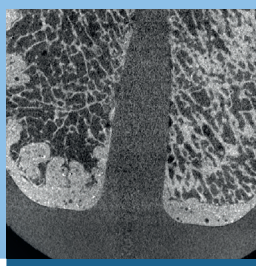
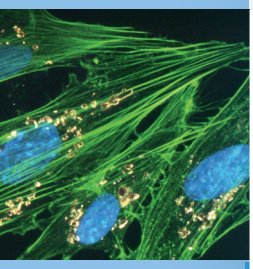
7th International Symposium

Interface Biology of Implants (IBI)





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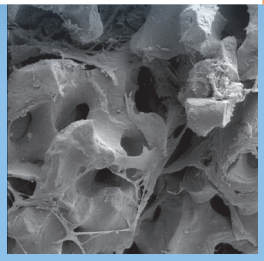

Abstracts

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Elaine electrically active implants SFB 1270

Abstracts

7th International Symposium Interface Biology of Implants

Congress Chairs
Prof. Dr. Rainer Bader
Biomechanics and Implant Technology Research Laboratory
Department of Orthopedics
Rostock University Medical Center

PD Dr. Kirsten Peters
Department of Cell Biology
Center for Medical Research (ZEMFO)
Rostock University Medical Center

Abstract Topics

- Biophysical Stimulation for Tissue Regeneration
- Innovative Biomaterials for Tissue Regeneration
- Cell-Biomaterial-Interaction
- Material-induced Immunomodulation
- Advanced Methods for Characterisation of Biomaterial-Tissue-Interface
- Complex in vitro Models for Biomaterial Testing

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Keynote lecture

KL 1

Use of electrical stimulus to influence mesenchyme cell activity in vitro

J. A Cosme[1]

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Electrical stimulation (ES), also known as e-stim, is the application of specific signals to cells to influence proliferation, differentiation, and maturation, leading to enhanced wound healing. Direct ES and indirect ES (capacitive coupling - CES) have shown promising results in biomedical applications, being used in fields like dentistry, dentistry, physical therapy, and orthopaedics. Most importantly, ES efficiency is highly dependent on the stimulation parameters, cell types, and environmental factors, requiring precise optimization and characterization for each application. Our work focuses on elucidating the variety of cellular responses in key relevant cell populations for tissue engineering and regenerative medicine, with a particular focus on stem cells. We design and build our custom ES bioreactors to deliver a range of clinically relevant electrical stimuli using models ranging from cell monolayers to multicellular spheroids, and an ex vivo murine bone model. We are also interested in evaluating conductive and piezoelectric materials like conductive hydrogels, fibrous scaffolds, and emulsion-templated emulsions for biomedical applications. Our work provides insights into the underlying mechanisms by which ES regulates cell function and matrix interactions, highlighting the versatility and potential of ES in the development of novel therapeutic strategies.

Keynote lecture
KL 2
Synthetic Hydrogels for Cell Therapy for Type 1 Diabetes
A. J. García[1]

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Hydrogels have emerged as promising biomaterials for regenerative medicine. We have engineered poly(ethylene glycol) [PEG]-maleimide hydrogels that support improved pancreatic islet functionalities, engraftment, vascularization and function in diabetic models. Two biomaterial strategies have been pursued. We have developed proteolytically degradable synthetic hydrogels, functionalized with vasculogenic factors for localized delivery, engineered to deliver pancreatic islets or human stem cell-islets to extrahepatic transplant sites via in situ gelation. These hydrogels induce differences in vascularization and innate immune responses among transplant sites. This biomaterial-based strategy improves the survival, engraftment, and function of a single pancreatic donor islet mass graft compared to the current clinical intraportal delivery technique. In a second application, we have developed a localized immunomodulation strategy using hydrogels presenting an apoptotic form of Fas ligand (SA-FasL) that results in prolonged survival of allogeneic islet grafts in diabetic mice. Survivors generate normal systemic responses to donor antigens, implying immune privilege of the graft, and have increased T-regulatory cells in the graft. Notably, allogeneic islet grafts exhibited long-term performance in diabetic non-human primates in the absence of systemic immunosuppression. This localized immunomodulatory biomaterial-enabled approach may provide an alternative to chronic immunosuppression for clinical islet transplantation.

Conclusion: Hydrogel-based strategies to promote islet engraftment and vascularization as well as local immune acceptance in diabetic models have been established. These strategies may significantly impact islet transplantation as a treatment for type 1 diabetes.

This research was funded by the Juvenile Diabetes Research Foundation and the U.S. National Institutes of Health.

Keynote lecture
KL 3
Tissue regeneration approaches using ion releasing bioactive glasses
A. R. Boccaccini[1]

[1]University of Erlangen-Nuremberg, Erlangen, Germany

It is well-known that biochemical reactions occurring at the interface between bioactive glasses (BGs) and the biological environment, which involve the release of BG dissolution products, are relevant for both hard and soft tissue regeneration. In this presentation, the development of biologically active ion doped BGs for bone and soft tissue engineering will be discussed, comprehensively covering current research in the field and considering silicate, phosphate and borate BG compositions. Results of both in-vitro and in-vivo studies will be presented that demonstrate the high potential of inorganic ions (or bioinorganics) and combination of ions in specific concentrations to stimulate relevant cellular pathways leading to important regenerative outcomes: from osteogenesis and tissue vascularization, to wound healing, nerve and muscle tissue repair. Moreover, the results of in-vitro studies which confirm the effects of BG dissolution products on cell behavior in relation to angiogenesis will be discussed in detail considering BG compositions incorporating ions such as Cu, Sr, Li and B. The effect of BG dissolution products on vascular endothelial growth factor release, induction of hypoxia conditions and endothelial cell behavior when exposed to different (time-dependent) ionic concentrations will be covered. Potentially active mechanisms of interaction of BGs and tissues based on the surface bioreactivity of BGs will be presented and the development of specific combinations of BGs and biopolymers to create flexible bioactive composites for soft tissue repair will be shown. Finally, incorporation of mesoporous BG nanoparticles in 3D printable hydrogels to develop multifunctional composite bioinks for biofabrication and tissue engineering will be discussed highlighting open areas for future research.

Keynote lecture
KL 4
How blood clotting initiates the first steps in the healing response
S. Lickert[1], V. Vogel[1]

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Blood has a plethora of properties that by far exceed those of any manmade biomaterial. It is the living material that Nature evolved to rapidly stop bleeding, but equally important, to then steer the subsequent regenerative processes. Any attempts to make artificial blood that at least mimics blood's most crucial functions have failed so far. While blood and blood born cells have been in the spotlight of research since decades, we still know rather little about the mechanobiological aspects and how they steer regenerative processes. From platelets to the extracellular matrix which they assemble and remodel, to the formation of a hematoma, from clot entrapped blood born cells to invading cells, a blood-filled site of injury defines highly complex multicellular niches that undergo rapid transformations. As bioengineers, we need to learn more about the underpinning mechanisms, as they ultimately define whether scar free healing can occur or gets obstructed by manmade material we generate. Our most recent results will be discussed.

Keynote lecture
KL 5
Different biomaterial-based strategies to promote vascularization
R. Perez Antoñanzas[1]

[1]Universitat Politècnica de Catalunya Llicenciat Química, Barcelona, Spain

The innate ability of bone to regenerate itself following injury is widely recognized. However, this natural process can be hindered when there is significant damage, often due to the failure to establish a new vascular network necessary for oxygen and nutrient transport. Originally, bone tissue engineering (BTE) focused on using inert biomaterials solely to fill bone defects. However, it has since advanced to mimic the structure of bone extracellular matrix and promote the physiological regeneration process of bone. Consequently, there has been a growing emphasis on stimulating osteogenesis, particularly through the promotion of angiogenesis, which is crucial for successful bone regeneration. Furthermore, creating vascular structures that resemble native blood vessels is also essential to allow prevascularization of scaffolds as well allow to find alternative strategies to test vascularization, avoiding the use of animals.

Keynote lecture
KL 6
The role of Interleukin-6 in immune modulation

S. Rose-John[1]

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The inflammatory cytokine Interleukin-6 (IL-6) is involved not only in most inflammatory states. It also plays a prominent role in the development of inflammation associated cancers. On the other hand, IL-6 is also involved in the defence of the human body from infections [1, 2]. The response of target cells to IL-6 depends on the presence of the membrane-bound IL-6 receptor (IL-6R), which on the cell membrane presents IL-6 to the signal transducing receptor subunit gp130, which is expressed on all cells of the body. Interestingly, the expression of IL-6R is limited to only few cells including hepatocytes, some immune cells and epithelial cells. These cells are therefore direct IL-6 target cells for IL-6. The IL-6R can be cleaved by proteases and the thus generated soluble IL-6R (sIL-6R) still binds the ligand IL-6. The complex of IL-6 and sIL-6R can bind to gp130 on any cell, induce dimerization of gp130 and intracellular signaling. This process has been named IL-6 *trans-signaling*. Importantly, this process drastically enlarges the spectrum of IL-6 target cells to virtually all cells of the body. It turned out that the activities of IL-6 mediated by the sIL-6R are the pro-inflammatory activities of the cytokine whereas activities of IL-6 mediated by the membrane-bound IL-6R are rather protective and regenerative [1-3]. In the blood large amounts of sIL-6R and even larger amounts of a soluble form of gp130 (sgp130) act as a buffer for IL-6, which under non-inflammatory conditions prevents systemic IL-6 *trans-signaling* [1, 2] The biologic relevance of the sIL-6R/sgp130 buffer in the blood during regenerative and inflammatory states will be discussed.

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1. Rose-John S, Jenkins BJ, Garbers C, Moll JM Scheller J (2023) Targeting IL-6 trans-signaling: past, present and future prospects. Nat Rev Immunol 17: 1-16
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Keynote lecture
KL 7
3D-characterisation of the biomaterials-tissue interaction on the (sub-) micron length-scale using synchrotron-imaging

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The successful integration of any material introduced to bone depends on effective interaction with the surrounding primary bone tissue. This interaction is facilitated by the bone's ability to adapt to changing external stimuli through a continuous remodeling process. This process balances material formation and resorption to ensure mechanical competence, maintain well-balanced mineral homeostasis, and integrate newly formed bone tissue or biomaterials.

All bone cells are involved in the remodeling process, but the key coordinators are the most frequent bone cells, the osteocytes. These cells are embedded in the mineralized tissue and interconnected via their dendrites, forming a complex 3D osteocyte-lacunar canalicular pore network (OLCN). Due to the small diameter of the canalicular channels, only a few hundred nanometers, the morphological assessment of the OLCN in sufficiently large volumes was not possible in the past. However, with advances in synchrotron nano-CT imaging, the required spatial resolution and sensitivity to robustly characterize the OLCN in mineralized bone tissue in 3D has been achieved.

Exploiting this technique, we demonstrate and discuss how newly formed cellular dendrites actively connect to the OLCN of pre-existing bone tissue. We hypothesize that the available OLCN contributes to the mineralization of newly formed bone as well as to other transport processes via these canalicular links. Consequently, alterations of these links may also help explain pathological or age-related declines in bone quality, impaired bone healing, and biomaterial integration. We propose this newly discovered continuity of the OLCN as a decisive factor for maintaining bone quality and potentially for the integration of bone replacement materials. Additionally, we will outline how synchrotron nano-CT imaging can be better utilized by the medical community.

Keynote lecture
KL 8
Complex in vitro-Models for Preclinical Testing of Biomaterials

Thomas Hartung[1]

[1]The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, US

Science has become a central societal driver; in turn, substantial funding but also enormous expectations as to the benefit from science have been created. These include expectations to provide us with safe and effective drugs and medical devices as well as protect us against toxicants. While drug development is based on a competition of ideas, the safety sciences largely lack this competition employing often protocols in essence unchanged for more than 50 years. Bioengineering and stem cell technologies have changed the way cell culture can be carried out. This is increasingly embraced in the product development process. It is timely, to leverage these technologies also for the safety of patients and consumers, where both an enormous ignorance of the potential risks of large numbers of substances and chemophobia fueled by precautionary approaches and disproportionate risk communication are the consequence. The advent of microphysiological systems (MPS), i.e., cell cultures replicating aspects of organ architecture and functionality represent a key scientific opportunity to develop more relevant test systems and serve society. In combination with the novel computational tools from machine learning (A.I.) and improved objective and transparent handling of the resulting evidence, science, economical applications, regulation and policy-making are served. The field of MPS is forming with dedicated conferences, societies, journals, best practices for culture (GCCP 2.0) and reporting standards, and educational offers.

O 1

Impact of deep brain stimulation on neuronal network mechanisms in generalised dystonia

F. Santana Kragelund[1], D. Franz[1], M. Heerdegen[1], A. Lüttig[2], S. Perl[2], A. Richter[2], R. Köhling[1]

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[2]Leipzig University, Institute of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Leipzig, Germany

Introduction: Pallidal deep brain stimulation (DBS) is an effective treatment for generalised, which improves dystonia severity by up to 60 %. However, the outcome for the individual patient remains unpredictable due to unknown mechanisms of the DBS treatment. With our electrophysiological and immunohistochemical experiments, we want to clarify the mechanism of DBS dystonia to enable higher treatment success rates.

Methods: We are investigating the synaptic transmission and network activities within the cortico-basal ganglia-thalamo-cortical as well as the cerebello-thalamo-cortical pathways in an animal model of paroxysmal generalised dystonia affected by DBS. For this purpose, we implanted bipolar stimulation electrodes bilaterally into the dtsz mutant hamster's globus pallidus internus. We fully implanted the STELLA stimulation system in the hamster's flank for continuous long-term DBS (130 Hz, 50 µA) over 11 days.
We defined two experimental groups: : (1) native dtsz mutant hamsters (dtsz), (2) non-dystonic control hamsters (WT), (3) dtsz mutant hamsters continuously stimulated for 11 days (dtsz -DBS), and (4) dtsz mutant hamsters undergoing the surgery, however with no active DBS (dtsz -Sham).. We use field potential, high-density microelectrode array for our electrophysiological recordings of acute cerebellar slices using micro electrode array.

Results: Our results indicated unexpected effects of pallidal DBS on the thalamus by upregulating the excitatory tone rather than direct inhibitory projections. O measurements demonstrated a gained mean firing rate to the range of healthy control in the spinocerebellar cortical network, while the neuronal activity within the deep cerebellar nuclei was decreased after short-term DBS.

Conclusion: Our results confirmed our hypothesis of a global network effect of DBS rather than a local impact on the stimulation target.

O 2

The Role of PTHrP for the Mechanoresponse of Mesenchymal Stromal Cell-Derived Chondrocytes

J. Lueckgen[1], J. Buchert[1], S. Diederichs[1], W. Richter[1]

[1]Heidelberg University Hospital, Research Centre for Experimental Orthopaedics, Heidelberg, Germany

Introduction: We have previously shown that physiological mechanical loading can reduce extracellular matrix (ECM) synthesis in mesenchymal stem cell (MSC)-derived neocartilage, which may severely limit their use for regeneration of high load-bearing articular cartilage. The biochemical signals that control this unfavourable mechanoresponse are poorly understood. Parathyroid hormone-related protein (PTHrP) is a known regulator of chondrocyte ECM synthesis and is induced by load in many other cell types, but whether it regulates MSC mechanoresponse remains unknown.

Objective: To assess whether PTHrP could be the factor responsible for the unfavourable load response, we tested its i) mechanoinduction and ii) its ability to mimic load-effects on mechanoresponse genes and ECM synthesis. Identification of PTHrP as target to rescue detrimental load effects may significantly improve current biomechanically inadequate MSC-based cartilage engineering approaches.

Materials and methods: Cartilage constructs were engineered from human bone marrow-derived MSC in collagen scaffolds. After 21 days effects of physiological loading (25% compression, 1Hz, 3h) or treatment with PTHrP(1-34), cAMP analogue or adenylate cyclase inhibitor MDL-12,330A were analyzed by radiolabeling, ELISA, qPCR or Western Blot.

Results: Load significantly stimulated levels of PTHrP mRNA and its second messenger cAMP in MSC-derived neocartilage constructs. PTHrP treatment increased a subset of mechanoresponse mRNAs (FOS, FOSB, BMP6) and, like load, decreased ECM de-novo synthesis. Inhibiting cAMP production during loading preserved GAG synthesis rates. Additionally, both load and PTHrP treatment were able to reduce expression of the hypertrophy markers MEF2C and PTH1R.

Conclusion: Our data indicate for the first time PTHrP-cAMP signalling as mechanotransducer in human MSC-derived neocartilage and PTHrP inhibition as a promising tool to enhance the mechanocompetence of MSC-based neocartilage for regenerative medicine.

O 3

Electrical stimulation of degenerative and non-degenerative chondrocytes for articular cartilage regeneration

J. Waletzko-Hellwig[1], N. Abroug[2], H. Seitz[2], L. Schöbel[3], A. R. Boccaccini[3], A. Jonitz-Heincke[1], R. Bader[1]

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[2]University, Microfluidics, Rostock, Germany
[3]University, Materials Science and Engineering, Erlangen, Germany

(1) Once damaged, cartilage cannot regenerate itself due to the lack of vascularization. Furthermore, degenerative diseases are accompanied by a de-differentiation of chondrocytes into hypertrophic cells. These cells cannot provide an appropriate hyaline extracellular matrix that can withstand common physiological loads. This, in turn, leads to further impairment of the joint, also affecting the subchondral bone.

(2) In this study, the effect of electrical stimulation (ES) on chondrocytes cultivated in ADA-GEL was evaluated. The response of human cartilaginous non-degenerative (ND) as well as osteoarthritic (OA) cells was examined to analyze the effect of ES therapy on cell-type specific reactions.

(3) OA or ND cells were added with a concentration of 10^6 cells per ml ADA-GEL. Following the addition of microbial transglutaminase scaffolds with Æ 10 mm and 1 mm height were prepared. After the incubation with 0.1 M CaCl2 solution, scaffolds were transferred and coated with cell culture medium. ES was applied for 7 days (1 kHz and 0.7 Vpp; 3 times 45 min/day). Deposited cartilage matrix proteins were analyzed.

