Cells and Extracellular Molecules Involved in Bone Fracture Healing

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INTRODUCTION: Bones are unique tissues that can heal without scar formation. It is well established that fracture healing begins with inflammation, which must subsequently subside for the successful repair of the damaged bone. A myriad of signalling molecules and cells are involved at various stages of fracture healing. Immune cells work along with osteoclasts, mesenchymal stem cells, osteoblasts and many other cells in a closely coordinated fashion. In our poster, we have attempted to visualise known interdependencies between signalling molecules and cells involved in fracture healing.

METHODS: We have searched SCOPUS and Google Scholar databases using terms "bone fracture healing", "bone repair", "bone fracture signalling", "fracture signalling pathways", "fracture inflammation", "fracture healing cells", while limiting the search to the past decade (2011-2021). The most relevant reviews and original research articles were selected and analysed, to extract the information about signalling molecules, pathways and cells that govern the bone fracture healing process.

RESULTS: Significant progress has been made over the last ten years to understand the intricate details of bone healing process, but the current literature agrees that the picture is far from being complete. Multiple cells are involved in the response to bone fracture. Immune system plays a crucial role in bone repair. Platelets, neutrophiles, macrophages (classically and alternatively activated, also osteomacs), monocytes, eosinophils, mast cells, B and T lymphocytes, contribute to the successful healing. Apart from the immune cells, chondrocytes, osteocytes, osteoblasts, osteoclasts, and mesenchymal stem cells drive fracture site 'clean-up', repair and remodelling. Although it was discovered that T-lymphocytes communicate with both osteoclasts and osteoblasts via direct cell-cell contacts, most of the communication and coordination proceeds by means of signalling molecules. Multiple signalling molecules and corresponding pathways were identified to be at play during the fracture healing: interleukins (ILs), transforming growth factor β superfamily (TGFβ), bone morphogenic proteins (BMPs), growth differentiation factors (GDFs), fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), platelet-derived growth factor (PDGF), tumor necrosis factors (TNFs), vascular endothelial growth factor (VEGF), interferons (IFNs) and others.

DISCUSSION & CONCLUSIONS: Bone fracture is accompanied by rapture of the blood vessels and clot formation, cell damage and death. Inflammation starts immediately upon fracture and is characterized by infiltration and activation of neutrophiles and macrophages to the site of injury. A snapshot of communication between the first responders is shown in Figure1.

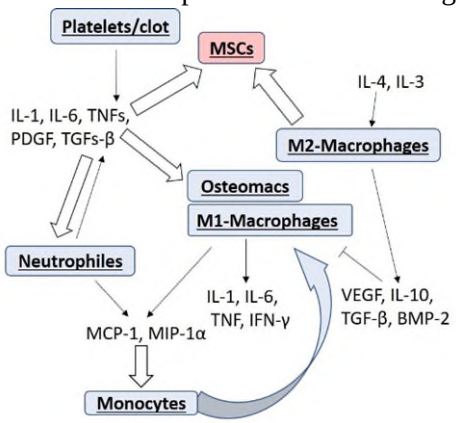

Fig. 1: A snapshot of non-lymphocytic immune cells and signalling molecules during the early response to bone fracture.

This figure depicts only a small fraction of complexity that is associated with bone healing process. The wealth of data published over the last decade, revealed many intricate details that are not easy to comprehend. This has motivated us to summarize and chart the well-established knowledge and recent discoveries, to assist researchers in understanding of the biology of bone fracture healing.

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Resin Cement Residue Removal Techniques: Analysis of Marginal Defects and Discolouration Intensity Using Micro-CT and Stereomicroscopy

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INTRODUCTION: Adhesively cemented porcelain veneers are widely used in aesthetic dentistry. Different cementation protocols and cement removal techniques are used, which could influence long term results of cementation. The objective of this study was to compare marginal defects and evaluate connection between defect types and the intensity of discolouration for adhesively cemented veneers, when using two cement removal techniques.

METHODS: Twenty premolars were included, all prepared similarly: 0.5 mm deep, rounded corners, chamfer finish line, borders in enamel. Ceramic (*IPS e.max CAD*) veneers were made using a scanner *Ceramill Map 600* and mill *Ceramill Motion 2*. Cement gap was set to 0.02 mm and *PANAVIA V5 (Kuraray, Noritake)* cement was used. Teeth were divided in 2 groups. For the first group (n = 10) cement excess was removed with a probe, after 3 – 5 second polymerization, continuing to a complete polymerization. For the second group (n = 10) excess was removed with a brush, then completely polymerized. Teeth were stored in an alginate gel until micro-CT examination (*Scanco medical µCT50*). Scanning was done twice: directly after the cementation and after the thermocycling (*Thermocycler 1100/1200, SD Mechatronik, Germany*) in distilled water for 5'000 cycles between 5ºC and 55ºC. For each tooth 413 micro-CT slices were obtained. To analyze discolouration, teeth were coloured in 0.5% basic fuchsine, cut in a vestibulo-lingual direction and examined under stereomicroscope. Discolouration depth was scored with score 0 (no discolouration) to 5 (discolouration along the entire margin). For statistical analysis Mann-Whitney, Wilcoxon signed rank, Fischer exact and Kruskal-Wallis tests were used.

RESULTS: Before thermo-cycling (TC) group 2 exhibited more defects overall than group 1 ($p = 0.0161$). After TC, there were no statistically significant differences between groups. Overall count of defects increased after TC for both groups ($p < 0.05$). After colouring 55% of the specimens in group 1 exhibited extensive discolouration and 90% in group 2 exhibited slight discolouration ($p = 0.008$). Group 2 had more defects initially, group 1 had bigger increase in the count of defects after TC. Analysis of different defect types did not show statistically significant connection between the type of defect and the depth of discolouration.

DISCUSSION & CONCLUSIONS: There have been studies on the effect of different bonding techniques on microleakage[1], however different cement removal techniques have not been investigated. The limitations of this study include not taking into account possible differences in the cervical region of the veneer, as well as possible measurement errors in the repeated micro-CT images.

Conclusions:

1. Removing unpolymerized cement with a brush initially caused more defects in cement layer when compared to light curing for 3-5 seconds and removing excess cement with a probe.
2. Count of defects increased in both groups after thermocycling.
3. Cement removal with a brush showed less discolouration after thermocycling.
4. No connection was found between the type of defect and the depth of discolouration.

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Effect of Initial Temporary Cementation on Adhesively Bonded Overlays Using a Tensile Strength Test. An *In-Vitro* Study

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INTRODUCTION: Adhesive cementation is widely used in aesthetic dental restorations. In routine, prior to adhesive cementation, temporary cements are used, which could adversely affect the bonding strength. The objective of this study was to determine whether temporary cement affects the tensile bond strength of adhesively cemented restorations.

METHODS: Twenty-six extracted intact premolars were included in this study.
Six teeth were grounded to expose dentine surface and PMMA overlays were cemented with *TempBond NE (Kerr Dental)* on them. Temporary cement residues were polished for three samples and left for the other three. All specimens were broken along vertical axis to undergo SEM/EDX (*SEM, Tescan Mira/ LMU; EDX, Oxford Instruments X-Max^N* detector size 150 mm^2) analysis to detect Zn ions that are present in temporary cement.
Remaining twenty teeth were embedded in 16x25mm PMMA cylinder, perpendicular to vertical tooth axis. The teeth were grounded to expose dentine surface. Twenty zirconia overlays (*Katana Zirconia STML, Kuraray Noritake*) (13.15x3.2 mm) and ten PMMA overlays were fabricated to simulate temporary restoration. All teeth were divided in two equal groups. In the first group temporary PMMA overlays were cemented on each tooth surfaces by *TempBond NE*, subsequently cleaned by rotary polishing brush and pumice, and sequentially zirconia overlays were cemented adhesively by *Panavia V5* (*Kuraray Noritake*) – (PV5-1) according to the protocol. For the second group, zirconia overlays were directly cemented by *PanaviaV5* to teeth surfaces – (PV5-2). After cementation, the bond strength was evaluated by tensile bond strength test (*Instron Universal Machine, Norwood, USA*) with a head speed of 0.5mm/min. Three groups were created to compare sample fracture types after tensile bond test.

RESULTS: In unpolished group, Zn ions were detected in 100% of specimens. In polished group, Zn ions were detected in 33% of specimens. Mean dentine area in TEST group was 30.48 mm^2 (SD=4.57), in CONTROL group- 33.43 mm^2 (SD=7.89). Mean enamel area in TEST group was 29.57 mm^2 (SD=7.37), in CONTROL group-25.96 mm^2 (SD=9.19). Tensile strength (TS) was higher in TEST group, and there is statistically significant difference between both groups (TEST - mean TS=337.43N, SD=116.67; CONTROL- mean 203.57N, SD=59.16; p=0.0102). A two-way ANOVA was run to examine the effect of enamel area and study group on the TS. There was a significant effect of enamel area and study group on the TS value (p=0.0005). With the increased enamel area, the TS was significantly higher (p=0.008). Dentine area did not influence TS. Enamel area and dentine area impact, on failure mode, were assessed. Enamel area has an impact on fractures after TS test (p=0.007).

DISCUSSION & CONCLUSIONS: Despite the TEST group gave a higher tensile strength, it was obvious that dentine has no influence on it. However, the intensified treatment of the test group tooth surface before cementation could have influenced the results. Since the enamel and dentine proportion was taken into account and teeth were so different, more samples are needed to make the group more homogeneous. Within the limitations of this *in vitro* study, the following conclusions can be drawn: Residual temporary cement may remain in the dentin tubes after polishing, but this does not affect the cementation result, as dentin has no effect on tensile strength. The size of the enamel area affects the tensile strength - larger enamel area could give greater tensile strength. To confirm the results larger sample size is necessary.

A Comparative Study of Strontium-Substituted and Strontium Ranelate - Loaded Nanocrystalline Hydroxyapatite

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INTRODUCTION: Repair and healing fractures caused by osteoporosis remains a major clinical challenge in orthopaedic surgery. It has been confirmed that Sr ions have the ability to enhance new bone formation by activating Ca-sensing receptors, while inhibiting bone resorption. For instance, anti-osteoporotic drug strontium ranelate (SrRAN) is a dual action bone agent, which reduces bone resorption and increases bone formation [1]. While negative systemic effects have been reported upon oral administration of SrRAN, we hypothesized that a sustained delivery system for local release of SrRAN can obviate systemic complications. Ionic Sr can be added to hydroxyapatite (HAp) instead of SrRAN, potentially stimulating bone formation locally. By using these alternatives instead of oral administration of SrRAN, it may be possible to avoid the negative systemic side effects. Therefore, the comparative study of Sr substituted and SrRAN loaded nanocrystalline HAp (nHAp), with a focus on the physicochemical properties and *in vitro* Sr ion release tests, was performed.

METHODS: Both nHAp powders were synthesized via wet chemical precipitation method from CaO and H_3PO_4 as described in [2]. SrO and SrRAN were Sr sources for the synthesis of Sr-nHAp and SrRAN-nHAp, respectively. Theoretical load of Sr was up to 10wt%. The powders were comprehensively characterized using several techniques of physicochemical analysis. Sr ion release profile in TRIS-HCl buffer solution (pH 7.4) from Sr-nHAp and SrRAN-nHAp was monitored for up to 30 days.

RESULTS: XRD patterns with broad and overlapping characteristic HAp peaks indicated that both synthesized Sr-nHAp and SrRAN-nHAp materials are nanocrystalline. High specific surface area (SSA) for each synthesised powder was confirmed by BET analysis. SSA of SrRAN-nHAp and Sr-nHAp (Sr load 10 wt%) was 119 m^2/g and 75 m^2/g, respectively. Phase purity of the materials was also confirmed using FTIR analysis to detect characteristic absorbance bands related to SrRAN and HAp (for SrRAN-nHAp) or solely HAp (for Sr-nHAp). SEM micrographs revealed that Sr-nHAp and SrRAN-nHAp powders consist of nano-sized needle-like particles. A steady release of Sr ions in the TRIS-HCl medium was observed during all 30 days.

DISCUSSION & CONCLUSIONS: The results show that the properties of materials having analogous Sr content depend on the chosen Sr source in the synthesis medium. Namely, the crystallinity of nHAp produced with SrRAN is lower, compared to nHAp in which Ca ions are replaced with Sr ions. As expected, two different Sr release profiles upon degradation of the materials were obtained.

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Silver and Titanium Co-doped Calcium Phosphate Bioceramics: Thermal Stability, Mechanical and Antibacterial Properties

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INTRODUCTION: Calcium phosphate (CaP) bioceramics, due to the biocompatibility, bioactivity or bioresorbtion, have been widely used for regeneration of the bone tissues. Characterization of the bioceramics can be tailored by adjusting the chemical formulations of precursors. Ionic substitutions are considered as a promising strategy to improve biological properties of CaP on the basis of the vicarious ions kinds and amounts. The strategy of combining Ag with Ti ions is a potential way to promote bone-implant integration with reduced bacterial infection and enhanced bioactivity. Unlike antibiotics, Ag is a non-specific bactericide and exerts bactericidal activity on a broad spectrum of fungal and bacterial species [1]. While studies have been conducted, where Ti(IV) containing HAp was formed for photocatalysis [2], the effects of Ti(IV) addition are yet undiscovered. However, it has been suggested that Ti(IV) containing CaP might have bioactive potential [3].

METHODS: Ag and Ti co-doped CaP bioceramic were prepared using synthesized Ag and Ti co-doped HAp powders as the precursors. Synthesis of Ag and Ti co-doped HAp, with a (Ca+Ti+Ag)/P molar ratio of 1.67, powders were performed using an aqueous precipitation method. The nominal Ag and Ti content of the precursor powders was changed systematically: 0.5 and 0.5, 0.5 and 2, 1 and 0.5,1 and 2 wt%, respectively. Ca, Ti and Ag precursors, used in the method, were CaO, TiO_2 and Ag_2O. Starting suspensions were prepared by dispersing oxides in deionized H_2O. Orthophosphoric aqueous solution (2 mol/L) was added dropwise to the starting suspensions until a final pH of 8.8±0.2 was reached. The precipitates were filtered, dried and crushed in a mortar to give a fine powder. Bioceramic samples were prepared by uniaxial compaction and sintering at 1000, 1100, 1200 and 1300 ^{o}C (holding for 1 h). Ag is known to evaporate at high temperatures, so the effect of high temperature treatment was studied to maintain the antibacterial efficiency of the bioceramics. The precursor powders and bioceramic samples were characterized in terms of phase (XRD) and chemical composition (EDX, XPS), structural (SEM) and mechanical properties. Antibacterial properties were determined using *E.coli* and *S.aureus* bacterial suspensions.

RESULTS & DISCUSSION: All synthesized precursor powders were identified as nano-HAp, though did not follow the expected ratios. Co-doping promotes the formation of biphasic CaP after high temperature treatment, with variable HAp/β-TCP ratios, on the ground of the substitution level. Doping with Ti slightly increases the mechanical strength of the bioceramics.

CONCLUSIONS: Optimization of antibacterial efficacy of the CaP bioceramics by simultaneous doping of Ag and Ti and assessment of a synergy and cumulative effect of the dopants was performed. By combining concentration of the dopants and high temperature processing temperature, it is possible to obtain bioceramics that can inhibit bacteria growth.

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Attachment of Chitosan to Ti6Al4V, Bioglass and Hydroxyapatite for Improved Biological Response

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INTRODUCTION: The objective of the work is to make use of chitosan as a potential bioactive coating material for implantable biomaterials. The aim is a modulation of inflammatory response and a utilization of chitosan‘s antibacterial and bone stimulating activity [1, 2] in the implant-bone interfacial area. Three biomaterials were used as substrates: Ti6Al4V, bioglass and hydroxyapatite. The Ti6Al4V alloy was pre-treated according to a specific, patented protocol [3], the bioglass was lab-made and the hydroxyapatite was a commercial product.

METHODS: The chitosan (CTS) was obtained from Genis hf. Following coating methods were examined:
a) Direct coating with CTS (CTS/AA): Coating was done by dipping into 0.75% CTS in acetic acid solution, followed by drying and neutralization.
b) Tresyl chloride (TC) treatment (TC/CTS/PBS): TC (100 µL) was evenly loaded onto the material surface and allowed to dry at 37°C overnight. Coating was done by dipping the TC treated surface into 0.75% CTS in PBS solution and dried overnight at 20°C.
c) Polydopamine (PD) treatment (PD/CTS/PBS): The material was submersed in PD solution, with stirring for 4h, and dried at 37°C overnight, then dipped into 0.75% CTS in PBS solution for 5 minutes and dried at 20°C.
FTIR, SEM, AFM, contact angle and zeta potential measurements were performed to examine the physical-chemical properties of the surfaces. Bioactivity was examined by soaking in SBF at 36.5°C for 14 days at pH 7.4. First *in vitro* cell culture tests, with UMR cells, were performed.

RESULTS: FTIR analysis confirmed that all surfaces were covered with CTS. Characteristic FTIR peaks including Amide I and Amide II groups were detected. SEM showed that the physical appearance of the coated film varied among treatments. Coated area coverage varied significantly between the three treatments as well. CTS/AA treatments achieved 100% surface coverage on all surfaces. CTS surface coverage on TC/CTS/PBS and PD/CTS/PBS was lower. AFM analysis showed that all treatments with CTS/AA developed topography with finer grains than with CTS in PBS solution. The AFM observations are consistent with the SEM analysis. Bioactivity trials showed formation of hydroxyapatite with different morphological expressions depending on coating method. Initial results on cell proliferation (MTT), cell morphology and mineralization will be presented.

DISCUSSION & CONCLUSIONS: CTS was successfully attached to all surfaces. The presence of CTS on all biomaterials was confirmed and clear differences in surface qualities depending on substrate were revealed. The results suggest that the coatings will withstand mechanical and chemical stresses, exerted under physiological conditions and improve the biological response.

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Microwave-assisted Synthesis of Nanomaterials. The Series of MSS Reactors for Medical Applications

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INTRODUCTION: Bioactive materials which can support bone ingrowth and osseointegration are commonly used in many medical applications. Hydroxyapatite (HAP) is a calcium phosphate compound and it is one of the inorganic components of hard tissues. In the Institute of High Pressure Physics (IHPP PAS), where is manufactured, it has name GoHAP^{TM}. Microwave solvothermal synthesis (MSS) is an example of microwave assisted wet synthesis process and nowadays it is counted as one of the most popular chemical methods of obtaining nanomaterials, like HAP, ZnO, ZrO_2 and others.

