties. These properties have led to their incorporation into various commercial products across diverse fields such as sports and electronics. Notably, CNTs have shown particular promise in tissue repair and regeneration when integrated into hydrogels[1]. They pose the unique ability to promote cell alignment, a crucial factor for mimicking the structure and function of various tissues, including cardiac, muscle, neural, and cartilage tissues[2]. Previously, we have successfully employed CNT micropillars to achieve the alignment of neurons [3] and chondrocytes on 2D template [4]. We have also demonstrated the feasibility of transferring CNTs to Gellan gum hydrogels and aligning them using electromagnetic fields [5]. In this study, we present a novel approach for the integration and long-term alignment of MWCNTs within GelMA hydrogels by combining electromagnetic and neodymium magnetic fields.

METHODS: MWCNTs were synthesized via catalytic chemical vapor deposition in a quartz tube reactor using ferrocene as a catalyst. The reduced ferrocene to iron within the walls of MWCNTs imparted magnetic properties. These MWCNTs were functionalized with carboxylic acid to create a homogeneously dispersed, MWCNT water-based ink. GelMA was prepared using Photogel® 50% DS purchased from Cellink. For the GelMA/MWCNT composite, equal mixture of LAP solution and MWCNT water-based ink were combined, resulting in final concentrations of 0.02 mg/mL MWCNT and 0.05% LAP. This MWCNT/LAP solution was subsequently added to the GelMA to create 5% GelMA/MWCNT solution. Crosslinking was initiated by exposure to 405 nm LED light for 2 minutes positioned at 5 cm from the samples. In case of alignment studies, two platforms were used. First, a stationary platform that can generate high magnetic field (800 mT) was employed for 10 min during composite crosslinking. Second, a portable magnetic stimuli-platform was built combining 3D printing and neodymium magnetic. This platform could be placed in CO_2 incubator, keeping the alignment of GelMA/MWCNT under 150 mT for 7 days.

RESULTS: The alignment of MWCNT in GelMA by combining high (800 mT) and low (150 mT) magnetic field was confirmed via optical measurement setup, which displayed maximum transmittance when laser polarization plan field E is perpendicular and minimum when it is parallel. This alignment was seen to extend over 7 days when the aligned composite is continuously exposed to 150 mT either at room temperature or in CO_2 incubator.

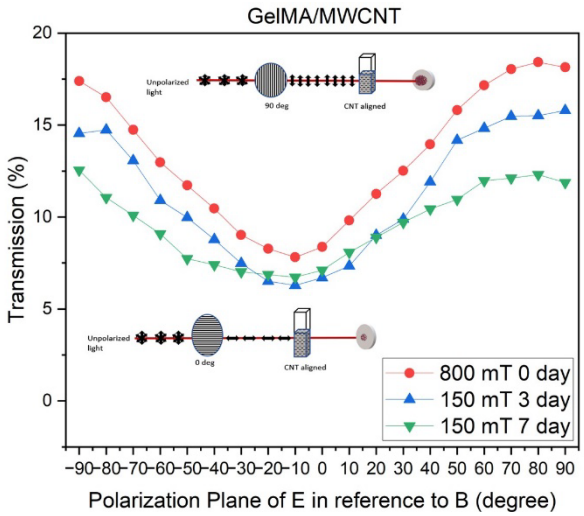

Fig. 1: Long-term alignment of MWCNT in GelMA under 800 mT (electromagnetic) for 10 min followed by 150 mT (magnetic-stimuli platform) for 7 days.

DISCUSSION & CONCLUSIONS: We have successfully demonstrated the long-term alignment of MWCNT in GelMA by combining stationary and portable magnetic stimuli platforms. The method overcomes the shortcoming of previous studies on Gellan Gum/MWCNTs composite where the alignment is lost over time [5]. This strategy will allow to proceed with cell studies aiming to induce cell alignment. Future work includes investigation of the MWCNT alignment up to 21 days and cell studies.

REFERENCES: [1] Ravanbakhsh, H.et al., *Sci. Rep.* **10**, 2543 (2020). [2]Li et al. *Biotechnol Adv.* 2014;32(2):347-365.[3]Lorite et al. *Nano Res.* 2019;12:2894–2899.[4]Janssen et al. *Carbon*. 2020; 158:681-689. [5]Saranya et al. *Compos Part B Eng*. 2022; 248:110398.

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Exploring the Benefits of SaOS-2 and HUVEC Co-Culture on Collagen Scaffolds for Bone Healing

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INTRODUCTION: The search for effective biomaterials in bone healing is crucial in the field of regenerative medicine, especially as the incidence of bone-related injuries and diseases continues to rise. Among various biomaterials, collagen has emerged as a prominent choice due to its natural abundance in bone tissue and its ability to facilitate cell adhesion and proliferation. Recent studies have shown that collagen-hydroxyapatite (HA) composites can significantly enhance osteogenic differentiation and vascularization, both essential for successful bone regeneration [1, 2]. The integration of HA into collagen scaffolds not only improves mechanical properties but also mimics the mineral composition of natural bone, thereby promoting better cellular interactions and tissue integration [3].

This study investigates the efficacy of collagen foams and collagen/HA composite foams in promoting osteogenic differentiation of SaOS-2 cells and angiogenic potential in HUVECs, hypothesizing that the incorporation of HA will enhance these cellular responses.

METHODS: To evaluate the biological performance, SaOS-2 and HUVECs were co-cultured in a 2:1 ratio on cylindrical scaffolds (7 mm × 3 mm) with 30,000 cells total, while control groups on TCP contained 4,500 cells per well. The study was conducted over 21 days with measurements on days 1, 7, 14, and 21.

Cell viability was assessed using the MTS assay, while alkaline phosphatase (ALP) activity and gene expression analysis of osteogenic markers (COL1, BGLAP) were employed to evaluate osteogenic differentiation. Cellular proliferation was determined by quantifying DNA content using the Quant-iT PicoGreen assay. Gene expression analysis was also performed to assess the expression of endothelial cell markers (PECAM1 and KDR). Confocal microscopy was utilized to visualize cellular morphology and investigate cell-biomaterial interactions.

RESULTS: Gene expression analysis revealed the highest COL1 expression at day 1 in all conditions, with TCP showing significantly higher levels compared to collagen-based scaffolds. OCN and ALP peaked at different time points; OCN

showed a distinct peak in TCP on day 7, while ALP showed the highest peak on day 1 for TCP. Endothelial markers showed varying patterns; with PECAM1 maintaining higher expression in collagen scaffolds throughout the culture period compared to consistently low levels in TCP.

Alkaline phosphatase activity peaked at day 1 for both collagen-based scaffolds with a gradual decrease over time. Metabolic activity increased throughout the culture period across all conditions, with TCP showing the highest activity by day 21, however, collagen-based scaffolds maintained more stable levels after day 14. Visualization using confocal microscopy demonstrated an even distribution of the cells on the scaffolds along with protein production.

DISCUSSION & CONCLUSIONS: The elevated expression of endothelial markers in collagen-based scaffolds, particularly PECAM1, suggests their superior ability to support vascular cell behavior compared to TCP, which is crucial for bone tissue vascularization. While TCP initially exhibited higher osteogenic marker expression, the sustained cellular response and stable metabolic activity observed in collagen-based scaffolds indicate their potential for long-term tissue support.

These findings demonstrate that collagen-based scaffolds, both with and without HA, provide a more physiologically relevant environment for co-culture systems, however, further optimization may be necessary to enhance their osteogenic potential to match or exceed that observed on TCP surfaces.

REFERENCES: [1]JZugravu et al. (2023) *Sci. Rep.*, **13**:28 [2]Bertolo et al. (2024) *J. Biomed. Mater. Res.*, **112**:456-467. [3]Scabbia et al. (2024) *Int. J. Morphol.*, **42**:567-578.

ACKNOWLEDGEMENTS:

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Decellularised Omentum-Derived Extracellular Matrix for the Culture of Next-Generation High-Grade Serous Ovarian Cancer Organoids: A Methodological Comparison

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INTRODUCTION: Ovarian cancer (OC) is acknowledged as the most aggressive gynaecological malignancy and stands as the fifth leading cause of cancer-related mortality in women [1]. Among the different types of OC, high-grade serous ovarian cancer (HGSOC) constitutes the most prevalent type, representing 75% of all identified epithelial OCs [2]. While two-dimensional *in vitro* models remain widely employed in diseases such as OC, 3D models such as patient-derived organoids (PDO) have gained prominence in recent decades, offering a significant advantage by replicating both the structure and function of tumours as they occur *in vivo* and recapitulating their histological and genomic features [3]. Despite their potential, the current derivation efficiency of OC PDO is one of the main limitations of their research and clinical use, partly due to their culture in commercial mouse-derived extracellular matrix (ECM) such as Matrigel™ [4]. Thus, next-generation organoid culture gets behind tailored ECMs that resemble the tumour microenvironment (TME) more accurately, such as decellularised tissues. In this study, we have compared different methodologies for obtaining decellularised omentum, a common site of peritoneal metastasis in advanced HGSOC, for its use in HGSOC PDO culture, to improve organoid derivation efficiency, as well as to better recreate the TME.

METHODS: Frozen omenta without tumoral nodules from donor HGSOC patients were obtained from the IdISBa Biobank under informed consent and ethical approval (CEI: IB 5535/24 PI). Omenta from three patients were submitted to decellularisation following two well-described protocols in parallel [5,6] with minor modifications. After decellularisation, tissues were frozen at –80°C and freeze-dried. Decellularisation was confirmed through total DNA extraction and quantification, as well as DAPI staining. For protocol comparison, tissues were characterised through scanning electron microscopy (SEM), histopathological analysis, total glycosaminoglycans (GAG) and collagen quantification.

RESULTS: Both protocols achieved an optimal degree of decellularisation, macro and microscopically preserving the extracellular architecture of the native tissue. The protocol with a major degree of preservation of the extracellular components (GAG and collagen) was selected to form an ECM-derived hydrogel for further studies with established HGSOC PDO lines.

DISCUSSION & CONCLUSIONS: ECM based on decellularised tissues constitute a good candidate for modelling cancer *in vitro*, serving as scaffolds or deriving hydrogels suitable for cell culture. Our results indicate that decellularised omentum preserves the necessary ECM components and thus could be exploited as a biomaterial for next-generation OC organoid culture.

REFERENCES: [1]U.A. Matulonis et al. (2016) *Nat Rev Dis Primers*. **2**:16061.[2]S. Lheureux et al. (2019) *Lancet*. **393** (10177):1240-1253.[3]B.L. LeSavage et al. (2022) *Nat Mater*. **21**(2):143-159. [4]E.A. Aisenbrey et al. (2020) *Nat Rev Mater*. **5**(7):539-551. [5]L.E. Flynn (2010) *Biomaterials*. **31**(17):4715-4724. [6]N. Soffer-Tsur et al. (2014) *Biofabrication*. **6**(3):035023.

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Enhancing Personalized Drug Delivery: Investigating SA/V Ratio Effects on Dexamethasone Release in 3D-Printed EVA Implants

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INTRODUCTION: Personalized medicine tailors treatments to patients' genetic, demographic, and lifestyle factors, but conventional drug production limits tablet geometry and dosage flexibility [1]. 3D printing offers a transformative solution, enabling small-scale production, customisable drug-dosage combinations, and precise control over drug release [2]. This study investigates the impact of surface area/volume (SA/V) ratio on drug release in ethylene vinyl acetate (EVA) implants fabricated using Fused Granular Fabrication (FGF).

METHODS: The dexamethasone-loaded (10% drug loading) EVA (Sigma-Aldrich) filaments were prepared by an Xplore MC15 conical twin-screw micro-compounder extruder at 85 °C. The filaments were cut into pellets for 3D printing with Brinter® One printer's Granu Tool granulate print head. Hemispherical implants of different sizes (D = 3, 4 and 5 mm) were printed using a custom GCode on a mould using a nozzle of 0.8 mm. The print head temperature was set to 197 °C and the print bed to 60 °C. After printing, the excess material was trimmed by pressing the closed mould.

In vitro drug release of dexamethasone from hemispherical implants with SA/V ratios of 3.0, 2.25 and 1.8 was studied by placing the implants under sink conditions in ultra-pure water at 37 °C with a shaking speed of 60 rpm for 35 days. Dexamethasone was quantified on an HPLC system with a ZORBAX SB-C18, 5µm, 3x150 mm column. 65% MeOH was used as the mobile phase. Detection occurred with a UV-Detector at λ = 239 nm. For release kinetics of dexamethasone, zero-order, first-order, Higuchi and Korsmeyer-Peppas models were evaluated.

RESULTS: The cumulative drug release from the 3D-printed EVA implant prototypes is shown in Fig. 1. The release data from hemispherical implants with different diameters highlights the influence of the SA/V ratio on drug release rates. A higher SA/V ratio of 3.0 leads to faster and higher drug release, reaching approximately 22.8% at day 35. On the contrary, the largest implant with SA/V of 1.8 has the lowest cumulative release, reaching only 13.1% at day 35.

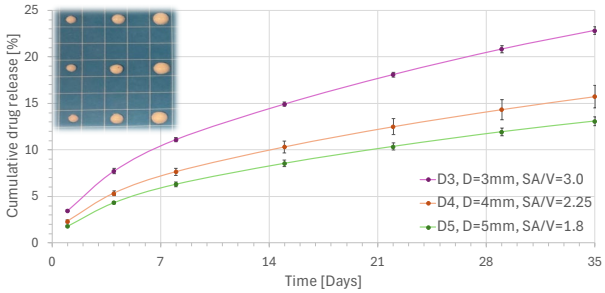

Fig. 1: The cumulative dexamethasone release from three hemispherical EVA implants. Data is presented as mean ± standard deviation, n=3.

To further understand dexamethasone release mechanisms, various release models were fitted into data, and the rate constants (k) and correlation coefficients (R^2) are listed in Table 1. For all implants, the best fit was the Korsmeyer–Peppas model ($R^2 \geq 0.998$). The release exponent values of $0.45 \leq n < 0.89$ referred to non-Fickian diffusion as the mass transport mechanism in the implants.