(4) ND and OA cells showed a similar deposition of glycosaminoglycans (GAGs) and collagen II after 7 days of cultivation. While ES promoted GAG synthesis in OA cells, a higher deposition of collagen II in ND cells was measured. Furthermore, the content of inflammation-associated lumican and VEGF was similar for OA and ND cells. Following ES, OA cells showed a significant reduction of both factors ($p=0.0078$).

(5) Our data point out the positive effect of ES on OA chondrocytes and underline the different responses of OA and ND cells to biophysical stimuli. The anti-inflammatory effect and formation of collagen II matrix showed, that ES may be feasible for both *ex vivo* application to induce re-differentiation of human chondrocytes and *in situ* application.
This study was funded by the Deutsche Forschungsgemeinschaft (German Research Foundation)– SFB 1270/2-299150580.

O 4

Quantitative imaging based on deep learning reveals fat infiltration in nerve-damaged muscles not to be triggered by electrical stimulation

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[2]Friedrich Schiller University Jena, Jena, Germany, Faculty of Biological Sciences, Jena, Germany
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[4]Friedrich Schiller University Jena, Jena, Germany, Institute of Microbiology, Jena, Germany

Quantitative imaging has become an essential approach in life sciences. It combines microscopy acquisition using numerous imaging techniques with automated analysis based on software frameworks. Automated image analysis is a powerful tool for analyzing the effects of functional electrical stimulation (FES) from an implanted laryngeal pacemaker (LP) on muscle tissue.

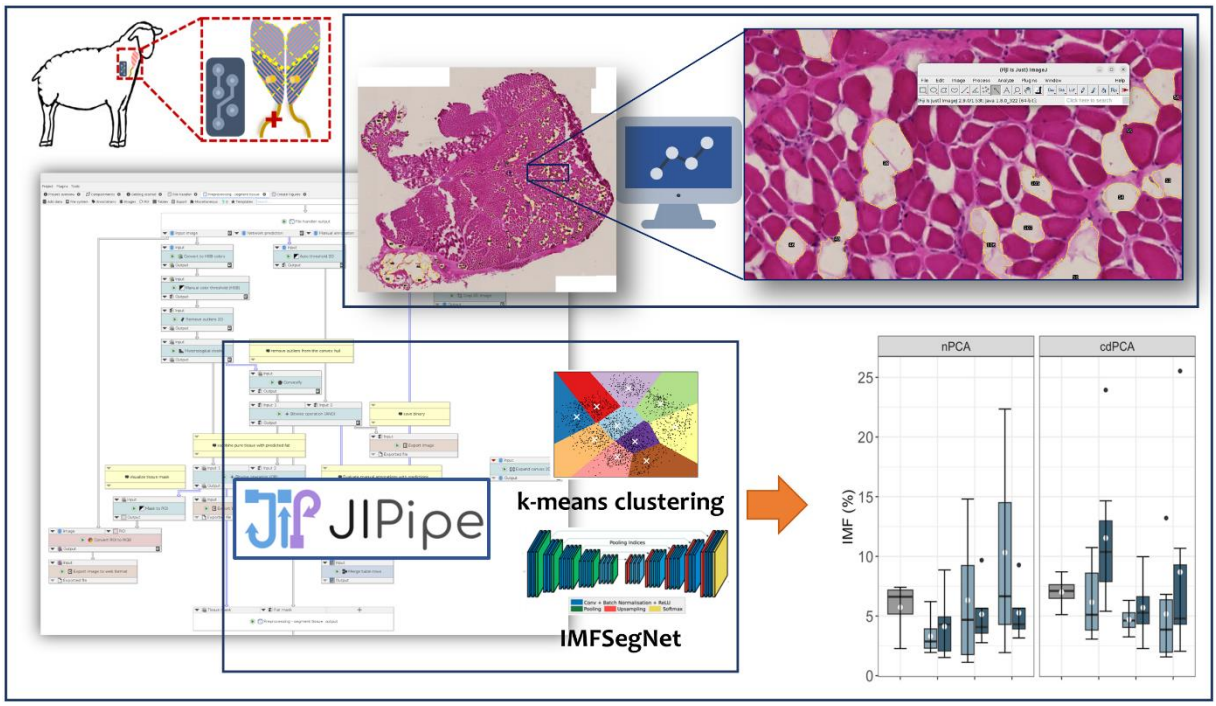
The present study focuses on the assessment of muscle tissue affected by an implanted LP that may support inspiration in patients with bilateral vocal fold paresis. The posterior cricoarytenoid muscles (PCA) of sheep were quantified to evaluate the effects of LP after long-term FES with different stimulation parameters.

Due to the large sample size and time constraints, a qualitative analysis by a pathologist is not feasible. Therefore, it is crucial to ensure that the image processing is as precise, fast, and objective as possible. To demonstrate the validity of an automated analysis, a comparison was made on 17 images using various image analysis approaches across five different metrics [1]. After determining that the neural network-based SegNet [2] was the most suitable, we utilized the pre-trained model, IMFSegNet, to quantify about 400 H&E-stained muscle sections. We then integrated the entire comparison, including IMFSegNet, into the open-source visual programming language JIPipe [3]. This allows for use by individuals without programming skills.

Previous studies have shown no evidence of atrophy or fibrosis resulting from functional electrical stimulation by LP on fiber types, muscle fiber size, and collagen quantity [4]. This study hypothesized that FES would not significantly alter the amount of fat in muscle cross-sections, which we can confirm using quantitative imaging and deep learning. Our findings extend previous studies on intramuscular fat in cross-sections.

This study confirms the safety of using LP in clinical applications and demonstrates the effectiveness of combining deep learning-based techniques with cost-effective quantitative imaging for objective and automated analysis.

Fig. 1


JIPipe
k-means clustering
IMFSegNet
nPCA
cdPCA
IMF (%)
0
5
10
15
20
25

O 5

Blood Vessels Regeneration using Magnetic Fields

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The design of physiologically relevant blood vessel in vitro models has been impaired by the difficulty to reproduce the complex architecture of native blood vessels and the mechanisms mediating key cellular functions within miniaturized perfusable systems. Aiming to simulate blood vessel walls, in this work innovative 2D platforms are designed and patterned with magnetic-responsive gelatin for enabling in situ co-culture of mesenchymal stromal cells (MSCs) and human umbilical vein endothelial cells (HUVECs) within confined compartments. The performance of the 2D chips is evaluated based on HUVECs migration, adherence, and angiogenic behavior (proliferation and sprouting), as well as production of Endothelin-1 (endothelium marker), and compared with the results of 3D single channel models, designed to mimic the morphology of native arteries and veins. The 2D chips obtain better cell adhesion and angiogenic performance, which is attributed to flow profiles and VEGF concentration gradients. Magnetic stimulation is then used as a novel strategy to increase cell sprouting and endothelization ≈1.5 times above the control condition. These bio-inspired devices advance the exploration of magnetism for a finer convergence to the native vascular conditions in vitro and improved modulation of angiogenesis, showing promising contributions to the development of sophisticated therapeutics for vascular ischemia-related diseases.

Fig. 1

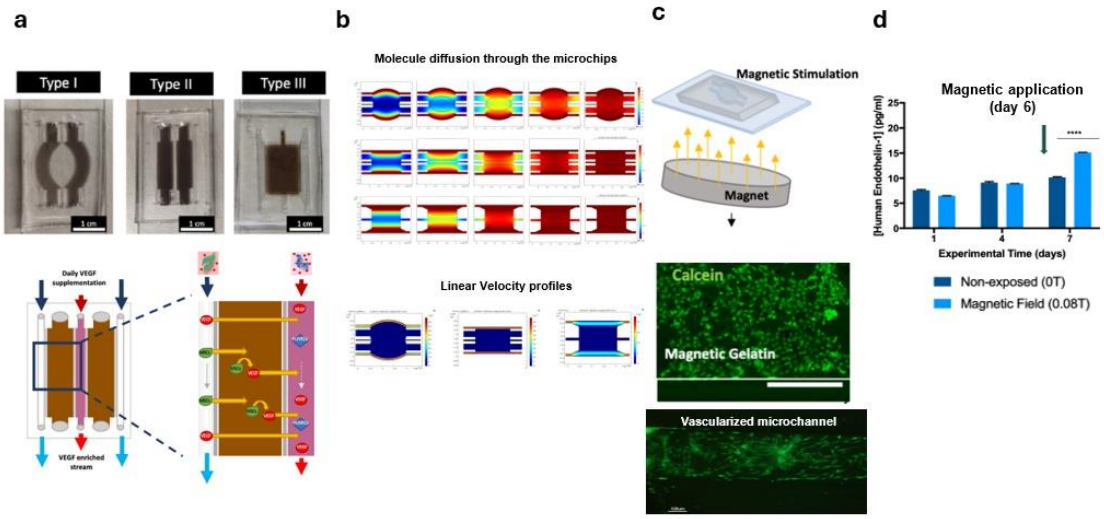

Figure 1. *Representation of the microchip design (a), molecule diffusion and velocity profile predicted by fluid dynamics simulation (b), confocal imaging of microvessel layer formed in the presence of magnetic actuation (c) and endothelium biomarker quantification (d). Scale bar: 1000 μm.*

Fig. 2

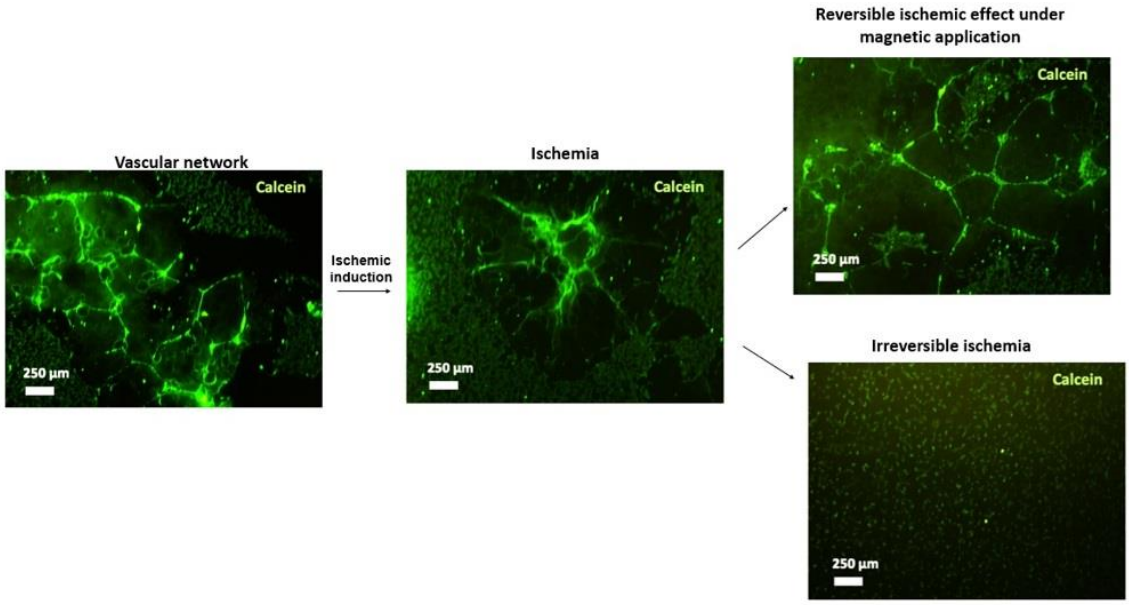

Figure 2. *Microscopic calcein stained images of the living cells in ischemia model. Sequential representation of the ischemic induction-on chip and the reversible effect obtained upon magnetic application, with microvessel network restored, in comparison with the control condition showing irreversible ischemic effect.*

O 6

Laser patterning of 3D printed near-beta Ti-13Nb-13Zr alloy for guided response of human bone marrow stromal cells

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Introduction: Additive Manufacturing (AM) of low modulus beta-Ti alloys paves the way for patient-specific implants which reduce stress shielding. Laser surface functionalization can further improve bone healing.

Objectives: The aim is to design a laser-based processing chain for a beta-type Ti alloy with (i) AM of 3D parts with defined microstructures and (ii) surface texturing with direct laser interference patterning (DLIP). *In vitro* studies with hBMSC help to clarify how those tailored textures guide the cell response.

Materials & methods: Ti-13Nb-13Zr parts are fabricated by laser powder bed fusion (LPBF) and microstructure - mechanical performance relations are studied [1]. DLIP with nanosecond (ns) and picosecond (ps) pulses generates single-scale and multi-scale topographies, respectively. These surface textures are analysed with LSM, SEM, TEM, AES and their impact on wettability and corrosion in PBS is studied. Their impact on the hBMSC behavior is assessed with fluorescence microscopy, SEM and MTS assay; TNAP activity serves as early differentiation marker [2,3].

Results: LPBF and 900°C HT samples exhibit high tensile strengths and low elastic moduli (~75 GPa). The ns-DLIP textured surface exhibit high beta-phase fractions and thick passive films that enhance the corrosion stability compared to ps-DLIP. The ns-DLIP textures encourage cell extensions anchored in grooves, ps-DLIP textures promote cell extensions attaching to nanostructures on walls. The groove width and nanotopography facilitate cell spreading. Topography and chemistry influence cell adhesion, proliferation, and differentiation.

Conclusions: Surface-functionalized 3D printed Ti alloys hold potential for a novel generation of biocompatible implants. Funded by EFRE, Parliament of Saxony (100382988/-89), EC (H2020-BIOREMIA, GA 861046).

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P. Goldberg et al., *Corros. Sci.* **2023**, 219, 111230
A. Hariharan et al., *Adv. Funct. Mater.* **2023,** 2310607

O 7

Dual chitosan hydrogel and polylactic acid microparticle biomaterial system for enhanced antimicrobial efficacy against Staphylococcus aureus infection

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Introduction: Osteomyelitis is an often-chronic infection of bone and/or bone marrow. Bone and orthopedic hardware are particularly susceptible to biofilm-forming strains of *S. aureus*. Typical treatment is debridement and prolonged, systemic antibiotics, which can lead to organ toxicity and antimicrobial resistant bacterial strains. Thus, biomaterials with inherent antimicrobial properties for local delivery are of dire need.

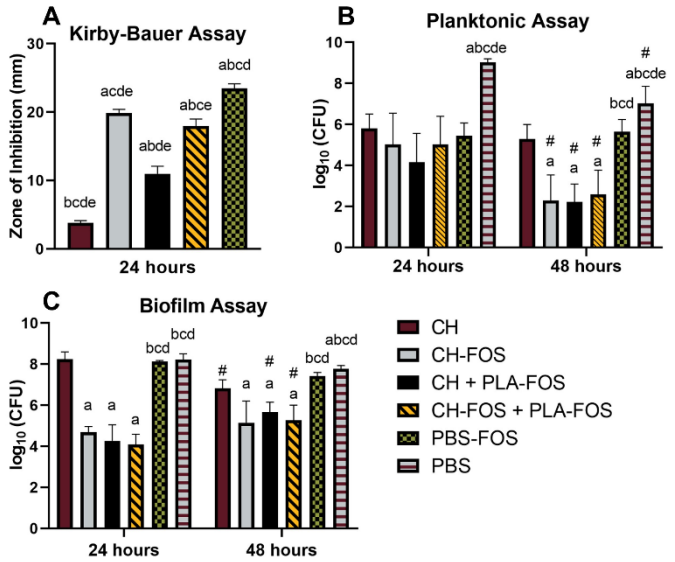
Objective: The objectives of this work were to evaluate the antimicrobial efficacy of a dual biomaterial system: polylactic acid (PLA) microparticles loaded within antimicrobial chitosan hydrogel (CH), each containing fosfomycin (FOS) antibiotic, against *S. aureus in vitro* and *in vivo*.

Material & methods: FOS was loaded in CH, in PLA microparticles within CH, or divided equally between CH and PLA microparticles (negative control: blank CH). Antimicrobial efficacy was evaluated against *S. aureus in vitro* using Kirby-Bauer, planktonic, and biofilm assays, and *in vivo* using an implant-based rat model of femoral and soft tissue chronic infection. A colonized screw was placed into a bicortical femoral defect; at 7 days, the screw was removed and treatments were applied locally. Radiographs and serum haptoglobin were evaluated through day 35. CFU counts of bone and soft tissue were quantified at day 35.

Results: In Kirby-Bauer, the CH with FOS groups were more effective than blank CH. Antimicrobial effects of CH and FOS were additive against *S. aureus* in planktonic and biofilm forms, with time dependent effects seen on planktonic cultures. *In vivo*, defect area, haptoglobin level, and bacterial burden in bone and soft tissue were lowest in the dual biomaterial group compared to all other groups.

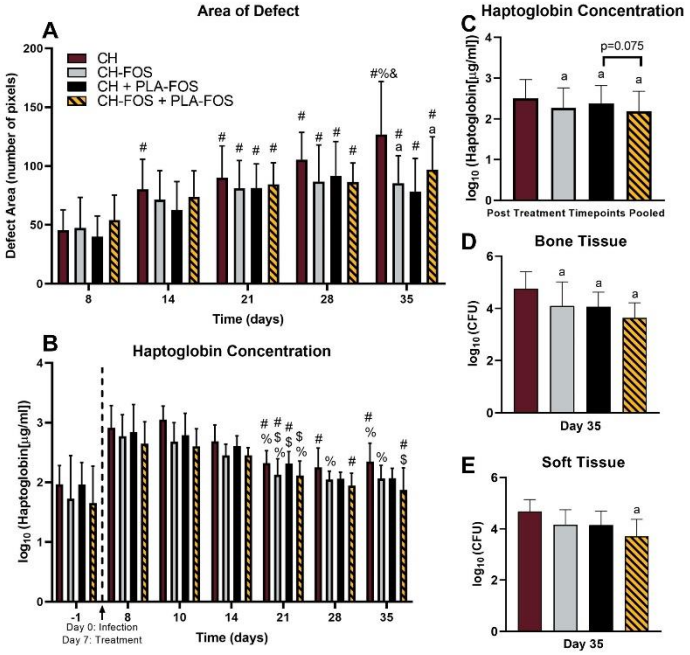
Conclusion: FOS in chitosan hydrogel and PLA microparticles mitigated *S. aureus in vitro* and in a clinically relevant osteomyelitis model. Multifunctional antimicrobial therapeutics such as these may improve targeted delivery against challenging, recalcitrant infections.