METHODS: Microwave heating enables a better control of the reaction time, fast heating and reducing the thermal gradients. The morphology, grain size and specific surface area of the nanopowder can be controlled by the microwave reactor and the high pressure consolidation technology for ceramic materials. This also results in a better crystallinity comparing to the precipitation process. At the Laboratory of Nanostructures, IHPP PAS, we have been developing new type microwave reactors for nanomaterial synthesis for more than 15 years. The use of the microwave radiation and the unique design of the reactors permit the precise pressure control during quick synthesis processes, controlled with the accuracy of one second.

RESULTS: The MSS2 reactor presents the control system which allows for an automatic operation in the stop flow mode or use the batch (closed vessel) mode in bigger production scale than in other commercial equipment. [1-3] The MSS4 reactor is a new reactor manufactured by the co-operation of IHPP PAS and Łukasiewicz Research Network – The Institute for Sustainable Technologies, and it is a bigger device, consisting of two independent microwave systems to make two batch syntheses in one time. The MSS4 reactor has also a connected robotic system, the device is plugged in with a robot from Universal Robots Company, which is connected to the Labview software, that is controlled from a control cabinet and from the additional PC.

DISCUSSION & CONCLUSIONS: The reactors like MSS1 or MSS2 also present a control system which allows for an automatic operation in the stop flow mode or use the batch (closed vessel) mode in bigger production scale than in other commercial equipment. The batch system brings inertial purity and repeatability of the process. It brings the same concentration of each batch. [3] MSS4 reactor has a scale-up potential for medical grade syntheses, with an automation system.

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Calcium Phosphate Nanoparticles for Tooth Enamel Protection

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INTRODUCTION: The most common oral disease is caries, which causes the loss of dental hard tissue. Preventive dentistry is the most effective strategy against caries, therefore calcium phosphates (CaP) have been added as remineralization agents to toothpastes, dentifrices, varnishes and mouthwash suspensions. In this study we evaluated the ability of substituted CaP nanoparticles to increase the pH of suspensions after incremental addition of acid.

METHODS: CaP nanoparticles substituted with Sr^{2+} and F^- ions were synthesized *via* wet precipitation method. Synthesis was carried out at 45°C and the end pH, in the diapason from 7.2 to 9.0, was controlled. Physio-chemical and morphological characterization was done by X-ray diffractometry (XRD), Fourier transform infrared spectrometry (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy and Brunauer–Emmett–Teller (BET) by N_2 absorption. Titration station *Mettler Toledo* was used to evaluate the ability of CaP nanoparticles to increase the pH of suspensions over time (pH-t test), after incremental addition of citric acid. At first 0.62 g/mL suspension of CaP nanoparticles was heated to 36°C, then 10 µL of 0.05 M citric acid was added to the suspension every 10 minutes. In addition, bovine enamel block was used to simulate real tooth in the same conditions.

RESULTS: XRD patterns and FTIR spectra showed typical CaP maximums. The shifting of [OH] mode to the new band (3543-3547 cm^{-1}) confirmed OH···F bonding, thus indicating fluoride incorporation into FCDHAp and SrFCDHAp structure. The incorporated Sr^{2+} and F^- amount was up to 7.8 wt% and 1.9 wt%, respectively. Specific surface area of CaP samples is in range from 71.1±0.2 to 140.0±1.5 m^2/g and particle size from 15.2±0.2 nm to 30.0±0.1 nm. SEM micrographs revealed needle-like morphology of CaP particles. Fig. 1 demonstrated the results of pH-t test.

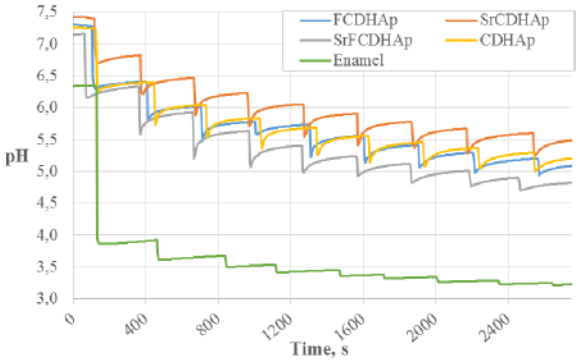

Fig. 1: The function of pH in time for CaP suspensions after incremental addition of acid.

DISCUSSION & CONCLUSIONS: Current research showed that after addition of acid, all samples initially showed a rapid decrease of pH. In the case of synthesized CaP, the pH reduction was about 1.0 to 1.2, but for the enamel, it was 2.5, which means that critical pH was reached (pH≤5.5) when enamel starts to dissolute irreversibly. Comparing the samples, the SrCDHAp suspension was able to maintain a relatively higher pH, but it should be noted, that initial pH of this suspension was about 0.2 higher than that of CDHAp, FCDHAp and SrFCDHAp. Additionally, Sr^{2+} and F^- incorporation into CDHAp structure did not show diverse results in the pH-t test. Based on the results it can be said, that CaP nanoparticles deposited on the tooth enamel could be a good candidate as pH increasing agent in the oral cavity, thus protecting enamel from dissolution, however, further studies are needed.

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Densification of Amorphous Calcium Phosphate by Cold Sintering Process

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INTRODUCTION: Sintering of amorphous calcium phosphate (ACP) is challenging because of its metastable structure. This structure is irreversibly altered when ACP is heated to temperatures above a few hundred degrees Celsius that leads to crystallization and transformation of ACP to other less bioactive phases. Therefore, only sintering techniques that enhances mass transfer at very low temperatures can potentially be used for ACP densification [1]. In this study, for densification of ACP, we used the so-called cold sintering process (CSP) [2]. We investigated the effect of CSP parameters such as sintering temperature and presence or absence of transient liquid (water) on densification and structure of ACP.

METHODS: ACP was synthesized by dissolution-precipitation method [3]. Dry or moistened (20 wt% H_2O) ACP powder was used as a starting powder. CSP was done at room temperature, 100, 120 or 150 °C for 30 min. During the CSP, the powder was held under the 500 MPa pressure. After CSP, bulk and true density of the samples were determined. In addition, they were characterized by X-ray diffraction, Fourier-transform infrared spectroscopy, scanning electron microscopy and thermogravimetry.

RESULTS AND DISCUSSION: X-ray diffraction patterns and Fourier-transform infrared spectrums of the CSP-sintered samples that were produced from the dry ACP powder indicated that the samples remained amorphous if they were sintered at room temperature, 100 or 120 °C. If CSP was done at 150 °C, the samples transformed to nanocrystalline hydroxyapatite phase. If moistened ACP powder was used as a starting powder, only the samples sintered at room temperature remained amorphous. Grain size of all CSP-sintered samples remained in the nanometer range regardless of the sintering temperature. Relative density of all samples were in the range of ~76 to ~87%. Relative density for the samples that remained amorphous after the CSP was close to 80%. Thermogravimetry analysis showed that CSP reduced water content in ACP. After CSP at room temperature, the samples produced both from the dry and moistened ACP powder lost approximately ~17 % of the water content that was present in the structure ofthe starting ACP powder. The samples that were produced from the dry ACP powder at 120 °C lost approximately ~23 % of the initial water content.

CONCLUSIONS: Sintering at elevated temperatures (in which ACP phase still remains stable) and use of transient liquid had no significant effect on ACP densification. By applying moderate uniaxial pressure, ACP could be sintered to relatively high relative density (~76 %) already at room temperature.

ACKNOWLEDGEMENTS: This work has been supported by the European Regional Development Fund within the Activity 1.1.1.2 “Post-doctoral Research Aid” of the Specific Aid Objective 1.1.1 “To increase the research and innovative capacity of scientific institutions of Latvia and the ability to attract external financing, investing in human resources and infrastructure” of the Operational Programme “Growth and Employment” (No.1.1.1.2/VIAA/2/18/318).

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Nano-particle Size as a Factor with High Impact on The Kinetics of The Sonocoating Process and Properties of Hydroxyapatite Layers

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INTRODUCTION: Millions of bone reconstruction operations are conducted every year. A promising alternative to autologous bone grafts are individual bone scaffolds made of bioresorbable polymers. Biofunctional implants fill the defect in the tissue and carry the loads. The presence of groups with hydrophilic properties (e.g., -OH) on biomaterial surface is preferred. For this reason, the modification of the surface of polymeric and metal materials is becoming more and more popular. In the field of regenerative medicine, homogeneous, biocompatible, bioactive coatings stimulate the regeneration of bone or cartilage tissue. All these conditions are met by nano-hydroxyapatite layers deposited by ultrasound. Hydroxyapatite (HAP) is the main mineral component of the bone, responsible for the stiffness and mechanical strength of the bones.

METHODS: Substrates were made from PCL granules (Sigma Aldrich) that were vitrified at 80°C and compressed to form flat pellets (ø11 mm). The substrate surface had defects in the form of pores, corrugations, and imperfections. GoHAP NPs were obtained using the hydrothermal microwave synthesis method described in detail by Kusnieruk et al. [1]. Two types of NPs, differing in particle size, were separately selected for coating: GoHAP with the particle size about 15 nm and GoHAP with the particle size about 45 nm. [2]

RESULTS: This work presents the mechanism of formation of the NPs layer deposited by sonocoating, as well as the relationship between the size of NPs used in the coating process and the properties of the deposited layer. The results obtained for 2 types of layers deposited by sonocoating on the surface of biodegradable polymer (PCL) are presented - the first type of layer was deposited with the use of GoHAP NPs with a size of 15 nm, and the second type with the use of GoHAP NPs with a size of 45 nm. The poster presents the kinetics of the NPs layer deposition process depending on the GoHAP NPs size, as well as the properties of the obtained layers, such as morphology or contact angle.

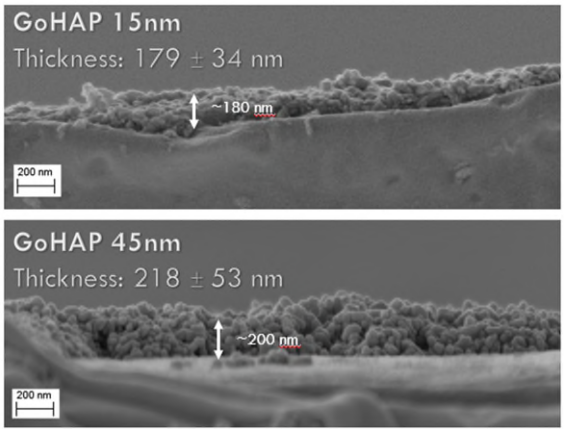

Fig. 1: Layer thickness investigations - SEM image analysis.

DISCUSSION & CONCLUSIONS: In case of hydroxyapatite nanoparticles, the particle size affects the efficiency of the sonocoating deposition process, as well as the properties of the hydroxyapatite layers. For this reason, special attention should be paid to the nanoparticle size in the design of new materials for bone tissue regeneration.

ACKNOWLEDGEMENTS: The following research was funded by the Centre for Preclinical Research and Technology -CePT II from the Operational Program of the Masovian Voivodship (RPMA.01.01.00-14-8476/17-01).

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Design of Experiments (DoE) – an Effective Approach in the Synthesis and Analysis of Doped Calcium Phosphates

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INTRODUCTION: Calcium phosphate-based bioceramics have been extensively used as implant materials due to their chemical composition and high biocompatibility with natural bones.

There are numerous studies available on synthesis methodologies for producing calcium phosphates. In recent years, an active research has been carried out on calcium phosphates doped with various ions. The synthesis of calcium phosphate can be conducted under a very wide range of processing conditions to produce calcium phosphate powders of desired characteristics (size, crystallinity, Ca/P ratio, concentration of doped ions). It is known that the final product can be affected by many factors, like pH, temperature, mixing speed, ageing time, reagent concentration, drying conditions, etc. Most studies are carried out by studying one-variable-at-a-time (OVAT). The main disadvantages of this method: many experiments are required and it do not consider interactions between variables.

The design of experiment (DoE) approach is a powerful tool to measure and understand the effects of several specific independent variables, to estimate interactions between the variables on the experimental response with a smaller number of experiments. It is quick, as well as cost-effective. The crucial role of the DoE method – it allows predicting system properties in non-performed experiments.

The aim of this work is to study the DoE approach using different DoE types, examine the effect of the most relevant factors of the synthesis, and optimize the number of experimental variables and experiments.

METHODS: The most common software that provide a set of tools for multivariate design and analysis of experiments, were studied: R, R-CAT, MATLAB, Minitab. The Pareto charts and main effect plots were used to determine the relationship between the input variables and the system responses.

The advantages and disadvantages of several DoE techniques (Full factorial, Box-Behnken Design, Taguchi, Central Composite) and their suitability for the synthesis of doped calcium phosphates were analysed in the literature.

DISCUSSION & CONCLUSIONS: Laboratory scientists and industries need a method of conducting experiments that optimise variables, number of experiments and increase credibility and quality of products. The correct selection of DoE technique depends on the problem to be investigated and the aim of the experiment. Meaningful application of the DoE experiment planning strategy to study biomaterial syntheses reduces usage of human resources and benefits from introducing the 3R principle: Replace, Reduce, Refine in a University laboratory.

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Synthesis of Hydroxyapatite Granules with Antibacterial Properties

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INTRODUCTION: Hydroxyapatite (HA) is a well-known material for its many beneficial properties, e.g., osteoconductivity, non-toxicity and biocompatibility with human bone tissue. Therefore, it is frequently used as a ceramic biomaterial in dentistry and orthopedic surgery [1]. It should be noted that the last decades have brought a huge development of implantology and thus an increased number of surgical procedures [2]. Unfortunately, they carry a risk of bone infections. The best way to resolve this problem seems to be a direct implementation of antibiotics and/or other antibacterial compounds into the bone tissue. Hydroxyapatite, as a material with a great ability of adsorption of many compounds, may be used as bone drug delivery system [1]. Application of HA porous granules or blocks would help in sustained release of antibacterial substances. Our work is focused on the preparation of different HA granules doped with silver or gallium ions and used as gentamicin delivery system.

METHODS: HA powders doped with gallium or silver ions were synthesized using wet method, described in the previous work [3]. Afterwards they were used for preparation of 3 types of granules for both ions (six different materials in total): microgranules (Ø 0.2-1 mm) synthesized using camphene emulsion, granules (Ø 2-5 mm) synthesized using alginate sodium solution and the same type of granules (Ø 2-5 mm) made of microgranules and HA powder in 1:1 ratio. Obtained granules were also enriched in gentamicin and lastly, they were coated with biodegradable polymer polycaprolactone (PCL), in order to improve extended release time of gentamicin and antibacterial ions. Several physicochemical and biological studies were used to investigate properties of prepared granules, e.g., scanning electron microscopy (SEM), mercury intrusion porosimetry, for porosity study, and MTT assay for cytotoxicity study.

RESULTS: The physicochemical studies FT-IR and PXRD allowed to confirm the identity of the synthesized materials and successful incorporation of gentamicin and PCL. Due to the mercury intrusion porosimetry, it was possible to determine the porosity of the obtained granules and to compare various methods of granules fabrication. Cytotoxicity of the synthesized granules and samples from *in vitro* release study was determined thanks to MTT assay. SEM representative images of different granules are presented in *Fig. 1*.

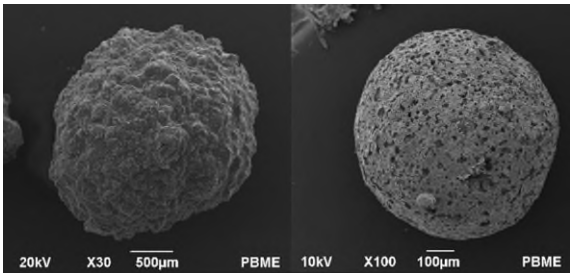

Fig. 1: SEM representative images of synthesized granules (left) and microgranules (right).

DISCUSSION & CONCLUSIONS: In this project various types of HA granules with antibacterial properties were successfully obtained. In future we are planning the preparation of granules with different calcium phosphates, modified with various antibacterial ions (e.g. HA and tricalcium phosphate) in order to achieve an efficient antibacterial activity.

ACKNOWLEDGEMENTS: This work was supported by the research programme (UMO-2016/22/E/ST5/00564) of the National Science Center, Poland**.**

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Foamed Calcium Phosphate Cements Obtained by the Surfactant-Assisted Process

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INTRODUCTION: Foamed calcium phosphate cements (fCPCs), due to the simple preparation and lack of heat treatment, are gaining in popularity among highly porous biomaterials for bone tissue engineering. Many approaches to the preparation of highly porous cements have been applied, such as the addition of water-soluble and biocompatible crystals (sugars, mineral salts) or the use of effervescent additives [1,2]. The first strategy tends to reduce the binding capacity of cements, while the second one leads to the risk of a sudden gas release after immersion in body fluids. A third, less often used strategy is use of surfactants as the foaming agents which seems to be an interesting alternative.
In this study, to obtain fCPCs, three different nonionic surfactants were applied as foaming agents. The physicochemical properties of the prepared materials have been evaluated.

METHODS: The solid phase of obtained cements was α-tricalcium phosphate (α-TCP) synthesized by the wet chemical method. The liquid phase of the cements was 2 wt.% Na_2HPO_4 aqueous solutions with 10 $g \cdot L^{-1}$ of selected surfactant. The surfactants used in the study were: Tween 20, Tween 80 and Tetronic 90R4 (Sigma Aldrich). The liquid to powder ratio (L/P) was 0.7 $g \cdot g^{-1}$. Cements fTW20, fTW80 and f90R4 were obtained by mixing the powder with a previously foamed surfactant solution. The control samples did not contain any surfactant (fCTRL). The prepared cements were used for further research. The phase composition of the obtained materials, after 7 days, was studied with the use of a diffractometer D2 Phaser (Bruker). Porosity was measured by mercury intrusion porosimeter Autopore IV 9500 (Micrometrics). fCPCs microstructures were evaluated using scanning electron microscope Nova Nano SEM (FEI Europe Company) and confocal laser microscope (Olympus LEXT OLS 4000).

RESULTS: Studies have shown that the phase composition of cements did not differ significantly from each other. Two crystalline phases were present: α-TCP and hydroxyapatite (HAp). The porosity of fCTRL, fTW20, fTW80 and f90R4 equaled 54.8 ± 0.7, 74.8 ± 1.7, 78.8 ± 2.1, 57.5 ± 1.0, respectively. The surface of the foamed cements is shown in Fig. 1.