Table 1: In vitro drug release constants.

Geometry	Zero-order $M_t=k_0t$		First-order $M_t=M_0(1-e^{-k_1t})$		Higuchi $M_t= k_Ht^{1/2}$		Korsmeyer-Peppas $M_t/M_\infty=kt^n$		
	k_0	R^2	k_1	R^2	k_H	R^2	k	n	R^2
Hemisphere D=3 mm	0.7518	0.7211	0.0086	0.7826	3.8596	0.9993	3.7962	0.5053	0.9994
Hemisphere D=4 mm	0.5182	0.7206	0.0057	0.7626	2.6606	0.9991	2.6172	0.5053	1.0000
Hemisphere D=5 mm	0.4309	0.7410	0.0046	0.7742	2.2095	0.9982	2.1046	0.5157	0.9987

DISCUSSION & CONCLUSIONS: The drug release primarily occurred at the surface of the EVA implants [3]. Therefore, implants with a larger SA/V ratio allowed for more drug to diffuse out over the same time. As the diameter increased, the surface area grew slower relative to the volume, which reduced the release rate. Hence, the biocompatible FDA-approved EVA is ideal for controlled-release applications, offering a promising avenue for personalised drug delivery with a desired release rate and total release tuneable by adjusting the implant's geometry.

REFERENCES: [1]S.A. Khaled et al. (2018) *AAPS PharmSciTech*, **19**: 3403-3413. [2]A. Goyanes et al. (2015) *Eur. J. Pharm. Biopharm.*, **89**: 157-162. [3]N. Genina et al. (2016) *Eur. J. Pharm. Sci.*, **90**: 53-63.

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A porous membrane for encapsulating engineered ARPE-19 cells to treat drug-resistant epilepsy

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INTRODUCTION: Neurological diseases represent one of the most complex medical challenges of this century, with epilepsy being one of the most common prevalent and serious conditions. Affecting approximately 1% of the global population, i.e. about 70 million people worldwide, (6 million in Europe), epilepsy remains a significant health concern. [1] Current treatments, such as anti-epileptic drugs and brain surgery, are effective for only about 2/3 of patients, leaving the remaining third still suffering from recurrent, unprovoked epileptic seizures. [2] This highlights the urgent need for new approaches to treat and simultaneously to improve the quality of life of those patients suffering from epilepsy.

The PRIME project, funded by the European Union, aims to develop an implantable device that detects and controls impending epileptic seizures. The ultimate goal is to prevent the onset of seizures, allowing patients to live seizure-free. The device is designed to use engineered ARPE-19 retinal cells that will detect elevated levels of epilepsy biomarkers and, in response, secrete seizure-suppressing molecules, such as glial-derived neurotrophic factor (GDNF). Currently, the ARPE-19 cells have been modified to secrete GDNF constantly without the need for a trigger.

METHODS: The device developed in PRIME consists of a porous polyethersulfone (PES) encapsulating membrane filled with engineered ARPE-19 cells, surrounded by a supportive 3D scaffold (GrowDex-T), enabling stable environment for cell survival and growth. A viability assay (RealTime-Glo™) is used to measure the viability of the cells inside the encapsulating membrane, with measurements taken weekly. In addition, the GDNF concentration is measured using ELISA assay to verify the GDNF secretion by the encapsulated ARPE-19 cells through the encapsulating membrane.

RESULTS: The ARPE-19 cells inside the encapsulating membrane were cultured for 15 weeks, during which viability assays confirmed cell survival. In addition, the ELISA assays showed clear GDNF secretion through the PES membrane by the encapsulated cells.

DISCUSSION & CONCLUSIONS: The results show promising advancements in the development of an implantable device for epilepsy treatment and management. The survival and functionality of the engineered ARPE-19 cells within the PES membrane were successful. However, currently, the ARPE-19 cells are engineered to secrete GDNF molecules constantly. Thus, further engineering of the cells to recognize epilepsy biomarkers and, in response to that, initiate the GDNF production is required.

REFERENCES:

[1]E. Beghi, (2019) *Neuroepidemiology,* **54**: 185-191.

[2]W. Löscher et al. (2020) *Pharmacol. Rev.*, 72: 606-638.

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Positive Effects of Biogenic Aminoacid Metabolites on Self-Setting, Degradation and Mechanical Properties of Phosphate Cements for Mini-Invasive Surgeries

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INTRODUCTION: Calcium phosphate cement (CPC) has been, for the last several decades, a promising material for bone tissue engineering due to its resorbability, biocompatibility and potential to support bone regeneration. As a hydraulic cement, CPC is created by mixing powder phase and aqueous solution, forming a paste with self-setting ability[1]. The CPC can be modified by adding various substances to improve its chemical, physical, and biological[2]. One of these substances is a phosphorylated biogenic amino acid metabolite found in human biofluids that can significantly affect the CPC's self-setting rate, accelerate its degradation, and enhance mechanical properties.

METHODS: Our developed CPC, consisting of tricalcium phosphate powder and a thermosensitive biodegradable copolymer, was prepared according to Vojtova et al.[2]. Prepared and bioactive-modified calcium phosphate cement materials were tested and optimized. To investigate the structural and mechanical properties of the cement, two experimental methods were employed: X-ray diffraction for the conversion of tricalcium phosphate to calcium-deficient hydroxyapatite, and static mechanical compression tests. Moreover, degradation in different media having neutral or acidic pH was evaluated.

RESULTS: The optimization of the aminoacid metabolite concentration was crucial for handling, injecting, the structure of fresh paste, self-setting, mechanical properties and degradation rate. The results showed a significant deceleration of the self-setting rate by using the biogenic additive metabolites. The X-ray diffraction indirectly confirmed the presence of the additive by affecting conversion and enhancing the characteristic peak for calcium-deficient hydroxyapatite. However, the bioadditive increased the compressive strength of the reference CPC more than twice (from 7 up to 16 MPa) when using only small concentrations of the additive up to 0.3%. A higher additive concentration caused a decline in this property to a value even lower than the control sample since the paste become very stiff and hardly injectable. Moreover, bioadditives significantly accelerate the degradation rates of CPC.

DISCUSSION & CONCLUSIONS: The amount of biogenic aminoacid metabolite could prevent cement paste from developing hydroxyapatite crystals and shift crystalline tricalcium phosphate to an amorphous state. The effects of biogenic metabolite additives addition play a key role in the fresh cement paste behavior, conversion of tricalcium phosphate, degradation rate and mechanical properties of final modified cement materials. Due to the positive effect of used metabolite on mechanical properties (both compressive strength and elastic modulus), and fastening the degradation rates, this material seems to be attractive for future use as bone fillers or bone adhesives provided by mini-invasive surgeries.

REFERENCES: . [1]M.-P. Ginebra et al. *Adv. Drug Delivery Reviews* (2012), **64** (12):1090-1110; [2]L. Vojtová et al. *Int. J. Mol. Sci.* (2019), **20**(2), 391

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Ascorbic acid 2-phosphate embedded poly(trimethylene carbonate) biomaterials for pelvic organ prolapse tissue engineering

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INTRODUCTION: Pelvic organ prolapse (POP) occurs when pelvic organs descend into or through the vagina due to weakened supportive tissues [1]. Lowered collagen content of the connective tissues is associated with POP [2]. The ideal biomaterial for POP treatment should provide mechanical support and promote tissue remodeling [3]. Ascorbic acid 2-phosphate (A2P) stimulates the collagen production and proliferation of different cell types [4]. Therefore, A2P embedded elastic poly(trimethylene carbonate) [5] could be a promising candidate for POP tissue engineering.

METHODS: PTMC membranes were prepared by co-precipitation of polymer and A2P (0, 5 or 10 wt%) followed by compression moulding. Tensile and suture retention tests were conducted to membranes.

Human vaginal stromal cells (hVSCs) and human adipose-derived stromal cells (ASCs) were cultured on membranes. Proliferation and collagen production of the cells were analysed during 14 d culture.

RESULTS: PTMC membranes had good handling characteristics in addition to appropriate tensile- and suture retention strength. Proliferation of both hASCs and hVSCs (Fig. 1) increased on PTMC membranes containing A2P compared to membranes without A2P.

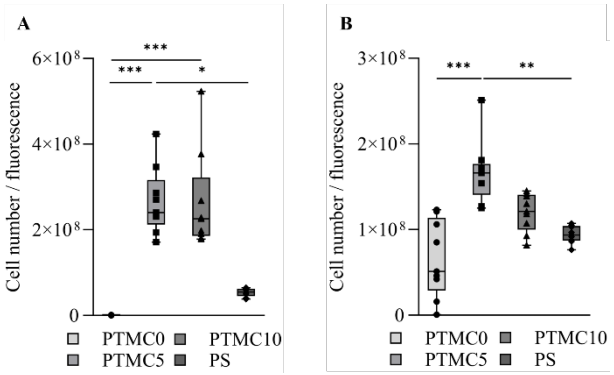

*Fig. 1: Cell numbers of A: hASCs and B: hVSCs at 14 d. *P < 0.05, **P < 0.01, ***P < 0.001.*

Further, the total collagen content (Fig. 2 A-B) increased on A2P embedded membranes. Collagen type I was also visible in immunocytochemical staining on PTMC5 (Fig. 2 C).

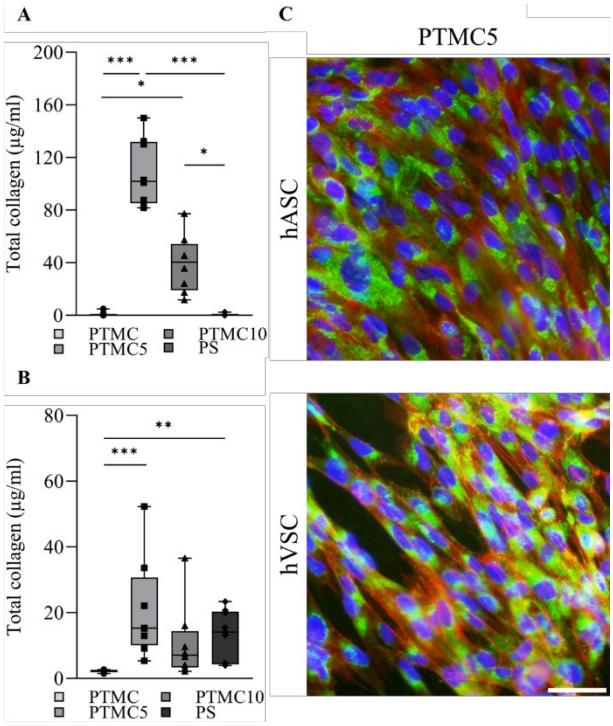

*Fig. 2: Total collagen content (µg/ml) A: hASCs and B: hVSCs at 14 d. *P < 0.05, **P < 0.01, ***P < 0.001. C: Collagen type I staining on PTMC5 at 7 d, scale bar 50 µm.*

DISCUSSION & CONCLUSIONS: A2P embedded PTMC promotes the proliferation and collagen production of both hASCs and hVSCs. Increasing effect of A2P on proliferation and collagen production has been detected previously [4, 6]. However, this is the first time A2P has been added to PTMC, and it seems to be a promising candidate for POP tissue engineering.

REFERENCES: [1]D. Chow et al. (2013) *Curr. Opin. Urol.*, **23**:293-298. [2]M. H. Kerkhof et al. (2009) *Int. Urogynecology*, **20**:461-474. [3]D. Yang et al. (2021) *J. Biomater. Sci. Polym. Ed*, **32**:2118-22143. [4]R. Sartoneva et al. (2024) *Tissue Eng. Regen. Med.*, **21**:81-96 [5]Y. Song et al. (2010) *Acta Biomater.*, **6**:1269-1277. [6]J. Yu et al. (2014) *Biomaterials*, **35**:3516-3526.

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Gallol-functionalized pH-responsive gellan gum-based biomaterial ink for extrusion-based 3D bioprinting

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INTRODUCTION: Bioprinting is rapidly advancing in tissue engineering, regenerative medicine, personalized medicine, and organ-on-chip technology. A critical factor for the success is bioink, which enables the creation of physiologically relevant 3D scaffolds for in vitro models. Ideal bioinks must possess suitable mechanical, rheological, chemical, and biological properties. The choice of printing technique depends on the biopolymers used, the crosslinking chemistry, and the complexity of the scaffold. Gellan gum (GG) is a naturally derived polysaccharide with free carboxylic and hydroxyl groups, allowing versatile chemical functionalization. The remarkable viscoelastic properties of GG-based hydrogels make them promising candidates for bioinks. In our previous work, we developed gallic acid-functionalized GelMA (GelMA-GA)[1] and GGMA-based bioinks[2] using a dual crosslinking strategy. In this study, we functionalized the GG with gallic acid to enable viscosity modulation through pH adjustment, ensuring optimal printability.

METHODS: The GG was methacrylated at basic pH, and methacrylated GG (GGMA) was functionalized with gallol (GA) using EDC coupling chemistry (Figure 1). ^{1}H-NMR spectroscopy confirmed the degree of methacrylation and conjugation of GA to the GG backbone. The flow behaviour of biomaterials inks and viscoelastic properties of hydrogels were determined from the rheological measurements.

RESULTS: The methacrylation was performed at pH 8–9. The degree of methacrylation obtained was ~21%, and gallic acid modification was obtained at ~10%. The hydrogel precursors were prepared by dissolving 2% w/v GGMA in 0.1% LAP (lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate) solution and were viscous liquid under acidic conditions (pH 3.5) but did not form a continuous fiber. They showed increased viscosity at a pH of 6–7, facilitating fiber formation (Figure 1) and making them suitable for bioprinting applications. However, under basic conditions (pH 8.5–9), the precursors transitioned into true hydrogels and hence were unsuitable for bioprinting.