Fig. 1



A) Kirby-Bauer diffusion assay showed all groups were different from one another (n=6). B) The planktonic assay showed the groups containing chitosan (CH) and fosfomycin (FOS) had lower bacterial counts than all other groups at 48 hours (n=8). C) The biofilm assay showed the groups containing CH and FOS had lower bacterial counts than all other groups at 24 and 48 hours (n=6-8). a=different than CH, b=different than CH-FOS, c=different than CH + PLA-FOS, d=different than CH-FOS + PLA-FOS, e=different than PBS-FOS, and #=different than 24 hours (α=0.05).

Fig. 2



A) Defect area was larger for all groups at day 21 compared to day 8. By day 35, defect area for the blank chitosan group (CH) was larger than on days 8, 14, and 21, and larger than CH-FOS and CH-FOS + PLA-FOS at day 35 (n=11). B) Pro-inflammatory haptoglobin peaked 1-3 days after treatment surgery and decreased over time for all groups (n=10-11). C) Pooled across days 8, 10, 14, 21, 28, and 35, haptoglobin was lower in all groups containing FOS compared to blank CH. D) Likewise, bacterial burden in bone tissue was lower in all FOS groups compared to blank CH (n=11). E) In adjacent soft tissue, only the dual CH-FOS + PLA-FOS group had a lower bacterial burden than blank CH (n=11). a=different than CH, #=different than day 8, $=different than day 10, %=different than day 14, and &=different than day 21 (α=0.05).

O 8

Developing a Glycocalyx-inspired Polyelectrolyte Multilayer Model to establish Intermediate Water Structures for anti-thrombogenic Biomaterials

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Despite the enormous advances in medicine in recent years, currently available biomaterials still cannot optimally support therapeutic approaches. Especially, the hemocompatibility of materials and coatings is not sufficient to prevent additional treatment with anti-coagulants.
A new approach in research is needed to close this gap of knowledge. In our project, the charge of polyelectrolyte multilayers (PEMs) is precisely varied to get a systematic model system for investigating the influence of physicochemical properties on the hydration state of the PEMs considering the known ´three-state´ model of water and its impact on the hemocompatibility.

Inspired by nature, the charges of anionic glycosaminoglycans from the endothelial glycocalyx are manipulated by changing chemically the number of sulfation groups per subunit. Combined with the biocompatible cationic natural polymer chitosan PEMs result, that exhibit a gradually varied charge density. The PEMs are analyzed for surface and bulk properties such as charge, stiffness and hydrophilicity while a key aspect of the characterization is the detection of surface associated water layers by advanced infrared reflection absorbance spectroscopy. The found properties are correlated with the biological answer defined by protein adsorption, platelet adhesion and cytotoxicity.
The results of this project facilitate the establishment of a "tailor-made" polyelectrolyte multilayer as a mimicry of the endothelial glycocalyx (eGCX) that forms a stable water barrier consisting of intermediate water in order to acquire an excellent anti-thrombogenicity.

O 9

Osseous integration of newly established porous 3D polyamide-ε-caprolactone scaffolds

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Introduction: Recently established polymer polyamide-ε-caprolactone (ACM) is suitable for generation of biodegradable 3D-scaffolds that mimics the osteochondral region. Cartilage regeneration is necessary during healing of osteochondral defects. Besides, bony integration is important to prevent the sinking of the articular surface. Thus, we implanted the scaffolds into a sheep model of osteochondral knee defects and analyzed the bony biomaterial-tissue-interface.

Objectives: The study aimed to determine the bony integration of the osseous implant phase especially at the interface.

Materials & methods: ACM scaffolds with 7 mm diameter and 10 mm height were filled with biogel prior to implantation into osteochondral knee defects in the distal femur of adult female sheep (n=5). After 3 months, animals were sacrificed, implant region extracted, and prepared for paraffin-embedded histology, immuno- and, enzyme histochemistry followed by histomorphometrical surveying. As controls, implants generated by poly-((D,L)-lactide-ε-caprolactone)-dimethacrylat (LCM) were used.

Results: After 3 months, the implants were fully integrated into the bony tissue. Cells adhered to the scaffold and all pores were filled with connective tissue and sometimes already mineralized bone. Blood vessels grew into the porous structures. Formation of osteoclasts were observed using TRAP enzyme histochemistry. Osteoblasts were detected by runt-related transcription factor 2 (RUNX2) immunohistochemistry and its up-regulation at ACM compared to LCM implants was determined by means of histomorphometry ($p<0,05$, Kruskal-Wallis, Mann-Whitney).

Conclusion: The results showed that biomimetic ACM as well as LCM scaffolds are suitable implants for the subchondral region of osteochondral defects. An increase in RUNX2 immunopositive area at the ACM scaffolds might be a first sign for stimulation of bone formation. However, this needs to be proven carefully by further investigations.
Funded by BMBF (FKZ13XP5089)

O 10

Repair of full-thickness osteochondral defects with printed absorbable zinc-biogel scaffolds

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[2]University of Science and Technology, Beijing, China
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Introduction: Upon suboptimal treatment, deep chondral/osteochondral defects (OCD) accelerate progression of osteoarthritis (OA) with a **severe socioeconomic burden**. Current repair techniques suffer from several limitations.

Objective: A bilayered medical device (MD) of an absorbable porous zinc (Zn) scaffold with a chondroitin sulfate (CS) hydrogel as top layer may be a novel treatment option.

Materials & methods: A diamond unit cell-based porous scaffold was printed by Laser powder bed fusion from pure Zn powder (15-53µm, 30µm layer thickness, 40 J/mm3 energy density). Upon cleaning, quality controls (Electron back-scattered diffraction and JEOL JSM-6500 F), and mechanical characterization (2 mm/min compression; ISO 13,314:2011), MDs were overlayed with chondroitin sulphate hydrogels. Chondro- and osteogenesis were monitored by RT-qPCR (seven markers), histochemical assays (ALP, Alizarin Red), and ELISA (IL-1β, TNF-α), respectively. Cytocompatibility was assessed through CCK-8 assays with rBMSCs and biocompatibility was evaluated in a porcine trochlear groove OCD model (n=8/group) with histological and mCT/MRI analyses. Statistics comprised t-tests and one-way ANOVA.

Results: With 82 kPa, the compressive strength of the CS hydrogel was similar to that of native articular cartilage. The Zn scaffold exhibited a yield strength of 11 MPa and a stiffness of 0.8 GPa, similar to cancellous bone. *In vitro*, the bilayered medical device promoted chondrogenic and osteogenic differentiation of rat bone marrow stem cells (rBMSC). In the porcine OCD model, the bilayered medical device resulted in the convincing formation of both cartilage and bone tissue, respectively. As compared to controls, a smoother cartilage surface, a more hyaline-like cartilage morphology, and a superior integration into the host tissue was found.

Conclusion: Our findings suggest that the novel bilayered Zn-CS hydrogel MDs hold potential as clinically viable solution for osteochondral repair.

Fig. 1

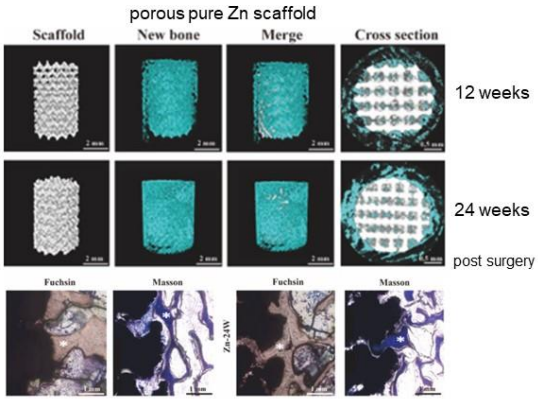
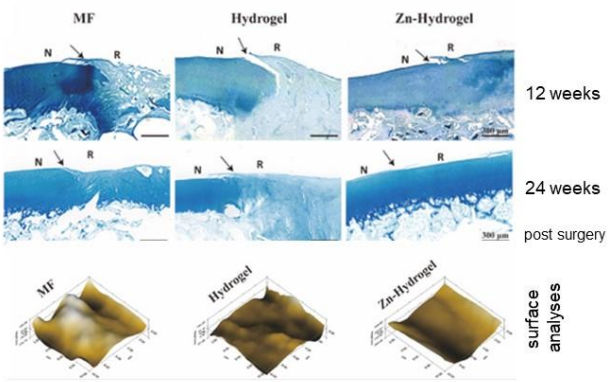

porous pure Zn scaffold
Scaffold
New bone
Merge
Cross section
12 weeks
24 weeks
post surgery
Fuchsin
Masson
Fuchsin
Masson
Zn-24W

Fig. 2


MF
Hydrogel
Zn-Hydrogel
N
R
12 weeks
24 weeks
post surgery
MF
Hydrogel
Zn-Hydrogel
surface analyses

O 11

Micro- and nanostructuring of polymer-based biomaterials for regenerative medicine

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[2]Fraunhofer Institute for Cell Therapy and Immunology IZI, Halle, Germany

Introduction: Polymers are used as implant materials in many medical applications. It is known that the adhesion and proliferation of cells depends on the physico-chemical properties of the raw material and the surface properties of the polymers. [1,2]

Objectives: It would therefore be desirable to have a biomaterial that provokes a specific bio-response through the targeted design of the physico-chemical properties of the surface.

Materials & methods: A new approach to surface modification of polymer biomaterials is thermoforming using micro- and nanostructured moulding tools. These are produced using laser structuring and electrochemical anodization. In addition to the surface structure, the chemical properties of the polymers were optimised using physical plasma processes. The evaluation of the cell behaviour was carried out by cell growth studies.

Results: The plastic surfaces could be structured with hierarchical structures in micro- and nanometer range (Fig. 1). The hydrophilicity and the antistatic and adhesive properties could be optimised by the plasma-chemical processes through activation with allylamine, hydroxyl and carboxyl groups. The cell growth experiments showed that cell adhesion and proliferation could be specifically influenced by the structural and chemical surface properties.

Conclusion: In summary, it can be said that the developed process enables a previously unattainable, targeted surface structuring and modification of polymers and opens new avenues in the provision of polymer biomaterials for therapeutic approaches in regenerative medicine.

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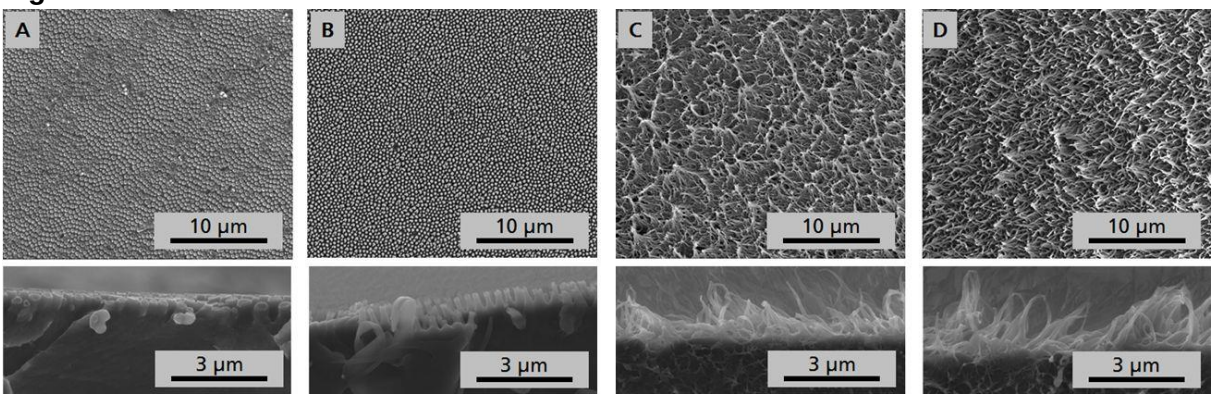
[1] Xiang, Tao, et al. "Biomimetic micro/nano structures for biomedical applications." Nano Today 35 (2020): 100980.
[2] Limongi, Tania, et al. "Fabrication and applications of micro/nanostructured devices for tissue engineering." Nano-micro letters 9 (2017): 1-13.

Fig. 1



O 12

Osteogenic potential of molybdenum doped mesoporous bioactive glass nanoparticles

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Mesoporous bioactive glass nanoparticles (MBGNs) are promising carriers for delivering biologically active ions and enhancing osteogenic properties. Molybdenum (Mo) is commonly utilized in orthopedic surgery within Cobalt-Chromium-Molybdenum (Co-Cr-Mo) alloys. Nevertheless, there is no substantial data illustrating Mo's effect on human bone marrow-derived mesenchymal stromal cells (BMSCs). Therefore, in this study, MBGNs (nominal composition in mol%: 70SiO2, 30CaO) and its Molybdenum doped MBGNs (nominal composition in mol%: 70SiO2, 25CaO, 5MoO3) synthesized using micro-emulsion based sol-gel process. The synthesized MBGNs (doped and undoped) were characterized by morphology, composition, and in vitro bioactivity. Scanning electron microscopy (SEM) and Brunauer-Emmett-Teller (BET) analysis confirmed that the particles had spherical morphology with disordered mesoporous structure. Energy dispersive X-ray spectroscopy (EDX) confirmed the presence of Si, Ca and Mo in the synthesized molybdenum-doped MBGNs. Further, the impact of molybdenum-doped MBGNs on the viability, proliferation and osteogenic differentiation of human BMSCs was investigated to assess the general suitability of Molybdenum as a part of MBGNs for use in bone tissue engineering applications. Initial results revealed a dose-dependent positive effect towards osteogenic differentiation.

O 13

Innovating TPU Scaffolds: A Green Synthesis Approach for Nonisocyanate Polyurethanes in Cardiac Tissue Engineering

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[2]NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany

Introduction: Traditional synthesis of thermoplastic polyurethanes (TPUs) often employs hazardous isocyanates and tin-based catalysts, posing environmental and health risks. A green synthesis approach is vital for safer, biocompatible TPU scaffolds.

Objectives: Our objective is to develop nonisocyanate polyurethanes (NIPUs) via an eco-friendly process, aiming to reduce ecological impact and improve biocompatibility for biomedical applications, particularly in cardiac tissue engineering.

Materials & Methods: We synthesized high-molecular-weight NIPUs through transurethanization of 1,6-hexanedicarbamate with various molecular weights of polycarbonate diols (PCDLs). Characterization involved nuclear magnetic resonance, Fourier-transform infrared spectroscopy, gel permeation chromatography, and differential scanning calorimetry. Electrospinning was utilized to create fibrous scaffolds.

Results: The highest molecular weight achieved was 58,600 g/mol. Scanning electron microscopy confirmed the formation of submicron-sized fibers in the scaffolds. Preliminary biocompatibility was assessed using primary human fibroblasts and a human epithelial cell line, showing promising results for biomimetic applications.

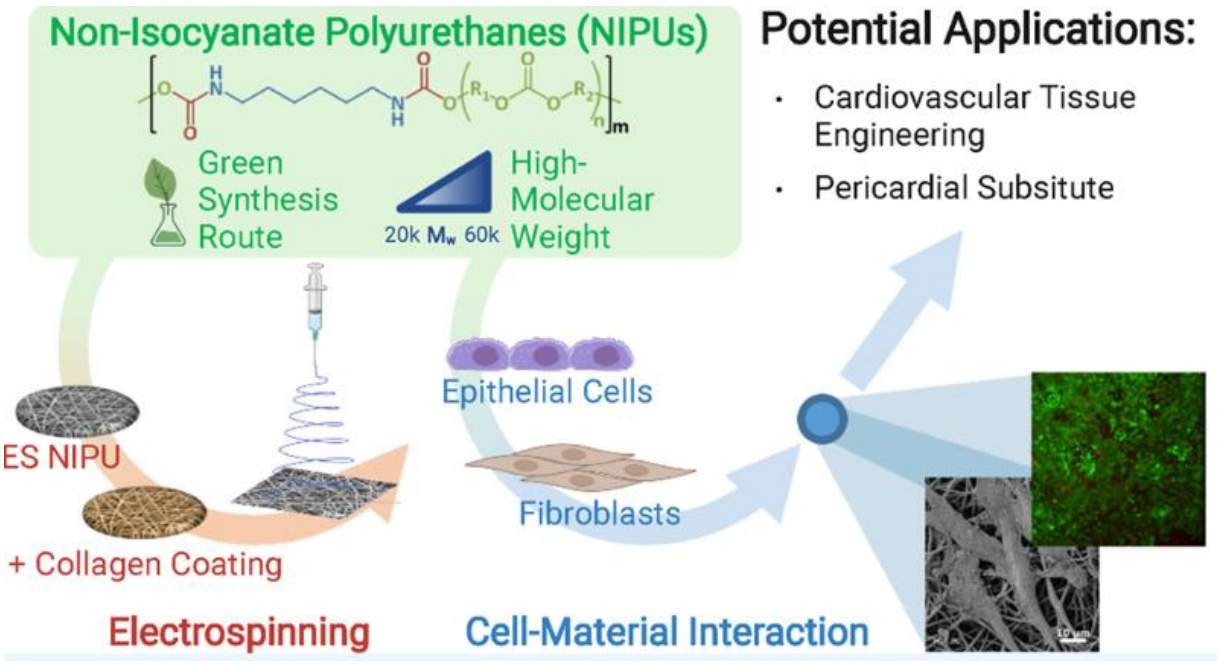
Conclusion: Our study demonstrates a successful green synthesis route for NIPUs, leading to the production of biocompatible, high-molecular-weight materials suitable for electrospinning into scaffolds. These NIPUs show significant potential for use in cardiac tissue engineering as biomimetic, load-bearing scaffolds.

Financial support: Ministry of Education and Research of Germany in the framework of "ProMatLeben − Polymere"

Visser, D.; Bakhshi, H.; Rogg, K.; Fuhrmann, E.; Wieland, F.; Schenke-Layland, K.; Meyer, W.; Hartmann, H. Green Chemistry for Biomimetic Materials: Synthesis and Electrospinning of High-Molecular-Weight Polycarbonate-Based Nonisocyanate Polyurethanes. *ACS Omega* **2022**, doi:10.1021/acsomega.2c03731.

Singh, N.; Bakhshi, H.; Meyer, W. Developing Non-Isocyanate Urethane-Methacrylate Photo-Monomers for 3D Printing Application. *RSC Adv.* **2020**, *10*, 44103–44110, doi:10.1039/D0RA06388F.