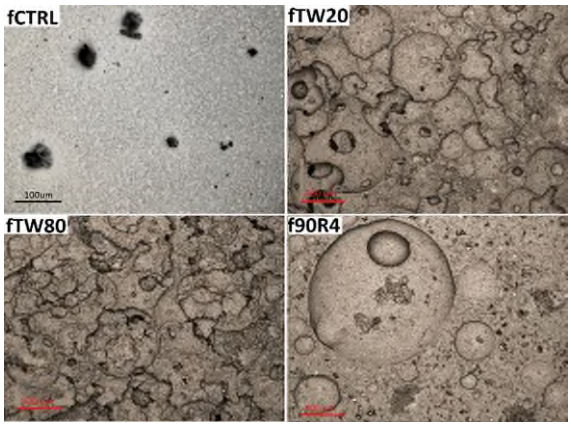

Fig. 1: Surfaces of the obtained fCPCs. Images obtained using confocal microscopy

DISCUSSION & CONCLUSIONS: The addition of the surfactants did not affect the phase composition of the final materials 7 days after setting. This is consistent with previous studies [3]. The porosity was much higher for materials fTW20 and fTW80, than in the case of fCTRL and f90R4. However, microscopic observations revealed a highly porous surface for all cements obtained by the surfactant-assisted process. Lower open porosity measured for f90R4, may resulted from the presence of numerous closed pores. Obtained fCPCs need further research.

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The Influence of β-Tricalcium Phosphate Powder Preparation on Manufacturing of Bioceramic Scaffolds via the Foam Replica Method

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INTRODUCTION: Recently, β-tricalcium phosphate (β-TCP) scaffolds have attracted much attention as artificial bone substitutes, due to the excellent biocompatibility and osteoconductivity [1]. Highly porous β-TCP based scaffolds can be used for a wide range of medical applications. The foam replica method, similar to slip casting, can produce complex shaped, porous scaffolds. The key steps in this method are: preparation of ceramic powder, coating step and thermal treatment. In this research the impact of preparation method on β-TCP powder properties, was investigated. In order to optimize the properties of β-TCP powder and its behavior during preparation of the ceramic slurry, three methods of manufacturing of the initial powder were used.

METHODS: β-TCP powder was synthesized by the wet chemical method, according to patent PL 190486. H_3PO_4 solution was dropped into $Ca(OH)_2$ suspension (Ca/P=1.5) and the pH was kept between 5-7. The precipitate was aged, centrifuged and dried. Initial powder was prepared in three different ways: a) β-TCP1 was grounded in ball mill, calcinated at 900 °C and grounded in an attritor mill, b) β-TCP2 was grounded in ball mill, c) β-TCP3 was calcinated at 900 °C and grounded in an attritor mill. All powders were sieved to grain size below 63 μm. β-TCP powders were characterized by different techniques such as X-ray diffraction, transmission electron microscopy, scanning electron microscopy and Brunauer–Emmett–Teller (BET) method. The foam replica method was used to obtain bioceramic scaffolds.

RESULTS: XRD analysis revealed that all powders consisted of β-TCP crystalline phase. Non calcinated powder was less crystalline than thermally treated powders. Calculated specific surface area for βTCP1 and βTCP3 was: 5.29 ± 0.02m²/g and 4.12 ± 0.08m²/g respectively. The specific surface area of βTCP2 powder was 71.76 ± 0.13 m²/g. Thermally treated powders also had a different morphology compared to the non-calcined βTCP2 powder. In the case of βTCP2 a clear tendency to combine the individual grains into large agglomerates was observed in comparison to βTCP1 and βTCP3 powders (Fig. 1). All initial β-TCP powders were used to prepare the bioceramic scaffolds via the replica method (Fig. 2).

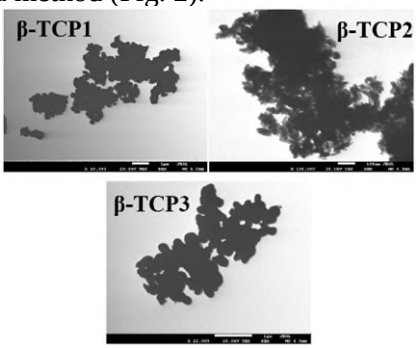

Fig. 1: TEM images of β-TCP powder morphology.

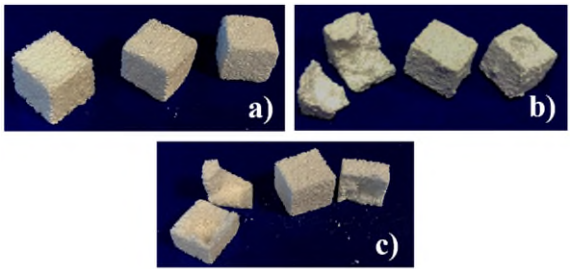

Fig. 2: Bioceramic scaffolds on the basis of: a) β-TCP1, b) β-TCP2, c) β-TCP3.

DISCUSSION & CONCLUSIONS: The obtained results indicate that the thermal treatment at 900 °C increased crystallinity and grain size of βTCP powder, thus reducing its specific surface area. It is known that presence of large agglomerates in the powder may have a negative impact on rheological properties of the slurry [2]. It has been demonstrated that β-TCP1 powder was the most suitable for constructing 3D scaffolds via foam replication method.

ACKNOWLEDGEMENTS: Research funded by National Centre for Research and Development (NCBiR), Poland project no. TECHMATSTRATEG2/407507/1/NCBR/2019

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Synthesis and Characterization of Low Temperature Alpha-TCP Cements

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INTRODUCTION: α-tricalcium phosphate (α-TCP) is a bioactive, biocompatible and osteoconductive material that reacts rapidly and hardens in the presence of aqueous salt solutions, to form calcium phosphate bone cement (CPC). During this reaction, α-TCP transitions into a calcium-deficient hydroxyapatite (CDHA) phase, with chemical composition and morphology similar to that of a natural bone mineral. Cement based on α-TCP is widely used to treat bone defects and regenerate damaged bone tissue [1], [2]. Within the current research, the impact of low-temperature synthesised α-TCP powder properties, like particle size, specific surface area and density, on the resulting CPC was evaluated in terms of setting time and porosity. CPC setting reaction kinetics and pH change in deionised water and simulated body fluid (SBF) of CPC samples, depending on time, was studied and evaluated.

METHODS: α-TCP was prepared using low-temperature synthesis at 630 - 850°C. The phase composition of prepared powder was analysed using XRD, the specific surface area of α-TCP was determined using the BET method (ISO 9277:2010). The value of specific surface area found was used to calculate the average α-TCP particle size. α-TCP powder density was measured using a gas pycnometer method. CPC samples were prepared by mixing the α-TCP with liquid phase (a mixture of Na_2HPO_4 and NaH_2PO_4 solutions), and setting time, as well as CPC setting reaction kinetics, porosity and pH change in deionised water and SBF (ISO 23317), depending on time of resulting CPCs, were determined.

RESULTS: It was determined that α-TCP particles specific surface area is 8.6 m^2/g and average particle size (calculated from BET results) equals to 245 nm. Measured α-TCP particle density is 2.871 ± 0.011 g/cm^3. For the cement obtained from the α-TCP, setting time is 85 ± 1 min and porosity is 80.92 ± 0.21 %. To study changes in the pH value of the set and dried cement in the surrounding media (deionised water or SBF), cement samples were placed in 50 mL of test solution in an incubator-shaker (50 rpm, 37°C) and the changes of the surrounding environment were monitored (using pH-meter). The results are presented in Figure 1.

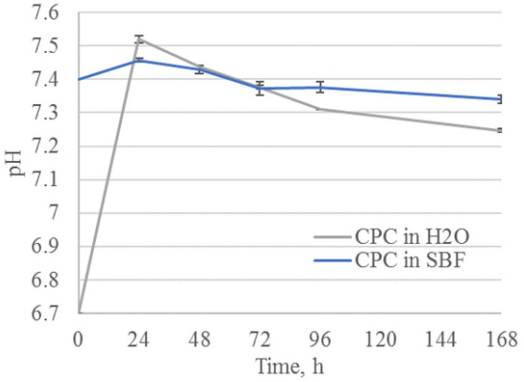

Fig. 1: pH as a function of time.

DISCUSSION & CONCLUSIONS: During the research, it was established that due to the low solid to liquid phase ratio, the cement porosity is relatively high. The CPC pH monitoring test showed that the surrounding media pH value did not change dramatically, so the material is promising to be used as an implant material within the body.

ACKNOWLEDGEMENTS: The authors acknowledge financial support from the European Union's Horizon 2020 research and innovation programme project RISEus2 under the grant agreement No 952347.

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Controlled Synthesis of Amorphous Calcium Phosphate

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INTRODUCTION: Amorphous calcium phosphate (ACP) serves as a precursor phase in bone biomineralization. The ACP has promising forthcoming application as the third-generation biomaterial in bone regeneration. It has excellent properties such as high solubility, bioactivity, biodegradability and osteoconductivity, that are better compared to other calcium phosphates[1]. ACP usually has Ca/P molar ratio of 1.5. Aim of the study was to obtain ACP with higher Ca/P molar ratio than 1.5 and to study the influence of Ca/P molar ratio and pH on properties of obtained ACP.

METHODS: ACP samples with different Ca/P molar ratios (1.5, 1.58 and 1.67) of raw materials were synthesised via chemical re-precipitation method[2]. Calcium oxide and orthophosphoric acid were used as raw materials, that were dissolved in hydrochloric acid to obtain calcium and phosphate ion rich solution. Precipitation of ACP was done by rapidly adding a strong base to this solution. Synthesis was done at different end pH values (9.5 and 11.5). The ACP precipitates were centrifuged, washed with deionised water, and lyophilized. In order to determine Ca/P molar ratio, ACP was calcinated at 1100 °C and respective phase compositions were detected using x-ray diffraction analysis (XRD) and Rietveld analysis. Phase stability at the room temperature of dried samples was studied with XRD. Chemical groups were characterized with Fourier transform infrared spectroscopy (FT-IR). Specific surface area (SSA) was determined by BET method.

RESULTS: ACP was successfully prepared at all different synthesis end pH values and Ca/P molar ratios of raw materials. Amorphous nature of the samples was observed on XRD patterns and FT-IR spectra (not shown). XRD analysis of calcinated ACP, revealed formation of β-tricalcium phosphate (β-TCP) or biphasic mixture of β-TCP/hydroxyapatite. The Ca/P molar ratio of the product was in range from 1.50 to 1.55. SSA values of the ACP samples are shown in Table 1.

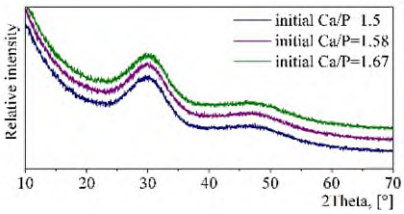

Fig. 1: XRD patterns of the ACP with different Ca/P molar ratio of raw materials (synthesis end pH=9.5).

Table 1. SSA of the ACP synthesized at different end pH values and with different Ca/P molar ratios of raw materials, m^2/g.

	1.5	**1.58**	**1.67**
9.5	103 ± 1	111 ± 3	123 ± 14
11.5	110 ± 21	117 ± 30	139 ± 6

DISCUSSION & CONCLUSIONS: Certain correlation between SSA of the ACP and synthesis end pH was not observed. This is due to the fact that other parameters of synthesis could have influenced the properties of ACP. Ca/P molar ratio of the obtained ACP was lower (1.50-1.57) than the initial one (1.5-1.67). However higher synthesis end pH (11.5) lead to the increased Ca/P molar ratio in the product. The obtained ACPs with different Ca/P molar ratios are structurally stable in dried state, up to 3 months in the room temperature. Further investigation on ACP properties and different parameters should be done. It will open opportunity to obtain products with better features, like higher SSA.

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Zinc-Doped Sol-gel Coating for Bone Tissue Regeneration: Protein Adsorption and Cellular Responses

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INTRODUCTION: Zinc is mainly stored in the calcified matrix of bone tissue, playing in bone metabolism and remodelling (1). In the last few years, the intrinsic physiological relevance of this element has attracted the interest of researchers in the biomaterial field, considering it the 'calcium of the twenty-first century' (2). Upon implantation, a material interacts with blood, leading to the adherence of many proteins that adhere to its surface (3). This phenomenon will influence how the consequent regenerative process is carried out. This study aimed to obtain a new organic-inorganic sol-gel release vehicle, doped with Zn, to be applied as coatings onto titanium. The physicochemical characterization was carried out and the effects of Zn^{2+} on protein adsorption were studied using proteomic analysis. The *in vitro* characterization was done with MC3T3-E1 osteoblasts and RAW 264.7 macrophages. These results will allow to better understand the role of this element in bone tissue regeneration.

METHODS: The materials were synthesized through the sol-gel route using organically modified alkoxysilanes, methyltrimethoxysilane (M), and tetraethyl orthosilicate (T), as precursors, and increasing amounts of $ZnCl_2$. Wettability, roughness, SEM, FT-IR, NMR, hydrolytic degradation, and Zn^{2+} release kinetics were studied to physicochemically characterize the materials. For *in vitro* assays, cytotoxicity, ALP activity, gene expression (ALP, RUNX2, iNOS, RANKL, RANK, TNF-α, IL-1β, TGF-β, and IL-4), and cytokine secretion by ELISA (TNF-α) were measured in MC3T3-E1 osteoblasts and RAW264.7 macrophages. For protein adsorption, the materials were incubated with human serum for 3 h and the analysis was employed using Orbitrap LC-MS.

RESULTS: The coatings obtained were uniform without $ZnCl_2$ precipitates. The incorporation of the salt did not affect the degree of crosslinking of the sol-gel network or did change the surface roughness. A controlled Zn^{2+} release was obtained and did not reveal cytotoxic effects on the cells. On osteoblasts, the materials increased the gene expression of TGF-β, RUNX2, and ALP. On macrophages, there was an increment on the expression of TNF-α, IL-1β, TGF-β and IL-4, and a reduction of TNF-α secretion. Proteomic analysis revealed that Zn-doped materials adsorbed more proteins related to inflammation, coagulation, and tissue regeneration processes.

DISCUSSION & CONCLUSIONS: A sol-gel materials doped with Zn was successfully obtained and applied as coatings onto Ti discs, allowing controlled release kinetics of this ion. The presence of Zn leads to an increase of ALP, TGF-β, and RUNX2 gene expression in osteoblasts showing the osteogenic potential of these materials. The increment in TNF-α, IL-1β, TGF-β, and IL-4 shows that the inflammatory responses are dependent on the amount of Zn incorporated into the material. The proteomic analysis revealed that the addition of Zn increased the adsorption of proteins regulating the immune reaction, such as VTNC, IC1, FHR1, CLUS, and KAIN. On the other hand, Zn-doped coatings showed less affinity to CATB, which is associated with chronic inflammation and delayed healing and increased adherence of proteins with osteogenic properties, such as VTNC.

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Synthesis and Characterization of Nanohydroxyapatite for Biomedical Application

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INTRODUCTION: Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is a major component and an essential ingredient of hard tissue such as bones and teeth. Hydroxyapatite (HA) shows good biocompatibility and it is an excellent candidate for bone repair. Nanosized biomaterials promote osteoblast adhesion, proliferation, osseointegration and the deposition of calcium-containing minerals on the surface of these materials. HA is often the material of choice for biomedical applications, e.g., bone implants, scaffold layers, drug delivery agent, dental materials. There are many ways to obtain synthetic hydroxyapatite.

METHODS: Nanohydroxyapatite was synthesized by hydrothermal synthesis using microwave reactor MSS (Microwave Solvothermal Synthesis). The starting materials include only two pharmaceutical grade substrates: calcium hydroxide $Ca(OH)_2$ and orthophosphoric acid H_3PO_4, as substrates to obtain ceramic nanoparticles.

RESULTS: Received nanoparticles have wide variety of average grain size, in range of 10 - 42 nm. Thanks to the different grain size and crystallinity HA can be used in many applications depending on desired resorption time of hydroxyapatite. Production is waste-free because the water is the single by-product. Nanopowder has been characterized by several methods such as: X-ray diffraction (phase purity), SEM (morphology), BET (Specific Surface Area) and Helium Pycnometry (Skeleton Density).

DISCUSSION & CONCLUSIONS: In conclusion, thanks to the hydrothermal microwave synthesis we can easily and precisely control properties of nanoparticles. Different hydroxyapatite powders were obtained by controlling synthesis conditions, such as time, pressure and temperature. Obtained nanohydroxyapatite is similar with human hard tissue.

ACKNOWLEDGEMENTS: The following research was funded by the Centre for Preclinical Research and Technology- CePT II from the Operational Program of the Masovian Voivodship (RPMA.01.01.00-14-8476/17-01).

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Antibacterial Properties of Sharkskin Mimicked Polymeric Membranes

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INTRODUCTION: Recent advancements of science and technology resulted in new discoveries, which show that the nature has provided every means for wellbeing of humans. One of these means is antifouling micro-structures such as skin of sharks.[1,2] Investigations among marine animals showed that sharks stay totally free of any microorganism attachment, hence completely immune from microbial infections. Further research revealed that sharkskin reduces drag force due to its surface microstructure, which led to some speculations among scientists that the unique morphology might be responsible for prevention of microorganism attachment. Herein we decided to investigate the biological properties of sharkskin morphology by mimicking its surface micro-topography using polymeric membranes.

In this regard, we have fabricated bio-mimicked structures using soft lithography and solvent casting methods. We aimed to investigate physicochemical, mechanical, and antibacterial properties of sharkskin mimicked micro-patterned polymeric membranes. Among commonly used polymers, Chitosan (CH) was chosen due to its potential for mimicking micro and nano-structures along with its excellent biological properties. Caffeic Acid Phenethyl Ester (C) and Ampicillin Sodium Salt (A) were used as model antibacterial agents in form of composite.[3] Obtained results of this study so far revealed that sharkskin polymeric membrane have indeed potential for reducing bacterial biofilm formation.

METHODS: Low molecular weight CH was prepared at 2.5% w/v concentration in 3% v/v Lactic Acid solution. Solvent casting technique was used to fabricate sharkskin-mimicked membranes. So far, our results have shown significant differences among our groups. Additionally, in vitro experiments were conducted in order to evaluate bactericidal properties using Gram-positive *Staphylococcus aureus* bacterial strain as model bacteria.

RESULTS: Based on obtained results, we conclude that Sharkskin surface topography is replicable, with a very high precision and sharkskin micropatterns, change phisicochemical properties of chitosan based membranes significantly. Moreover, bacterial attachment on smooth membranes occurs in a multilayered form which is different than that of sharkskin micropatterned membranes appearing in form of a mono layer in most groups.

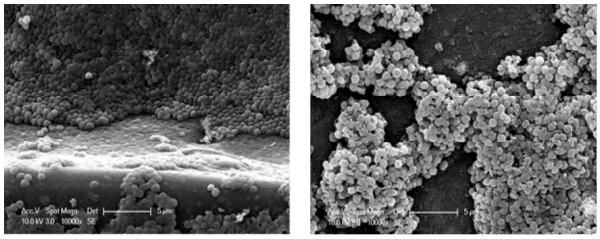

Fig. 1: SEM images of S. aureus adhesion on CH based membranes. Sharkskin mimicked (left) and Smooth (right).