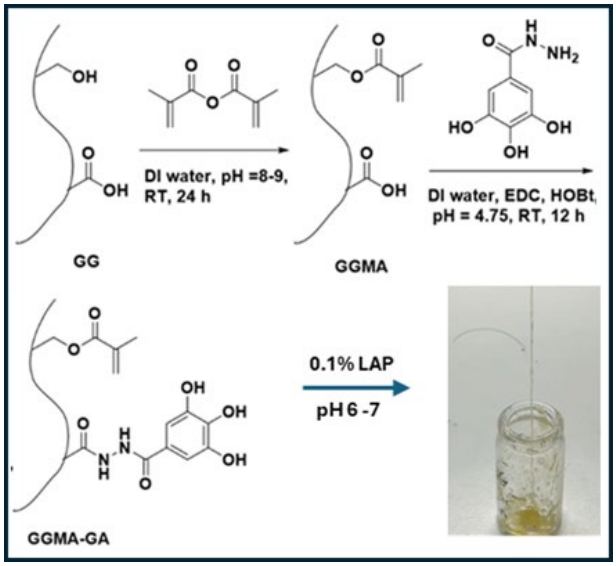

Fig. 1: Synthesis of gellan gum methacrylate (GGMA) and GGMA-GA and development of a biomaterial ink through pH modulation.

DISCUSSION & CONCLUSIONS: The pH-responsive viscosity modulation significantly influenced hydrogel printability, with pH 6–7 providing optimal shear-thinning behaviour. Our study demonstrated controlled shear-thinning properties in gallol-functionalized GG hydrogels through pH modulation. Post-printing photocrosslinking further enhanced the stability of the scaffolds. This two-step crosslinking approach offers a promising strategy for developing a diverse library of bioinks.

REFERENCES: [1]H. Jongprasitkul, et al. (2023) *Biomacromolecules*, **24**, 1: 502–514. [2]H. Jongprasitkul, et. al. (2022) *Bioprinting*, **25**: e00185.

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Alginate-encapsulated bone spheroids: approaches to study bone cells in 3D

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INTRODUCTION: The application of cellular spheroids for bone tissue engineering (BTE) purposes has gained a lot of interest. Spheroids with their extensive cell-cell and cell-extracellular matrix (ECM) interactions [1], are considered a suitable model for investigating bone ECM and bone mineralization process in vitro. Bone ECM is composed of an inorganic and organic part, made up mostly of carbonate-substituted calcium-deficient apatite and type I collagen respectively [2]. Adequate characterization techniques and proper optimization of the already existent methods are required to study these 3-dimensional cell models and the characteristics of the produced mineral.

METHODS: MC3T3-E1 spheroids were obtained using the micro-mold [3] technique and were subsequently embedded in an alginate hydrogel, in which ECM deposition and mineralization were followed. Second Harmonic Imaging Microscopy (SHIM), Transmission Electron Microscopy (TEM) and hydroxyproline assay were used to perform qualitative and quantitative analysis of the deposited matrix. Coherent Anti-Stokes Raman Scattering (CARS) was used to assess Calcium Phosphate (CaP) deposition on monolayer cell cultures.

RESULTS: Alginate-encapsulated spheroids were followed in culture for up to 4 weeks. SHIM and TEM revealed the presence of collagenous matrix in differentiation media (OM) cultured spheroids starting from week 2 (Fig.1 and 2). CARS revealed the presence of mineral deposition after 3 weeks of culture in OM cultured monolayer cell cultures (Fig. 3).

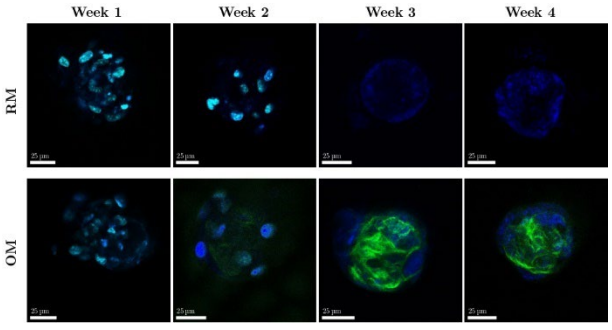

Fig. 1: SHIM of regular and differentiation media cultured spheroids. A progressive increase in collagen production (green) can be observed from week 2 to 4 in OM cultured spheroids.

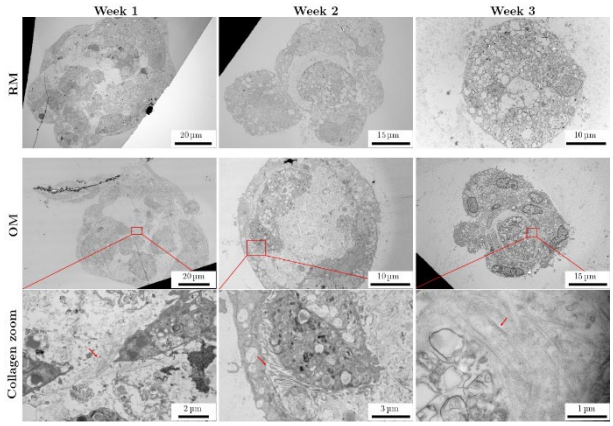

Fig. 2: TEM images of spheroids sections showing the deposited collagen fibrils.

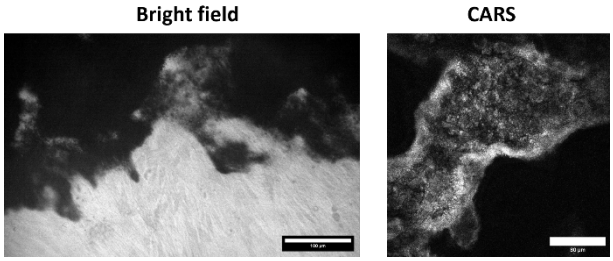

Fig. 3: On the left, bright field image after using Alizarin Red Staining, which stains CaP. On the right, CARS image of the stained CaP deposit.

DISCUSSION & CONCLUSIONS: The results show that alginate-encapsulated spheroids can be a suitable model for studying bone ECM deposition and mineralization. SHIM is an advantageous method that can be used to image intact, embedded spheroids. CARS will be further improved and tested for CaP detection in embedded spheroids.

REFERENCES: [1] S. J. Kim et al (2020) Advanced Healthcare Materials, 9.23: 2000608; [2] Mansour et al (2017), Tissue Engineering part A 23.23-24: 1436-1451; [3] A.P. Napolitano et al (2007) Biotechniques, 43.4:494-500.

ACKNOWLEDGEMENTS: We acknowledge Senior Engineer Astrid Bjørkøy and the Center for Advanced Microscopy (CAM) at the Department of Physics, Faculty of Natural Sciences, NTNU for technical assistance and access to confocal microscopy infrastructure. NTNU and NTNU Biotechnology are acknowledged for financial support. Nencki Institute (project No 2022/WK/05) for TEM sample preparation.

pHEMA Hydrogels as a Cartilage-Mimicking Material for Biotribological Studies

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INTRODUCTION: The human articular cartilage **(AC)** plays a crucial role in absorbing joint pressure during movement and reducing friction between bones. It is a complex connective tissue primarily composed of hyaline cartilage, which provides essential functions and joint lubrication to minimize friction. To better understand cartilage tribology and lubrication mechanisms, which are the key to developing AC with clinical applications, various materials have been explored as model systems for tribological studies. Among these, hydrogels based on **polyvinyl alcohol (PVA)** and its copolymers have gained significant attention in scientific research [1]. Poly(hydroxyethyl methacrylate) (**pHEMA**) based hydrogels, introduced in the 1960s by soft contact lenses, are widely used in biomedical applications. They offer excellent flexibility, transparency, mechanical strength, stability in aqueous environment, and biocompatibility. A key advantage is their customizable mechanical and optical properties, achievable by modifying parameters (ratio of chemicals inputs) [2].

METHODS: pHEMA hydrogels were prepared by free-radical polymerization of hydroxyethyl methacrylate (HEMA) monomer, crosslinker and photoinitiator [3]. The surface of the HEMA hydrogel was captured using ascanning electron microscope (MIRA3-XMU). To determine the frictional properties of pHEMA hydrogels, reciprocating sliding tests (pin-on-plate) were conducted using a universal tribometer (UMT Tribolab, Bruker, USA). The swelling properties of pHEMA and AC using gravimetric analysis were investigated.

RESULTS: Surface analysis of bovine AC and pHEMA showed that its surface exhibits a wrinkling surface. The swelling of the AC samples was significantly greater than that of pHEMA hydrogel. The initial increase in fluid content was evident during the 30 minute swelling period, and the maximum swelling ratio was approximately 50% for pHEMA hydrogels and over 200% for AC.

The frictional behaviour of pHEMA in contact with AC lubricated with artificial synovial fluid (SF) was investigated. To assess the ability of the pHEMA hydrogels to mimic AC behaviour, the results were compared with measurements of cartilage-on-cartilage contact under the same conditions. AC reported the lowest initial coefficient of friction (COF) value of 0.0159 while the COF value of pHEMA was 0.0299. Additionally, surface wrinkles create a larger surface area, from which the pressurized interstitial fluid can be squeezed into the contact area.

DISCUSSION & CONCLUSIONS: Our research has shown that a well-known and simple polymeric material, pHEMA hydrogel, can serve as a viable alternative to commonly used PVA hydrogels in tribological evaluations. By optimizing specific conditions, such as a controlled atmosphere and the initial composition of the pHEMA hydrogel, it is possible to create a transparent material that closely mimics the surface morphology, and friction coefficient of articular cartilage.

REFERENCES: [1]N. Sakai et al. (2019) *Biosurf Biotribol*, **5:** 13-19. [2]M. Zare et al (2021*] Int J Mol Sci* **22**, 6376. [3]T. Krajňák et al (2022) *Sci Rep* **12**: 10

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Effects of CoCr- and SiN-derived particles on glial cells: implications for spinal implant safety

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INTRODUCTION: Spinal implants are commonly used to treat conditions such as spinal fractures and degenerative disc disease. However, concerns persist about the release of ions and particles that may trigger adverse host responses [1]. Glial cells, including astrocytes and microglia, are essential for spinal cord health and could be affected by implant-derived particles [2], yet their interactions with these materials have not been comprehensively studied. This work examines the effects of particles from cobalt chromium (CoCr) alloys and silicon nitride (SiN) on glial cell viability and activation. The findings aim to improve understanding of biocompatibility and to inform spinal implant design to minimize adverse cell responses.

METHODS: SiN (≤50 nm), cobalt oxide (CoO; ≤50 nm), and chromium oxide (CrO; ≤100 nm) particles were suspended in cell media at concentrations ranging from 0.05 to 50 µm³ particles per cell. These model particles were characterized using dynamic light scattering (DLS), and ion release was quantified via inductively coupled plasma optical emission spectroscopy (ICP-OES). Murine C8-D1A astrocytes and C8-B4 microglia (ATCC) were exposed to the selected doses of particles. Glial metabolic activity was assessed using Alamar Blue, and viability using calcein-AM staining, while inflammatory activation was evaluated by measuring release of the cytokines TNFα and IL-6 (ELISA), and astrocyte reactivity was assessed by GFAP and vimentin transcript expression (RT-qPCR).

RESULTS: DLS measured mean hydrodynamic diameters of 267.0 ± 9.2 nm for SiN, 115.1 ± 99.2 nm for CoO, and 473.4 ± 56.4 nm for CrO. Ion release from CoO and CrO remained near the detection limit over three days, while Si ions from SiN increased over time, reaching 0.27–0.47 ± 0.07 mg/L at day 1 due to slow dissolution. Early exposure resulted in increased astrocyte metabolic activity, but the absence of GFAP and vimentin mRNA upregulation suggests that this increase was not associated with astrocyte reactivity. TNFα and IL-6 levels in microglial supernatants remained undetectable, indicating no inflammatory stimulation. Microglial metabolic activity was unaffected by CoO and CrO particles; however, SiN exposure caused decreased viability and reduced cell numbers between day 1 and 3, with calcein-AM-negative cells clustered around SiN agglomerates (Fig 1).

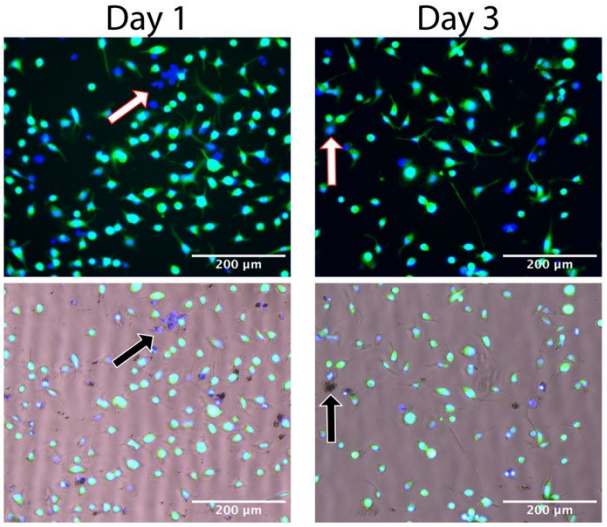

Fig. 1: Microglial viability assessed by calcein-AM staining. Green (Calcein-AM) for living cells and blue (Hoechst 33342) for cellular nuclei. White arrows point to the presence of calcein-AM negative cells and black arrows point to SiN agglomerates.

DISCUSSION & CONCLUSIONS: The tested particles did not induce astrocyte reactivity or stimulate inflammatory cytokine release by microglia, suggesting that their release from spinal implants is unlikely to trigger inflammation in the spinal cord. High doses of SiN particles negatively affected microglial cell viability, potentially due to their agglomerate size or high dissolution rate, which results in ammonia production and a consequent increase in local pH. These findings highlight the need for further studies to assess the safety of SiN in spinal implants.

REFERENCES: [1]Y. Shimamura et al (2008) *Spine.* **33**:351–355. [2] Lee H et al. (2020) *Eur. Spine J.*, **29(11)**:2701–12.