Fig. 1


Non-Isocyanate Polyurethanes (NIPUs)
Green Synthesis Route
High-Molecular Weight
20k Mw 60k
Potential Applications:
Cardiovascular Tissue Engineering
Pericardial Subsitute
ES NIPU
+ Collagen Coating
Epithelial Cells
Fibroblasts
Electrospinning
Cell-Material Interaction

O 14

Investigation of the ultra-structure of bone around Mg implant alloys and the impact on the mechanical properties

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[1]Helmholtz Zentrum Hereon, Institute for metallic Biomaterials, Department for Imaging and Data science, Geesthacht, Germany

Introduction: The potential of biodegradable magnesium implants for treating bone fractures is gaining attention due to their excellent biocompatibility and mechanical properties. Despite the importance of understanding the regeneration process and bone tissue response, there are limited studies on the ultrastructure reaction in bone.

Objectives: We investigated the influence of ZX00 bone implants (Ti served as a control) on the ultrastructure of the bone surrounding the implants using synchrotron radiation-based scanning small and wide-angle X-ray scattering (SAXS/WAXS). We performed correlative SAXS/WAXS measurements during nano-indentation to reveal differences in the strain distribution in the bone tissue. Additionally, bone maturity and osteon density were studied using histology.

Material and Methods: Sheep bone explants with Ti and ZX00 Mg alloy screws (3.5 mm diameter, 16 mm length) were examined after 6, 12, and 24 weeks. High-resolution SAXS/WAXS experiments and nanoindentation were conducted at the P03 beamline at PETRA III, Hamburg.

Results: The analysis revealed a significant decrease in the lattice spacing of the (002) Bragg"s peak closer to the ZX00 implant compared to Ti. The Crystallite size showed no significant difference. The hydroxyapatite platelet thickness and osteon density decreased near the ZX00 implant interface. Correlative indentation and strain maps indicated higher stiffness and quicker mechanical adaptation for the bone surrounding Ti implants compared to Mg.

Conclusions: The HAp (002) plane's d-spacing and platelet thickness decrease under the influence of ZX00, with crystallinity and platelet thickness correlating with bone remodeling and maturity. This is further supported by larger strains and lower stiffness observed in the bone surrounding ZX00 implants. Histological findings, such as lower osteon density in the presence of ZX00 implants, align with the hypothesis that bone maturation is slower around ZX00 than Ti implants.

O 15

Innovative Plasma-Enhanced Collagen Coating for Improved Implant Performance

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Introduction: The successful integration of implants into the surrounding bone tissue is critical for the long-term stability and functionality of orthopedic and dental implants. Although collagen coatings have exhibited promising outcomes in animal models, conventional wet chemical methods for collagen attachment are associated with manufacturing challenges and poor adherence, which hinder their approval for clinical use.

Objectives: Addressing these challenges, we present an innovative approach utilizing a simple inhalation device in combination with non-thermal atmospheric pressure plasma to deposit an extra-thin collagen coating on titanium and polymer implants. The direct deposition of the collagen solution onto the implant surfaces enables the creation of a mechanically stable layer without the need for primers or linkers.

Materials & methods: Our approach involved a commercially available nebulization device integrated into a cold plasma system (Fig. 1). The collagen layer, with a thickness of less than 100 nanometers, was characterized for surface morphology, composition, and mechanical properties. We investigated the adhesion, proliferation, and differentiation of osteoblasts on the collagen-coated surfaces in *in vitro* studies.

Results: The collagen coating demonstrated excellent adhesion to the surfaces. No adverse effects on the metabolic activity of the cells and improved spreading of osteoblasts on the hydrophilic surfaces were observed (Fig. 2). Despite contact with reactive plasma species during coating, no denaturation or cross-linking of collagen was measured, preserving the extracellular matrix (ECM) protein in its native form. Furthermore, the coating showed promising results in osteogenic differentiation.

Conclusion: The thin collagen coating presents a promising avenue for reducing implant failure risks, offering improved mechanical stability while preserving the native form and biological activity of collagen.

Fig. 1

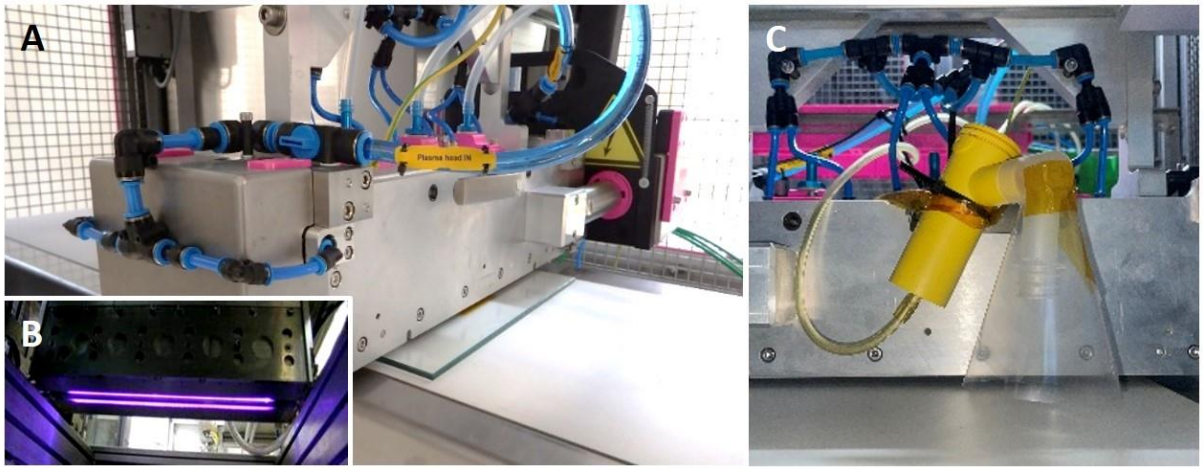
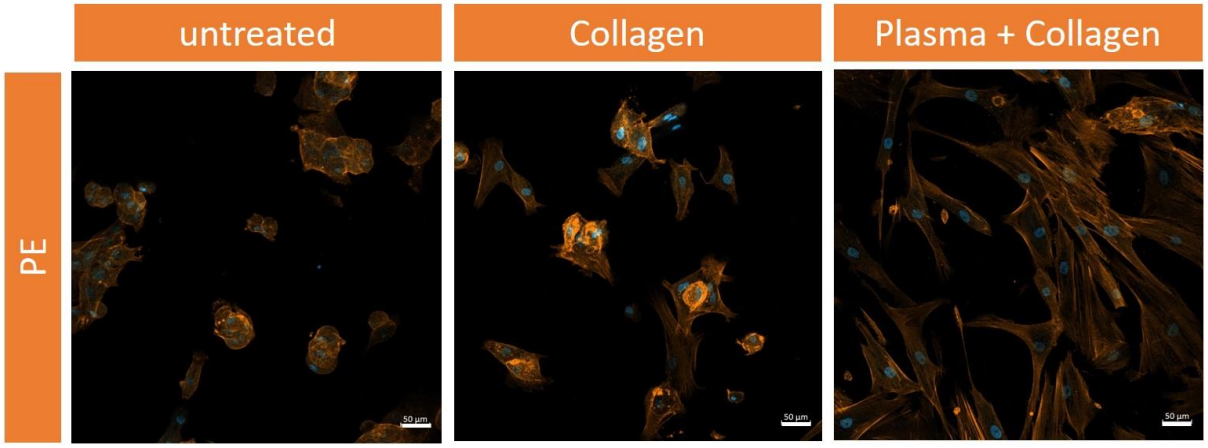


Fig. 2



O 16

Drug delivery systems for polycaprolactone-based growth factor releasing implants

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[2]TU Braunschweig, Institut für Pharmazeutische Technologie, Braunschweig, Germany
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Introduction: Functionalization of implants with drug delivery systems that allow a spatio-temporal control of growth factor release is an approach towards in-situ tissue engineering.
Objectives: A method for implant modification was developed for Polycaprolacton (PCL) based structures.

Materials & methods: PCL textiles were produced by spinning highly oriented fibers and a subsequent braiding process. Porous PCL structures were obtained by blending PCL with polyethylenoxide (PEO), annealing the mixture to induce controlled phase separation and subsequently leaching the PEO.
Chitosan (CS) was grafted with PCL; the resulting CS-g-PCL was used to modify the PCL implants.1 Adhesion of the graft polymer results from surface-induced crystallization of the PCL grafts on the fiber surfaces and brings cationic chitosan to the surface.1 Nanoparticular hydrogels can be formed by ionotropic gelation upon mixing solutions of chitosan with e.g. TGF-b and tripolyphosphate.2 The dispersions of the nanoparticles were used to functionalize the modified implants.

Results: Implants modified with CS-g-PCL have cationic charges.1 By alternating application of polyanions and dispersions of the nanoparticles the implants can be functionalized.2 It was shown that the method not only works on electrospun fiber mats but also on mechanically more stable implants and despite the lower specific surface sufficient amounts of protein can be released.

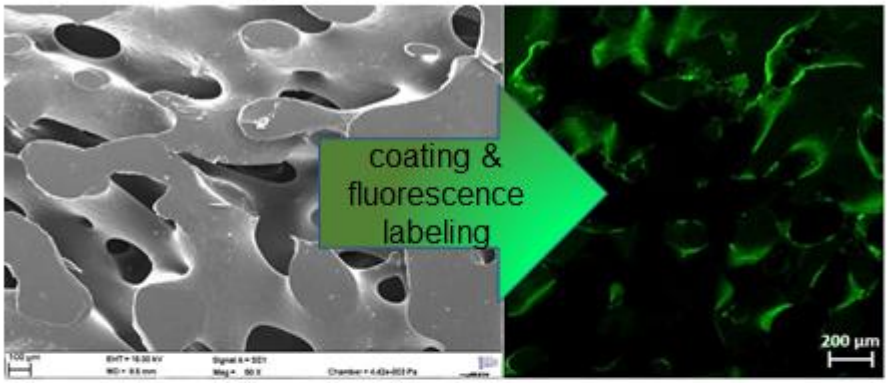
Conclusion: Modification and functionalization of PCL implants can be carried out in an easy and scalable way using CS-g-PCL for surface modification and nanoparticle suspensions for functionalization with drug delivery systems. In this way PCL implants can be rendered bioactive.

References: 1 de Cassan et al, Colloids Surf. B Biointerfaces, 163, 309-320 (2018)
2 Sydow et al. Biomater. Sci. 7, 233 - 246 (2019)

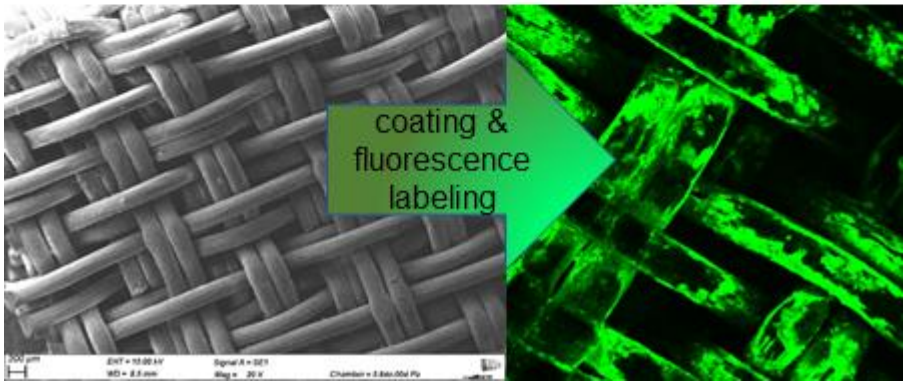
Acknowledgement: The project supported by the DFG via the Research Unit 2180 "Graded Implants for Tendon-Bone Junctions".

Fig. 1

3D porous structures



braided and knitted textiles



O 17

Bone-mimicking degradable jawbone replacements for application in load-bearing critical bone defects

M. Ahlhelm[1], E. Schwarzer-Fischer[2], N. Kaube[1], S. Kurz[3], K. Loger[4], A. Jonitz-Heincke[5], L. Christin-Ingwersen[5], R. Bader[5], J. Wiltfang[4], H. Naujokat[4]

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[3]ZESBO – Center for Research on Musculoskeletal Systems; Department of Orthopedic Surgery, Traumatology and Plastic Surgery, Leipzig University, Leipzig, Germany
[4]Klinik für Mund-, Kiefer- & Gesichtschirurgie Universitätsklinikum Schleswig-Holstein, Campus Kiel, Kiel, Germany
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Introduction: Bone defects in the craniofacial region are often associated with aesthetic and functional impairments. Until now, autologous bone grafts, for example from the fibula or pelvic bone, have been used for reconstruction but they provide an inadequate reproduction of the complex anatomy of the facial skull.

Objectives: The BMBF-funded "HybridBone" project (03VP07633) aimed for a holistic approach that takes shape complexity into account and unites it with the natural distribution of forces as well as biodegradability and new bone formation capability.

Materials and Methods: Based on the combination of the CerAM VPP (Ceramic Additive Manufacturing via Vat Photopolymerization) process and Freeze Foaming, precision-fit, mechanically stable tricalcium phosphate (TCP)-based bone substitute materials were developed. As a result of animal studies in the mandible, a generic hybrid implant has been developed as a demonstrator for the porcine jaw that was implanted in animal studies (Fig.1).

Results: Mechanical and biological suitability have been demonstrated in preclinical large animal studies (Ellegaard Göttingen Minipigs). The proportion of regenerated bone is well above 40% in all groups. The proportion of artificial TCP of the original scaffold has decreased to about 20 %. The contact ratio of newly formed bone and to the CerAM VPP-manufactured support struts is 37 - 61 %.

Conclusion: At the end of "HybridBone" project, a prototype of a topology-optimized hybrid implant for the human jaw was designed and manufactured (Fig.2). In the not-too-distant future such a bone graft substitute might serve as a load-bearing and degradable jawbone replacement suitable for critical maxillary bone defects. Furthermore, the presented combination of processes and materials are adaptable to other defect sites, and therefore, might be the long-desired way for tissue regeneration approaches of critical bone defects.

Fig. 1

Fig. 2

O 18

Synergistic Design of a Multi flexible Bioink Platform for Advanced Drop on Demand Bioprinting

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[1]Institute of Biomaterials/University of Erlangen-Nuremberg, Department of Materials Science and Engineering, Erlangen, Germany

Introduction: Biofabrication has been entering several research fields, like regenerative medicine, cancer research, the lab on a chip and synthetic biology. Especially, cell on-demand printing, an emerging additive manufacturing technology, holds promise for various life science applications [1].

Objectives: The primary goal is to create a bioink with ADA, incorporating adhesion motifs (AdM) through reversible imine bonds or irreversible carbodiimide reactions. Materials derived from PEG, biomolecularly functionalised with YIGSR, IKVAV (from laminin), and RRETEWA (from fibronectin) were developed and assessed for cross-linking, degradation, stiffness, printability, and cell behaviour.

Materials & methods: Various ADA-based bioinks were created, incorporating peptides. The extensive analysis covered cross-linking, physicochemical properties, degradation, stiffness, printability, and cell behaviour using reporter cells.

Results: Important insights into the chemical and physical properties have been gained through extensive analysis. [1,2]. Although there was no significant difference in biocompatibility between bioinks with irreversible and reversible bonds when modified with peptides, bioinks with reversible imine bonds showed higher cell viability after printing, which was attributed to their self-healing properties.The study revealed that peptide combinations synergistically affected the interaction between cells and the bioink material.

Conclusion: Peptide selection and combination play a crucial role in enhancing cell interactions. The engineered bioink formulations, featuring novel peptide mixtures, demonstrate expandable properties and are considered highly responsive materials.

Acknowledgements: This project is funded by the Deutsche Forschungsgemeinschaft (German Research Foundation) project number 326998133-TRR-225 (subproject B06 and A01).

References:

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[2] E. Karakaya, et. al., Biomacromolecules (2023)

O 19

Spontaneous *in vivo* repopulation of decellularized porcine vena cava grafts

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Introduction: Organ decellularization is a method with great potential in tissue engineering. Decellularized extracellular matrix or so-called scaffold has the potential to be repopulated with cells to form an artificial tissue or organ. However, *in vitro* repopulation could be extremely demanding. In theory, spontaneous *in vivo* recellularization could facilitate this process.

Objectives: A functional vascular system is a cornerstone of every organ. Therefore, we decided to study the possibilities of spontaneous *in vivo* repopulation of decellularized blood vessels.

Materials and methods: Porcine inferior vena cava (IVC) were decellularized by perfusion of detergents and then orthotopically implanted in recipient pigs (n=12). To evaluate the immunogenicity and the thrombogenic potential, allogeneic cellular grafts were implanted in animals from control group (n=8).

Results: Endothelial (ECs) and smooth muscle cells successfully repopulated the decellularized IVC grafts. The lumen was fully covered by ECs 28 days after implantation even with endothelization of vasa vasorum. Quantitative histological analysis showed a comparable amount of smooth muscle actin in both implanted groups. Lymphocyte infiltrates representing signs of rejection were present only in the allogeneic cellular grafts. The decellularized grafts had a higher incidence of thrombosis in comparison with allogeneic grafts (33.3 vs. 12.5%). No anticoagulants were used in the postoperative period.

Conclusions: The experiment documents in detail spontaneous *in vivo* recellularization of the decellularized vein. We conclude that *in vivo* recellularization process has potential to greatly contribute to the development of artificial organs based on decellularized ECM.

Funding: This work was supported by the projects of Charles University (GAUK No. 462520, START/MED/027 and COOPERATIO-207043 ""Surgical disciplines"") and the project of the Ministry of Health of the Czech Republic (AZV NU22J-06-00058).

O 20

Superior hemocompatibility of genetically modified porcine pericardium for aortic valve prosthesis fabrication

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Introduction: Xenogeneic pericardium is the main source for biological aortic valve (AV) prostheses. Epitopes such as αGal (synthetized by alpha-1,3-galactosyltransferase/GGTA1) are almost completely inactivated by state-of-the-art glutaraldehyde (GA) tissue fixation. Remaining immunological activation by αGal and GA-fixation are responsible for deterioration and the need for reoperation.