DISCUSSION & CONCLUSIONS: It can be concluded that sharkskin polymeric membrane has remarkable potential for reducing bacterial biofilm formation. Hence, they have numerous applications in infection control.

ACKNOWLEDGEMENTS: Authors would like to thank TUBITAK project No. 117R055 for providing the financial support to this project.

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Modeling Myonuclear Domains using Nonviral Gene Delivery

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INTRODUCTION: Skeletal muscle cells contain many nuclei in a shared cytoplasm, but little is known about how their gene expression is coordinated. The transport behaviour of nuclear proteins, such as transcription factors, is poorly understood. Here, we used polymer-mediated gene delivery to help identify factors that control the distribution of nuclear proteins in skeletal muscle cells.

METHODS: Using polymer nanoparticles made from a bioreducible linear poly (amido amine), we transfected primary mouse myoblasts with inducible constructs encoding nuclear proteins. Transfection levels were tuned to yield expression from a single nucleus, in each cell, following differentiation into multinucleated myotubes. We used multimeric variants of red fluorescent protein (RFP), bearing a nuclear localization signal (NLS), to study the role of molecular weight on distribution. We treated myotubes with drugs to simulate muscle atrophy (dexamethasone), hypertrophy (capsaicin), and to diminish nuclear import (importazole), to probe how each affects the transport of nuclear proteins. We then generated a computational model to help understand the relative contributions of various protein and cell characteristics on nuclear protein distribution. Finally, we validated these model system findings by quantifying distributions of actual muscle-related transcription factors.

RESULTS: Quantification of RFP-NLS distributions revealed an inverse relationship between molecular weight and propagation. Monomeric mCherry (29 kDa) showed the broadest distribution, followed by dimeric tdTomato (55 kDa), while tetrameric DsRed (110 kDa) was the most restricted. Inhibition of active nuclear import with the drug importazole extended the distributions of the larger two RFPs, while the smallest was unaffected. Myotube thickening in the presence of capsaicin extended the transport of larger RFPs, while thinner cells cultured with dexamethasone exhibited diminished propagation of smaller RFPs. Using our empirical measurements along with values from the literature, we developed a simulation of nuclear protein transport that accounts for protein molecular weight, active and passive nuclear import rates, cell width, and protein half-life. Finally, we validated the results from our RFP-NLS model system and the computational simulations using representative small (Six1, 36 kDa) and large (ARNT-CFP, 118 kDa) transcription factors known to be expressed in skeletal muscle.

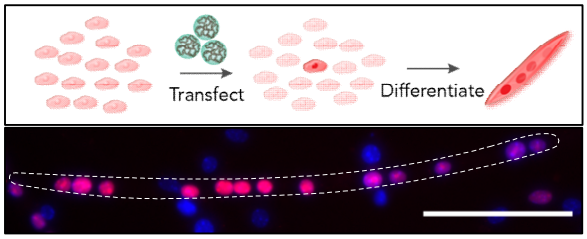

Fig. 1: Transfection of myoblasts enables the study of nuclear protein transport in myotubes after differentiation. RFP-NLS propagates from the transfected nucleus. Scale bar is 100 μm.

DISCUSSION & CONCLUSIONS: Understanding nuclear protein behaviour is critical to understand how gene expression is coordinated in skeletal muscle and to design optimized gene therapies. Here, we used a polymer nanoparticle gene delivery method to identify a set of parameters that govern nuclear protein transport in skeletal muscle cells.

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Efficacy of Various Antimicrobial Agents Loaded in Resorbable Porous Bone Cement Useful in the Treatment of Osteomyelitis

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INTRODUCTION: All surgeries carry the risk of complications due to bacterial infections. Infected hip or knee revisions are generally accompanied with local and systemic antibiotic therapy. Generally, in two-stage revisions, non-degradable poly(methyl methacrylate) spacers loaded with antibiotics (ATBs) are used, however, reports show that only 3 - 5 % of ATBs are released in vivo [1]. To overcome this issue, we have developed a biodegradable, "self-setting", thixotropic and osteoconductive polymer/calcium phosphate bone cement [2]. In the presented work, we loaded the cement with either vancomycin or biogenic selenium nanoparticles (SeNPs) to compare their release and antimicrobial efficiency in vitro.

METHODS: The cement consists of alpha tricalcium phosphate powder mixed with a thermosensitive biodegradable copolymer according to [2]. The cement was loaded either with vancomycin (1-10 hm%) or SeNPS (0.1-100 ppm) during mixing to study their release kinetics at physiological conditions by HPLC or ICP-OES, respectively. Antibacterial properties were evaluated by disk diffusion method against Staphylococcus aureus (SA), methicillin-resistant SA (MRSA), and E-Coli. The cytocompatibility of bone cement was evaluated in vitro using human mesenchymal stem cells and the biocompatibility and osteointegration was studied on Vistar rats by filling an iatrogenic bone defect in femoral bone.

RESULTS: The in vivo study shows that the cement was set at physiological conditions with no signals of chronic inflammation. In contrast, slower cell growth was observed in vitro compared to plastic. Both vancomycin and SeNPs loaded cements were very effective against the tested bacteria strains. The antimicrobial capacity was associated with the resorption of the cement, generating pores on the surface that accelerate the release of antimicrobial agents.

DISCUSSION & CONCLUSIONS: The new bone cement is an excellent excipient for the controlled release of the two antibacterial agents studied. SeNPs may be as effective as vancomycin for preventing or healing musculoskeletal infections. Therefore, SeNPs may be used to avoid ATBs resistance. Furthermore, the osteoconductivity of the cement will support the repair of the bone defect. The osteomyelitis treatment by novel antibacterial cement on an infected animal model is in progress.

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Octacalcium Phosphate: A Contemporary Drug Delivery System for Local Biologically Active Substances – A Review

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INTRODUCTION: As it has been extensively researched, calcium phosphates (CaPs) have shown an unparalleled result when it comes to inducement of bone formation. Being one of the members, with the capacity to convert to hydroxyapatite (HAp) readily and irreversibly[1], octacalcium phosphate (OCP) has been more frequently in the scientific foreground in recent years. OCP, due to the particular arrangement of the structure and a fairly empty water layer, has a higher affinity towards the incorporation of biologically active molecules than other calcium phosphates[2]. Enlistment of OCP for drug delivery purposes is still a novel concept and a lot more *in vitro* and *in vivo* tests are required.

OCP STRUCTURE: Alternately packed structure, made of apatite layer and water layer, is what makes OCP, to some extent, comparable to HAp and it is the reason behind why OCP is presumed to be the precursor of biologically formed apatite. Positions of Ca^{2+}, HPO_4^{2-} and PO_4^{3-} and their certain connections, coupled with further bonding interactions enable the possibility of ion or drug incorporation among them[2].

OCP as a DDS: OCP has been proposed as a promising direction in incorporating different set of active substances, ranging from ions such as magnesium (Mg^{2+}), zinc (Zn^{2+}), carbonate (CO_3^{2-}), strontium (Sr^{2+}), iron (Fe^{3+}), succinate and fluoride (F^-) ions, however, at the moment, the spectra of researched drugs is still not vast. Synergistic outcomes of intercalated OCP are considerably influential and have a wide array of beneficial effects on improvement of osteoblast differentiation, as well as on inhibition of osteoclast proliferation[3]. Providing the careful control of concentration of the incorporated substance, the structure of the OCP itself can be ameliorated.

DISCUSSION & CONCLUSIONS: Conventionally used OCP has displayed promising properties as one of the biocompatible inorganic biomaterials, with the potential of different set of incorporations of foreign ions and drugs. Nevertheless, accomplishing satisfying loading capacity and a controllable drug-release, still remains a significant challenge. Until now, the biggest research done on this subject was in relation to bisphosphonates (BPs), but as we progress in this open-minded field, increase of the examined drugs is imminent and the demonstrated high potential of OCP is thought to be the future of personalized drug delivery systems.

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Antibacterial Properties of Platelet-Rich Fibrin Matrices Saturated With Vancomycin

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INTRODUCTION: Platelet-rich fibrin (PRF) is an autologous material derived from a patient blood with a high concentration of platelets and leukocytes. It contains high concentrations of growth factors and biologically active substances. Platelet-rich fibrin matrices, together with delivery system of antibiotic drug, may enhance tissue regeneration and decrease risk of surgical site infection. The aim of the study was to evaluate the antibacterial effect of autologous platelet-rich fibrin matrices and its combination with vancomycin.

METHODS: Bacterial suspensions were prepared from 1 mL trypticase soy broth (TSB) (Oxoid, UK) and 1 mL *Staphylococcus aureus* (ATCC 25923) reference culture with an optic density of 0.5, according to the McFarland standard. Fibrin/MK_Blank (Blank micro) and Fibrin Blank Blank (Blank O) and Fibrin/MK_Vancomycin (VANC) samples were placed in bacterial suspension, and incubated for 24 hours in 37°C. After 24-hour incubation, 0.1 mL of bacterial suspension was inoculated on trypticase soy agar (TSA) (Oxoid, UK). A new bacterial suspension was prepared at the same time, and with the help of sterile pincers, samples of the studied group were transferred into a new TSB and bacterial culture suspension for the next 24 hours. These actions were repeated every 24 hours, until no trace of antibacterial characteristics was found in the studied sample groups for two days in a row, and TSA colony number was equal to the colony forming units (CFU) in the control group on TSA.

RESULTS: More significant antibacterial effect was observed in VANC samples, after first 24 hours incubation, because CFU count on TSA plate reached 255, what is significantly less compared to control samples. After 48 hours incubation antibacterial activity in VANC samples was not observed. Blank micro and Blank O did not show any antibacterial activity.

DISCUSSION & CONCLUSIONS: Maximal antibacterial activity of VANC samples was for 24 h. Samples did not show antibacterial activity after 48 hours incubation. Further release of Vancomycin may be hindered by the fact that TSB may build a shell around Vancomycin.

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Antibiofilm Properties of Nanostructured Silver-Based Coatings Made by Flame Spray Pyrolysis

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INTRODUCTION: Implant infections are responsible for a high number of revision surgeries each year. The biofilm formation and increasing antibiotic resistance of common bacteria causing them, leads to an urgent need of alternative antibacterial agents. In this study, the antibiofilm behaviour of a coating consisting of silver (Ag) nanoparticles (NPs) and a support ceramic (S) is studied. Different silver compositions and sizes are tested against *S. aureus* biofilm formation and compared to the coating without silver nanoparticles.

METHODS: Flame spray pyrolysis (FSP) is used to synthesize a multicomponent nanosized coating consisting of Ag NPs and ceramic, and to deposit it on silicon or medical grade titanium (implant). Four different mass fractions of Ag (stated as xAg) are prepared: x = 20, 30, 40 and 50 wt%. As a positive control (biofilm growth), only the ceramic is coated on the substrates (0Ag).

The nanostructure of the coatings is evaluated using X-ray diffraction (XRD) and scanning electron microscopy (SEM).

S. aureus is chosen as a relevant and common representative of the infection causing microbes, related to orthopaedic implants. An overnight culture is incubated together with the chips for 24 hours in tryptic soy broth (TSB). After that, the chips are rinsed in phosphate buffered saline (PBS) to remove the planktonic bacteria and the biofilm is evaluated using the following methods: 1) live/dead staining followed by fluorescence microscopy, 2) live/dead staining followed by fluorescent activated cell sorting (FACS), 3) recovery of the biofilm and plating dilutions for colony forming units (CFU)-counts on agar.

RESULTS: It was possible to completely inhibit the biofilm growth with some compositions of the NPs coatings. On the 50wt% chip there were 0 colonies found after 24 hours incubation. This was also confirmed by the live/dead images (Fig. 1), where a biofilm grows on the positive control (top left), but not on the 50wt% Ag chip (bottom left).

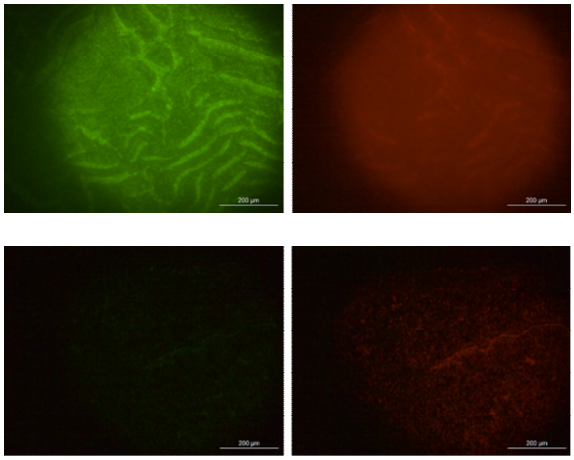

Fig. 1: Fluorescence microscopy images of positive control (top) and 50 wt% of Ag (bottom); the top left image shows green labelled living bacteria cells forming a biofilm; in contrast, the bottom left image shows almost no living cells on the Ag containing chip; the two images on the right show red labelled dead bacteria.

DISCUSSION & CONCLUSIONS: With this study we present a nanostructured implant coating, which has high antibiofilm properties and is reproducible / scalable at the same time. It was possible to completely inhibit biofilm formation of *S. aureus*, on a coated silicon wafer after 24 hours of incubation in TSB.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (ERC Grant agreement n˚ 758705). Funding from the Karolinska Institutet Board of Research, the Swedish Research Council (2016-03471), the Jeansson Foundations (JS2016-0029) and the Åke Wiberg Foundation (M16-0098) is kindly acknowledged.

"Smart" Biodegradable Hydrogel as Carrier for Pro-healing Proteins Applicable in Regenerative Medicine: Synthesis, Characterization and Controlled Release

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INTRODUCTION: There is a strong emphasis on controlled drug delivery systems at this time. In this work, stable Fibroblast growth factor 2 (FGF2-stab), developed by the Enantis company (Brno, CZ), was used as a protein-based drug. FGF2-stab stimulates the growth and the development of new vessels (angiogenesis), leading to improved wound healing, tissue development and contributes to the pathogenesis of several diseases (cancer or atherosclerosis)[1]. Amphiphilic PLGA-PEG-PLGA triblock copolymer was used as the biodegradable water-soluble copolymer, that undergoes to a gel at physiological temperature. Along with increasing temperature in an aqueous environment, the PLGA-PEG-PLGA copolymer forms micelles having the hydrophobic core (PLGA) and the hydrophilic shell surface (PEG)[2]. Nowadays, drug carriers based on PLGA-PEG-PLGA copolymers are already commercially available under the names ReGel® and OncoGel® loaded with insulin or paclitaxel, respectively.

METHODS: Different concentrations of PLGA-PEG-PLGA hydrogel, synthesized in our lab, were used as matrices. FGF2-stab was added to the matrix at room temperature, which is interesting due to its longer stability (20 days at 37 °C). Subsequently, the entire system was heated to the temperature of the human body, thereby causing gelation of the hydrogel. The samples were stored in a membrane-containing insert, to avoid affecting the amount of released protein by the degrading polymer over time. Finally, protein release was monitored by UV-VIS spectrophotometer in the presence of Bradford reagent and SDS-page electrophoresis.

RESULTS: Model proteins, lysozyme and albumin, were firstly used to set-up the method instead of expensive FGF2-stab. For these proteins, two-step release from the PLGA-PEG-PLGA hydrogel was observed. The two-step release was due to the polarity, when lysozyme and albumin showed both polar and surface non-polar character. Since PLGA-PEG-PLGA is an amphiphilic hydrogel and forms micelles, a portion of the proteins was bound to the micelle surface and part of the proteins into their nuclei. Subsequently, FGF2-stab was measured using inserts. Contrary to albumin with lysozyme, one-step well-controlled first-order release was observed. Release of FGF2-stab. was established only by diffusion and the rate depends on the concentration of the prepared hydrogel.

DISCUSSION & CONCLUSIONS: It has been found that a hydrogel matrix based on PLGA-PEG-PLGA copolymer is suitable as a matrix for pro-healing proteins, thanks to the ability to adjust the gel concentration according to the user's needs. Furthermore, it has been found that the controlled release of FGF2-stabs occurs via first order kinetics and the rate can be slowed by increasing the concentration of the hydrogel, optionally by modifying the copolymer itself with functional groups, or by cross-linking.

ACKNOWLEDGEMENTS: Grant Low temperature 3D printed bioresorbable hydrogels as carriers of pro-healing protein for tissue regeneration is realized within the project Quality Internal Grants of BUT (KInG BUT), Reg. No. CZ.02.2.69 / 0.0 / 0.0 / 19_073 / 0016948, which is financed from the OP RDE. The authors greatly appreciate the collaboration with Enantis, s.r.o., which provided the stabilized protein FGF2-STAB® for this project.

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Hyaluronic Acid Based Composites for Local Drug Delivery

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INTRODUCTION: Site specific drug delivery systems (DDS) are often developed to overcome high drug dosages, side effects and even toxicity of conventional ones (e.g., injections or oral ingestions). By creating smart drug delivery vehicles, with greater efficiency, it is possible to predict the therapeutic response, as well as ensure controlled and prolonged drug release periods [1]. Post-traumatic and post-operative tissue damage often carries potential infection risks, usually precluded through local antibiotic therapy. Microencapsulation of gentamicin sulphate (GENTA) could overcome possible infections due to its wide range of antibacterial spectrum [2]. Effective strategy to provide an optimal drug release rate and to ensure the necessary therapeutic levels over the certain period of time, is to load the microencapsulated active substances into the hydrogel matrix [3]. DDS made of hyaluronic acid (HA) and ε-poly(-L-lysine) (ε-PL) has shown high biocompatibility and increased antibacterial activity [4]. Thus, the aim of the current study was to prepare HA/ε-PL composites for controlled drug delivery and to analyze the active substance release profiles. For this purpose, GENTA was microencapsulated in poly-L-lactic acid (PLA) matrix and prepared vehicles were incorporated in HS/ε-PL hydrogels.

METHODS: HA/ε-PL GENTA loaded hydrogels and HA/ε-LL hydrogels, loaded with GENTA/PLA microcapsules, were prepared with solid to liquid phase ratio 1:5 and 2:5 (mass:volume). GENTA containing microcapsules were prepared by water-in oil-in water ($W_1/O/W_2$), double emulsion technique. GENTA release kinetics from modified HA/ε-PL hydrogels were studied using Ultra Performance Liquid Chromatography, equipped with Evaporative Light Scattering Detector (UPLC-ELSD) at λ=650 nm, using Aquity UPLC BEH C18 column.