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Autologous vs Commercial Fibrin: Material Perspectives for Controlled Drug Delivery

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INTRODUCTION: Fibrin, a natural biopolymer formed during blood clotting, plays a crucial role in tissue regeneration and is widely used in biomedicine due to its excellent biocompatibility and bioactivity. Commercially available fibrin (C-fibrin) matrices are standardized and easy to use, but coagulate quickly (2–3 minutes), limiting their versatility [1]. Autologous fibrin (A-fibrin), derived from the patient blood, offers longer coagulation time (30–40 minutes), providing greater flexibility for controlled drug delivery [2]. Vancomycin hydrochloride (V-HCl) is a glycopeptide antibiotic that blocks bacterial cell wall synthesis without cross-resistance with other antibiotics [3]. The aim of this study was to compare the performance of commercial and autologous fibrin matrices for controlled V-HCl release. Samples were prepared using donor blood and commercial thrombin, fibrinogen, and calcium chloride ($CaCl_2$) and V-HCl was encapsulated in microcapsules to ensure sustained release kinetics.

METHODS: Written consent from all donors was obtained for their blood samples to be used in the research study (ethical approval No. 6-2/10/53).

A. Preparation of V-HCl carriers

Microcapsules were prepared using the w/o/w method. Poly (lactic-co-glycolic acid) / dichloromethane solution (20 wt%) was homogenized with V-HCl solution (100 mg/mL), mixed with PVA (4 wt%), stirred in water for 1 hour, centrifuged, frozen, and lyophilized.

B. *Preparation of the A-fibrin and C-fibrin matrices*

A-fibrin matrices (Fig. 1A) were prepared by centrifuging donor blood at 700 rpm for 3 minutes to obtain platelet-rich fibrin (PRF). PRF was separated with a syringe, and V-HCl carriers were suspended in the PRF using an automatic pipette before clot formation. For C-fibrin matrices (Fig. 1B), a two-syringe mixing method was used, combining fibrinogen, thrombin, and $CaCl_2$ in various sequences to achieve a homogeneous mass. The mixtures were then shaped in steel molds, allowed to cross-link at room temperature for 2–3 minutes. Samples were assessed for gel fraction, coagulation time, and drug release.

RESULTS: To prepare A-fibrin matrices, shorter centrifugation times produce less PRF, while longer centrifugation encourages clot formation. For C-fibrin matrices, thrombin should first be mixed with a 40 mM $CaCl_2$ solution before combining it with fibrinogen to achieve a uniform and homogeneous mass. When comparing V-HCl release from carriers in A-fibrin and C-fibrin matrices, the highest concentrations (7.23 µg/mL for A-fibrin and 2.34 µg/mL for C-fibrin) were observed within the first few hours. A-fibrin is ideal for rapid drug release in the first hours to reduce infection risk.

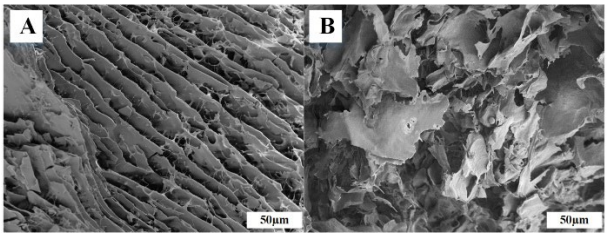

Fig. 1: SEM images of A-fibrin matrice (A) and C-fibrin matrice (B).

DISCUSSION & CONCLUSIONS: When medical personnel need to combine fibrin matrices with other materials or drugs, A-fibrin matrices are more suitable due to their longer coagulation time (30–40 minutes) than C-fibrin matrices (2–3 minutes). In terms of gel fraction, C-fibrin matrices exhibit a higher value (84.01 ± 0.54%) compared to A-fibrin matrices (65.14 ± 0.60%).

REFERENCES: [1]J.W. Weisel & R. I. Litvinov (2017) *Subcell Biochem.,* **82**: 405-456. [2]N.Laurens et al. (2006) *J Thromb Haemost*., **4**(*5*): 932-939. [3] Z. Yang et.al. (2015) *Int J Pharm*., 495(1): 508-515.

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Effects of biomimicking the physical and biochemical microenvironment of bone tissue on cellular behavior

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INTRODUCTION: Physical and biochemical cues from cellular microenvironment are sensed by the adherent cells and relayed into the nucleus for triggering intra- and intercellular signaling pathways. Recently, cell derived decellularized extracellular matrices (cd-dECMs) gained traction due to its inherent ability to support cell functionality ranging from adhesion and migration to proliferation and differentiation. However, utilization of cd-dECMs in tandem with biomaterials is still rare and the potential benefits of combining designable biomaterials with cd-dECMs is mostly undiscovered. [1] In this study, PDMS was selected as a model polymer due to its functionality of transferring topographical patterns high resolution through soft lithography. Bone surface mimicked (BSM) PDMS surfaces were prepared from decellularized bovine femur and modified with fibronectin to enhance initial cell attachment and cultured with MC3T3-E1 pre-osteoblast cells in order to deposit cd-ECMs. These samples were later decellularized and recellularized to investigate the potentially synergistic effects of combining a native-like bone topography with a native-like dECM that retains its biochemical and architectural complexity.

METHODS: PDMS and BSM PDMS were prepared with 10:1 monomer to curing agent ratio and bovine femur bone cut into well plate sizes were used as negative master molds. Surfaces were activated with corona discharging and fibronectin was cross-linked to PDMS surfaces with glutaraldehyde to increase cell retention after initial seeding. MC3T3-E1 cells were seeded onto PDMS surfaces and cultured for a week. Surfaces were decellularized with ammonium hydroxide and Triton X-100. Nuclear content was removed with a DNAse I solution. Pre-osteoblasts were later seeded onto PDMS + dECM surfaces and cultured again. Cell proliferation was assessed with a resazurin assay. Expression of markers for osteogenic differentiation (RUNX2 and ALP) as well as a mechanotransduction marker (YAP1) were investigated with Western blotting. Localization of YAP1 in the cells on PDMS surfaces was also investigated with immunocytochemistry. Presence and structural integrity of cd-dECMs were characterized with Sirius Red staining and quantification for collagens, Alcian Blue for GAGs, SEM for surface coverage, and DAPI staining for nuclear content removal.

RESULTS: Cell derived dECMs were successfully retained after decellularization, shown by collagen and GAG staining. Pre-osteoblast proliferation was shown to be significantly affected in the presence of dECM and osteoblastic differentiation media, separately. YAP signal was observed on PDMS membranes and enhanced with the presence of dECM. Protein expressions for RUNX2 and ALP is observed to be more dependent on surface topography but YAP1 was observed to be more media dependent.

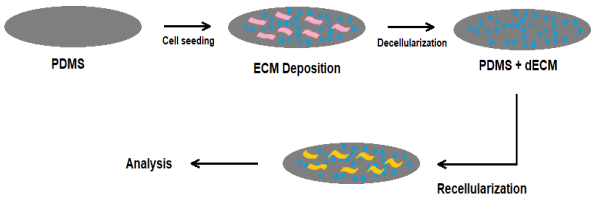

Fig. 1: General workflow for preparing PDMS + dECM surfaces.

DISCUSSION & CONCLUSIONS: Deposition of ECM resulted in statistically significant differences in cell proliferation, especially on earlier days. Presence of dECM on the surfaces also affected the intracellular location as well as the amount of expressed proteins, shown with ICC and Western blotting. Investigation cell behavior at different days of culture might shed more light on understanding the potential synergies more extensively.

REFERENCES: [1] N.S Sharma et al. (2024) *Acta Biomater.*, **184**: 81-97.

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Modelling Native-like Bone Tissues for Breast Cancer Bone Metastasis

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INTRODUCTION: Bone is one of the frequently targeted sites for cancer metastasis, particularly for breast cancer, often leading to poor clinical outcomes due to limited treatment options[1,2]. Early detection and improved prognostic tools are essential to mitigate the devastating effects of breast cancer bone metastasis. To address this need, we introduce a novel approach to model bone-like microenvironments, enabling the study of invasion and extravasation of breast cancer cells using two customized lab-on-a-chip (LOC) platforms: invasion/chemotaxis (IC-) and extravasation (EX-) chips.

METHODS: Our models were designed to simulate native-like bone microenvironment by incorporating osteoblasts, bone marrow stromal cells, and monocytes embedded in three-dimensional (3D) collagen I-based extracellular matrices with varying compositions and stiffness levels. Matrix compositions? included collagen I alone or in combination with agarose or chitosan to tune the stiffness. *In situ* contactless rheological measurements were performed under cell culture conditions to monitor matrix stiffness over five days, revealing the dynamic impact of cellular activity on mechanical properties. The invasion potential of two metastatic clones of the triple-negative, highly metastatic MDA-MB-231 breast cancer cell line—BoM 1833 and LM2, which predominantly metastasize to bone and lung, respectively, in vivo—was analyzed using the IC-chip, while their extravasation potential was examined on the EX-chip.

RESULTS: We showed that collagen I matrices supported robust growth of bone cells, creating a more physiologically relevant bone-like environment to study breast cancer bone metastasis. Matrix composition and stiffness of the developed bone microenvironments significantly influenced the invasive behavior of breast cancer cells. Over five days, cellular activity increased matrix stiffness from 1750 Pa to 2960 Pa, highlighting the reciprocal interaction between bone-related cells and collagen I-based extracellular matrices. Breast cancer cells exhibited behaviors that aligned with their known *in vivo* metastatic potentials on the IC- and EX-chip models, underscoring the platform's predictive capabilities.

DISCUSSION & CONCLUSIONS: Our findings demonstrate the critical role of extracellular matrix properties, including composition and stiffness, in regulating breast cancer cell invasiveness. The developed approaches successfully mimic the bone microenvironment and provide a versatile system for studying the cellular and molecular mechanisms underlying breast cancer bone metastasis. By bridging the gap between *in vitro* models and *in vivo* conditions, these tools lay the foundation for potential preclinical applications to predict bone metastasis risk and optimize therapeutic strategies.

REFERENCES: [1]B.Firatligil-Yildirir et al. (2024), *PLoS ONE,* 19(10): e0309285, [2]B.Firatligil-Yildirir et al. (2021), *Biotechnology and Bioengineering,* 118, 3799–3810.

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Protein Adsorption and Cell Behavior on Laser-Textured Titanium Surfaces

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INTRODUCTION: Implants offer treatments for many malfunctions in the body. When an implant is introduced into the body, blood proteins immediately cover the implant surface to make the implant recognizable. The formed protein layer around the implant depends both on the proteins' and implant surface properties. Surface texturing by different methods such as laser texturing enables the evaluation of protein layer and surface relationship. This study focused on distinct surface patterns on titanium (Ti), a widely used bone implant material, and the surfaces formed by the microsecond (mTi) and femtosecond (fTi) lasers. The protein adsorption and cell behavior corresponding to the surface patterns were investigated. [1-2]

METHODS: Modification of Ti surfaces was done by microsecond (1800 nm) and femtosecond lasers (1030 nm), forming hemi-cycle like patterns on mTi and laser-induced periodic surface structures (LIPSS)on fTi as shown in Figure 1.

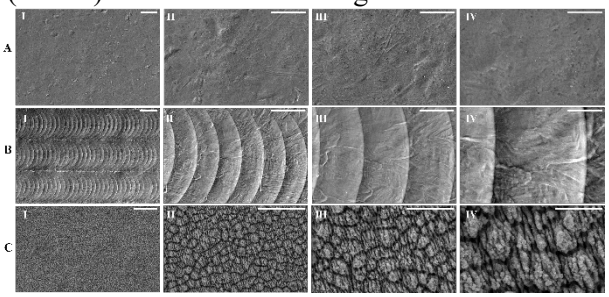

Fig 1.: SEM images of A) pTi, B) mTi, and C)fTi.

Prior to laser texturing the Ti samples were polished, pTi group used as the control. Surface characterization performed by using SEM, WCA, and AFM for roughness. Protein adsorption amounts were analyzed by XPS, and FTIR for identifying the conformational changes of proteins upon adsorption. Surface-cell interactions was assessed through direct and indirect MTT assays, alamarBlue cell proliferation assay, SEM and fluorescence imaging to evaluate cell morphology and adhesion.

RESULTS: The WCAs for pTi, mTi, and fTi were calculated as 54.36±3.76◦, 73.55±6.04◦ and 113.60±13.49◦, respectively, with average roughness (Sa) following the order fTi>mTi>pTi. The surface root mean square (Sq), surface skewness (Ssk) and surface kurtosis (Sku) were derived from AFM images. Protein adsorption, based on N1s atomic percentage, was highest on pTi and decreased in the order pTi> mTi > fTi. Secondary structures of BSA upon adsorption, analyzed via the amide-I band, revealed β-sheet, β-turn, intermolecular β-sheet, α-helix, and random coil conformations. Indirect MTT assay indicated higher cell viability for all Ti groups compared to positive control, while direct MTT assay showed reduced viability across all groups. Over 7 days, cells proliferation on the fTi was observed but remained lower than the control. SEM images revealed that more contact points, elongated morphology, and dense actin filaments on mTi and fTi surfaces compared to pTi.

DISCUSSION & CONCLUSIONS: The adsorption of BSA was highest on pTi surfaces, while fTi, with the highest roughness and WCA, exhibited the lowest adsorption. Denaturation of BSA upon adsorption was higher on mTi and fTi compared to pTi surface. Laser texturing of Ti samples showed no cytotoxicity in indirect MTT assay; however, direct MTT assays indicated cytotoxic effects. Cell distribution on mTi and fTi surface are more organized with actin filaments aligning to the surface pattern. These results indicated that surface nanomorphology significantly influences protein adsorption, conformation, cell proliferation and morphology but it might not be sufficiently enhancing viability.

REFERENCES: 1Barberi, J.; Spriano, S. Titanium and Protein Adsorption: An Overview of Mechanisms and Effects of Surface Features. Materials (Basel) 2021, 14 (7), 1590. 2Barberi, J.; Mandrile, L.; Napione, L.; Giovannozzi, A. M.; Rossi, A. M.; Vitale, A.; Yamaguchi, S.; Spriano, S. Albumin and Fibronectin Adsorption on Treated Titanium Surfaces for Osseointegration: An Advanced Investigation. Applied Surface Science 2022, 599, 154023.