Objective: Hemocompatibility of pericardia of different genetically multi-modified pigs is presented as a basis for a GA-free preparation without prior decellularization.

Materials and methods: Pericardia of different genetically multi-modified (gm) pigs (gm group (A): *GGTA1*-knockout (KO) with additional *CCL2*-KO and transgenes for human PD-L1 and CD47; (B): *GGTA1*-KO with transgene for human CD46) were implemented (native or GA-fixed). Pericardia from slaughterhouse wild-type pigs were used as controls. Tissues were incubated with fresh human blood (1.5 U/ml heparin, 2h, 37°C). Inflammation (complement C5a, granulocyte activation [CD11b]) and hemostasis (prothrombin fragment F1+2, platelet activation [CD62P]) were analyzed via flow cytometry or ELISA. αGal epitope was verified via IHC and uniaxial tensile testing was applied to determine elastic moduli.

Results: Complement and granulocyte activation were significantly lower for the native gm pericardia compared to WT control, but increased after GA-fixation independent from source. Granulocyte loss tended to differentiate between the two gm pericardia, with a trend for a reduced granulocyte loss in gm group (A), compared to group (B). Coagulation activation of native tissues was excessively high, but low after GA-fixation. Elastic moduli did not differ in modified tissues and αGal expression was not detectable.

Conclusions: The low inflammatory potential of the gm pericardium favors this for AV bioprostheses. This initial data suggest that the inflammatory reaction depends on genetic modifications besides αGal depletion.

O 21

The microarchitecture of 3D-printed ceramic scaffolds orchestrates bone healing on the cellular level

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Introduction: In the last decades, advances in bone tissue engineering mainly based on osteoinduction and on stem cell research. Only recently, new efforts focused on the micro- and nanoarchitecture of bone substitutes to improve and accelerate bone regeneration.

Objectives: To test the effect of diverse microarchitectures on the biological response and the interaction between scaffold and bone tissue in osteoconduction and bone augmentation.

Materials and Metods: To produce scaffolds, we applied the CeraFab 7500 from Lithoz, a lithography-based additive manufacturing machine. Hydroxyapatite-based and tri-calcium-phosphate-based scaffolds were produced with Lithoz TCP 300 or HA 400 slurries. The evaluation of triply periodic minimal surface (TPMS) microarchitectures was performed with diamond, gyroid, primitive in comparison to a lattice microarchitecture. Filament based microarchitectures were designed with identical amount of material, porosity, microporosity and directionality. Two filament-based microarchitectures were used to study the effect of microarchitectures on gene expression for differentiation of mesenchymal stem cells and angiogenesis in vivo in a cranial defect model in rabbits.

Results: The evaluation of the gene expression profiles at early bone healing leading to osteoconduction revealed that for filament-based microarchitectures the reduction of the filament diameter and distance from 1.25 mm to 0.50 mm induced differentiation of mesenchymal stromal cell, angiogenesis and osteoconductivity significantly. TPMS and lattice microarchitectures revealed that bone to implant contact is affected by transparency of the scaffold in vivo.

Conclusions: Micro- and nanoarchitectures are key driving forces for osteoconduction and bone augmentation and affect gene expression for differentiation of mesenchymal stem cells and angiogenesis in vivo.

Acknowledgements: This work was supported by a grant from the Swiss National Science Foundation and Innosuisse.

O 22

Enhancing soft and hard tissue integration of titanium implants with laser-textured designer surface topographies

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Introduction: Implant surface design plays a critical role for the integration of dental implants with the surrounding tissue. Traditional techniques, such as sandblasting and acid-etching (e.g. SLA), are purely stochastic and do not offer control over uniformity, consistency, and reproducibility of implant surface designs.

Objectives: This study aimed at designing implant surfaces with improved integration into bone and gingival tissue using femtosecond laser-texturing, which allows precise control over surface characteristics, including chemistry, roughness, and design, down to the nanoscale.

Materials & methods: TiZr samples (laser-textured or SLA treated) were assessed for blood protein adsorption and fibrin network formation. Bone progenitor cells, and a newly developed 3D gingiva model were used to assess their hard and soft tissue integration potential, respectively. Osseointegration was tested in rabbits, complemented with abiological pull-out tests to decouple mechanical interlocking from the biological response.

Results: Mimicking features of bone tissue, laser-textured surfaces optimized for osseointegration presented nano-scale periodic surface structures on a trabeculae-like microarchitecture. While blood-material interaction was similar on SLA and laser-textured surfaces, *in vitro* and *in vivo*osseointegration was significantly enhanced with the latter group. Interestingly, abiological pull-out tests showed weaker mechanical interlocking compared to SLA, indicating that the enhanced osseointegration was primarily due to the biological response to the laser-textured surface design. Surface designs optimized for integration with gingival tissue promoted organized gingival cell growth and matrix deposition, and enhanced attachment with the 3D gingival model.

Conclusion: In conclusion, femtosecond laser-textured implants promote superior biological integration, highlighting the pivotal role of surface design in advancing dental implant success.

O 23

Study of the dynamics of cell-matrix interactions and mechanics in photopolymerized 3D fiber networks

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Introduction: Cell migration, morphology and fate are highly dependent on the geometrical, mechanical and chemical properties of the 3D fibrillar extracellular matrix, that is strongly reorganized in some pathophysiological processes or following implant insertion. To understand the mechanisms at play, a general approach has been to reconstitute architectures mimicking generic features of the matrix. However, the control of local physical properties (stiffness of individual fibers, local density, geometry) as well as the measurement of 3D cellular traction forces on individual fibers call for the development of new nano- and micro-fabrication techniques and analyses approaches.

Objectives: We propose an innovative technology to study cell-matrix interactions, with the development of networks of deformable fibers with controlled 3D architectures and local mechanical and chemical properties, and the quantification of cell dynamics and mechanics in these systems.

Materials & methods: 3D fiber arrays were built by two-photon polymerization of polyethelene glycol diacrylate, with local control of adhesive properties. On them, fluorescent fibroblasts, endothelial and immune cells were imaged by spinning disk or Lattice Light Sheet Microscopy. For 3D traction forces, fiber deflections were measured by automatic segmentation, fiber mechanical properties by AFM, and a Finite Element Model was developed.

Results: We developed a set of deformable fiber networks with controlled 3D architectures, studied the migration and morphological changes of fibroblasts, endothelial cells and immune cells in link with the local properties of this network, and proposed an original method to measure cellular traction forces in 3D.

Conclusion: These technological advances in microfabrication and in 3D forces measurement allow to finely characterize cell-matrix interactions and mechanics at the level of individual fibers, in a controlled and reproducible 3D context.

Fig. 1

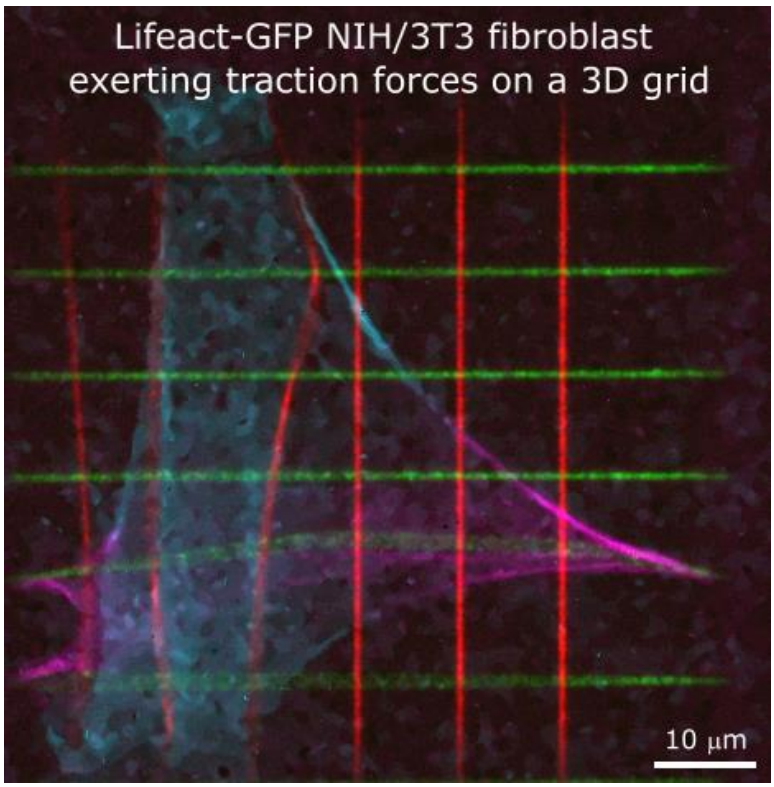
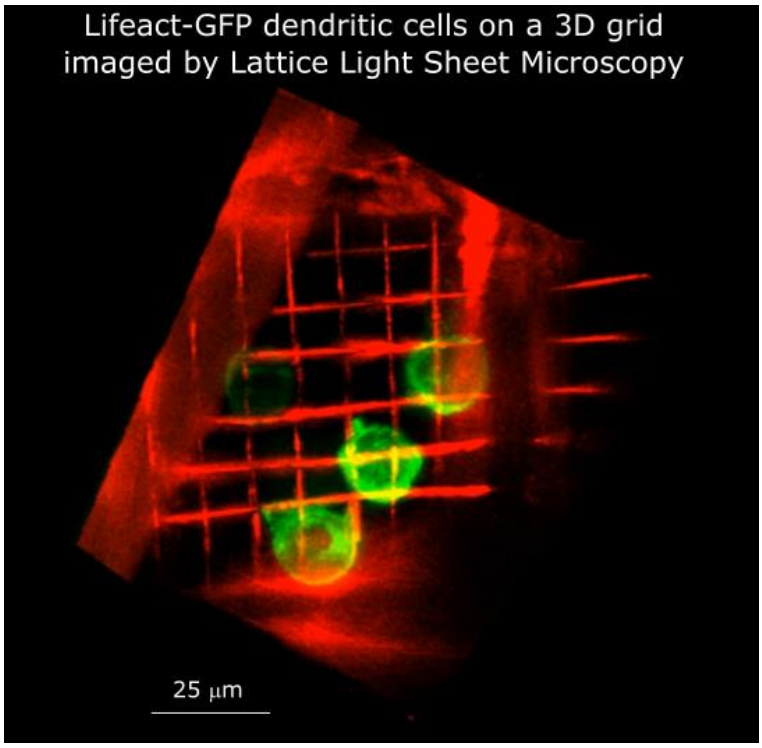

Lifeact-GFP NIH/3T3 fibroblast
exerting traction forces on a 3D grid
10 μm

Fig. 2


Lifeact-GFP dendritic cells on a 3D grid
imaged by Lattice Light Sheet Microscopy
25 μm

O 24

Decellularized porcine liver: Spontaneous *in vivo* recellularization

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Introduction: Due to the lack of donor organs for liver transplantation alternative strategies are being sought. Tissue engineering offers several directions where the use of decellularized liver tissue as a matrix for repopulation with human cells represents a promising approach. An efficient method for high quality pig liver scaffold preparation was developed [1], however, its suitability as an implant still needs to be verified as well as the potential to be recellularized.

Objectives: This study aim was a complex histological assessment focused on the interaction of decellularized pig liver scaffold with the porcine omentum after allogeneic implantation.

Materials & methods: Small pieces of decellularized pig liver scaffold were implanted in porcine omentum of the recipient pig in 2-week survival experiment. Samples were processed and different types of IF stainings applied to assess selected parameters. Quantitative analysis was done on slide scans using QuPath software.

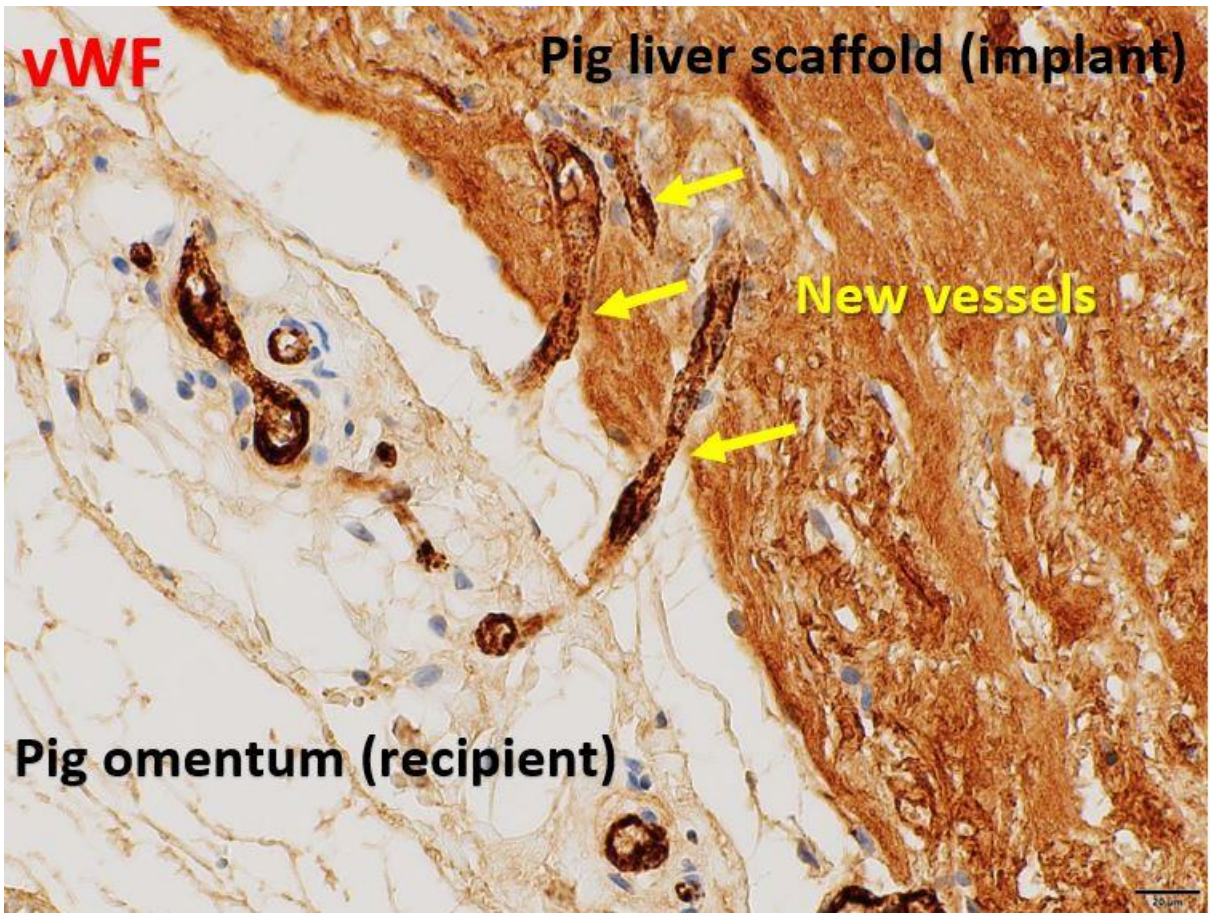
Results: In the explanted tissue no inflammatory changes were observed either in the scaffold or in surroundings. Cells of the recipient were present in the scaffold; their numbers were decreasing toward the central zone. Some of these cells expressed mitotic activity. Recreated small vessels were merging with the circulatory system of the recipient (Figure 1), some of them with clearly presented muscular layer.

Conclusion: It was confirmed the scaffold is biocompatible as no immune reaction was present. Implanted scaffold proved also as a favourable environment for cell migration, division, and growth. Partial revascularisation serves as an indirect proof of preserved signal molecules in the matrix.

Funding: The work was funded by projects UNCE/MED006 Center of Excellence, Charles University, COOPERATIO-207043, Charles University, and NUVR – NICR (No. LX22NPO5102), Next Generation EU.

Reference: [1] Moulisova V. et al (2020), Journal of Tissue Engineering, 11, 2041731420921121.

Fig. 1


vWF
Pig liver scaffold (implant)
New vessels
Pig omentum (recipient)

O 25

Fibronectin Conformations after Electrodeposition onto 316L Stainless-Steel Substrates Influenced Early-stage Osteoblasts' Behavior

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Introduction: Implantable materials, such as metallic orthopedic prostheses, continue to be increasingly used due to both an aging population and traumatology. There is a real need to implement new metal implants.

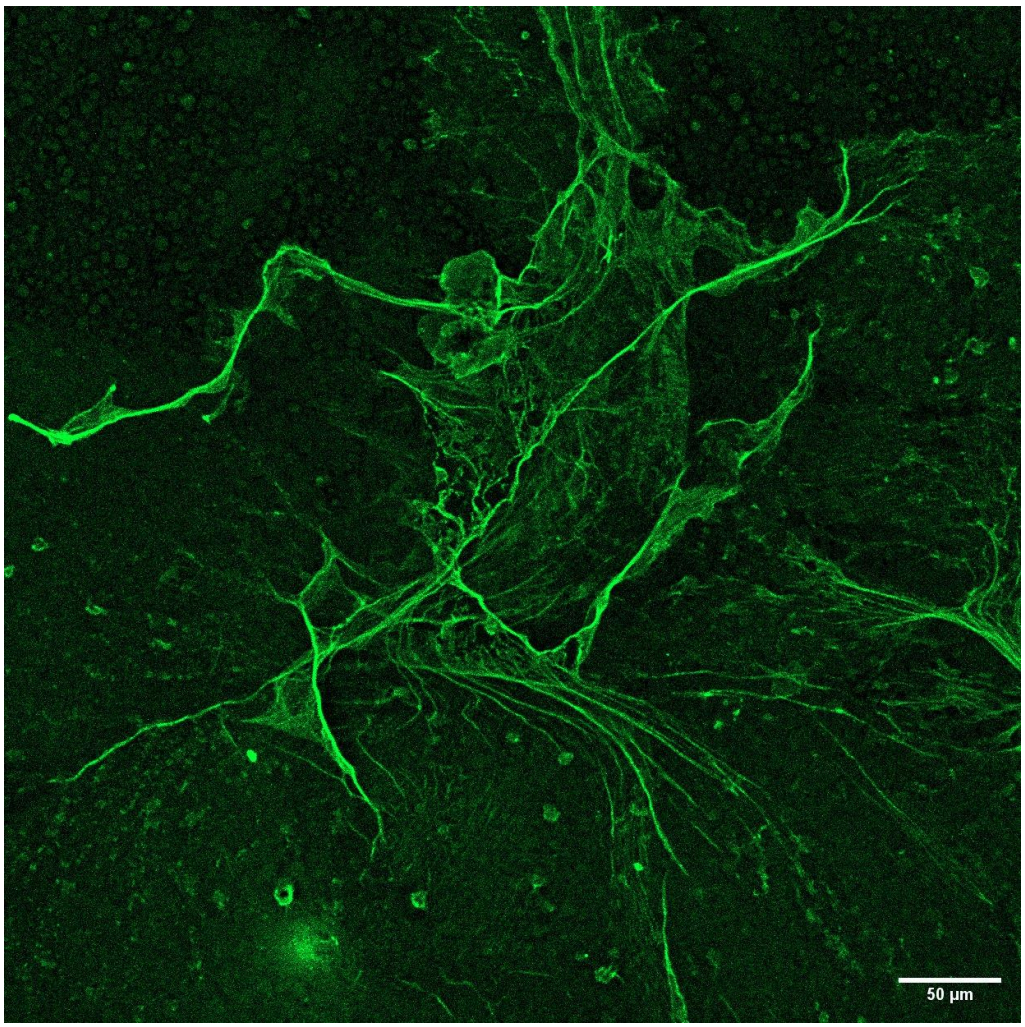
Objectives: The aim of this work is to functionalize 316L Stainless-steel (SS) supports through successive electrodepositions of a polypyrrole (PPy) film and fibronectin (Fn). The effects of the electrodeposition on the bioactivity of Fn were analyzed as well as the early response of pre-stenoblasts STRO-1+ A cultured on supports.