RESULTS: By analyzing drug release profiles from HA/ε-PL hydrogels, loaded with GENTA/PLA microcapsules (Fig.1), it was observed that within the first 24 h already 78 ± 3% (HA/ε-PL 1:5 mass:volume) and 59 ± 7 % (HA/ε-PL 2:5 mass:volume) of GENTA was released, while rest of the drug was transferred into the dissolution medium within next 144 h. If GENTA was directly incorporated into the HA/ε-PL hydrogel matrix, total drug release was already observed after 24 h.

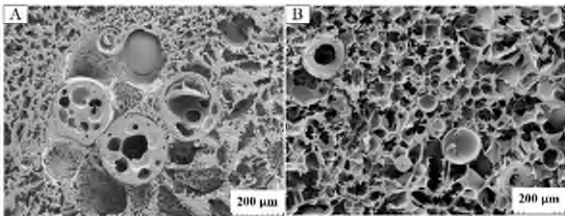

Fig. 1: SEM images of HA/ε-PL hydrogels loaded with GENTA/PLA microcapsules: A – HA/ε-PL 2:5 (mass:volume); B – HA/ε-PL 2:5 (mass:volume).

DISCUSSION & CONCLUSIONS: During the research, it was established that HA/ε-PL modification with GENTA loaded PLA microcapsules prolonged the total drug release up to one week.

ACKNOWLEDGEMENTS: The authors acknowledge financial support from the Latvian Council of Science research project No. lzp-2019/1-0005 "Injectable in situ self-crosslinking composite hydrogels for bone tissue regeneration (iBone)".

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Antibacterial Activity of Chemically Cross-linked Hydrogels Based on ε-Polylysine and Hyaluronic Acid

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INTRODUCTION: In recent years bacterial infections and antibiotic resistance are becoming a global problem in health care sector, and therefore design and development of hydrogels for tissue engineering with an antibacterial function are a main focus in biomedical research. The aim of this study is to develop and investigate novel antibacterial hydrogels based on natural biopolymers: antibacterial ε-polylysine (ε-PL) and intrinsic biocompatible hyaluronic acid (HA).

METHODS: The hydrogels based on ε-PL and HA (mass ratios of ε-PL and HA are 60:40; 70:30; 80:20 wt%) were *in situ* synthesized via chemical cross-linking using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) crosslinking agents (molar ratio of EDC and NHS is 1:1). The molecular structure, morphology, gel fraction and swelling analysis of the fabricated hydrogels were investigated. The minimal inhibitory concentrations (MIC) of ε-PL against *E.coli* and *S.aureus* were determined. The antibacterial activity of the fabricated ε-PL-HA hydrogels were tested against *E.coli* and *S.aureus* bacterial cultures.

RESULTS: FTIR spectra indicated interaction between ε-PL and HA and successful formation of cross-linked copolymer via amide bond linkage. SEM micrographs of the lyophilized ε-PL-HA hydrogels revealed highly porous structure. The obtained hydrogels were successfully sterilized by autoclave sterilization. The calculated value of gel fraction of the fabricated ε-PL-HA hydrogels is in the range of 62-65%. Swelling dynamics of ε-PL-HA hydrogels show that uptake of liquid occurs increasing their weight ten times. The MIC of ε-PL against *E.coli* and *S.aureus* were determined to be 25 µg/ mL.

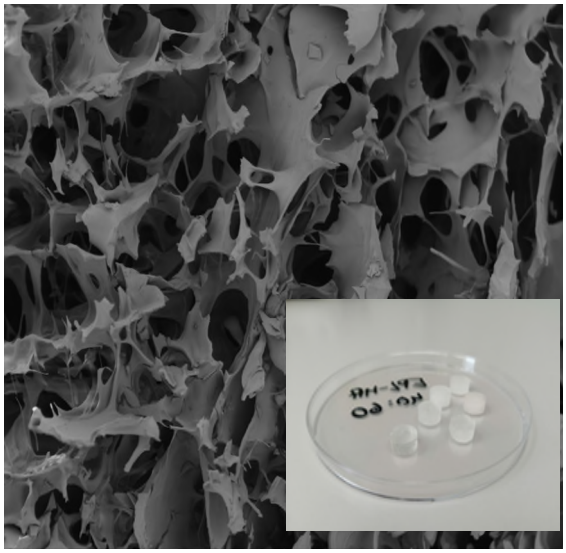

Fig. 1: SEM micrograph of lyophilized ε-PL-HA hydrogel (60:40 wt%).

DISCUSSION & CONCLUSIONS: In the present study, novel hydrogels based on ε-polylysine and hyaluronic acid copolymer systems were synthesized and investigated. The antibacterial tests indicated that the developed hydrogels can be considered as promising antibacterial biomaterials for tissue engineering.

ACKNOWLEDGEMENTS: The authors acknowledge financial support from the Latvian Council of Science research project No. lzp-2020/1-0072 "Injectable bioactive biocomposites for osteoporotic bone tissue regeneration (inBioBone)".
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Mesoporous Silica Based Protein Release Systems

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INTRODUCTION: Mesoporous silica nanoparticles (MSNs), such as MCM41, are effective support carriers with excellent adsorption properties and large surface areas [1-3]. Bioactive molecules like proteins, such as albumin, casein and collagen, are universally present in nature and products. Interaction of these proteins and MCM41 has not been widely explored. The aim of this study is to assess how these proteins behave in the presence of MCM41 and assess its protein release properties for the purpose of interdisciplinary applications.

METHODS: Commercial standards of ovalbumin (albumin from chicken egg white, A5378, Sigma-Aldrich), commercial collagen from rabbit skin (Type I, Sigma-Aldrich), and casein (C3400, Sigma-Aldrich) were procured. Coomassie blue dye G-250 (Acros Organics™) [0.6% (m/v) in Hydrochloric acid 0.6 M (HCl)] was used for protein quantification and was prepared in the lab. MCM41 was synthesized and calcined in a vacuum chamber at 550ºC for 6 hours, following the emulsion-condensation route reported by Cao et al [4]. The protein was extracted by 3 consecutive cycles of orbital agitation (at 27° C) and ultrasonication (at 37° C) of 1h each. Finally, the extracted protein was quantified by Bradford Assay, in the presence of Coomassie Blue solution, using BSA as standard solution (1–100 µg mL^{-1}).

RESULTS: MCM41 was characterized with SEM to X-Ray Diffraction to understand the morphological characteristics and the reflections within MCM41(Fig. 1). The protein extraction with MCM41 was compared with ordinary protein extraction in terms of a triple extraction process. As from Fig. 2, the protein supernatant was extracted three times, to procure the maximum output of extraction with and without MCM41 comparatively. After each extraction, the fractional % of protein extracted with MCM41 increases, while a decrease was observed w/o MCM41.

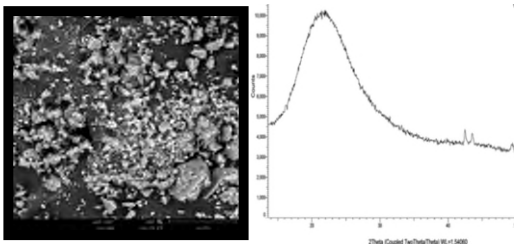

Fig. 1: SEM and XRD images of MCM41.

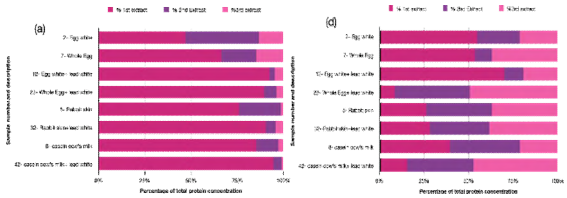

Fig. 2: Fractional % extraction (1st,2nd 3rd) a) w/o MCM41 d) with MCM41.

DISCUSSION & CONCLUSIONS: The above study shows that it is possible for MCM41 to increase protein extraction, fine-tune the sustained release of proteins, while also having a longer duration of protein recovery and a highly controlled release system. MSNs like MCM41 give a new dimension for proteomic studies.

ACKNOWLEDGEMENTS: ED-ARCHMAT grant agreement number 766311 (Project-ESR2). Marie Sklodowska-Curie Innovative Training Networks, Horizon 2020 and THE SCREAM Project – Touchstone for Heritage Endangered by Salt Crystallization, a Research Enterprise on the Art of Munch, Ref: ALT20-03-0145-FEDER-031577.

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Hyaluronic Acid Containing Hydrogels as Strontium Ranelate Delivery Systems

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INTRODUCTION: Hydrogels have attracted extended attention due to their ability to provide controlled release of a variety of therapeutic agents and easily modifiable properties to obtain the desired release kinetics of active substances [1]. The desired release of drug from hydrogels, can be obtained by controlling the size of the meshes of the polymer network, by establishing links between the active complex and the polymer chains, as well as by encapsulating another system, containing the active substance to the polymer network, thus slowing their release, such as micro- and nano-capsules [2].

Hyaluronic acid (HA) is an important component of the bone extracellular matrix (ECM), as it regulates cell adhesion, differentiation and proliferation, and controls cell-cell and cell-ECM interactions. Due to these functions, as well as their excellent biocompatibility and complete biodegradability, HA is widely used in the synthesis of hydrogels and development of the drug delivery systems. However, HA hydrogels are highly soluble and show very poor mechanical properties with rapid degradation *in vivo*. These disadvantages can be eliminated by adding an inorganic component – calcium phosphate (CaP) nanoparticles - to the HA during the preparation of hydrogels, which would not only promote bone regeneration, but at the same time increase the mechanical properties of hydrogels [3].

Combining the properties of HA and calcium phosphate composite materials, in the field of bone tissue engineering, were developed and modified with the anti-osteoporotic drug strontium ranelate (SrRan) [4] to create local, controlled release drug delivery systems. Within the current research HA/strontium ranelate (HA/SrRan) and HA/CaP/SrRan hydrogels were prepared and kinetics of SrRan release was evaluated.

METHODS: HA and HA/CaP hydrogels were prepared using 1,4-butanediol diglycidyl ether (BDDE) as the crosslinking agent. After preparation, hydrogels were lyophilized, impregnated with SrRan solution and lyophilized again. Ultraviolet-visible light spectrophotometry was used to evaluate the release of active substance from prepared hydrogels and results were expressed as the % of SrRan released from the total drug content in the samples. To analyze the molecular structure of hydrogels, to identify absorption bands of organic and inorganic phases, and to determine the possible molecular interactions, Fourier transform infrared spectroscopy (FTIR) was used.

RESULTS: Obtained results indicated that already after one hour 93% of SrRan was released in dissolution media in the case of HA and 87% in the case of HA/CaP hydrogels. Also, the FTIR analysis did not confirm any molecular interactions between the components of hydrogels.

DISCUSSION & CONCLUSIONS: During the research it was established that by impregnation approach it is not possible to obtain long-term controlled release from SrRan delivery systems, neither from pure HA hydrogels, nor from CaP containing ones.

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Antibacterial Hydrogel Particle Surface-Modified Poly(dimethylsiloxane)

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INTRODUCTION: Due to its excellent mechanical properties and biocompatibility poly(dimethysiloxane) (PDMS) elastomer is an indispensable tool in production of medical devices. However, the low surface energy renders PDMS prone to protein adsorption, bacterial attachment, and infection formation. Consequently, antibacterial surface modifications of PDMS are long sought after. Here, PDMS surface modification, with amphiphilic hydrogel microparticles, in combination with covalently immobilized antibacterial peptides (AMP) is introduced [1]. The study demonstrates the use of AMPs, broad-spectrum antibacterial agents, in coating formulation, as an alternative to conventional antibiotics, to prevent biomaterial-associated infections.

METHODS: Amphiphilic hydrogel microparticles were prepared by top-down methods, from self-assembled lyotropic liquid crystal hydrogels, based on polymerizable versions of triblock Pluronic F127 copolymers. The intrinsic amphiphilicity facilitated the particle immobilization onto solid PDMS substrates, via dip-coating the substrates in the PDMS prepolymer, followed by the subsequent freeze-dried particle deposition and heat curing. The formed particle coatings were rehydrated in aqueous media, followed by the covalent AMP attachment to the hydrogel network via carbodiimide crosslinker chemistry and peptide bond formation. The antibacterial activity of the formed coatings was evaluated against gram-positive *Staphylococcus Epidermidis* bacteria species. Coating properties were characterized with water contact angle measurements (WCA), x-ray photoelectron spectroscopy (XPS) and small-angle x-ray scattering (SAXS).

RESULTS: Coating characterization with WCA displayed contact angle reduction from 99.5 ± 3.4°, of pristine PDMS, to 30.3 ± 11.6° of hydrogel particle coated PDMS, resulting in surface wetting. XPS surface scans of AMP modified coatings exhibited presence of nitrogen (1s) when compared to AMP-free coatings, indicating peptide integration. SAXS data displayed distinct scattering signals, indicating mesoscale order in the hydrogel networks. Microbiological assessment resulted in the bacteria colony forming unit (CFU) count reduction on AMP modified surfaces, in liquid bacteria culture, with AMP pre-modified particle coatings, displaying highest efficiency of 99.3 % and 99.6 % bacterial load reduction, in comparison to control (AMP-free) coatings and pristine PDMS, respectively.

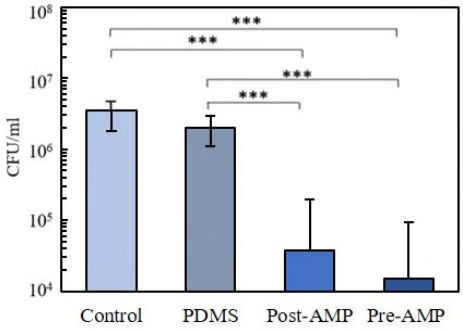

*Fig. 1: Antibacterial activity against S. Epidermidis, seeding density 10^6 (CFU)/mL. Pre- and post- AMP designates particle activation with AMP before or after deposition, respectively, control is AMP free particle coating and PDMS is nonmodified substrate. *** indicates $P \leq 0.001$.*

DISCUSSION & CONCLUSIONS: Early results show successful surface modification of pristine PDMS, proving to be highly efficient against gram-positive *S. Epidermidis* bacteria and demonstrating the applicability of hydrogel particle-AMP coatings.

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

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Study of Autologous Fibrin Matrices for Controlled Drug Delivery

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INTRODUCTION: Fibrin is a blood component involved in the clotting process and can be used as a matrix for the tissue engineering applications [1]. Vancomycin, a glycopeptide bactericidal antibiotic, has been used in human medicine for over 40 years [2]. The aim of this study was to develop autologous fibrin matrices for controlled vancomycin hydrochloride (V-HCl) delivery and determine their antibacterial properties against Staphylococcus aureus (ATCC 25923). Samples were prepared from donor blood, and V-HCl was encapsulated in microcapsules for ensuring the sustained release kinetics.

METHODS: Written consent from all of the donors was obtained for their blood samples to be used in the research study.

A) Preparation of V-HCl carriers

Microcapsules were prepared by w/o/w method. Briefly, poly (lactic-*co*-glycolic acid) (PLGA)/ dichloromethane (DHM) solution (17 wt %) was homogenized and mixed with V-HCl aqueous solution (100mg / mL). The resulting solution was transferred to Poly (vinyl alcohol) (PVA) (4 wt %) and homogenized, then the solution was transferred to 2.5 L water and stirred for 1h. The suspension was centrifuged, frozen and lyophilized, yielding microcapsules (see Fig.1A).

B) Preparation of fibrin matrices

Fibrin matrices (see Fig.1B), respectively, platelet-rich plasma (PRP) was obtained by centrifugation of the donor blood sample at 700 rpm for 3 min. Followed by the separation of PRP by syringe. V-HCl carriers were added to the separated PRP by suspending them in the PRP, using an automatic pipette before the clot forms.

C) Antibacterial properties with the disk diffusion method

S.aureus suspensions were prepared according to EUCAST standards and were inoculated onto a sterile Mueller-Hinton agar. After *S.aureus* inoculation, V-HCl samples were placed onto agar and incubated for 24 hours in 37°C. After 24 hours, antibacterial properties were analysed by measuring the sterile area (diameter) around the samples. After the measurements, samples were transferred to the new culture agar, and incubated for another 24 hours in 37°C.

RESULTS: Lower amounts of plasma were obtained by the centrifugation in shorter period of time. By increasing the time, plasma levels increased, but could promote the clot formation in the centrifuge tube, which could not be further used to develop drug delivery systems. Antibacterial properties of V-HCl samples were observed against *S.aureus* for 48 hours.

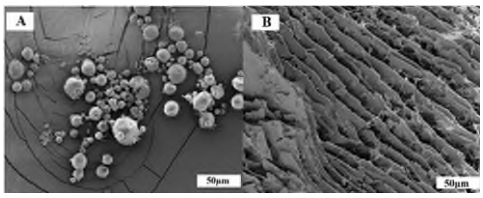

Fig. 1: SEM images of PLGA microcapsules (A); Fibrin matrices (B).

DISCUSSION & CONCLUSIONS: PLGA / V-HCl/fibrin matrices showed promising results and the highest concentration of V-HCl was released after 72h. This responds to the microbiologic inhibitory activity to the strains of methicillin-resistant *S. aureus* (0.125 µg/mL to 2 µg/mL) [3]. In addition, sample dissolution was not observed.

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Synergy of Recombinant Silk and Sodium Phosphate to Improve Macroporosity and Cell Adhesion in Apatite Cements

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INTRODUCTION: Calcium deficient hydroxyapatite (CDHA) is used in tissue regeneration applications due to its chemical and physical similarity to the inorganic phase of the bone. To enable internal cell colonization, interconnected macroporosity is however needed in the scaffold. CDHA can be prepared via a cementitious reaction, briefly mixing an aqueous solution with α-tricalcium phosphate (α-TCP). Macroporosity can be incorporated by foaming a surfactant added to the liquid phase, which maintains the porosity within the structure after setting [1]. Although CDHA has been proven to induce bone regeneration, its affinity to take up calcium ions hinders cell adhesion *in vitro* [2]. In this work, silk protein functionalized with an RGD-motif from fibronectin (FN-silk) [3] is used as a macroporosity template and a promoter of cell adhesion.

METHODS: The solid phase consisted of α-TCP [1] with 2% precipitated hydroxyapatite and 0 or 2.5% $Na_2HPO_4{\cdot}2H_2O$ (NaP). Two liquid phases were used as macroporous templates: FN-silk (2 mg/mL) [3] and Tween (0.05-0.4%). To prepare the templates, the liquid phase was foamed and mixed with the solid phase. The paste was transferred into moulds and allowed to set in a 100% humidity container for 1 day before being immersed in a PBS solution for 9 more days. The crystalline phases were measured by X-ray diffraction. The total macroporosity of the materials, was assessed by X-ray micro-tomography and the morphology was examined by scanning electron microscopy. Pre-osteoblast-like cells were cultured on the surface of CDHA over a period of 14 days. Cell adhesion and cell proliferation were monitored.