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Modifications of PDMS surfaces for human iPSC derived neuronal and cardiac cells

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INTRODUCTION: Polydimethylsiloxane (PDMS) is a popular material in Organ-on-a-chip devices. It provides good properties for microfluidics but performs poorly as a cell culture substrate. Surfaces can be modified with extracellular matrix (ECM) proteins to mimic the cell microenvironment *in vivo*. However, chemically inert PDMS requires prior functionalization to induce binding sites for crosslinking proteins to the surface. [1]

Herein, we have studied two different coatings to be employed in neuro-cardio applications. We have studied the applicability of a laminin coating method of Peng et al. [2] for human induced pluripotent stem cell (hiPSC) derived cortical neural cells typically grown on laminin surfaces [3] and a gelatin coating applicability for hiPSC derived cardiac cells. The coating method proposed by Peng et al. consists of crosslinking laminin to aminated PDMS surface using carbodiimide chemistry. In the second coating, modified gelatin is crosslinked to acrylated PDMS surface.

METHODS: Two crosslinking strategies were implemented on modified PDMS surfaces for two ECM proteins, laminin and gelatin. Changes on the PDMS surfaces after functionalization and protein immobilization were studied using contact angle measurements.

Cytocompatibility of the gelatin coatings was studied using PrestoBlue Cell Viability Reagent on mouse embryonic fibroblast (MEF) cells, a commercial cell line. Additionally, the cells were immunostained to study cell spreading on the coated surfaces. Finally, to demonstrate the applicability of the coatings, hiPSC derived cardiac cells were cultured on the gelatin coated surfaces and hiPSC derived cortical neurons on laminin coatings for 4 weeks.

RESULTS: According to contact angle measurements, the functionalization methods affected the surface properties resulting in hydrophilic coatings together with the ECM proteins. Both spreading and viability of MEF cells were improved when the modified gelatin was applied. Immunostained hiPSC derived cardiac cells cultured on these surfaces demonstrated better attachment and growth compared to only oxygen plasma treated PDMS with gelatin. On the contrary, the immunostained cortical neurons showed robust aggregation, suggesting that the laminin crosslinked to the aminated surface was not suitable culture substrate for cortical neurons.

DISCUSSION & CONCLUSIONS: We have studied here two different crosslinking strategies to modify PDMS with ECM proteins for Organ-on-a-chip applications. The crosslinking of gelatin was found to provide suitable substrate for hiPSC derived cardiac cells whereas the laminin coating by Peng et. al. was not applicable for culturing cortical neural cells in this study. The improved cell attachment and viability on the modified gelatin suggest that this method could be a viable solution for enhancing cell culture on PDMS substrates in microfluidic applications.

REFERENCES:

[1]S.-G. Hu et al, Carbohydr. Polym., (58) 2004 doi: 10.1016/j.carbpol.2004.06.039

[2]C.-H. Peng et al., Oncotarget, 7(40) (2016) doi: 10.18632/oncotarget.11502

[3]T. Hyvärinen et al., Scientific reports, 9 (2019) doi: 10.1038/s41598-019-53647-8

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Levan based hydrogel with insulin-like growth factor 1 to promote osteogenic differentiation

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INTRODUCTION: The main challenge in oral tissue regeneration is replicating the complex structure and function of oral tissues, including soft and hard tissues. The wound-healing process includes cell migration, proliferation and differentiation. One of the key signaling molecules regulating these responses is insulin-like growth factor 1 (IGF-1) [1]. Hydrogels, like levan, with their 3D porous structure, support cell growth and bioactive molecule retention, therefore making them ideal for tissue regeneration [2]. To ensure the delivery of IGF-1 directly into the cell, it will be encapsulated into liposomes that will then be incorporated into levan hydrogel.

METHODS: The hydrogel was fabricated from enzymatically obtained *Halomonas* levan and crosslinked with 1,4-butanediol diglycidyl ether. Liposomes were synthesized via thin-film hydration method using 2 phospholipids (DSPC and DSPE-PEG). IGF-1 was incorporated during hydration process of the liposomes. The release of IGF-1 was determined using ELISA method. The *in vitro* cytotoxicity tests were conducted for 24 and 48 h using various liposome concentrations (1, 3, 5 and 12 mg/mL) 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. IGF-1 influence on osteogenic, adipogenic and chondrogenic differentiation of GMSC's was evaluated for 21 days.

RESULTS: The encapsulation of IGF-1 alter the hydrodynamic diameter, decreasing it to 780 and 360 nm for DSPC and DSPC DSPE-PEG liposomes, respectively. The highest IGF-1 release was observed from DSPC DSPE-PEG liposomes, achieving 80% from the added IGF-1 amount after 10 days. Basically all levan hydrogel and liposome samples showed cell viability above 70% samples, where samples with IGF-1 showed the highest cell proliferation (Fig. 1). IGF-1 significantly enhanced the osteogenic differentiation of GMSCs.

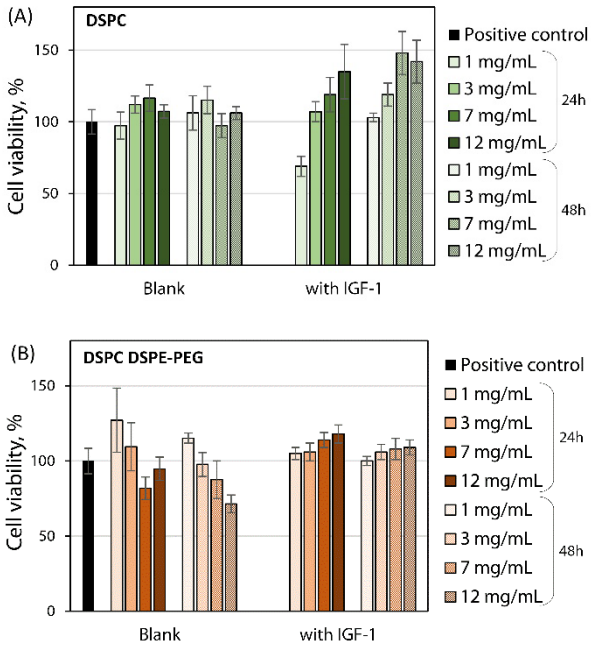

Fig. 1: The influence of (A) DSPC and (B) DSPC DSPE-PEG liposomes with and without IGF-1on GMSCs viability.

DISCUSSION & CONCLUSIONS: Liposomes and levan hydrogel showed biocompatibility with GMSC's and they are considered as non-cytotoxic, according to the ISO 10993-5:2009. The highest cell viability was observed for DSPC liposomes with IGF-1 due to the mitogenic effect of IGF-1 [3]. The observed osteogenic differentiation results indicate the possible application of the developed material for bone regeneration in the oral cavity.

REFERENCES: [1]C.-H. Tsai et al (2005) *Oral Oncol*, **41**:940-6. [2]E.T. Öner et al. (2016) *Biotechnol. Adv.,* **34**:827-844. [3]N.-Z. Bashir (2021) *Arch Oral Biol*, **122**:104993.

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Exploring Auxetic Design Principles for Medical Implants

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ABSTRACT: Auxetic structures, characterized by showing negative Poisson's ratio (υ), fit the characteristics of special bones and stents. Auxetics are cellular metamaterials consisting of interconnected struts in repeating unit cells. The mechanical behavior of auxetics depends on dimensions of the unit cell such as height and length of the unit cell, strut thickness, and orientation angle of the struts. Auxetic lattices could be additively manufactured by laser powder bed fusion (LPBF), offering flexible fabrication with minimized material waste. However, there is a lack in the literature on mechanical characteristics of auxetics produced by LPBF facility. In the present study, we explore the effect of strut thickness variation on the mechanical behavior of 316L stainless steel with auxetic structures fabricated by LPBF technique. The investigated auxetics had different thicknesses of 0.6-1.4 mm with same gauge volumes to manipulate structures' density. Mechanical behavior was investigated through micro-indentation hardness measurements, and tensile testing. Microstructural features were revealed through electron backscattered diffraction facility, while fracture features were examined by scanning electron microscopy. The results showed that hardness was increased with thickness reduction of the printed specimens. Remarkably, as the structure's density decreased, the absolute strength of auxetics decreased due to a deterioration of the surface roughness, exhibiting an inhibited initiation of ∑3 boundaries during tensile testing and promoted brittle fracture features in the thin-walled structures.

Evaluation of the therapeutic potential of lipophosphonoxins DR-6180 and DR-7072 in biodegradable nanofibrous carrier systems for the treatment of murine skin wounds experimentally infected with *Staphylococcus aureus*.

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INTRODUCTION: The presence of infection in wounds is a common cause of complications of healing and in most cases antimicrobial therapy is necessary. In view of the development of resistance, it is desirable to develop new antimicrobial agents useful for the control of localised bacterial infections of soft tissues. Lipophosphonoxins (LPPOs) appear to be a very promising group of agents suitable for such an indication. The aim of the study was to test the efficacy of LPPOs (molecules DR-6180 and DR-7072) in a biodegradable nanofibrous sandwich carrier system consisting of PCL and PVA layers for antimicrobial therapy of the wounds experimentally infected with *Staphylococcus aureus*.

METHODS: The CB6F1 mouse strain immunosuppressed with cyclophosphamide was used as an *in vivo* model. Wounds were covered with an occlusive membrane cover after application of bacterial inoculum of *S. aureus* and left for 3 days, after which treatment with experimental wound dressings was initiated. The result of the treatment was microbiologicaly evaluated after another 4 days.

RESULTS: The results show that the infection is localized in the wound bed and the animals do not show obvious clinical signs of systemic involvement. Experimental covers incorporating the tested LPPOs in a sandwich of PCL and PVA proved to be effective, with complete inhibition of infection in a number of cases.

DISCUSSION & CONCLUSIONS: The experiment demonstrated the advantage of incorporating LPOOs into PCL and PVA, whereby as the degradation of the nanofibers progressed, LPOOs were released into the wound with satisfactory kinetics.

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The copolymer of sebacic acid and poly(ethylene glycol) as azithromycin carrier in pulmonary drug delivery

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INTRODUCTION: Chronic lung diseases are often associated with permanent bacterial infections that frequently lead to exacerbations. The efficacy of systemic administration of antibiotics is limited due to the poor bioavailability in the lungs. Therefore, novel solutions to deliver drugs directly to the site of action are developed, e.g. dry powders for inhalation (DPIs) [1-2]. Studies confirmed that inhalable therapy with azithromycin (AZM) improves the patients' conditions and limits the lung's permanent colonization with bacteria [3]. Therefore, we propose the use of polyanhydride copolymer of sebacic acid (SA) and poly(ethylene glycol) with M_w = 250 Da (PEG250), namely PSAEG250, as a carrier of AZM in the form of microparticles (MPs) to be delivered to the lungs via inhalation in the form of a DPI.

METHODS: PSAEG250 (9:1 SA:PEG250 weight ratio) was synthesized by melt polycondensation. The chemical structure was assessed by ^{1}H NMR and FTIR, thermal properties by DSC, and the hydrophilicity and surface free energy (SFE) by contact angle measurements with water and diiodomethane. AZM-loaded MPs were fabricated by the emulsification/solvent evaporation method. Laser diffraction was used to design the setup to obtain MPs with defined size distributions. SEM and zeta potential measurements assessed the physicochemical properties of the MPs. The encapsulation efficiency (EE) and drug loading (DL) were evaluated with mass spectrometry (MS). Degradation and AZM release studies were carried out in phosphate-buffered saline using SEM and MS after contact with human liver microsomes (HLMs), and also monitoring the process by changing mass and pH. The aerodynamic properties of the powders were evaluated in terms of flowability (Carr's index, Hausner ratio), and aerodynamic size distribution along with its parameters (fine particle fraction and mass median aerodynamic diameter). Cytotoxicity of the MPs was tested with A549 and BEAS-2B lung epithelial cells, and the antibacterial efficacy with four strains of *Staphylococcus aureus* (ATCC 25923, ATCC BAA 2094, and clinical strains).

RESULTS: The obtained MPs were spherical and smooth with a slightly negative surface charge becoming closer to neutral at higher AZM content. The EE was almost 100% due to drug-polymer conjugate formation confirmed by MS. The presence of the drug improved the aerodynamic properties of the MPs providing adequate aerodynamic properties (fine particle fraction 66.3 ± 4.5%, mass median aerodynamic diameter 3.8 ± 0.4 μm) at fair flowability and good aerosolization due to their low density. The MPs interacted favourably in the simulated biological environment of HLMs, as the conjugated drug could be quickly released from the formulation thanks to: (1) fast polymer degradation and (2) enzymatic detachment of the covalent bond between AZM and the matrix. The bioactivity was preserved, as the formulation was bactericidal in the nontoxic concentration.

DISCUSSION & CONCLUSIONS: PSAEG250 appeared to be a proper material for the delivery of AZM due to conjugate formation which is quickly degradable in the biological environment. The superiority of the PSAEG250 was shown as the presence of AZM improved the MPs' zeta potential, aerodynamic properties, and interactions with the drug. Therefore, we claim it is promising for pulmonary drug delivery.

REFERENCES: [1]J. G. Weers and D. P. Miller (2015) *Journal of Pharmaceutical Sciences*, **104(10)**: 3259–3288. [2]A. I. Ritchie and J. A. Wedzicha (2020) *Clinics in Chest Medicine* **41(3)**: 421–438. [3]K. W. Southern et al. (2024) *Cochrane Database of Systematic Reviews* **2**: CD002203.

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Apatite/Chitosan Composites Synthesized from Mussel Shells for Bone Tissue Engineering and Drug Delivery Applications

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INTRODUCTION: The development of biomimetic materials for bone tissue engineering remains a significant challenge. Hydroxyapatite (HAp) is widely recognized as a suitable material due to its chemical similarity to bone mineral, bioactivity, and osteoconductivity. However, its low mechanical strength and lack of biodegradability necessitate the incorporation of bioactive polymers. This study explores the synthesis of nanocrystalline hydroxyapatite and apatite/chitosan composites derived from mussel shells via dissolution–precipitation synthesis. Cold sintering was employed to densify the composite while preserving its bioactive properties. The materials were further functionalized for drug delivery applications using strontium ranelate (SrRAN), a known anti-osteoporotic drug.