Materials & Methods: Electropolymerisation of pyrrole was performed onto stainless-steel supports to obtain a layer of PPy by cyclic voltammetry. Then three different methods are employed to deposit Fn on supports covered by a PPy film: Fn adsorption (AD), oxidation of precoated Fn (OX) and electrodeposition of Fn (ED).Deposits of Fn on supports were quantified and characterized. Early-stage behavior of osteoblastic cells (STRO-1A+) cultured on Fn functionalized supports was studied. Staphylococcus aureus attachment on bio functionalized support was quantified.

Results: Fn was successfully electrodeposited on supports, and presented typical reproducible fractal fibrillar structures (figure 1). The amount of Fn electrodeposited on 316L SS supports was higher but a strong diminution of Fn cell binding domain accessibility on ED and OX supports was observed.
Early-stage culture of pre-osteoblasts on supports revealed adhesion and spreading on all types of support, suggesting the absence of toxicity. S aureus attachment at the opposite was not affected neither by Fn electrodeposited nor Fn oxidized.

Conclusion: An easy, fast, and reproducible way to bio-functionalize 316L SS supports was developed using electrodeposition. This promising technique could also enable the simultaneous deposition of as matrix proteins and an anti-bacterial peptide, to improve implants" properties.

Fig. 1


50 µm

O 26

Gas bubbles, osteopromotion, and adipogenesis: Decoding the multifaceted cellular response of bone to biodegradable magnesium implants *in vivo*

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Introduction: Metallic magnesium (Mg) implants are increasingly used in orthopedic applications. They achieve bone formation while degrading *in situ* and release degradation products such as H2 that elicit gas bubbles in the peri-implant tissues. Mg degradation is known for its osteogenic properties and is presumed to have anti-inflammatory effects. However, in patients treated with Mg implants, peri-implant tissues may feature increased inflammation near gas bubbles, raising questions on immunomodulation by Mg degradation in bone.

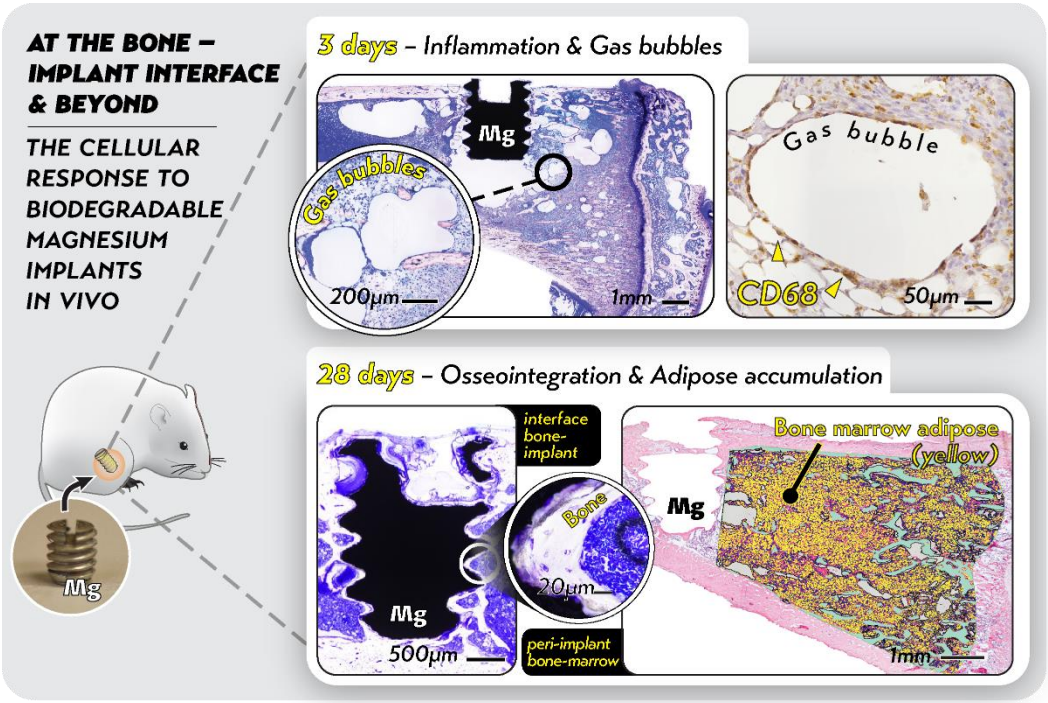
Objectives: to investigate cell regulation at the bone–implant interface by Mg degradation during inflammation and bone regeneration, and to determine if gas bubbles amplify the inflammatory response of the surrounding cells.

Materials and Methods: Pure Mg (99.998%) and Ti (grade 4) screws were implanted in rat tibiae. Implants and adjacent bone were retrieved at 3 d and 28 d and histomorphometry was conducted. Quantitative polymerase chain reaction and immunohistochemistry analyzed gene and protein expression of cells at the bone–implant interface and around gas bubbles. Mann-Whitney test was used for the statistics ($p<0.05$).

Results: Cells at the bone–Mg implant interface had an evident but transient gene upregulation linked to inflammatory cytokines, proinflammatory macrophage polarization, osteoclastogenesis, and neoangiogenesis versus Ti. At 28 d, Mg implants had a superior bone–implant contact. An upregulation of adipogenesis genes coincided with denser and larger adipocytes in the marrow, closely associated with gas bubbles that were surrounded by dense CD68-immunopositive inflammatory cells.

Conclusion: Mg implants initially trigger a transient proinflammatory response that fosters reparative osteogenesis. Yet, their degradation also induces a proadipogenic response and a persistent low-grade inflammation in peri-implant marrow closely linked with gas bubbles, with implications for Mg implant tailoring and clinical monitoring.

Fig. 1


AT THE BONE – IMPLANT INTERFACE & BEYOND
THE CELLULAR RESPONSE TO BIODEGRADABLE MAGNESIUM IMPLANTS IN VIVO
Mg
3 days – Inflammation & Gas bubbles
Mg
Gas bubbles
200µm
1mm
Gas bubble
CD68
50µm
28 days – Osseointegration & Adipose accumulation
interface bone-implant
Bone
20µm
Mg
500µm
peri-implant bone-marrow
Bone marrow adipose (yellow)
Mg
1mm

O 27

In vitro investigation of osteoblasts and macrophages behavior on anatase-coated titanium

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Introduction: Despite high success and survival rates, about 1-4% of dental implants fail due to poor osseointegration, infection, or inflammation in the peri-implant area. Different micro- and nanostructures have been applied to implant surfaces to improve their biocompatibility and osseointegration. These modifications can trigger different biological responses and modulate immune reactions.

Objectives: This study aims to investigate osteoblast responses and the immunomodulatory potential of anatase-modified blasted/etched titanium (SLA-anatase) surfaces.

Materials & methods: Unipolar pulsed direct current (DC) sputtering, was used to fabricate thin anatase layers on SLA specimens. Machined, SLA and SLA-anatase sample discs were studied regarding their surface characteristics (i.e. roughness, wettability, topography), osteoblast proliferation, and differentiation. Furthermore, the effects of different surfaces on blood monocyte-derived macrophages (MDMs) were evaluated using qRT-PCR, ELISA and immunostaining.

Results: Physico-chemical analyses reveal anatase surfaces to be more hydrophilic than SLA surfaces. Roughness parameters (Sa, Sz, and Sdr) also indicated the modified surfaces were rougher than machined control surfaces. As compared to SLA, SLA-anatase modifications significantly enhanced osteoblast proliferation in vitro, which could potentially contribute to implant success. In addition, anatase surface modification partially enhanced osteoblast mineralization in vitro, whereas bacterial colonization was not significantly affected. Additionally, we found that surface modification could modulate MDM's response. This was indicated by different expression profiles of anti-inflammatory and pro-inflammatory markers on anatase surfaces compared to control groups.

Conclusion: The anatase coating holds significant potential as a promising candidate for future advancements in dental implant surface modification for improving the initial stages of osseointegration.

O 28

Immune-instructive polymers for tissue regeneration and wound healing

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Introduction: The immune system plays a key role in response to foreign materials as well as orchestrating tissue homeostasis following different types of injury including wounds and medical device implantation. A close collaboration between macrophages and stromal cells is known to be crucial for a successful wound healing and integration of implants. Dysregulation in any of these processes could results in chronic inflammation.
Objectives: Discovery and characterisation of simple polymeric materials with distinct bio-instructive properties able to support a pro-healing phenotype in macrophage and stromal cells.

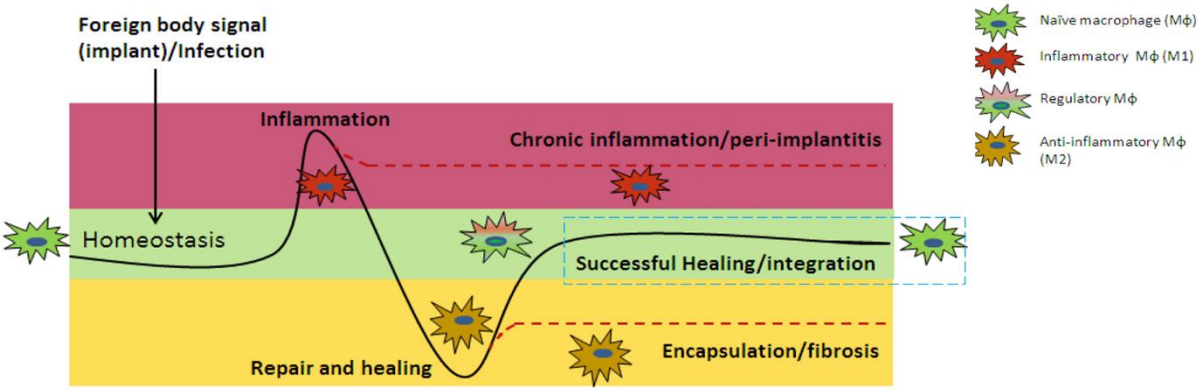
Methods: Screening of a polymer library using high content microscopy to assess their impact on macrophage polarisation, stromal cells phenotype using a mix of immunohistochemistry, PCR and ELISA followed by in vivo assement of their immune modulatory ability

Results: Several polymers were shown to support pro or anti-inflammatory phenotypes in macrophages, and modulate attachment, proliferation and differentiation of fibroblasts. The ability of these polymers to modulate foreign body response (FBR) against subcutaneous implants, or their impact on chronic wound healing were investigated in relevant in vivo models. These experiments revealed that these polymers can significantly suppress FBR and/or promote tissue regeneration and wound healing without the need to use any additional exogenous mediators.

Conclusion: Immune-instructive materials are powerful tools for modulating the phenotype and function of immune and stromal cells and able to reduce detrimental pro-inflammatory responses and promote beneficial pro-healing responses. We propose that it is possible to use immune-instructive materials to harness the immune system to regulate inflammation and promote pro-healing responses with potential applications in a variety of inflammatory conditions.

Ref: 1-Rostam et al. *Matter*. 2020:2(6):1564-1581
2-Latif et al. *Adv Mater*. 2022:e2208364

Fig. 1



Macrophages play a central role in orchestrating tissue homeostasis following injury and/or infection. They high plasticity in response to different stimuli provides opportunities for modulating their functional phenotype using physicochemical cues such as those provided by 'immune-instructive' materials described in this abstract.

O 29

Poly (Glycidyl Ether) Coatings as Functional Materials for Immunomodulation

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Introduction: The effective incorporation of artificial implants into the body is strongly influenced by its interaction with surrounding tissue. Cells sense their microenvironment through complex biophysical and biochemical signalling, prompting nanostructural reorganization.[1] While tissue regeneration involves multiple cell populations, dendritic cells (DCs), as mediators of innate and adaptive immunity, are crucial in triggering inflammatory response upon foreign body recognition.[2] However, a critical gap exists in the material-induced modulation of DCs, essential for advancing their application in regenerative medicine.

Objective: This explorative study aims at elucidating the *in vitro* potential of synthetic poly(glycidyl ether) (PGE)-based coatings for modulating human monocyte-derived DCs.
Materials & Methods: Polymeric coatings[3,4] were made in a "grafting-to" approach followed by UV- irradiation. Structural characterization employed spectroscopic ellipsometry, contact angle, AFM, QCM-D, XPS and ToF-SIMS. Culture of fibroblasts and human DCs on these coatings was used to study cytocompatibility, cell adhesiveness as well as DC activation via microscopy, FACS and ELISA.

Results: PGE-coatings ranging from self-assembled brushes to thin gels on plastic or brushes assembled on bioinert gels allowed control over the materials' mechanical properties and improved cell adhesion. Expressed surface markers, secreted growth factors and MMPs of DCs cultured on PGE coatings *in vitro* revealed a phenotypic shift towards immunotolerance, crucial for tissue regeneration.

Conclusion: PGE coatings of various architecture and stiffness activated DCs to a degree previously observed only *in vivo*. Successful PGE self-assembly on both surface-immobilized and bulk gels highlights them as functional, bioactive coatings for hard and soft implants to stimulate tissue regeneration.

1. S.A. Maynard, C.W. Winter, E.M. Cunnane, M.M. Stevens. Regen Eng Transl Med 2021:7;533.
2. S. Wang, Y. Chen, Z. Ling, J. Li, J. Hu, F. He, Q. Chen Int J Oral Sci 2022:14;52.
3. D.D. Stöbener, M. Weinhart. Polymers 2020:12;1899.
4. D.D. Stöbener, A. Cosimi, M. Weinhart, M. Peiser. Mater Today Bio 2023:23;100869.

O 30

Impact Of High Hydrostatic Pressure Treatment On Cytokine Milieu And Its Consequences On Cancer Cell Behavior

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The devitalization of tissue via high hydrostatic pressure (HHP) represents a promising approach for processing autologous tumor-infiltrated grafts in order to address tissue defects. While the structural and biomechanical properties of the tissue grafts are preserved, HHP affects cytokine release that can possibly influence remaining vital cancer cells in the surrounding tissue.

To gain a comprehensive understanding of the biological effects of HHP-treated tumor-infiltrated tissue, this study investigates the release of cytokines from HHP-treated cancer cells and their effects on cancer cell behavior.

Head and neck squamous cell cancer cell lines (HNSCC16, UT-SCC-14, HNSCC46 & PE/CA-PJ15-NIR-680) were subjected to 0, 300 and 450 MPa HHP for 10 minutes at 20 °C. After one hour of cultivation at 37 °C, supernatants were collected, screened for 42 cytokines, and quantified. Tumor cell proliferation was measured by cell counting after exposure to control or HHP-supernatants, and migration behavior of vital cancer cells was assessed using Boyden chamber and scratch assay.

IL-1α, IL-1β, MCP-1, SDF-1, IL-6 and IL-8 were identified as relevant cytokines in HHP-treated tumor cell supernatants. SDF-1, MCP-1, IL-1α and IL-1β showed an HHP-dependent release in all cell lines. In contrast, IL-6 and IL-8 release was increased in untreated controls. Tumor cells exposed to the HHP-derived supernatants showed increased proliferation and migration rates compared to unexposed controls.

With rising pressure, necrotic processes are induced, resulting in an increased release of intracellular cytokines. This cytokine milieu significantly influences the behavior of tumor cells. Our method provides a valuable approach for studying the impact of cytokine milieu on cell behavior, and sheds light on potential applications of HHP in modulating cellular responses.

This study is funded by the German Research Foundation (DFG project no. 456590961).

O 31

Effects of different metal ions on polarization and cytokine production in human macrophages

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The main factors that determine the polarization of macrophages upon contact with biomaterials are type, chemical composition, and surface texture. In aseptic implant loosening, the predominance of a macrophage phenotype has been controversially discussed with one study reporting a higher M1/M2 ratio in synovial membranes from revision patients while another study was unable to identify solely M1 or M2 typical cytokines in periprosthetic tissue. The discrepancy might partly be due to the various alloys used in arthroplasty and thus the different corrosion products derived from implant wear.
The study aimed to compare the effects of different metal ions on macrophage polarization and cytokine release in vitro.

Human macrophages were incubated with 100 µM solutions of different metal salts ($CoCl_2$, $NiCl_2$ and $CrCl_3$) for 48 h under standard culture conditions. Cell surface markers of M1 and M2 polarization were assessed with flow cytometry on a BD FACSAria™Illu, while the cytokine release was measured in a multiplex assay (LEGENDplex™ Human Essential Immune Response Panel) on a BD FACSVerse™.