RESULTS: After 10 days of setting, all samples transformed into CDHA, with residues of ~5% α-TCP. The addition of NaP increased the total porosity of the scaffolds, when using both FN-silk and Tween as foaming agents. This increase was more dramatic for Tween samples, where total porosity increased from 5.22% to 34.98%, for 0.1% Tween (Fig. 1). High magnification micrographs showed the presence of FN-silk on the surface of the scaffolds. Cells cultured on the surface of both scaffolds proliferated over time. In addition, silk enhanced the adhesion of cells, which showed a slightly more spread morphology than on the Tween scaffolds 3h after seeding. Furthermore, preliminary data suggested a more efficient migration into the FN-silk scaffolds compared to the Tween-scaffolds.

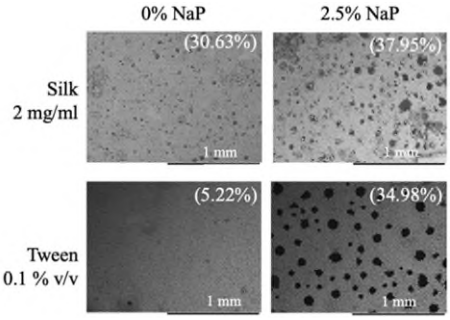

Fig. 1: Effect of NaP and surfactant agent (total macroporosity indicated in parenthesis).

DISCUSSION & CONCLUSIONS: The addition of NaP accelerated the setting reaction, keeping a higher quantity of porosity in the transition from paste to solid. This was especially relevant when Tween was used as foaming agent. The more spread morphology of cells was associated to the presence of RGD-motifs from the silk. FN-silk has proved to be an effective foaming agent to produce macroporous CDHA scaffolds with enhanced cell adhesion.

ACKNOWLEDGEMENTS: GM and CP acknowledge Göran Gustafsson's Foundation for supporting this research.

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Bloom Strength – A Rapid Method for Screening Hydrogels

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INTRODUCTION: Scaffolds are an integral part of tissue engineering. Hydrogel composition is of prime importance as properties of the scaffold, as well as parameters of biofabrication techniques are dependent on it. To date, the hydrogels are synthesized using the trial-and-error method to procure the optimized formulation for scaffold fabrication. Handling huge samples is a laborious and time-consuming operation. Hence, there is a pressing need for the development of a rapid screening technique for optimized hydrogel. Efforts are made in analyzing the viscoelastic properties of hydrogels, which are crucial, but again a time-consuming process. On the contrary, Bloom strength is a simple technique frequently used in industries for analyzing the strength of gelatin. This technique can potentially be employed for rapid analysis of hydrogels, and for finding an optimum window, which can be utilized as a standard framework in the development of new hydrogels. In this study gelatin (GE) and guar gum (GG) were used for formulating hydrogels. The scaffold formation potential of these hydrogels was analyzed by two different biofabrication techniques; bioprinting and freeze-drying. The data obtained can be used as a framework in the generation of new hydrogels.

METHODS: Hydrogels were formulated using different concentrations of (GE) and (GG)[1,2]. Oscillatory shear test of these formulations was performed using Physica MCR 301 rheometer (Anton Paar, Ostfildern, Germany), having a 25 mm diameter parallel plate test geometry. Further bloom strength of these samples was analyzed using Brookfield CT3 texture analyzer (AMETECK, Germany), using T10 probe using TA-RT-KIT fixture having load cell of 100g for compression type test.

RESULTS: The amplitude sweep analysis reveals that all the formulated hydrogels possess a linear viscoelastic region of 10% strain. Furthermore, the frequency sweep analysis indicates that the bioprinting requires hydrogel with high storage (G') and loss (G") modulus, whereas the freeze-drying requires hydrogels with low G' and G". Tan ∂ was calculated as G"/G', which shows that the hydrogel required in freeze-drying is close to 1 while for bioprinting is close to 0.1. Results from the bloom strength go in line with the rheological analysis and suggest that the scaffold formation via freeze-drying requires a minimum bloom of 30 whereas 700 Bloom is required for bioprinting.

DISCUSSION & CONCLUSIONS: In this study, same biomaterials were used in formulations of the hydrogels, but the different concentration is required by different bio-fabrication techniques. In bioprinting the filament formation of the hydrogel is a vital criterion, therefore the viscoelastic requirements are high. On the other hand, in freeze-drying, the scaffold is formed due to sublimation, so the viscoelastic requirements are low. Therefore, the bloom test imparts a number to hydrogel which can be used in the screening and development of new hydrogels. Additionally, the bloom strength can be used to compare two hydrogels.

ACKNOWLEDGEMENTS: Authors are thankful to funding agencies Department of Biotechnology BT/PR22446/MED/32/572/216 and BT/HRD/01/01/2015 for providing financial assistance. Further, the authors acknowledge financial support from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 857287.

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Synthesis of A Novel Bioinspired Apatite/Collagen Composite with Potential Use as a Drug Carrier

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INTRODUCTION: Type I collagen and nanocrystalline multisubstituted apatite are the major components of a natural composite - bone tissue. Since apatite and collagen provide hardness and flexibility, respectively, only when used together, they form a material with appropriate mechanical properties. Moreover, due to high biocompatibility and osteoinductivity, these composites play a significant role in orthopaedic surgery and implantology. In addition, the porous structure of the composites allows their use not only as bone defect fillers, but also as a drug delivery system providing controlled release of drugs directly to the bone.

The aim of this study was to develop a method for the synthesis of a novel bioinspired composite containing nanocrystalline apatite imitating the biological apatite and collagen type I. The next goal was to incorporate a drug substance (ciprofloxacin) into the composite structure and to study its release.

METHODS: The synthesis of the composite was carried out using two different methods. In the first method, biomimetic apatite was obtained by precipitation from modified simulated body fluid (SBF), containing ions such as: Na^+, K^+, Mg^{2+}, Cl^-, CO_3^{2-}, SO_4^{2-} and SeO_3^{2-}. Then, it was homogenized with a suspension of type I collagen in various proportions (50:50 and 80:20 respectively). In the other method, the biomimetic apatite was precipitated in the collagen suspension in various proportions (50:50 and 80:20 respectively). Then all materials were lyophilized, soaked in ciprofloxacin hydrochloride solution, some of them were covered with polycaprolactone (PCL) and lyophilized again.

RESULTS: The obtained composites were characterized using the following methods: FT-IR mid-infrared spectroscopy, Scanning Electron Microscopy – SEM, SEM with Energy Dispersive Spectroscopy (EDS) and Powder X-ray diffraction – PXRD. At the end the drug substance release profile was examined by liquid chromatography (HPLC). The obtained composites differed in the mutual arrangement of collagen fibres and apatite crystals, as well as in the mechanical strength of the samples depending on the method of synthesis. There were also differences in the amount of foreign ions in individual samples. Significant differences in the release profiles of ciprofloxacin were detected.

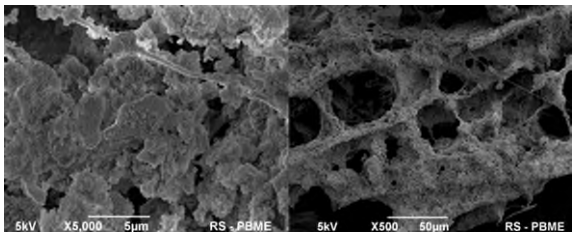

Fig. 1: SEM images of the analyzed apatite/collagen structure. Scale bars: 5 µm and 50 µm.

DISCUSSION & CONCLUSIONS: In the present study, eight biomimetic apatite/collagen composites were synthesized. All biomaterials were enriched in various ions, characteristic for biological apatites. Their composition and crystallinity are similar to bone tissue structure. Some differences in physicochemical properties depending on the synthesis method were observed. Appropriate modification of composites makes the materials a suitable carrier for various drug substances. *In vitro* studies on its biological properties are in progress and will contribute to the full evaluation of the obtained materials.

ACKNOWLEDGEMENTS: This study was financed from a research grant (Project FW23/F/MB1/N/20) from Medical University of Warsaw.

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Alginate Hydrogel Characteristics Regulate Dermal Fibroblasts Biology

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INTRODUCTION: Alginate is a polysaccharide of marine origin, which is considered an attractive biomaterial for fabrication of extracellular matrices for *in vitro* 3-dimensional (3D) cell culture [1,2]. In this work we investigated how 3D microenvironment provided by three different alginate scaffolds influences the viability, morphology and gene expression of normal human dermal fibroblasts (NHDF).

METHODS: The tested hydrogels consisted of 1% alginate, cross-linked with calcium ions, using internal gelation method [3]. Three gel types were tested: (A) UPLVG alginate (F_G 0.68, MW 237 kDa), (B) a 1:3 mix of UPLVG : POA (periodate oxidized UPLVG, D_{OX}=0.08), and (C) a 1:3 mix of UPLVG : POA-RGD (POA laterally substituted with a GRGDSP peptide, DS=0.05). NHDF were embedded into the gels and cultivated for 7 days. Viability and morphology of the cells, in gels, were examined using confocal laser scanning microscopy (CLSM); the obtained images were analysed with Imaris software. For RNA sequencing screening, RNA was isolated from cells growing within the gels at days 1 and 7. NHDF grown in conventional 2D culture were used as a reference.

RESULTS: It was shown that the hydrogels A, B and C promoted different cell morphologies (Fig.1). Preliminary analysis of the RNA sequencing data showed differences in gene expression levels between two timepoints (day 1 and day 7) and between 2D and 3D cultures. But most importantly different gene expression patterns were observed between different hydrogel types. The RNA sequencing data were further processed, and specific genes that influence fibroblast functions in the provided 3D environments, were identified.

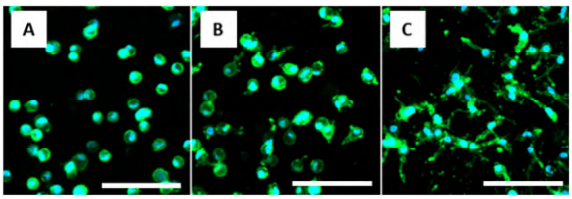

Fig. 1: Differences in morphology of NHDF cultured within 1% alginate gels A, B and C, respectively, for 7 days. F-actin (green), cells nuclei (blue). CLSM. Scale bar – 100 µm.

DISCUSSION & CONCLUSIONS: Evaluation of cell viability and morphology within the different alginate hydrogels, along with analysis of the RNA screening data, may provide a better understanding of how fibroblasts biology can be modulated by single characteristics of the alginate scaffolds.

ACKNOWLEDGEMENTS: This work is a part of 3DLife project within Centre of Digital Life Norway and is financed by the Research Council of Norway (project 269273). The RNA sequencing screening was provided by the Genomics Core Facility, Norwegian University of Science and Technology (NTNU). GCF is funded by the Faculty of Medicine and Health Sciences at NTNU and Central Norway Regional Health Authority.

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Acetylation of Alginate in the Gel State

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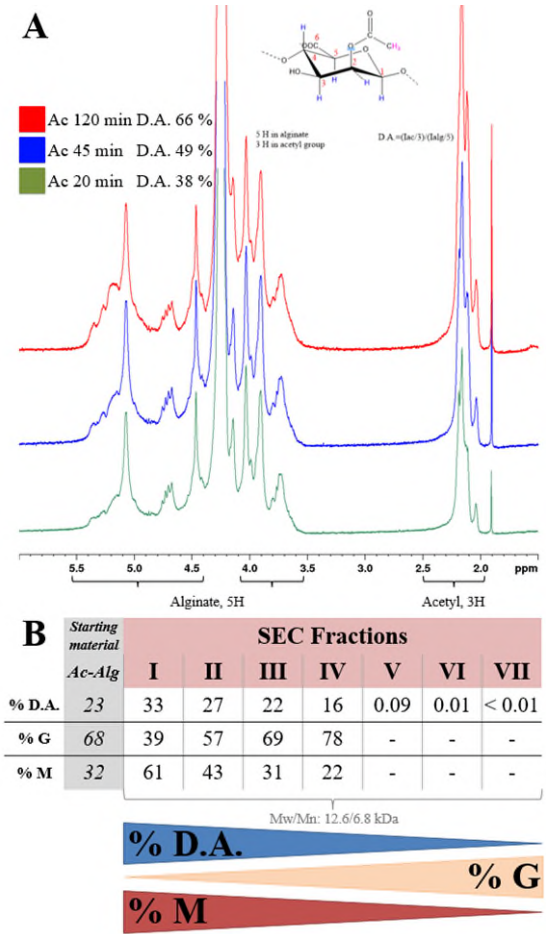
INTRODUCTION: Alginate is a polysaccharide which consists of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. Alginate produces viscous solutions in water and it forms hydrogels with divalent cations. Commercially available alginates are derived from brown seaweeds, such as *Laminaria hyperborea*. Alginates are also produced by bacteria such as *Pseudomonas spp.* and *Azotobacter vinelandii*. A distinction between alginate that is derived from seaweed and alginate of bacterial origin is the presence of acetyl groups on the M residues in the latter. O-acetylation (O-Ac) enhances chain extension, viscosity, and water binding capacity[1]. Acetylated alginates (Ac-Alg) are likely necessary for biofilm matrix formation and clustering of *P. aeruginosa*, and for the formation of desiccation resistant cysts in *A. vinelandii*[2,3]. Reactions with alginate in organic solvents are limited by solubility. Described herein, is the chemical O-Ac of ionically gelled alginate from *L. hyperborea* in organic solution. This approach also grants selective O-Ac of the M residues of alginate.

METHODS: Alginate hydrogel spheres were produced from solutions of 2 % (w/v) alginate, from *L. hyperborea* (68 % G), and gelled in 50 mM $CaCl_2$ or 20 mM $SrCl_2$. Water, in the alginate hydrogel spheres, was exchanged by exposure to three shifts in an excess volume of pyridine. Acetylation was performed in a solution of 1:1 pyridine-acetic anhydride and incubation at 38 °C. Degree of acetylation (D.A.) was regulated by time. Reactant was removed, and Ac-Alg was recovered by 0.15 M EDTA (pH = 7.5), dialysis and lyophilisation. The D.A. of Ac-Alg was determined with 400 Mhz ^{1}H NMR in D_2O. For determination of monomer selectivity of O-Ac, Ac-Alg was treated with a GG/GM selective lyase from *K. pneumoniae*. The product was fractioned using SEC. For each fraction, D.A. was determined using ^{1}H NMR. Following D.A. determination, the same fractions were deactylated by the addition of NaOD to 0.1 M for 15 minutes. Fractions were subsequently neutralized with DCl, and G/M percent was determined with ^{1}H NMR.

RESULTS:



B

	Starting material	SEC Fractions						
	Ac-Alg	I	II	III	IV	V	VI	VII
% D.A.	23	33	27	22	16	0.09	0.01	< 0.01
% G	68	39	57	69	78	-	-	-
% M	32	61	43	31	22	-	-	-

Mw/Mn: 12.6/6.8 kDa

Fig. 1: A, ^{1}H NMR of Ac-Alg showing D.A. at select reaction times. B, % G/M and D.A. of lyase treated Ac-Alg, fractioned with SEC.

DISCUSSION & CONCLUSIONS: Presented herein is a monomer (M) selective chemical modification of alginate in the gel state, performed in organic solvent. D.A. was regulated with reaction time and increased rapidly in the first minutes (Fig. 1A). O-Ac was selective for M residues (Fig. 1B). Using lyase treatment and SEC fractioning with a non-gel state modified Ac-Alg, might elucidate whether or not the selectivity for M is dependent on the gel state.

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Injectable Hydrogels for Bone Tissue Regeneration

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INTRODUCTION: Natural and synthetic polymers, such as poly(vinyl alcohol) (PVA), hyaluronic acid (HA), elastin, poly(ethylene glycol) (PEG) and chitosan, which have similar structure of cartilage extracellular matrix (ECM), may be used for the preparation of injectable hydrogels, able to repair irregular-shaped tissue defects [1]. Hydroxyapatite (HAp) is the most advanced bioactive filler because of its biocompatibility and osteoconductivity. Combination of hydrogels with HAp, could be a right solution to enhance the mechanical and biological properties of the final biomaterial, intended for the bone tissue regeneration [2].
Main preconditions for biomaterials, used in close contact with the human body are their biocompatibility, microbiological safety and sterility, which are the most important requirements to minimize the potential risks of infections. For hydrogel sterilization, physical, chemical and physicochemical approaches are often used. Although high temperature steam sterilization permanently destroys variety of microorganisms, it can also irreversibly influence the intrinsic properties of hydrogels [3]. Thus, the main objective of the current research was to prepare hyaluronic acid based HAp/ε-poly-L-lysine (ε-PL) hydrogels and characterized them towards the swelling behavior, gel fraction and resistance to the heat sterilization procedure.

METHODS: HA/ε-PL and HA/ε-PL/HAp (inorganic phase content 21wt%) hydrogels were physically crosslinked and characterized. Swelling behavior and integrity of prepared hydrogels were evaluated in 20 mL of deionized water at 37 °C for more than 3 months. Gel fraction of prepared sample was determined by measuring its insoluble part after immersing in 200 mL deionized water for 48 h at room temperature. Moreover, impact of different sterilization conditions (105 °C under 179.5 kPa for 4 min and at 121 °C under 101.3 kPa for 20 minutes) on hydrogel gel fraction was assessed.

RESULTS: Swelling equilibrium of HA/ε-PL hydrogels was reached within 5 weeks, and the swelling degree equalled to 275.13% ± 11.01%, while for HA/ε-PL/HAp hydrogels, swelling equilibrium was already reached at the 2nd week of experiment (223,92% ± 20.82%). Also, results indicated that, with an addition of inorganic phase, statistically significant increase in gel fraction results was observed (75.28% ± 1.57% in case of HA/ε-PL and 82.31% ± 1.97% in case of HA/ε-PLL/HAp). The suitability of steam sterilization method for the hydrogel treatment was analyzed and it was found that there are no statistically significant changes in gel fraction values before and after sterilization process, if 105 °C for 4 min was used. However, if 121°C was used for the sterilization purposes, gel fraction of HA/ε-PLL samples decreased by 50%, while for HA/ε-PL/HAp samples gel fraction decreased by ~25%.

DISCUSSION & CONCLUSIONS: During the research it was established that through addition of inorganic phase to HA/ε-PL hydrogels, degree of hydrogel crosslinking could be increased. Moreover, for hydrogel sterilization 105 °C for 4 minutes was found to be suitable, while sterilization temperature of 121°C facilitated the crosslinked structure disruption.