METHODS: Apatite/chitosan composites were synthesized using a dissolution–precipitation method from mussel shells as a calcium source. Chitosan was incorporated at various weight percentages to tailor the mechanical and biological properties of the composites. The powders were then subjected to cold sintering at room temperature under 1.5 GPa pressure for 10 min to obtain densified bulk samples. The materials were characterized using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The *in vitro* drug release profile of SrRAN from the composites was assessed in phosphate-buffered saline (PBS) over 35 days, and cytotoxicity was evaluated using MG-63 human osteoblastic-like cells.

RESULTS: The synthesized apatite/chitosan composites exhibited nanocrystalline morphology and were successfully densified using cold sintering, achieving up to 90% relative density. The inclusion of chitosan improved the mechanical properties, increasing the flexural strength from ~45 MPa to ~57 MPa while maintaining bioactivity[1]. The drug-loaded composites demonstrated a sustained release of SrRAN over 19 days, indicating potential for controlled drug delivery[2]. Cytotoxicity tests confirmed the biocompatibility of the materials, with no significant reduction in cell viability up to 72 hours.

DISCUSSION & CONCLUSIONS: This study highlights the potential of mussel shell-derived apatite/chitosan composites as biomimetic materials for bone tissue engineering and local drug delivery applications. The combination of cold sintering with dissolution–precipitation synthesis allows the fabrication of bioactive, mechanically stable materials that can support bone regeneration while enabling controlled drug release. Future work will focus on optimizing the composite formulation to further enhance osteoconductivity and mechanical performance.

REFERENCES:

[1]Galotta, A. et al. (2023) *Open Ceramics*, 15, 100418.

[2]Galotta, A. et al. (2024) *Nanomaterials*, 14, 441.

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Physiologically relevant sensor interfaces

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INTRODUCTION: Three physiologically relevant interfaces must be addressed for electrochemical sensors in medical applications: 1) The **analyte–material interface** is critical for sensitivity and selectivity in detecting target molecules. 2) The **protein–material interface** impacts biofouling, where proteins and other biomolecules adsorb onto the sensor surface, impairing its functionality. Addressing this interface is essential to maintain performance in biological environments. 3) The **cell–material interface** is key to implantable sensors, as scar tissue formation limits lifespan. The long-term success of the sensor depends on minimizing adverse cellular responses and ensuring biocompatibility.

METHODS: Analyte–surface interactions were assessed using electrochemical measurements in physiological saline, complemented by density functional theory simulations to study adsorption. The impact of biofouling was evaluated through electrochemical measurements with bovine serum albumin as the biofouling agent. Advanced insights into the protein-material interface under applied potential were gained using electrochemical quartz crystal microbalance (e-QCM) and scanning electrochemical microscopy (SECM).

RESULTS & DISCUSSION: Regarding the analyte–material interface, a strong emphasis has been placed on the interaction between surface chemistry and the analyte. Our research has demonstrated that surface geometry is critical in analyte-material interactions[1]. The current investigation highlights subtle differences in sensing chiral molecules and the influence of analyte structure—whether flexible or rigid—on sensing performance. Computational models are invaluable for verifying these findings. However, existing approaches often focus on the global minimum conformation of the analyte and a limited set of selected adsorption sites. To address these limitations, we recently demonstrated that Bayesian optimization offers a faster and more systematic approach for adsorption structure search[2]. This method minimizes reliance on manually selected candidate structures, making the process more efficient and comprehensive.

Considering protein–material interface, we have shown that biofouling significantly impacts the redox kinetics of outer- and inner-sphere probes on carbon surfaces in markedly different ways[3]. Developing tools for in situ observation of fouling is essential for advancing this research. We have previously demonstrated that SECM is an effective tool for investigating chemical fouling[4], and our current findings confirmed its applicability for studying biofouling. Additionally, the e-QCM proved to be a valuable technique for quantifying biofouling, providing complementary insights into fouling behaviour. The results indicate that the applied potential of the sensor significantly influences the extent of biofouling.

At the cell–material interface, an additional challenge in studying interactions arises from unexpected phenomena at the interface. For instance, we have observed that common cell viability dyes interact with high concentrations of carbon nanomaterials, leading to false cytotoxicity results. This highlights the urgent need to develop advanced in vitro tools for more accurate predictions of implant integration. One promising approach involves cell mechanical measurements using atomic force microscopy, which opens new possibilities in this field. We addressed the combination of geometrical softening and the effect of nanoscale topography on cell adhesion with neural cells[5]. These findings highlight the need to combine nanoscale characterization with biological assays to understand material-cell interactions.

CONCLUSIONS: Developing advanced tools and systematic approaches to address challenges at analyte–, protein–, and cell–material interfaces are essential for improving sensor performance, biocompatibility, and fouling resistance in medical applications. Leveraging computational models and in situ techniques can accelerate progress toward more reliable and efficient implantable sensors.

REFERENCES: [1]A. Kousar et al. *Analytical Chemistry* **95**:2983-2991. [2]S. Sippola et al. (2024) *ACS omega*, **9**:34684-34691. [3]E. Peltola et al. (2020) *Physical Chemistry Chemical Physics,* **22:**16630-16640. [4] E. Peltola et al. (2018) *Analytical chemistry*, **90**:1408-1416. [6]S. Rantataro (2022) *Acta Biomaterialia*, **146**:235-247.

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Medical device regulations: Comparative study between the EU and Asia

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INTRODUCTION: The growing demand for medical devices in Asia, driven by aging populations and the rising prevalence of chronic diseases, presents significant opportunities for foreign manufacturers[1]. However, regulations governing the manufacturing, import, and sale of medical devices in the region are complex, and information related to the requirements is scattered and sometimes limited. For manufacturers in the European Union (EU), knowledge of the area is important in identifying market opportunities and planning a go-to-market strategy.

This study reviewed the current state of medical device regulations in Asia and compared them with the Medical Device Regulation (MDR) of the EU. In order to do so, key regulatory elements namely definition of medical device, classification system, quality management system, pre-market approval procedures, and post-market controls were compared.

METHODS: The research was qualitative in nature and utilized a comparative study method. The study consisted of two phases. First, information on Asia as a whole was collected. This included the regulatory authorities, relevant regulations, and size of the medical device market in 48 countries. The collected data was further applied as the selection criteria for the countries included in the comparative analysis. As a result, the following countries were taken into closer examination: India, Pakistan, Japan, South Korea, Singapore, Malaysia, Thailand, and Saudi Arabia. For the second phase of the study, regulatory documents were collected from online sources, namely the official websites of the regulatory authorities of each country. The documents included laws, regulations, rules, notifications, and guidance documents. After extracting relevant information from the documents, a comparison between the countries and the EU was carried out.

RESULTS: Among the studied countries, variation was observed in the definitions of the term medical device. Although the definitions were generally similar to the EU one, differences were observed in the intended use and scope of the devices. While some countries had included veterinary products under the regulations, others lacked devices meant for support of conception or aid for disabilities. Consequently, not all products classified as medical devices in the EU constitute as such in the examined Asian countries. For classification systems, fewer differences were observed. All the countries had a four-tiered, risk-based system, like the EU. The most distinct differences were observed in the pre-market pathways which presented great variance among the countries. None of the countries had the same approach as the EU, although similarities were found, for instance from Malaysia. As for quality management, most countries recognize the ISO 13485 standard as a means of complying with requirements. However, some exceptions exist, and additional country-specific requirements, such as those in Japan, may apply. Of the post-market controls, adverse event reporting had a similar approach among the countries, but the identified post-market surveillance systems were estimated to be less extensive compared to the EU. Comparative findings indicate that while some countries such as Saudi Arabia and Malaysia shared similar traits with the EU, none of the countries had the same requirements to a larger extent.

DISCUSSION & CONCLUSIONS: During the study, it became evident that regulations in the EU and Asia differ in many respects, yet similarities also exist. The findings in this work essentially relate to the level of harmonization in Asia. A distinct difference between the implementation of pre-market and post-market elements among the countries was observed during the study, the latter being less frequently applied. In fact, Badnjević et al.[2] found that PMS activities worldwide tend to be less harmonized compared to pre-market controls. Although additional research is still needed to develop a full picture of the regulatory elements in these countries, this study adds to our understanding of the regulatory landscape in Asia and offers some insights into the varying practices and features found in the region.

REFERENCES: [1]A. Kaushik et al. (2010) *J. Young Pharm.,* **2**:101-106. [2]A. Badnjević et al. (2022) *Technol. Health Care,* **30**:1315-1329.

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Biocompatibility and antibacterial properties of alpha-mangostin cocrystal gel for periodontal disease: comparison with commercial gels

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INTRODUCTION: Periodontal disease (PD) is a prevalent and significant health issue, demanding the development of effective, antimicrobial, and biocompatible treatments. Traditional therapies, particularly commercial periodontal gels containing chlorhexidine (CHX) or other antiseptics, have raised concerns due to their cytotoxic effects on periodontal tissues and the growing resistance to these agents [1]. This has created a need for novel, safer oral antiseptics.

The pericarp of the mangosteen fruit is a rich source of xanthones, a class of polyphenolic compounds with potent antibacterial properties [2]. After screening various xanthones for biocompatibility and antibacterial effectiveness, we selected alpha-mangostin (aMG) for its high efficacy and abundance [3]. However, its low water solubility limits its application in water-based formulations. To overcome this challenge, we developed a soluble form of aMG by creating a cocrystal (aMG-CC), which was then incorporated into a chemically modified hyaluronic acid (HA) hydrogel [4].

The aim of this study was to compare the biocompatibility and antibacterial efficacy of commercial periodontal gels with our aMG-CC-HA hydrogel formulation, after its characterisation.

METHODS: Native HA was chemically modified incorporating amino and aldehyde groups in different batches of HA, allowing spontaneous crosslinking and gelation when combined at room temperature. aMG-CC at different concentrations was incorporated before gelation. The structure and rheological parameters of the loaded HA hydrogel were evaluated by SEM and reometry, and aMG-CC release by HPLC. The antimicrobial properties were tested on different oral bacteria and its biocompatibility on cell cultures of human gingival fibroblasts.

The antibacterial capacity and biocompatibility were compared with different commercial products.

RESULTS: aMG-CC HA hydrogel showed optimal rheological properties and release profile. Moreover, it showed equivalent antimicrobial activity compared to the commercial gels presenting active principles such as CHX or enoxolone. In contrast, while aMG-CC HA hydrogel was biocompatible, CHX or enoxolone commercial HA gels showed high cytotoxicity with human gingival fibroblasts.

DISCUSSION & CONCLUSIONS: The modified HA hydrogel allows the controlled release of aMG-CC, resulting in a hydrogel with antibacterial activity and highly biocompatible. This hydrogel can be an alternative therapy to prevent and treat PD to the products currently on the market that have antibacterial activity but are toxic to tissues.

REFERENCES: [1] M.Munar-Bestard et al. (2021) Pharmaceutics 13(9):1502. [2] M. Abate et al. (2022) Pharmaceuticals (Basel) 15(1):84. [3] Y.W.Chin et al (2008) Mini Rev Org Chem 5(4):355-364. [4] Yan et al. ACS Omega 2023 8(24):21522-21530.

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Evaluation of different biomaterials for human induced pluripotent stem cell derived airway epithelium patch engineering

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INTRODUCTION: Cystic fibrosis (CF) is the most common monogenic disease in Caucasians, caused by mutations in CF transmembrane conductance regulator (CFTR) gene. Despite it being a multisystem disease, pulmonary exacerbations represent the major cause of disability and mortality [1]. Although CFTR-modulator drugs development has meliorated many patients' symptoms and prognosis, this is a life-long expensive treatment that is not suitable for all CF patients [2]. Therefore, there is an urgent need to find new therapeutic approaches for airway regeneration. The use of human induced pluripotent stem cells (hiPSC) could be a promising approach, as they can replicate indefinitely providing an unlimited source of cells that can be differentiated to airway epithelial cells (AEC). Moreover, hiPSC can be obtained from both healthy donors and patients, and they can be easily genetically modified to correct CFTR mutations enabling the development of autologous therapies. Nevertheless, most of the existing protocols for hiPSC-AEC differentiation have failed to consistently produce rare AECs such as ionocytes and pulmonary neuroendocrine cells (PNEC). The use of biomaterials (natural or synthetic) could enhance hiPSC-AEC *in vitro* differentiation, also providing a structural support for the airway epithelium transplant. In the present study, we have developed an airway epithelium patch using hiPSC and different biomaterials that thanks to their properties could further improve the differentiation and function of the *in vitro* generated epithelium.

METHODS: AEC were derived from FS13B hiPSC cell line using our previously described protocol [3]. In brief, cells were driven through definitive endoderm and anterior foregut endoderm to reach a lung progenitor state. At day 16 of differentiation, cells were sorted by FACS (fluorescence activated cell sorting), and purified lung progenitors were differentiated to AECs in air-liquid interface (ALI) culture into temperature-responsive culture inserts (4.2 cm^2; CellSeed Inc., Tokyo, Japan) and on Corning® Transwell inserts with various biomaterials coatings. After 28 days of AEC maturation in ALI culture, airway epithelia were phenotypically characterised with different molecular biology techniques including immunofluorescent staining and functionality was assessed through techniques as transepithelial electrical resistance (TEER) measurement.

RESULTS: Different conditions have shown different properties of cell attachment and viability. We have obtained a polarised airway epithelium containing both abundant and rare AEC types. The biomaterial that had shown a major degree of cell viability and functionality was selected for further studies to assess their engraftment capacities.

DISCUSSION & CONCLUSIONS: Our results demonstrate the feasibility of a cell patch therapy with potential therapeutic applications to CF and other respiratory diseases. Further optimisation studies should be done to evaluate the engraftment of the cell patch and its ability to restore CFTR function in the airway epithelium.