While the fluorescence intensity (MFI) of CD80 on M1 differentiated macrophages was increased, the percentage of CD80+, CD86+ and double-positive cells significantly decreased after incubation with cobalt ions. Concurrently, MFI of CD163 was elevated on M1 macrophages after cobalt stimulation to M2 macrophage levels. The lack of pro-inflammatory activation in M1 macrophages was supported by the cytokine data as only IL-17A was increased after exposure to cobalt. M2 macrophages were mainly unaffected by metal ions, however, IL-8 release was increased in M1 and M2 macrophages after cobalt ion exposure.
Unexpectedly, metal ions did not promote M1 (pro-inflammatory) polarization in macrophages. This suggests that inflammation due to metal wear and corrosion products in aseptic loosening may not be induced via classical activation, but might depend on alternative mechanisms.

O 32

Rational engineering of glycosaminoglycan-based dickkopf-1 scavengers to improve bone regeneration

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Introduction: The WNT signaling pathway is a central regulator of bone development and regeneration. Functional alterations of WNT ligands and inhibitors are associated with a variety of bone diseases that affect bone fragility and result in a high medical and socioeconomic burden. Hence, this cellular pathway has emerged as a novel target for bone-protective therapies, e.g. in osteoporosis.

Objective: Here we investigated glycosaminoglycan (GAG) recognition by Dickkopf-1 (DKK1), a potent endogenous WNT inhibitor, and the underlying functional implications in order to develop WNT signaling regulators.

Material & Methods: In a multidisciplinary approach we applied in silico structure-based de novo design strategies and molecular dynamics simulations combined with synthetic chemistry and surface plasmon resonance spectroscopy to rationally engineer oligomeric glycosaminoglycan derivatives (REGAG) with improved neutralizing properties for DKK1.

Results: In vitro Alpl gene expression, Wnt reporter assay and in vivo analyses showed that the REGAGs modification translated into increased WNT pathway activity [+20%; $p<0.05$ and +40%; $p<0.05$ respectivly] and improved bone regeneration in a mouse calvaria defect model with critical size bone lesions. Importantly, the developed REGAG outperformed their precursor, polymeric high-sulfated hyaluronan (sHA3), in enhancing bone healing up to 50% due to their improved DKK1 binding properties.

Conclusion: Thus, rationally engineered GAG variants may represent an innovative strategy to develop novel therapeutic approaches for regenerative medicine.

O 33

CI electrode insertion force in human cadaveric ears at different insertion speeds

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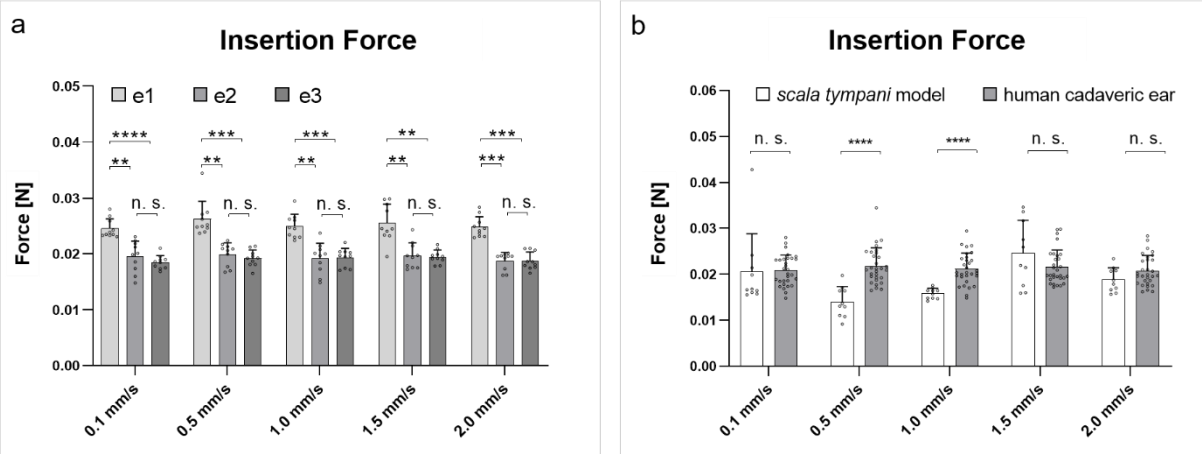
Introduction: The development of endocochlear trauma during cochlear implantation is influenced by a number of mechanical parameters. In numerous studies with artificial *scala tympani* models it was found that electrode insertion speed has a significant effect on the insertion force. However, insertion force at different insertion speeds in human cadaveric ears has not been studied in detail.

Material and Methods: In this study, standard 31.5 mm cochlear implant electrodes (MED-EL, Innsbruck, Austria) were inserted into three different human cadaveric ears and a *scala tympani* model. Insertion force was recorded and compared at five different insertion speeds (0.1, 0.5, 1.0, 1.5 and 2.0 mm/s). Additionally, one human cadaveric ear was histologically prepared after the insertion procedure, and the sections were analyzed for tissue integrity.

Results: The data show that there are differences in insertion force between the individual human cadaveric ears. However, insertion speed has no significant impact on electrode insertion force. It is further shown that at medium insertion speed (0.5 mm/s and 1.0 mm/s) insertion force in the human ears is significantly increased compared to the force in the *scala tympani* model. In addition, 50 times of electrode insertion caused no detectable trauma to the endocochlear structures, such as elevation or rupture of the basilar membrane.

Discussion: This data indicates that the individual shape of the cochlea appears to have a significant influence on the development of force during electrode insertion. Furthermore, repeated electrode insertion in a human cadaveric ear did not appear to increase intracochlear trauma. However, due to the individuality of the human cadaveric ear, larger studies need to be performed to confirm these findings.

Fig. 1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0.1 mm/s
0.5 mm/s
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1.5 mm/s
2.0 mm/s
b
Insertion Force
Force [N]
scala tympani model
human cadaveric ear
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0.05
0.04
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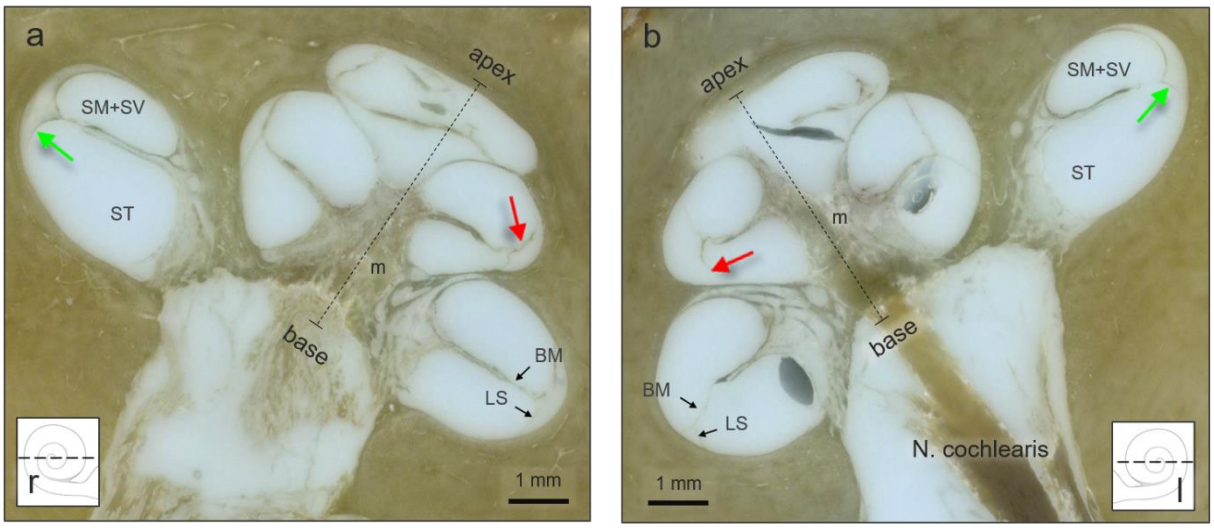
Fig. 2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O 34

Time-dependent, selective serum protein adsorption on degradable Mg-based biomaterials

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Introduction: Metallic implant materials interact with numerous proteins in a physiological setting. Protein adsorption plays a crucial role in influencing degradability, biocompatibility, and integration of temporary implants.

Objective: This study aims to provide a systematic evaluation of the protein-material interface considering the factors of time and material composition.

Materials & methods: We used nanoLC-MS/MS techniques to study the early time-dependent adsorption of human serum proteins on Magnesium (Mg)-based material. We compared the Mg-surfaces to titanium (Ti) at three time points of 3 min, 60 min and 24 h. Protein identification was performed with MaxQuant and PERSEUS. Functional annotation was performed via PANTHER, DAVID, and STRING databases.

Results: We identified 172 serum proteins that bind to both Ti and Mg surfaces. The highest number of distinct proteins adsorbed was observed at 3 min, with 73 proteins, decreasing to 42 proteins at 24 h. Initially, more proteins adsorbed on the Ti surface, but by 60 minutes, this trend reversed.. Our comprehensive analysis, using DAVID for Gene Ontology annotation, revealed the involvement of 161 distinct biological processes. These predominantly relate to complement activation, immune response, and blood coagulation. Notably, key blood coagulation components showed a higher affinity for the Mg surface within the first hour, including factor X, thrombin, and factor V. After 24 hours, proteins of the complement cascade (factor C3, enriched at Mg) and the innate immune system, particularly acute phase proteins like ceruloplasmin, were affected.

Conclusion: The study provides insights into how Mg-based materials affect protein adsorption dynamics over time. Future research will focus on examining the adhesion of various cell types in relation to this dynamic protein layer. The goal is to understand the integration of Mg-based implants and enhance the use of metallic implants in medical applications.

O 35

Evaluating the correlation between *in vitro* and *in vivo* biological responses to Ca-modified surfaces: a proteomic study

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[3]CicBiogune, Proteomics Platform, ..., Spain
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Introduction: The frequently weak correlation between *in vitro* and *in vivo* outcomes poses a challenge for the development of biomaterials. Therefore, it is crucial to find new methods that allow researchers to predict the biomaterial biological responses in a more efficient and ethical way.

Objectives: In this work, Ca-modified surfaces were characterized physical-chemically and biologically. Moreover, we used proteomics to analyze the effects of Ca-doped biomaterials in the *in vitro* surface protein adsorption and the tissue protein expression surrounding modified implants *in vivo*, examining their correlation.

Materials & Methods: Ti surfaces were modified with CaCl2 in different amounts. Their osteogenic and inflammatory potentials were tested *in vitro* using HOb and THP-1. The *in vivo* experimentation was carried out in a condyle rabbit model. One part of the samples was used for histology studies, and the other to elute the tissue for proteomics. To analyze surface protein adsorption, coatings were incubated with human serum and adsorbed proteins eluted. Both *in vitro* and *in vivo* samples were evaluated with nLC-MS/MS.

Results: The surfaces with CaCl2 increased TNF-α secretion and IL-1β gene expression. It also increases HOb proliferation and ALP. Proteomics showed that Ca doping increased the presence of coagulation proteins, both *in vitro* and *in vivo*. The higher adsorption of immune and osteogenic proteins onto the Ca-surfaces was accompanied by a higher expression of inflammatory and osteogenic proteins *in vivo*. Proteomic results were consistent with the biological responses.

Conclusion: Proteomics showed a good correlation between *in vitro* and *in vivo*. Consequently, conducting proteomic analyses to assess biological responses to biomaterials *in vitro* proves to be a valuable tool for predicting their effects *in vivo*. Therefore, it can be useful in developing new biomaterials.

O 36

Immunomodulatory effects on mesenchymal stem cells mediated by extracellular vesicles from different macrophage phenotypes

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[2]Sahlgrenska Academy, University of Gothenburg, Department of Oral Biochemistry, Institute of Odontology, Gothenburg, Sweden
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Introduction: Cell-material interactions and cell-cell communication are critical processes during inflammation and tissue repair at bio-interfaces, involving e.g. proinflammatory (M1) and proregenerative (M2) macrophages and mesenchymal stem cells(MSCs). At least partly, these processes can be mediated via extracellular vesicles (EVs), providing cargo transfer between cells, thus modulating signaling pathways in the recipient cells. Hitherto, little is known about the influence of EVs from different macrophage phenotypes on MSC behaviour.

Objectives: To determine the role of different macrophage EVs in the interplay between macrophages and MSCs in normal and simulated inflammatory environments *in vitro*.

Materials and Methods: Monocytes (THP1 cells) were differentiated to M0 macrophages using phorbol-myristate-acetate. Polarization of M0 to M1 phenotype was obtained using lipopolysaccharide (LPS) and to M2 phenotype using interleukin(IL)-4 and IL-13. Macrophage phenotypes were characterized using gene expression and flow cytometry analyses. Isolation and quantification of EVs from different macrophage phenotypes were performed using ultracentrifugation and nanoparticle tracking analysis, respectively. miRNA cargo of EVs was characterized by next generation sequencing. Adipose-derived human MSCs were treated with the EVs (± LPS addition). EV internalization, gene expression and protein secretion by MSCs were analysed after 2, 6, 12 and 24 h.

Results: EVs from different macrophages differently altered gene expression and cytokine production by MSCs. M1 macrophage EVs were more efficiently internalized by MSCs at earlier time periods (2 and 6 h) than other EVs. Moreover, the effect of M1 EVs on MSCs was independent of LPS. miRNAs were differentially expressed in EVs from the different macrophage phenotypes.

Conclusion: Depending on the macrophage phenotypes, the MSCs internalize and handle their EVs differently, resulting in cell-specific immunomodulatory effects.

O 37

Design and Synthesis of Bioactive Material via Two-Photon Polymerization for Studying Cell Interaction with Microenvironment

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Introduction: In summary, the utilization of two-photon polymerization (2PP) for microfabrication purposes provides a powerful means to (re)create the complex 3D cell environments essential for advancing our understanding of cellular biology and developing innovative solutions in the fields of tissue engineering and regenerative medicine.

Objectives: Our primary objective includes developing bioactive materials using 2PP to create well defined microstructures with adhesive and non-adhesive areas or controllable degradation properties. The second objective is the investigation of their potential for studying cell behavior within the designed microenvironment.

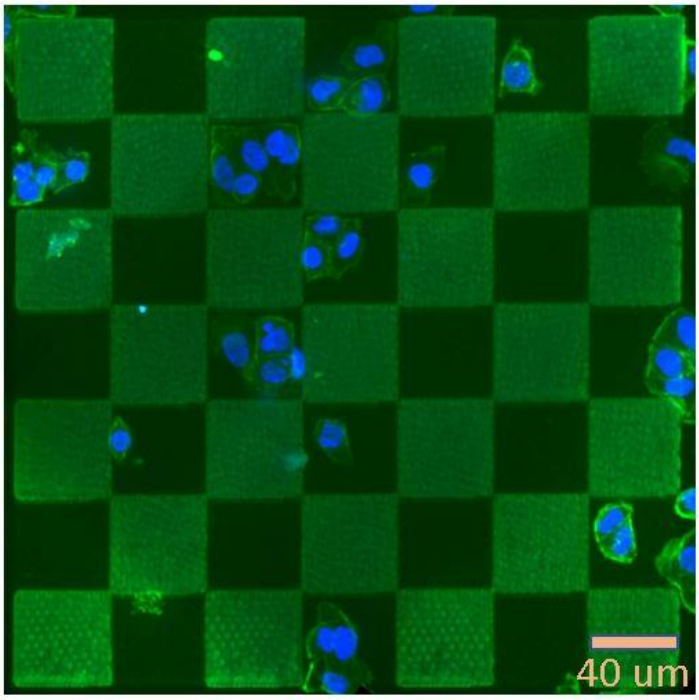
Materials & Methods: We have developed i) new Off-stoichiometry thiol-ene (OSTE) resins allowing the fabrication the direct laser writing of microstructures as well as direct laser grafting of non-adhesive polymers locally through 2PP and ii) protein-based formulation to control the biodegradation of microstructures.

Results: The 2PP-fabricated bioactive OSTE as well as protein materials demonstrated excellent precision and reproducibility in the production of well-defines microscaffolds. We were able to create microstructures allowing respectively the control of cell adhesion (Figure 1) or the biodegradation. These findings open avenues for tailoring microenvironments to control and manipulate cellular responses.

Conclusion: In conclusion, our study showcases the successful design and synthesis of bioactive materials through Two-Photon Polymerization, offering a platform for investigating cell interactions within precisely engineered microenvironments.

Figure 1. MDCK cell adhesion on microfabricated OSTE resin after Direct Laser Graftin

Fig. 1



O 38

High hydrostatic pressure (HHP) reduces Implant-based serological chronic inflammation markers in an in vivo rat-model

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1) Proper evaluation of the immunogenic potential of implants is crucial for clinical success. HHP is a gentle devitalization method that utilizes high pressure levels to induce cell death throughout the implant, while keeping matrix integrity undiminished. Cytokines are key parameters to evaluate immunogenicity.

2) This in vivo study aimed to investigate the immunogenic potential of HHP devitalized bone implants, regarding serum cytokine levels over an extended period of 56 days to identify the impact of HHP on serological host reaction.

3) Sixteen male Lewis rats received a single implantation of human bone, treated with either HHP (250MPa for 10 minutes) or an untreated control. Samples were implanted into the neck musculature. Blood was collected 1 day prior to implantation and weekly thereafter for a total of 56 days. Collected serum was analyzed with custom made multiplex analysis kit (Thermofisher) and measured using a Luminex system.