ACKNOWLEDGEMENTS: The authors acknowledge financial support from the Latvian Council of Science research project No. lzp-2019/1-0005 “Injectable in situ self-crosslinking composite hydrogels for bone tissue regeneration (iBone)”.

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In Situ Synthesis of Calcium Phosphate in Silk Solution

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INTRODUCTION: Silk fibroin (SF) composite materials are used for bone regeneration due to their elasticity, biocompatibility, and biodegradability [1], on the other hand calcium phosphates (CaP) increase bone growth and enhance bone mineralization, with ion release, from which hydroxyapatite (HAp) and beta-tricalciumphosphate (β-TCP) is widely used, as it is similar to human bone inorganic phase and can be used for bone regeneration in different ratios [2]. Hydroxyapatite is fragile; thus, creation of silk fibroin and hydroxyapatite composite material *in situ*, would combine the valuable properties of both materials by creating interaction between organic and inorganic phases [3]. Calcium phosphate *in situ* synthesis in SF is novelty in the field of biomaterials, the synthesis process and parameters are still poorly studied. The aim of this research is to fully study SF/CaP synthesis, its parameters, and products.

METHODS: SF solution was prepared from *Bombyx mori* silkworm cocoons accordingly: at first, degummed in 0.02 M Na_2CO_3, then the obtained fiber was rinsed with water to wash out wax and sericin. Then, fiber was dried and dissolved in $CaCl_2$:H_2O:C_2H_5OH (1:8:2). The obtained solution was dialyzed in deionized water through 14 kDa MWCO cellulose membranes and the concentration was determined by weighting dry film after evaporation at 60°C, for three days. CaP/SF composites were synthesised using wet precipitation synthesis method, shortly: in room temperature 0.5625±0.0005 g of CaO was added to 25 mL of SF solution and stirred at 150 rpm. H_3PO_4 was added dropwise and stirred at 300 rpm up untill the desired pH value. The pH range was from 4.0 to 11.0 with step of 0.5 and for even more accurate results, 0.1 step was studied. Synthesis was performed in water as well, to analyze SF effect on composite materials and synthesis parameters. The obtained suspensions were lyophilized for 72h and analyzed with XRD, FTIR and BET. [1]

RESULTS: Firstly, we evaluated extracted silk fibroin. The thin SF films after evaporation were examined to determine functional groups and sericin and $CaCl_2$ presence, and neither were present, which means that rinsing and dialysis was done properly. After examining the obtained CaP/SF composite materials with XRD Rietveld refinement phase analysis – monophasic, biphasic and polyphasic structures were observed. In SF at pH 10.0 pure HAp was obtained, if compared to water, pure HAp phase was obtained at pH 10.5. On the other hand, β-TCP was 99% pure at pH 5.9 for both solutions. SF contains both hydroxyl and carboxyl groups, which affect CaP crystal growth and nucleation. Else ways J. Mobika et.al., also synthesized HAp/SF composite at pH 10.0 and concluded that HAp/SF composite has poor crystalline nature, and has increased surface area [1]. Our studies led to the same conclusion for the surface area – SF/HAp (68.45±4.070 m^2/g) composite has slightly higher surface area, than H_2O/HAp (65.42±10.99 m^2/g), the opposite relevance can be observed with β-TCP - 47.71±21.17 m^2/g in SF and 65.13±11.72 m^2/g in water. Both SF composites have higher crystalline nature.

DISCUSSION & CONCLUSIONS: From the obtained results it can be concluded that *in situ* CaP synthesis can be done in SF solution, leading to different HAp and β-TCP ratios. Synthesis parameters, to achieve composite with different ratios, were developed. To obtain pure HAp and β-TCP in SF solution, pH must be 10.0 and 5.9, respectively.

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Investigating Biomaterials, Structure and Cell Mechanics in Tissue Engineering Scaffolds

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INTRODUCTION: Development of novel biomaterials for tissue engineering is driven by the biomechanical and molecular cues provided to cells by their surrounding environment. In particular, the topography and mechanical properties of biomaterials are crucial parameters that influence cell adhesion/motility, morphology and mechanics, as well as the fate of stem and progenitor cells (1,2). To this end, atomic force microscopy (AFM) is an advanced multiparametric technique that offers nanometer resolution of the analyzed biological systems, while being able to simultaneously acquire information about the sample's mechanical properties at near native sample conditions. Recent AFM technological advances, have made it further possible to resolve dynamic processes on the second and even millisecond scale (3). This offers an invaluable insight into processes such as the self-assembly of single matrix molecules, and adhesion-induced structural changes in living cells.

METHODS: Tissue engineering takes an inspiration from nature, where collagen type I is the most abundant extracellular matrix (ECM) protein, and a target for biomaterial scaffolds, due to its complex hierarchical structural and mechanical resilience. We have recently demonstrated, that by modifying the biochemical composition of the collagen solution and substrate, we can fine tune its self-assembly kinetics, structure, and even drive the directionality of fiber formation (4). We will provide a further insight into the structural formation of collagen type I, emphasizing the intermediate steps in fibrillogenesis, with a temporal resolution of one frame per second. The experimental setup allows to further extend the interactome investigation of collagen type I with other molecules, typically appearing in the ECM of specific cells.

Living cells are constantly interacting with their surroundings, exchanging a number of molecules and signals. This is mostly associated with membrane turnover for e.g., membrane ruffling, or vesiculation (exo-/endocytosis of metabolites and vesicles). We will demonstrate how such processes can be analyzed with a high temporal resolution during cell spreading and migration, in living KPG-7 fibroblasts and CHO cells.

RESULTS: External mechanical stress is known to influence cell mechanics in correlation to the differences in actin cytoskeleton dynamics. A crucial aspect of investigating cellular mechanobiology is to go beyond purely elastic models, which do not reflect their complex composition. We have therefore performed sine oscillations (up to 1 kHz, amplitude 5-60 nm) in Z while in contact with the surface, to probe the frequency-dependent response of the cells. This allowed to calculate the viscoelastic properties, characterized by the dynamic storage and loss modulus (E', E'') distribution in living fibroblast cells.

DISCUSSION & CONCLUSIONS: AFM has a wide range of applications. We will show how the above processes can be further associated with spatially resolved cytoskeletal reorganization events taking place under the cell membrane. This is instrumental for understanding cell behavior in novel tissue engineering scenarios.

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A Comparison of Two Approaches to Integrate Medical Grade Titanium On-Chip

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INTRODUCTION: Before entering the clinic, biomaterials need to be thoroughly evaluated, which requires accurate *in vitro* models. However, it has been proven that the currently used models correlate poorly with *in vivo* results [1]. In this work, two approaches to prepare microfluidic chips that integrate medical grade titanium (Ti6Al4V) are compared. In subsequent sections, these will be referred to as 'polydimethylsiloxane (PDMS) chip' and 'glass chip'. Cells were cultured on-chip and their viability and proliferation was assessed by microscopic imaging and a biochemical assay.

METHODS: The PDMS chip consisted of a PDMS channel and a Ti6Al4V disc, held together by a 3D printed fixture (Fig. 1A). The PDMS was cured on a silicon wafer master and punched to enable connection to the tubing. The glass chip consisted of a double-sided tape channel, sandwiched between a conventional microscopic glass slide and cover glass, the latter comprising a laser cut hole to which the Ti6Al4V disc was docked with another layer of double-sided tape (Fig. 1B). Prior to assembly, PDMS stubs were bonded to the cover glass, to connect tubing to the chip. MC3T3-E1 pre-osteoblast cells were seeded at 50,000 cells/cm^2 on the PDMS chip and 15,000 cells/cm^2 on the glass chip. After 1 day of flow (2 µL/min) cell viability was assessed with calcein/propidium iodide (PI), staining live and dead cells, respectively. In addition, on the glass chip, cell proliferation was monitored over a period of 9 days using the lactate dehydrogenase assay. As a control, MC3T3-E1 cells were seeded on Ti6Al4V discs off-chip.

RESULTS: Ti6Al4V was successfully integrated on both chips, and the cells growing on the biomaterial could be imaged. However, to obtain sharp images, the PDMS chip had to be opened. The potential to maintain cells on-chip was confirmed both by imaging and quantitative proliferation data. As is shown in Fig 1C/D, viable cells were present in both chips after 1 day, which had a similar morphology as the control.

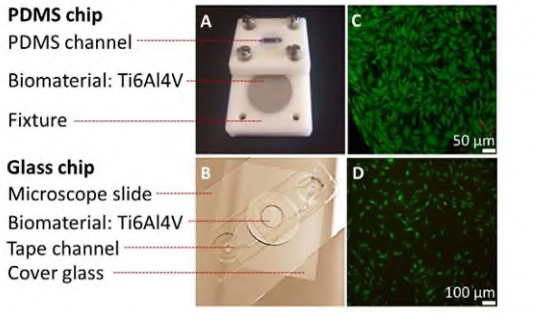

Fig. 1: (A) PDMS and (B) glass chip. Calcein/PI stained MC3T3-E1 cells (C) at 20x on PDMS chip and (D) at 10x on glass chip after 1 day.

DISCUSSION & CONCLUSIONS: MC3T3-E1 cells were successfully grown-on chip, as observed by imaging after 1 day and proliferation studies over a period of 9 days. Images could be obtained on both chips, however, for the PDMS chip, the Ti6Al4V disc had to be removed and transferred to a well plate to ensure sharp images. Whereas this brings the advantage of using standard off-chip assays and protocols, it requires an additional step. On the glass chip, images could be taken directly, which saves time and allows for continuous monitoring during the experiment. Moreover, in this chip, the area in which the cells are cultured does not contain PDMS. Although PDMS is a commonly used material in the field, controversy exists on its biological inertness. Given these findings and the debate around PDMS, the glass chip is considered to be more suitable and is being further examined, particularly for long-term cell differentiation studies.

ACKNOWLEDGEMENTS:

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Influence of Monovalent Ions on the Coating Formation of Tannic Acid

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INTRODUCTION: Naturally derived polyphenolic molecules, such as tannic acid (TA), are well known for their metal ion complexation and anti-microbial and anti-oxidant properties.[1] Therefore a rising interest emerged to use these characteristics for the modifications of biomaterials. For the deposition of a larger amount of TA, we described the use of silicic acid (Si_{aq}) to form silica-phenolic networks.[2] While the coating kinetic of this system can be regulated by the solution pH and its ionic strength, the type of monovalent cation was found to be important to control the electronic properties of intermediates.

METHODS: Coating formation was monitored using a quartz crystal microbalance (QCM-D). Radicals were characterized by electron paramagnetic resonance (EPR). Supplementary chemical analysis involved NMR, UV-vis, and FTIR spectroscopy.

RESULTS: Deposition kinetics of TA was determined to be optimal around 600 mM NaCl. Increased ionic strength led to the precipitation of TA. Substitution of NaCl with KCl and LiCl, interfered with the TA deposition process: (i) K^+ containing solutions instantly turned turbid in the range of pH 6 – 9. (ii) Li^+ suppressed both TA precipitation and coating formation. We could not detect any specific interaction of LiCl with TA or with Si_{aq} by means of ^{13}C and ^{29}Si NMR, and FTIR spectroscopy. In addition, the assessment of free silicic acid via a silico-molybdic assay did not result in a reduced amount of Si_{aq}. However, QCM-D experiments showed interaction of Si_{aq} with NaCl and KCl, which were reduced in presence of LiCl. Further, UV-vis spectra of TA, in presence of Li^+, indicated changes in the electronic properties. This result was corroborated by changes in the radical species observed in the presence of different alkali metal salts (Fig. 1).

DISCUSSION & CONCLUSIONS: Our results suggest that the ionic properties of Na^+ play an important role in the interaction with polyphenols. The reduced efficiency of the coating process in the presence of K^+ is likely to originate in the strong interaction with phenols.[3] In contrast, Li^+ has a lower affinity to the dihydroxy group compared to Na^+, but coordinates water more strongly. Although we could not directly prove interactions between Si_{aq} and Li^+, changes in the QCM-D response cannot dismiss this option. However, we could determine that the different salts have an influence in the radical formation of TA. These radicals are key intermediates in the oxidative polymerization and may play an important role in the formation of silica-phenolic networks.
In conclusion, primary and secondary salt effects of alkali metal ions are important in the formation of TA coatings and choosing the right conditions in biological applications has to be considered.

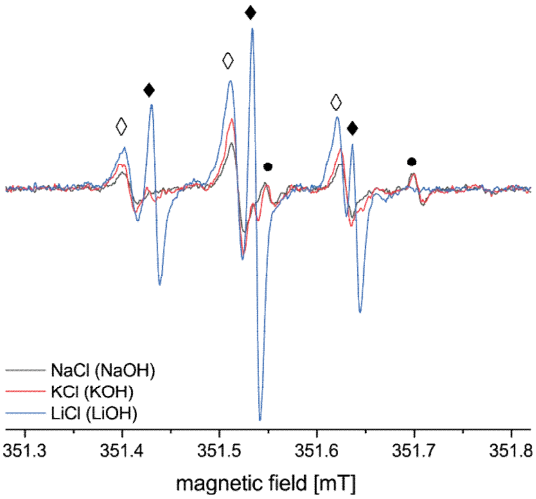

Fig. 1: EPR spectra showing radical formation of TA at pH = 11 with different alkali metal salts.

ACKNOWLEDGEMENTS: This work was funded by the Faculty of Dentistry, UiO.

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Building *In Vitro* Cell Models with HF Vibration Platforms

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INTRODUCTION: Mechanical stimulation systems are developed for studying cell mechanobiology and for directing cell physiology *in vitro*. In our previous study, we were able to stimulate osteogenic differentiation of human adipose stem cells (hASCs) with high magnitude and high frequency (HMHF) vibration [1]. Here, we introduce two stimulator systems designed for stimulating high sample numbers (high throughput system) and a system designed for studying stimulation responses with live cell imaging (microscopy system).

METHODS: All stimulator platforms consisted of 3D printed sample vehicles, compliant with five commercial multiwell plates (high throughput system) or an imaging chamber (microscopy system). The mechanic impact or high frequency movement of the sample vehicle is achieved in the multiwell systems by using subwoofers and in the microscopy setting by using miniaturized speaker. The movement is monitored by 3-axis accelerometer. As a result, the samples experience inertial -based dynamic stimulation (Fig. 1). [2 - 4]

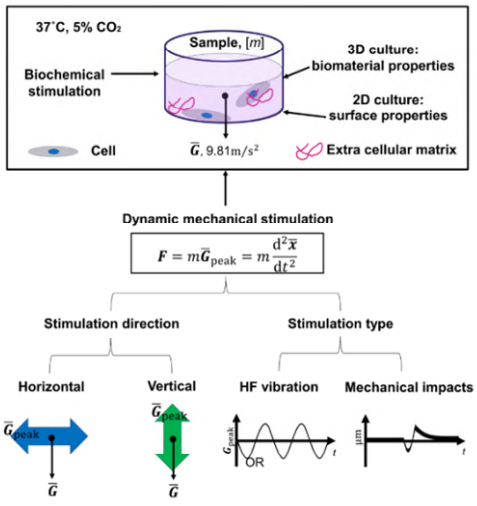

Fig. 1: The dynamic mechanical stimulation principle of the HF vibration systems.

The high throughput systems, consisted of both horizontal and vertical HMHF vibration devices, were used with the hASCs [2]. The microscopy system was used to study low magnitude HF (LMHF) vibration effects on the morphology of epithelial cells [4].

RESULTS: All stimulator systems were compatible with live cell stimulation experiments. Strong cell adhesion was found to be important for directing osteogenic differentiation and intracellular organization of the hASCs, with the HMHF vibration [2], whereas the LMHF vibration failed to alter the epithelial cell morphology [4].

DISCUSSION & CONCLUSIONS: 3D printing is a promising fabrication method for development of versatile HF vibration platforms. The microscopy system could be used for high-resolution light microscopy, studying fast cell responses and optimization of the stimulation parameters. After finding the most potential parameters, the high throughput systems could be used to study combined effects of the dynamic stimulation and the culture environment, for example, on the cell differentiation. Consequently, complementary mechanobiological knowledge could be obtained for different cell applications.

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Measuring the pH of Biofilms by Luminescent Calcium Phosphate Nanoparticle Films

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INTRODUCTION: Implant associated infections caused by biofilms are a major cause of implant rejection and, if not detected sufficiently early, may result in fatal sepsis. Biofilms are dense microcolonies of bacteria, which typically adhere to surfaces, therefore, their detection and diagnosis can be difficult. Their extracellular matrix and the diverse roles, which the bacteria can fulfil in the biofilm, also lead to them being more tolerant of antibiotics and more easily developing resistance against them. One promising strategy to help combat biofilms are "smart" anti-biofilm release surfaces. These surfaces are often loaded with antibiotics or other antimicrobials and respond to the creation of a microenvironment characteristic of biofilms by releasing their cargo. Such a characteristic biofilm microenvironment is low pH, in healthy tissue the pH should lie close to 7.4, but at the biofilm substrate interface the pH can decrease to 5 and below (Schlafer et al. 2015). Here, a robust pH responsive all-inorganic luminescent CaP:Eu^{3+} nanoparticle film is presented in order to probe the interfacial biofilm surface acidity in a well-plate reader friendly format.

METHODS: The europium doped calcium phosphate (CaP:Eu^{3+}) coatings were deposited onto silicon chips using flame spray pyrolysis and subsequently annealed in-situ. Their structure was analysed using SEM, XRD and FTIR and their luminescence evaluated with a spectrofluorometer. The sensor response was calibrated with pH buffers, and a variety of bacterial strains and species were allowed to form biofilms on the chips in M9 minimal media.

RESULTS: The deposited CaP:Eu^{3+} coatings exhibited a pH dependent change in their luminescence intensity, whereby the emission peak at 616 nm undergoes a strong decrease, compared with the 592 nm peak. This provides a ratiometric sensor response and known pH buffers were used as calibrants. A panel of

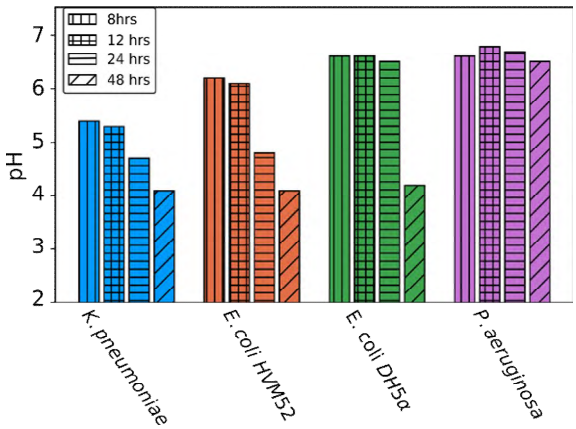

Fig. 1: Normalised sensor response (S_R) with 24 hr biofilm growth vs. clean growth medium (M9). Horizontal lines show the associated pHs derived from the calibration curve.

clinically relevant biofilm forming bacteria were selected to measure their interfacial pH. These bacteria grown in buffered media demonstrated low pH environments, however, differences between bacterial strains and species were observed.