REFERENCES: [1]N.L. Turcios (2020) *Resp Care*, **65(2)**: 233-251. [2]K.B. Hisert et al. (2023) *Lancet Respir Med*, **11**: 916-931. [3]M. Vilà-González et al. (2024) *Respir Res*, **25**: 180.

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Enhancing Reflective Holographic Microscopy Technique for Real-Time Cell Mass Analysis on Bulk Ceramic Substrates

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INTRODUCTION: Tissue engineering (TE) relies on biomaterials that not only exhibit biocompatibility but also actively support tissue regeneration. The assessment of cellular interactions with these materials traditionally involves metabolic activity measurements or staining techniques, which may interfere with real-time, in situ observations. Reflective Holographic Microscopy (RHM) emerges as a promising optical imaging approach for direct non-invasive, high-resolution analysis of live cells on opaque ceramic substrates, addressing a critical limitation of conventional techniques such as Differential Interference Contrast (DIC) and Transmission Holographic Microscopy (THM), which require transparent materials.

METHODS: This research enhances RHM for real-time in vitro monitoring of cellular behavior on dense hydroxyapatite (HA) and Al_2O_3 substrates. Samples were prepared using isostatic pressing to achieve high-density ceramic structures, followed by precise polishing (Tegramin 30) to obtain an optimal mirror-like surface for imaging. The study optimizes imaging resolution, refines sample preparation, and validates reproducibility by correlating RHM data with Scanning Electron Microscopy (SEM), Confocal Laser Scanning Microscopy (CLSM), and Atomic Force Microscopy (AFM). Comparative microscopy (CM) further establishes correlations between in vitro dynamic and fixed-state cellular observations.

RESULTS: Dense HAp samples were polished to roughness levels of 9 µm, 3 µm, 1 µm, and 0.25 µm to determine the minimum acceptable roughness for RHM. The highest image quality was achieved on surfaces polished below 1 µm. RHM data were complemented by CLSM, AFM, and SEM to assess surface roughness and compare imaging techniques (Fig. 1). Mathematical reconstruction of RHM images is ongoing in collaboration with the Institute of Physical Engineering at BUT. Fixed cell cultures on dense HAp substrates were successfully imaged, confirming the feasibility of FIB sectioning and Transmission Electron Microscopy (TEM) observation (Fig. 2). The first RHM image enabling real-time visualization of live cells is shown in Fig. 2.

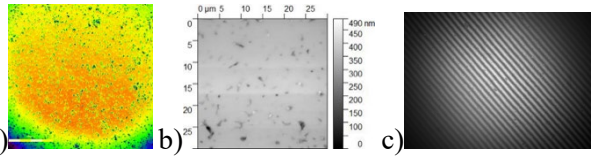

Fig. 1: Images of the surface of HA substrates polished using a 1 µm grit paste, captured by a) CLSM, b) AFM, and c) surface record of the polished sample obtained using RHM.

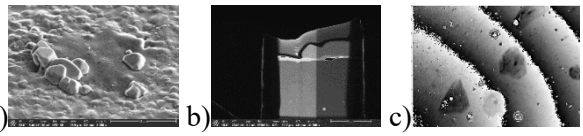

Fig. 2: a) Dehydrated and fixed cell on the HA substrate, b) Cross-section of this cell, and c) Cells on the surface of dense HAp samples via RHM.

DISCUSSION & CONCLUSIONS: The study confirms RHM as a viable method for real-time, non-invasive imaging of live cells on opaque ceramic substrates. Surface roughness significantly impacts image quality, with optimal results on substrates polished below 1 µm. Correlation with CLSM, AFM, and SEM strengthens RHM's reliability, while FIB sectioning and TEM provide deeper structural insights. The first real-time RHM image of live cells (Fig. 3) marks a key advancement, though challenges remain, including substrate preparation constraints and the need for further imaging optimization. Future work will refine imaging conditions, enhance processing algorithms, and expand studies to different cell types and ceramic materials, reinforcing RHM's role in biomaterial research and TE.

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Mechanical evaluation of the adhesion of injectable bone cement in pull-out experiments

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INTRODUCTION: Bone defects often require synthetic substitutes due to limitations of natural healing and complications with bone grafts, such as infection risk. Calcium phosphate cements (CPCs) offer biotolerance, biodegradability, and injectability, addressing issues of commonly used PMMA, which lacks these properties.

This study evaluates the adhesion performance of CPC enriched with different adhesives using a pull-out test. The results compared CPCs with PMMA to optimize CPC for clinical use.

METHODS: The objective of the study was to evaluate the mechanical properties of the bonded bone-cement interface by performing pull-out tests on 3D-printed cylindrical titanium implants (Fig. 1A; diameter 4 mm and length 8 mm), under the assumption of a perfectly rigid cement-implant bond. Bone fragments with cemented implants (Fig. 1B) were prepared and divided into 7 groups (*n=10*); one with PMMA-based Refobacin (reference) and six with CPCs of varying adhesive concentrations.

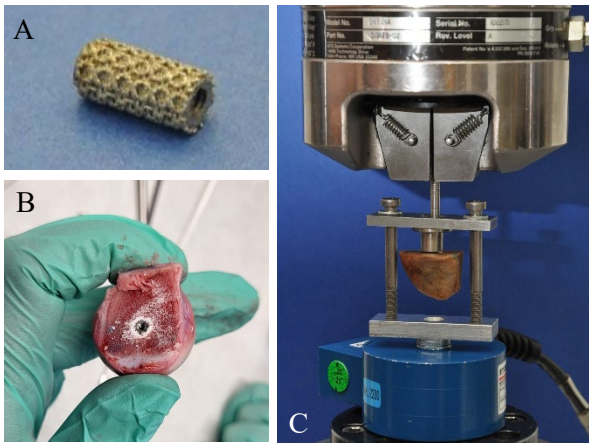

Fig. 1: A) Testing system with hydraulic jaws, fixture and load cell. B) 3D-printed cylindrical titanium implants. C) Bone fragment with implant.

The pull-out experiments were conducted using the MTS Mini Bionix 858.02 testing system (MTS, Minnesota, USA), with load cells (0–500 N and 0–2500 N), equipped with hydraulic jaws and special fixture for pull-out test (Fig. 1C). A constant feed rate of 10.0 mm/min was applied until implant extraction. The displacement, force and time. including their maximum values, were recorded during the experiment.

Evaluation parameters were the pull-out force, shear stress, displacement at failure, work of adhesion, and efficiency of adhesion work.

RESULTS: Compared to the reference Refobacin (PMMA), the values of the base cement are statistically significantly lower (except for the efficiency of adhesion work). The addition of adhesives to the base cement has only a small effect on adhesion. When comparing groups with adhesives to the base cement, no improvement or improvement in investigated mechanical properties was observed.

DISCUSSION & CONCLUSIONS: The developed CPCs have not achieved the properties of PMMA but are injectable and biodegradable, which might work better in physiological conditions and are indispensable prerequisites for specific clinical applications. The addition of a certain concentration of adhesives had a variable effect on the increase in adhesion. The effect of the addition of adhesives will be further investigated on a different concentration range of biomimetic additives and in vivo conditions on sheep animal models.

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Multi-Parametric Surface Plasmon Resonance (MP-SPR) – breaking the barriers of traditional SPR in biomaterial research

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INTRODUCTION: The new Multi-Parametric Surface Plasmon Resonance (MP-SPR) technology is a technique utilizing a complete SPR angular spectra in air and liquid environments at several laser wavelengths. This allows for the acquisition of several parameters (e.g., thickness, refractive index, optical dispersion, etc.) from the complete SPR curve at the same time. Furthermore, MP-SPR is not limited to tens of nanometers thickness layers and allows for monitoring of refractive index changes of micrometer thick layers and adsorbates (e.g., micrometer thick chitin layer [1], cells [2-3], bacteria [4], etc.).

By identifying the Total Internal Reflection (TIR) angle along with SPR Peak Minimum Position in the complete SPR curve (*Fig. 1*), MP-SPR instruments are capable of employing a unique PureKinetics™ feature [5]. It can remove the so-called bulk effect seen during the data acquisition in real-time. In this case, there is no need for a reference channel (a necessity in traditional SPR), because the same measurement spot is used for "bulk signal" subtraction. This tool allows for monitoring of material coated sensor chips in serum [6], cell media [7] or other complex samples.

RESULTS: MP-SPR has been an irreplaceable tool in biomaterial research due to above mentioned reasons. Some examples of MP-SPR in biomaterial studies include promotion of cell adhesion on flat substrates via Layer-by-Layer glycoprotein and gelatin based nanofilms [8], self-assembly of cellulose nanomaterials [9], protein adsorption on cellulose [10], enzymatic degradation of modified cellulose [11], etc. Furthermore, the investigation of adsorption of human hepatocellular carcinoma cells to cellulose nanofibrils [7] or cell binding on hydroxyapatite coatings [12] would not have been possible without additional features provided by MP-SPR.

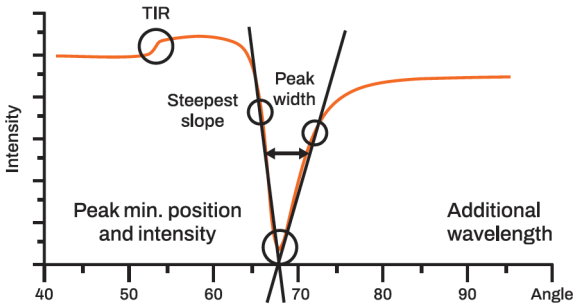

Fig. 1: Complete SPR curve measured with a unique MP-SPR goniometric setup.

DISCUSSION & CONCLUSIONS: The ability to measure affinity and kinetics of molecules in a label-free manner brings MP-SPR at the forefront of analytical tools in research. In addition, the method allows to characterize the adsorbed layer thickness, refractive index and dispersion independently, therefore we foresee the expansion of application areas of MP-SPR in multidisciplinary sciences.

REFERENCES: [1]M.G. Casteleijn, et al. (2018) *Colloids and surfaces*, 539: 261-272.

[2]M. Soler, et al. (2018) *ACS Sens.*, 3, 11: 2286:2295.
[3]A. Koponen, et al. (2020) *Biosensors and Bioelectronics*, 168: 112510.

[4]B.B. Tasbasi, et al. (2019) *Analytical Biochemistry*, 587:113449.

[5]Å. Frostell-Karlsson, et al. (2000) *Journal of Medicinal Chemistry*, 43, 10: 1986-1992.

[6]G. Emilsson, et al. (2015) *ACS Applied Materials & Interfaces*, 7, 14: 7505-7515.

[7]X. Zhang, et al. (2021) *Journal of Colloid and Interface Science*, 584: 310-319.

[8]A. Nishiguchi, et al. (2015) *ACS Biomaterials Science Engineering*, 1, 9: 816-824.

[9]K. Solin, et al. (2020) *Small*, 16, 50: 2004702.

[10]H. Orelma, et al. (2011) *Biomacromolecules*, 12, 12: 4311-4318.

[11]J. B. Schaubeder, et al. (2022) *Carbohydrate Polymers*, 294: 119737.

[12]A. M. Vilardell, et al. (2016) *Journal of Functional Biomaterials*, 7, 3: 23.

Centre of Excellence in Materials-Driven Solutions for Combatting Antimicrobial Resistance (MADNESS)

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INTRODUCTION: Antimicrobial resistance (AMR) poses a serious threat to public health, as it makes it difficult or even impossible to treat infectious diseases. World Health Organization listed AMR in top 10 global health threats. Furthermore, biofilm-forming bacteria pose significant challenges and are associated with the majority of chronic and device-related infections. Development of new antimicrobials is not a top priority for the pharmaceutical industry as it requires enormous economic and labour investment with uncertain commercial return. Thus, alternative approaches are urgently sought to combat AMR. Our Centre of Excellence (CoE) in Materials-driven Solutions for Combatting Antimicrobial Resistance (MADNESS) at Åbo Akademi University was established to generate alternative solutions for AMR, by joining expert forces from the fields of pharmacy, materials chemistry, and artificial intelligence (AI).

METHODS: We employ several methodologies to create novel materials that are either inherently antimicrobial or have been loaded with drugs. The approaches we use:

1. Integration of AI in materials design for predicting and maximizing the antimicrobial activity and to shorten the design life cycle.

2. Nanoparticles (NPs) based on – (a) Woody polyphenols as inherently antimicrobial NPs, (b) Functional polymeric NPs as antimicrobial drug carriers, and (c) Inorganic NPs as carriers for genetic constructs.

3. Developing antimicrobial textiles for AMR therapy utilising flexible cellulose nanofibers with polypyrrole nanocoatings.

4. Development of antimicrobial, functional composite materials and 3D-printed templates for tissue regeneration.

5. Implementing new real-time label-free analytical methods for measurements of bacterial adhesion and biofilm growth kinetics via surface plasmon resonance.

RESULTS: Throughout the study, we will deepen our knowledge on the impact of distinct materials on AMR. We expect to create a "toolbox" for treating infectious diseases and other medical conditions where traditional antimicrobials are ineffective (Fig. 1).

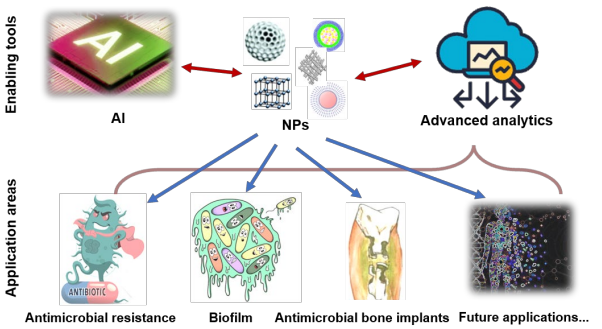

Fig. 1: Assisted by AI and advanced analytics, we develop NPs that are applied to overcome biofilm-forming resistant microbes in diseases as well as in wounds and in tissue defects.

DISCUSSION & CONCLUSIONS: We envision to create highly specialized solutions that have great potential for practical implementation and can have an impact on the pharmaceutical industries, while creating opportunities for entrepreneurs. In future, we believe to aid people in managing prevalent microorganisms in a financially viable manner.

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Histocompatibility evaluation of biodegradable polymer/calcium phosphate bone paste with prolonged injectability

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INTRODUCTION: Clinicians in orthopaedics seek resorbable bone defect fillers that stimulate new bone formation, prevent infections and accomplish bone mechanical load. Concurrently, easy going handling and application into complicated defects are practical demands on the bone fillers. The aim of this work is to histologically evaluate the ability of new injectable and resorbable bone paste to promote bone defect restoration.

METHODS: The bone paste consists of tricalcium phosphate powder mixed with a thermosensitive biodegradable copolymer[1].The ability of the bone paste (iBP) to heal bone defects was evaluated in a standardized rat model. 96 adult Wistar rats (body weight > 400g) were randomly divided into 12 groups according the experimental design (Table 1).

Table 1: *Summary of experimental groups.*

	day 1	week 3	week 6	week 12
iBP	8	8	8	8
Stimulan®	8	8	8	8
woTRT	8	8	8	8

The hole (2 x 5 mm) was drilled into lateral condyle of the femur and after stopping the bleeding the bone paste (iBP) was injected into the defect by 1mL syringe and G20 cannula. The commercially available bone filler based on calcium sulphate Stimulan® was applied according to manufacturer. The no treatment control (woTRT) was left without the filler. The animals were sacrificed after 1 day, 3, 6 and 12 weeks from the operations and the explanted femoral bones were fixed in 4% formaldehyde for micro-CT and histological evaluation. The quantitative histology analyzed the area fractions of the i) new bone; ii) remaining bone filler; iii) connective tissue; and iv) bone marrow/adipose tissue.

RESULTS: The histological analysis revealed the favourable osseointegration of the bone paste and its consecutive replacement by new bone tissue (Fig. 1 – black arrows). On the other hand, Stimulan® was mostly replaced by bone marrow and adipose tissue (Fig. 1 – black lines) and the defect without any treatment healed in the slowest rate, but by means of new bone formation (Fig. 1 – black asterisks).

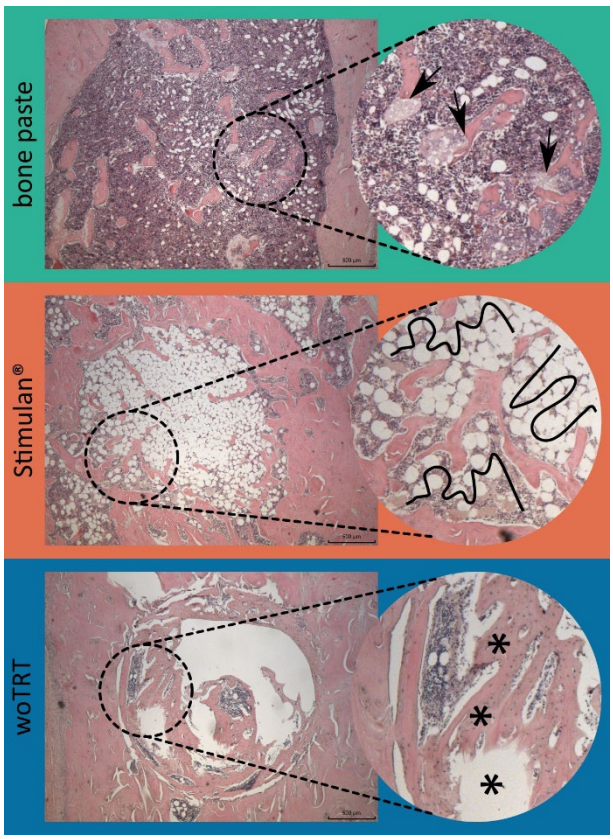

Fig. 1: *The histological evaluation of bone paste osseointegration after 12 weeks from operation.*

DISCUSSION & CONCLUSIONS: Our bone paste shows safe osseointegration and degradation rate comparable with other studies[2]. Our novelty consists in its long term injectability and possibility to add bioactive molecules which is valued by orthopaedists.

REFERENCES: [1]L. Vojtová et al. *Int. J. Mol. Sci.* (2019), **20**(2), 391. [2] G. Qian et al. Ceram. Int. (2024), 50(20), 39581.

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Fabricating hyaluronic acid-based hydrogels

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INTRODUCTION: The regeneration process of cartilage tissue is severely hampered by the fact that cartilage is avascular and innervated. Currently available methods of treating cartilage damage are based mainly on alleviating pain in patients usually allowing them to return to daily activities, but this solution provides relief for a limited time. Above all, they do not stimulate regeneration of cartilage tissue, and as a result, endoprosthesis surgery is necessary after some time[1]. Hydrogels are one of the numerous biomaterials that have drawn a lot of attention and gained popularity in the field of tissue regeneration, particularly cartilage tissue. Hyaluronic acid (HA) is one of the several polysaccharides that are commonly employed as a hydrogel matrix. It is defined as the primary component of synovial fluid and a naturally occurring polymer found in the human body[2]. Despite its versatility as a biomaterial, it cannot be used in hydrogel preparation without functionalization. Partial oxidation introduces aldehyde groups that allow click-reaction cross-linking with small biodegradable molecules such as adipic acid dihydrazide (ADH)[3]. The aim of this study was to functionalize hyaluronic acid by introducing new functional groups during partial oxidation and to optimize the fabrication of hydrogels with adipic acid as a cross-linker.

METHODS: HA was dissolved in distilled water, combined with aqueous solution of sodium periodate ($NaIO_4$) and placed on the magnetic stirrer for 24 h. The reaction was stopped by adding glycol ethylene. The solution was dialyzed against distilled water for 5 days, followed by freeze-drying. Oxidized HA (oxi-HA) is a product of this reaction. Respectively, oxi-HA and ADH were dissolved in phosphate-buffered saline (PBS) at 4 ºC, combined at the volume ratio of 4:1 (oxi-HA/ADH), and poured onto the Petri dishes, which acted as a mould for a cross-linking process. Then the samples were cut out by the circular puncher with a diameter of 12 mm and freeze-dried. Self-healing properties were assessed by cutting freshly cross-linked samples in half and measuring time required to full recovery. Optical microscopy and scanning electron microscopy (SEM) were used to describe microstructure of freeze-dried hydrogels. Swelling properties and mass changes were tested in the course of time via incubation in PBS, whereas Fourier-transform Infrared Spectroscopy (FTIR) enabled the recognition of newly formed functional groups in both, oxi-HA and oxi-HA/ADH.

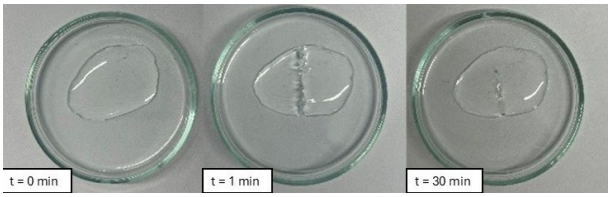

Fig.1. Pictures representing self-healing assay: before cutting (t = 0 min), after cutting (t = 1 min), and after 30 minutes from cutting (t = 30 min).

RESULTS: FTIR spectrum for oxi-HA proved that the aldehyde groups were formed, whereas spectra for oxi-HA/ADH hydrogels confirmed the presence of a new N-H bond. The hydrogels exhibited self-healing properties, as just 30 minutes after cutting the samples were fully recovered (Fig.1). Microscopic observations confirmed the porous nature of the samples. Moreover, oxi-HA/ADH hydrogel has high water capacity, observed by rapid water absorption during the first 24 hours after immersion in PBS.

DISCUSSION & CONCLUSIONS: The presence of dynamic hydrazone bonds makes oxi-HA/ADH hydrogels very attractive in a form of injectable materials (due to self-healing properties). This material appears to have the potential to be formed into microgels. However, further investigation concerning injectability, mechanical and biological properties is required. Based on that results it is possible to design a process of obtaining hydrogel in a form of injectable microspheres (microgels).

REFERENCES: [1]M.Liu et al. (2017) *Bone Research*, **5**: 17014 [2]Y. Luo et al. (2023) *Int. J. Of Biol. Macromol.* 231: 123308 [3]C. França et al. (2021) *Polysaccharides*, **2**: 477-496.

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Preparation of dense and porous monetite ceramics using the cold sintering process

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INTRODUCTION: Over the years, the rising number of orthopedic disease cases has led to growing interest in calcium phosphate biomaterials. Previously overlooked calcium phosphate phase – dicalcium phosphate anhydrous, also known as monetite, has been proven to promote osteoinduction and osteoconduction. Monetite also exhibits a greater balance between implant resorption and bone formation than conventionally used calcium phosphate phases [1]. Currently, the use of monetite is limited to powder form due to its instability at temperatures above 400 °C, which complicates its densification and potential applications in bulk form. The cold sintering process presents a promising solution to overcome this limitation.

METHODS: A fractional factorial experimental design was employed to identify the sintering parameters that most significantly impact the densification of monetite. The influence of five parameters – temperature, pressure, sintering time, amount of transient liquid (H_3PO_4 solution), and molarity of H_3PO_4 solution were investigated. To each parameter, low and high values were assigned, and a chosen number of tests was conducted to assess the impact of each factor. The sintered materials were characterized by XRD and FTIR, while their fracture surface morphology was examined via SEM. Additionally, their density and flexural strength were determined. By comparing the density and flexural strength of the sintered samples, optimal sintering conditions were determined. Porous monetite ceramic scaffolds were fabricated under the optimal sintering conditions using the space holder technique.

RESULTS: Dense monetite ceramics with relative densities exceeding 95% were successfully fabricated using the optimized cold sintering process parameters. XRD and FTIR spectroscopy analysis confirmed that the cold sintering process did not lead to the formation of any other phases. The flexural strength of the samples fabricated with the optimized sintering parameters exceeded 30 MPa. Monetite ceramics with 60% porosity (pore size ranging from 200 to 500 µm) were successfully fabricated using the space holder technique (Fig. 1).

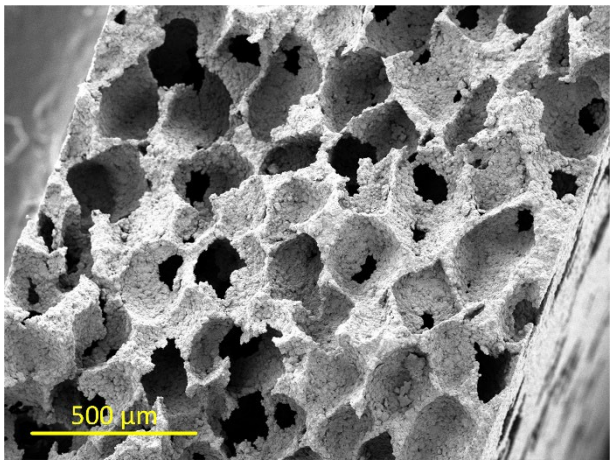

Fig. 1: Fracture surface of a monetite ceramic scaffold with 60% porosity.

DISCUSSION & CONCLUSIONS: The cold sintering process can be effectively utilized for the densification of monetite. The space holder technique can be successfully utilized to produce porous bioceramics with controlled porosity using the cold sintering process.

REFERENCES:

[1]H. Zhou et al. (2021) *Acta Biomater,* **127**: 41-55.

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Biocompatibility of CMC-gel with PEG using keratinocytes (HaCaT) & fibroblasts (HDFn)

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INTRODUCTION:

Carboxymethyl cellulose (CMC) hydrogels incorporating polyethylene glycol (PEG) have emerged as promising candidates for advanced wound dressings. This abstract explores the potential of CMC-PEG hydrogels in wound management.

CMC, a natural polymer, provides a biocompatible and hydrophilic matrix for hydrogel formation. The incorporation of PEG enhances hydrophilicity, promotes cell proliferation, and can improve the mechanical properties of the hydrogel.

CMC-PEG hydrogels offer several potential advantages as wound dressings, including moist wound healing [1], exudate management [2], protection [3], pain mitigation [1], and drug delivery [1,3].

Further research is warranted to fully evaluate the clinical efficacy and optimize the formulation of CMC-PEG hydrogels for various wound types.

METHODS: HACAT (density 21,875 cells/cm^2) and HDFn (density 15,625 cells/cm^2) were seeded to tissue culture plastic, and the CMC+PEG gel was added to an insert above the cells (i.e. there was no direct contact between the cells and the gel). Three types of gels with different concentration of CMC and PEG were used.

MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay; Promega) was used to analyze cell metabolic activity.

PI/DIOC staining was applied to detect the morphology of cells using fluorescent microscope.

Statistical evaluation was performed using GraphPad Prism 8. Normality was tested by the Shapiro-Wilk test for each experiment and each experimental time point. Since none of the experimental days passed the normality test, Kruskal-Wallis with Dunn's multiple comparisons test was performed. The statistical difference (p-value $\leq$ 0.05) of any material to the control is marked as an asterisk above the column of the material for the specific time point.

RESULTS: Results of HDFn cell viability showed significantly decreased metabolic activity on day first compared to the control group. From day 1 to 3, cells increased their viability which was even better in group 1 in comparison with untreated cells. The viability of HACAT cells on day 1 was lower in all groups with CMC-PEG in comparison with untreated cells and continued to decrease until day 3 except for the group with 1. PI/DIOC showed typical morphology of HACAT and HDFn cells.

DISCUSSION & CONCLUSIONS: In this study, we investigated the efficacy of various gel formulations containing different concentrations of carboxymethylcellulose (CMC) and polyethylene glycol (PEG) as potential wound dressings. Overall, the results of this study suggest that the group 1 of gel formulation is the most promising in terms of its biocompatibility for medical applications.

REFERENCES: [1]S. Nasra et al. (2023) *Life Sciences*, **334**:122232. [2]A.M. Hosseini et al. (2023) *J. Lab. Anim. Res.*, **2**:41-50. [3]J. Xiao et al. (2024) *Sci. Rep.*, **14**:26854.

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