DISCUSSION & CONCLUSIONS: In this work, a novel ratiometric sensor of biofilm interfacial pH is presented. The sensor is synthesised in a fast and scalable manner and biofilms grown in a high-throughput friendly manner. The sensor response is measured for a variety of bacterial strains and species, and differences in the pH of their interfacial microenvironment is observed.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (ERC Grant agreement n˚ 758705). Funding from the Karolinska Institutet Board of Research, the Swedish Research Council (2016-03471), the Jeansson Foundations (JS2016-0029) and the Åke Wiberg Foundation (M16-0098) is kindly acknowledged.

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Novel Method to Produce a Layered 3D Scaffold for Human Pluripotent Stem Cell-Derived Neuronal Cells

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INTRODUCTION: Degenerative disorders or traumas in the central nervous system (CNS) occur often as a consequence of damage and loss of axons. Adult human brain and spinal cord have limited ability to regenerate after injury [1] but regeneration and restoration of the function of injured neural tissue requires regrowth of axons. *In vivo*, axons form aligned bundles between different areas of the brain and in innervation to target tissues. The formation of this kind of cellular organization is enabled by guidance cues, including extracellular matrix (ECM), surrounding cells and tissue structures, which can be mimicked in *in vitro* models, with biomaterials. Ability to offer guiding cues for neuronal cells, and thus direct neuronal growth three-dimensionally (3D) *in vitro*, opens new possibilities to model defected neuronal tissue in the future. In particular, there is a need for human-based models for neuronal tissue engineering (TE). Here, we describe a method to create 3D, layered fiber-hydrogel scaffold for *in vitro* cell culture by combining electrospun, align oriented poly (D,L-lactide) (PLA) fibers [2] and collagen I hydrogel. The aim of the work is to study parallel alignment of neuronal cells relative to fibers in 3D composite scaffold.

METHODS: In this work 3D composite scaffold consists of electrospun fiber layers and hydrogel layers. The scaffold was created by the novel method of alternating electrospinning of aligned oriented PLA fibers [2] and cell laden collagen hydrogel gelation, in one process. Cultured cells were human pluripotent stem cell (hPSC) derived neuronal cells.

Diameter and surface topography of the fibers were studied using scanning electron microscope (SEM). Expression of neuron specific proteins was investigated using the immunocytochemical staining. Scaffolds were imaged using confocal microscope. Data obtained from the images were analysed with ImageJ software. 3D reconstructions of the layered structure were made with Imaris software.

RESULTS: Optimization of the electrospinning process has been successfully performed. We have managed to produce fluorescent PLA fibers (600 nm in diameter) in order to help visualisation of 3D layered structure of the scaffold.

We have managed to construct layered scaffold of three fiber layers and two hydrogel layers, in which neuronal cells grow in every layer.

Neuronal orientation along fibers was observed after two weeks culturing of hPSC derived neurons on 2D align oriented fiber layer and in 3D composite scaffold.

DISCUSSION & CONCLUSIONS: We successfully prepared 3D layered nanofiber-hydrogel scaffold for neural cell orientation. Our results indicate the ability to guide neuronal orientation in relation to fiber orientation. This electrospinning method enables us to spin fibers on freshly gelated, cell laden collagen hydrogel, therefore distributing fibers better into the hydrogel and bringing fibres and cells in closer contact to each other.

This novel method allows to create 3D scaffold containing fibres as guiding component for neuronal *in vitro* models that better mimic 3D fibrous nature of natural guidance cues.

ACKNOWLEDGEMENTS: This work was funded by Academy of Finland 286990, 326436, 312414, 311017 and Instrumentarium Science Foundation 200011.

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Design and Characterization of Biomimetic Ca-P Coated 3D Printed Scaffolds for Bone Tissue Engineering

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INTRODUCTION: 3D printing technology, in the bone tissue engineering, holds promising perspective which permits to manipulate bone scaffolds to patients' specific needs. Among other important properties, osteoconductivity is also essential parameter for creating successful bone tissue scaffold to enhance the attachment and migration of osteoblasts and osteoprogenitor cells. Biomimetic coating, using simulated body fluid (SBF), is a simple alternative method to the harsh apatite coating processes. In the present study, we aimed to obtain bone-like apatite layer onto 3D printed polymeric scaffolds by means of using a biomimetic approach and to investigate the effects of different surface treatment and immersion conditions.

METHODS: 3D design of the scaffolds was created using SolidWorks® and imported into the printing software CURA as an STL. Scaffolds with dimensions of 1.6 x 1.6 x 0.6 cm have 3 distinct layers, that first layer stays in 90° angular to y axis, then second layer stays bellow it, with 0° angle to y axis. Third layer positioned at 45° angular position to y axis, then the sequence continues with first layer. They were fabricated from filaments of poly(L-lactide) and polycaprolactone blend (PLLA/PCL, 70/30 w/w) using a 3D printer (Ultimaker 2-Go, Ultimaker, Netherlands). Prior to biomimetic coating, the surfaces of the scaffolds were modified by dipping them into 1M NaOH, saturated $CaCl_2$ and saturated K_2HPO_4 solutions, consecutively. After drying at 37°C, the 3D constructs were placed into falcon tubes containing 1x SBF solutions and incubated at 37°C for up to 21 days. To investigate the effect of immersion conditions, 3 groups were determined: samples with continuous agitation, solution renewed and still. The scaffolds were then removed from the solutions, rinsed with distilled water, dried, and characterized by different methods, including scanning electron microscopy (SEM), Electron Dispersive Spectroscopy (EDS) and X-ray Diffraction (XRD) analysis.

RESULTS: The SEM images revealed that scaffolds, pre-treated with 1M NaOH alkaline solution for 3 hours, and then followed by that 0.2M $CaCl_2$ and 0.2M K_2HPO_4 solutions, were able to induce the formation of biomimetic Ca-P layer on scaffold surfaces.

Three distinct groups with different immersion conditions were tested for biomimetic Ca-P coating approach. Despite EDX results represented clear Ca-P layer formation for all groups, needle-like crystal formation was observed for the group with continuous agitation unlike the others. Regarding XRD results, both frequent solution renewals and 100 rpm agitation, resulted to decrease in diffraction peaks which correspond to apatite. Nonetheless, its characteristic peaks were apparent for all groups at the end of 21 days of immersion in SBF, indicating the successful biomimetic coating.

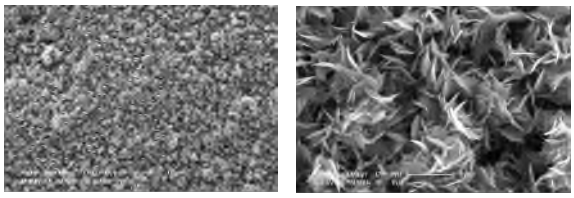

Fig. 1: SEM micrographs of PCL/PLA scaffold immersed in SBF with agitation for 21 days; x5000 (left) and x50000 (right).

DISCUSSION & CONCLUSIONS: By using 3D printing and biomimetic coating approach, bone-like apatite structure was successfully formed on printed PLLA/PCL scaffolds. These observations were confirmed by SEM and EDS and XRD analysis. Moreover, it was found that immersion conditions could affect the crystalline structure of formed apatite. The further investigations will be more focused on *in vitro* and *in vivo* biocompatibility of the produced structures to confirm their potential as a preferable alternative to existing bone tissue engineering scaffolds.

Thixotropic Copolymer as Binder for the Robocasting of Self-Setting Bone Tissue Engineering Scaffolds

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INTRODUCTION: Additive manufacturing technologies are becoming very popular for the fabrication of artificial bone substitutes. These substitutes have potential mainly in reconstructive medicine, to heal complicated critical sized bone defects. Nowadays, many studies have been known to deal with development of materials for robocasting of bone substitutes. The most popular inks are based on calcium phosphate powders in combination with an organic binder, which ensures good printability of the material [1,2]. Traditionally, the robocast scaffolds are consolidated by sintering, but recent works have shown the possibility to obtain calcium deficient hydroxyapatite (CDHA) scaffolds at the room temperature, due to the hydrolysis reaction of alpha tricalcium phosphate (α-TCP) [3,2]. This new biomimetic route requires the organic binder to be biocompatible and biodegradable because it will not be removed by sintering and will therefore remain within the structure of the scaffold. This restriction limits the number of binders that can be used for robocasting of self-setting CDHA scaffolds. Another limitation is still the poor mechanical properties of the scaffolds, that prevents the use in load-bearing conditions [1,2]. The aim of this work is to study the PLGA/PEG tixotropic and biodegradable copolymer [4], as binder for the robocasting of CDHA scaffolds, with particular focus on the effects on the mechanical properties in comparison with the previously used Pluronic binder [2].

METHODS: α-TCP inks were prepared with aqueous solutions of either Plutonic F127 [3] or the PLGA/PEG copolymer [4]. The inks were introduced into the cartridge of the robotic deposition device to print cylindrical scaffolds with Cartesian pattern. Post-printing treatment was performed for one week at biomimetic conditions [2]. The phase composition, after setting, was verified using XRD, TGA and FTIR. MIP and SEM were used to analyse porosity. Finally, the compressive strength was tested in dry and wet state, using a universal testing machine.

RESULTS: The transformation of α-TCP to CDHA during setting was not affected by the polymeric binders, and in both cases the phase composition of the final scaffold was mostly CDHA. In both cases the total porosity was in the range of 45-55%. Scaffolds containing PLGA/PEG copolymer exhibited almost two times higher compressive strength (11.2 ± 2.4 MPa) in comparison to scaffold printed with Pluronic (6.3 ± 1.6 MPa). In wet state the scaffolds were, in general, less strong and presented a quasi-brittle behavior.

DISCUSSION & CONCLUSIONS: PLGA/PEG copolymer is a promising binder for robocasting of CDHA scaffolds. In contrast to Pluronic, it is biodegradable and not soluble in the physiological environment. Therefore, PLGA/PEG copolymer is expected to be slowly degraded, reinforcing the scaffold for longer time, even in wet state.

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Down the Rabbit Hole: The Journey to Reproducible Biofilm Experimental Methods

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THE ISSUE: Lack of reproducibility among published studies is one of the biggest issues facing science today. Different factors contribute to this e.g., selective reporting, unreliable methods and lack of data sharing among many others.[1] This project uses four different approaches to accomplish the ultimate goal of reproducible biofilm research.

GUIDELINE: Minimum information guidelines instruct authors and reviewers on the necessary information that a manuscript should include for experiments to be clearly interpreted and independently reproduced. An international consortium was consulted to create "Minimum information guideline for spectrophotometric and fluorometric methods to assess biofilm formation in microplates", within the MIABiE framework. (http://miabie.org/introduction.php) The intention of the guideline is to improve reporting for these methods and as a result reproducibility.[2]

METHODS EVALUATION: A ring trial was performed in 5 laboratories to evaluate the reproducibility of three microplate-based biofilm quantification methods: CFU counts, resazurin, and crystal violet. Experiments were divided into control and treatment. A standard biofilm growth protocol and standard protocols (STM), for the three quantification methods, were developed. For treatment experiments, the efficacy of sodium hypochlorite (NaOCl) measured using *S. aureus* biofilms, was evaluated. Control experiments showed that crystal violet was the most reproducible method with the lowest standard deviation (SD). (Table 1) In the treatment experiments, CFU counts had the best reproducibility with respect to the responsiveness (SD/slope), making it the more reliable method to use in a disinfectant efficacy test. (Table 2)

Table 1. Summary of results for the STM control data for each method.

Method	Mean Log ± SE	Units	Reproducibility SD
CFU count	7.32 ± 0.40	CFU/well	0.92
Resazurin	0.71 ± 0.22	µg/mL	0.53
Crystal violet	1.13 ± 0.19	µg/mL	0.44

Table 2. Summary of reproducibility with respect to responsiveness for each method.

Method	SD/slope
CFU count	0.98
Resazurin	1.15
Crystal violet	3.22

MULTISPECIES BIOFILM METHOD ASSESSMENT: To investigate the repeatability of the formation of mixed strain biofilms on surfaces, a Fluorescence in-situ hybridization (FISH) based imaging method will be tested. This should allow the identification of individual bacterial strains within one biofilm and their spatial arrangement on the chosen surface.

DATA SHARING: The data from this project will be stored in open access repositories such as Zenodo.org under a creative commons license, to promote data sharing.

CONCLUSIONS: The reproducibility issue is complex and multifaceted. The work presented in this abstract offers possible solutions to tackling this problem in biofilm research. The guideline, and ring trial provide a better understanding of microplate methods and an option for data comparability across labs. The FISH method could help the study of complex biofilm formation on surfaces, and data sharing can help improve communication and dissemination in the field.

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Correlation Between Proteomics, Macrophage Polarization, and Oxidative Stress in Biomaterials

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INTRODUCTION: Macrophages represent the first line of immunological defense and present great plasticity, adopting a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype depending on microenvironmental cues and stimulus. The differential adsorption of proteins from biological fluids onto a material surface can regulate its activation and it has been suggested that the regulation of the M2:M1 ratio may be the key to the positive outcome of a material. Additionally, many studies identified a correlation between oxidative stress, inflammation, and healing, indicating that the production of ROS and lipid peroxidation act as chemo-attracts to immune cells. With this, the focus of this study was the characterization of the polarization of macrophages when exposed to the sol-gel hybrid biomaterials with known *in vivo* outcomes (two compositions with a bad biological response and two with good osseointegration properties) and correlate with oxidative stress responses and proteomic analysis.

METHODS: The sol-gel route was used to synthesize materials with distinct *in vivo* outcomes. These compositions were applied as coatings onto titanium discs. RAW264.7 macrophages were used to study cell polarization. Gene expression of M1 and M2 markers (TGF-β, IL10, EGR2, TNFα IL1β, iNOS) was measured using qRT-PCR. Liberation of pro-inflammatory (TNF-α, IL1β) and anti-inflammatory (IL-10, TGF-β) cytokines was measured by ELISA. Macrophage polarization was analyzed with immunostaining using IL7-R (M1) and CD206 (M2) markers. For proteomic analysis, samples were incubated in human serum. Proteins adsorbed onto the materials were analyzed through LC-MS/MS. MDA, GPX, and GR were measured to determine oxidative stress damage.

RESULTS: Materials with the worst compatibility showed a higher production of pro-inflammatory cytokines and higher IL7-R fluorescence when compared to materials with good compatibility. No changes could be observed in the oxidative stress measurements. Proteins related to the immune/inflammatory response were found predominantly on the materials with poor biocompatibility.

DISCUSSION & CONCLUSIONS: Materials with poor compatibility showed more production of pro-inflammatory cytokines and a higher IL7-R fluorescence. Additionally, these materials also showed higher adsorption of proteins that activate the immune response. As shown in previous studies (1, 2), a higher deposition of complement proteins onto a biomaterial is linked with biomaterial outcome *in vivo*. Thus, protein deposition can be associated with the macrophage polarization and ultimately modulate biological responses.

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Local Delivery of the Antimicrobial Peptide SAAP-148 from PLGA to Prevent Orthopaedic Infections

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INTRODUCTION: Fracture fixation devices (FFD) have infection rates ranging from 5 to 10%, for closed fractures and even higher rates up to 30% for open fractures[1], causing devastating complications. Clinically, implant infections are prevented by systemic antibiotic prophylaxis, the application of antibiotic loaded bone cements, and the use of minimally invasive surgical procedures[2]. However, conventional biomaterials such as bone cements are compatible with only a limited number of antibiotics and they show poor antibiotic release profiles which can lead to antimicrobial resistant development. Cationic antimicrobial peptides (AMPs) have shown to successfully kill antibiotic resistant bacteria. However, the application of AMPs in FFD is quite limited. We previously designed the potent Synthetic Antimicrobial and Antibiofilm Peptide (SAAP)-148[3]. Here, we studied incorporation of SAAP-148 in tailored poly-lactide-co-glycolic acid (PLGA) formulations as a coating of titanium bone fixation plates made by powder-bed selective laser melting (SLM) technology. The fit of the bone fixation plates to mouse femurs was assessed, and release of the SAAP-148 from the PLGA was characterized.

METHODS: The coatings were prepared by the solvent casting method using chloroform as a solvent. SAAP-148 was loaded into PLGA with 3 different molecular weights: 5 KDa, 15 KDa and 40 KDa. Eight layers with 40 µL of each solution were added to glass slides. The release of the peptide was studied by incubating the slides in 500 µL of phosphate buffer solution at 37 ºC and 120 rpm. The concentration of the peptide was measured by a protein quantification assay. In addition, SLM printed titanium bone fixation plates made with a particle size of <45 µm were chemically polished and applied *ex vivo* to an explanted mouse femur to confirm the dimensions (Fig. 1 C).

RESULTS: The release assay showed a burst release of SAAP-148 from the 15 KDa and 40 KDa PLGA coatings at the first 24 hours followed by a sustained release for the next 36 days (Fig. 1A). The SLM printed devices fit to the size femur of the mouse (Fig. 1 D).

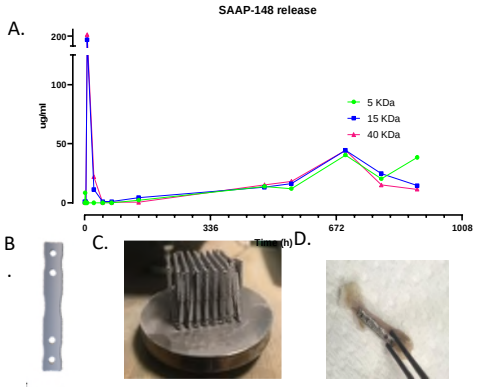

Fig. 1: (A) Drug release kinetics of the SAAP-148 PLGA coatings with different molecular weights. (B) CAD design of the fixation plate. (C) SLM printed bone fixation devices. (D)Ex vivo application of the fixation plate on the femur.

DISCUSSION & CONCLUSIONS: The AMP SAAP-148 was successfully incorporated in the 15 KDa and 40 KDa PLGA showing desirable release kinetics. The antimicrobial activity and biocompatibility of the printed Ti devices with the SAAP-148 PLGA coating will be further evaluated *in vitro* and *in vivo*.

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