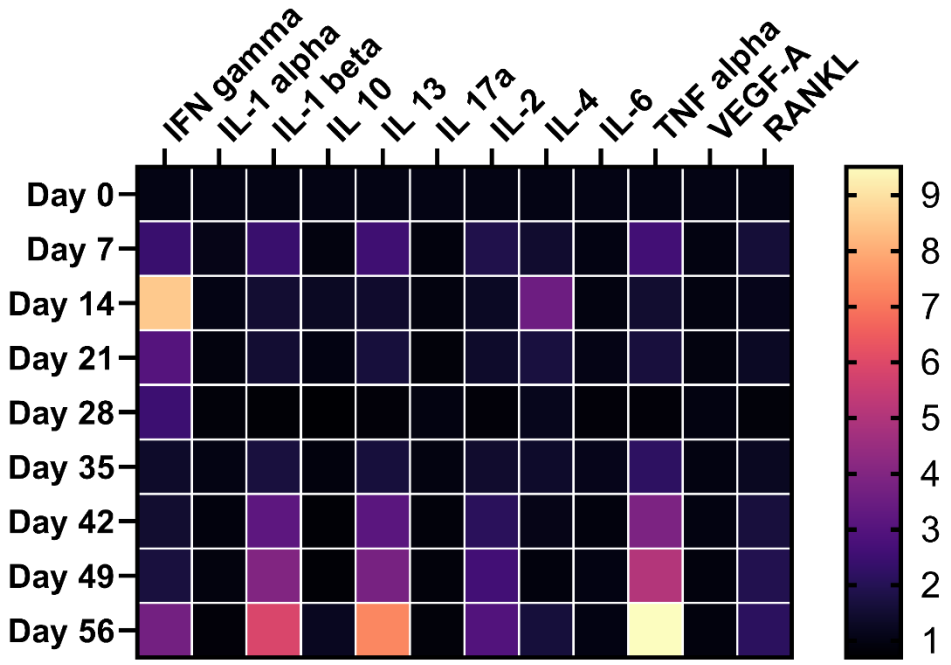
4) We were able to highlight a different reaction pattern in serological cytokine levels of implants treated with HHP compared to untreated control implant. HHP treatment showed a generally higher immunological response on day 7 during the acute phase. No similar effect seen in the control implants. The chronical phase revealed no increases in cytokine levels in the HHP group, however the control group expressed notable increases in serum cytokine levels.

5) The results indicate that a HHP treatment induces a desired moderate acute reaction to the implant dominated by anti-inflammatory cytokines, benefitting implant ingrowth, as well as proper bone remodeling effects. It decreases chronic inflammation serological parameters, therefore reducing the risk of chronic inflammation of the implant. The data show promising results for HHP as a devitalization method for xenogenic implant materials.

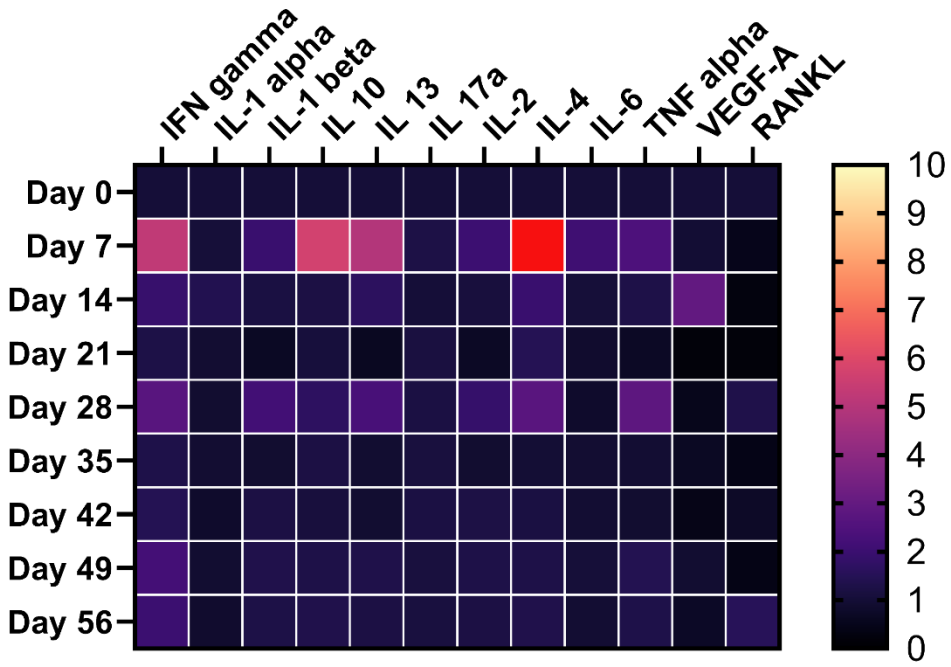
Figure 1: Fold change of inflammatory markers after implantation of a either HHP treated sample or untreated control.

Fig. 1

Heatmap Mean Fold Change Control



Heatmap Mean Fold Change HHP



O 39

In Vitro Testing Method for Evaluation of Ion Emission from Biomaterials

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Introduction: Biomaterials are developed to withstand severe conditions in the human body. Interaction of a biomaterial with the surrounding tissue and, especially, salt containing body fluids can destructively influence metallic biomaterials causing corrosion that results in emission of metal ions and corrosion products. This may lead to elevated serum metal ion levels that can cause cell death and systemic toxic and inflammatory reactions. Testing methods simulating the ion emission from these materials can help understand the underlying mechanisms and develop improved biomaterials.

Objectives: An in vitro testing method was developed to evaluate the ion emission from metallic biomaterials. The method should mimic conditions of a human body that can induce corrosion of an implanted biomaterial in vivo.

Materials & Methods: A special testing device was developed and additively manufactured using chemically stable materials. It provides fixation of a biomaterial sample with defined contact to the testing fluid – calf serum with an optional addition of HCl and H_2O_2 for simulation of inflammatory conditions. The testing is performed by incubation of the samples for seven days on an orbital shaker at 37°C. After the incubation time, the ion concentration in the test fluid is measured using inductively coupled plasma mass spectrometry (ICP-MS).

Results: The functionality of the developed method is represented using experiments on un- and ta-C-coated CoCrMo samples. The results showed that the coating worked as a barrier preventing emission of metal ions from the substrate. A significant reduction of ion concentration was found for Co and Mo, whereby the concentration of Cr and Ni was not significantly higher compared to a negative control.

Conclusion: The developed method showed a high potential in simulation of in vivo conditions and proved its capability to deliver quantitative results for evaluation of corrosion mediated ion emission from metallic biomaterials.

O 40

Biotribological Functionality: Evaluating Cartilage Interaction with Additive Manufactured and Non-Metal Partial Implants

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[2]University for Continuing Education, Department for Health Sciences, Medicine and Research, Krems, Austria

Introduction: Partial implants offer a less invasive alternative to total knee replacement for unicompartimental osteoarthritis. However, its higher revision rates require a deeper understanding of the interaction between biomaterials used for partial implants and articular cartilage. This study assesses patient specific CoCrMo biomaterial manufactured by 3D printing versus non-metallic alternatives.

Objectives: The study aims at extending the lifetime of partial implants currently made of CoCrMo by using more patient friendly materials and understanding their interaction against articular cartilage. The final goal for using additive manufacturing techniques is the development of patient-specific implants.

Material & Methods: Analyses of cast and 3D-printed CoCrMo samples included SEM/EDS for microstructural characterization. Non-metallic alternatives, a high-performance polymer, and a 3D-printed ceramic were examined. Biotribological in vitro tests, under physiological conditions, involved a 1 N load, sliding at 8 mm/s in 37°C phosphate-buffered saline. Osteochondral plugs were scrutinized for chondrocyte activity, gene expression, and proteoglycan content. The surface characteristics of selected bovine plugs were assessed using SEM cryo-cell (see figure 1).

Results: In vitro experiments show material differences in friction and gene expression (see figure 2). This study analyzed and compared heavy metal ion content (Co, Cr, Mo) in metal partial implants after biotribological tests. Assessments of gene expression and metabolic activity revealed heightened metabolic activity across all materials due to chondrocyte stimulation from sliding action.

Conclusions: This study seeks to advance individualized patient treatment by utilizing 3D-printed partial implants made from both metal and non-metal materials. Ongoing animal in vitro biotribological testing aims to thoroughly assess their clinical viability, considering the influence of synovial fluid components.

Fig. 1

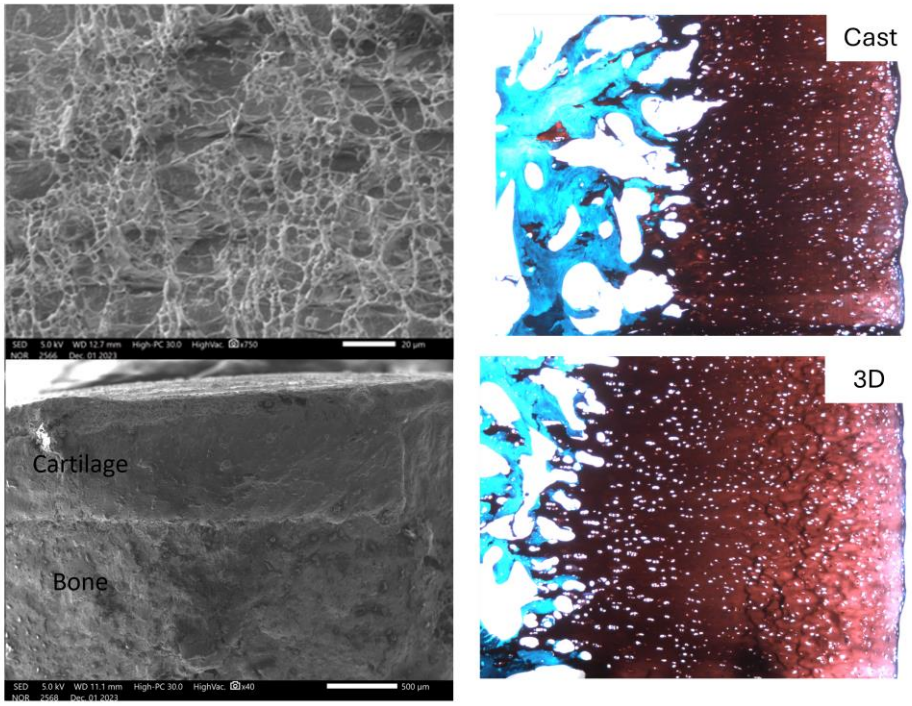

Figure 1: SEM cryo cell (left) and Histology (right)

Fig. 2

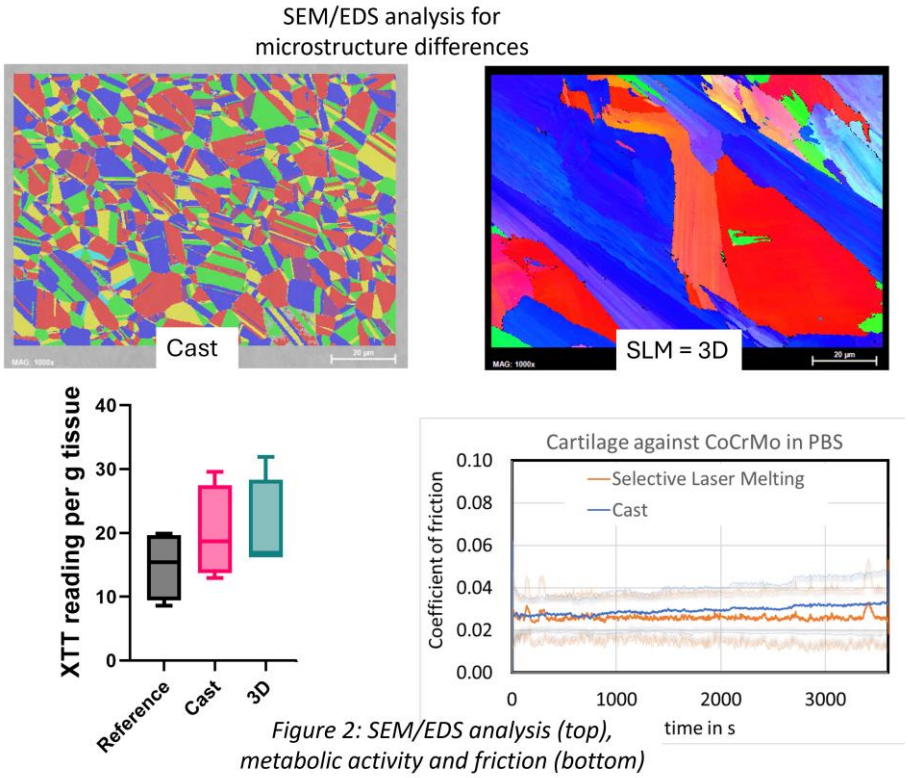

Figure 2: SEM/EDS analysis (top), metabolic activity and friction (bottom)

O 41

Enzyme-triggered drug delivery system for peri-implantitis prevention

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Introduction: Osseointegrated implants replacing lost teeth have shown significant advancement, with more than 89 % success rate. However, bacterial infections from biofilm formation leading to peri-implantitis which results in the undesired loss of implants remain a limitation. Due to the resistance of bacteria in the biofilm against antimicrobial agents, different approaches have been investigated to prevent the invasion of implants surrounding by pathogenic bacteria. Among them are static antibacterial implant coatings as well as the development of drug delivery systems.

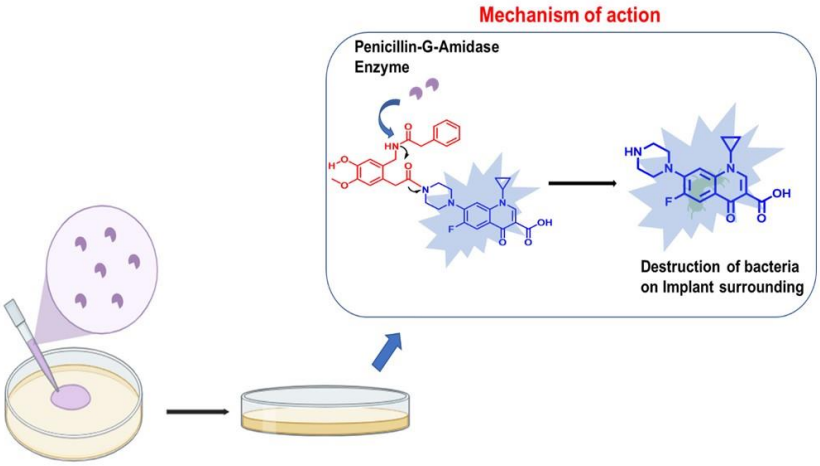
Objective: This work aims to synthesize a chemical sensor-actuator (CSA) system which can detect the presence of bacteria on implants surrounding at an early stage of Mucositis, thereby releasing anti-bacterial drugs and hence, preventing bacteria-associated infections. An enzyme-sensitive linker to bind the drug to a polymer chain was synthesized in a multi-step synthesis.

Method and Material: The enzyme-sensitive linker was synthesized in 7 synthetic steps. The cleavability of the system and the viability of bacteria upon drug release in the presence of Penicillin-G-amidase will be tested in a 2D and 3D cell culture model. In the future, the linker-drug system will be coupled to the backbone of Chitosan polymer and coated on implant surfaces.

Results: The successful synthesis of the enzyme-sensitive linker system was synthesized and confirmed by NMR and IR spectrometer. The obtained system will be tested for cleavability in the presence of Peniciline-G-amidase and analyzed using a Mass spectrometer. Further tests on the viability of bacterial on implant surround will be conducted in 2D and 3D cell culture model.

Conclusion: Synthesis of the Enzymatic sensitive linker system was achieved through a multi-synthesis step. The obtained system is currently tested for cleavability upon enzyme action and viability upon drug release in 2D and 3D cell culture models.

Fig. 1


Mechanism of action
Penicillin-G-Amidase
Enzyme
Destruction of bacteria
on Implant surrounding

O 42

Low immunogenic decellularized biomaterials

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Introduction: Bone and cartilage repair have always been a clinical problem. At present, the methods of treatment defects are mainly autologous and allogeneic tissues. Autologous transplantation has difficulties in obtaining materials and causing new defects. Allogeneic repair has problems such as strong antigenicity, strong immune response, insufficient donor sources, complicated operation, and long healing cycle.

Objectives: Exnotransplantation tissue repair materials can not only retain the original natural matrix of the tissue, such as the cytokines that make biomaterials have two important characteristics of bone/cartilage conduction and bone/cartilage induction, but also change the plight of the scarcity of donors. This type of biomaterials is an ideal implantable material with great potential.

Materials & methods: In this work, a novel scaffold using porcine bone/cartilage components and polycaprolactone (PCL) or GelMA, HAMA was prepared by our research team. Decellularized matrix from α1,3-galactosyltransferase gene knockout (GTKO) porcine tissues with attenuated immunogenicity were combined with biopolymers, wherein advanced 3D printing technology was used to prepare novel composite scaffolds.

Results & Conclusion: The mechanical property, in vitro degradation of scaffolds and their interaction with stem cells were studied. Different animal models (rat cranial defect model, knee cartilage defect moderal and tympanic membrane perforation model) were applied to evaluate the feasibility of tissue repair using these composites in vivo. The biograft developed is intended for use in patients undergoing bone/cartilage defect healing to expedite the tissue defect reconstruction and reduce morbidity as harvesting tissues from the donor sites are no longer required.

Fig. 1

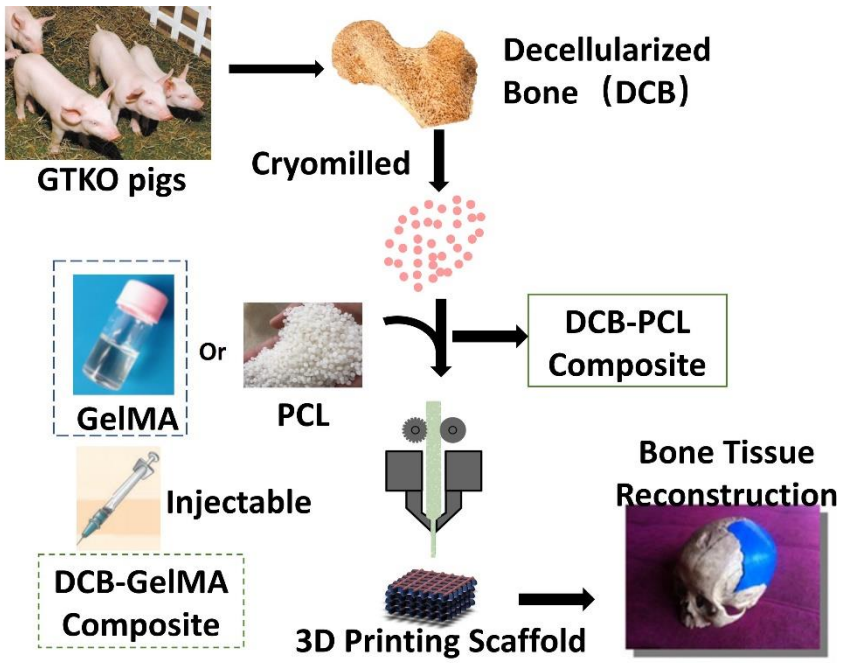
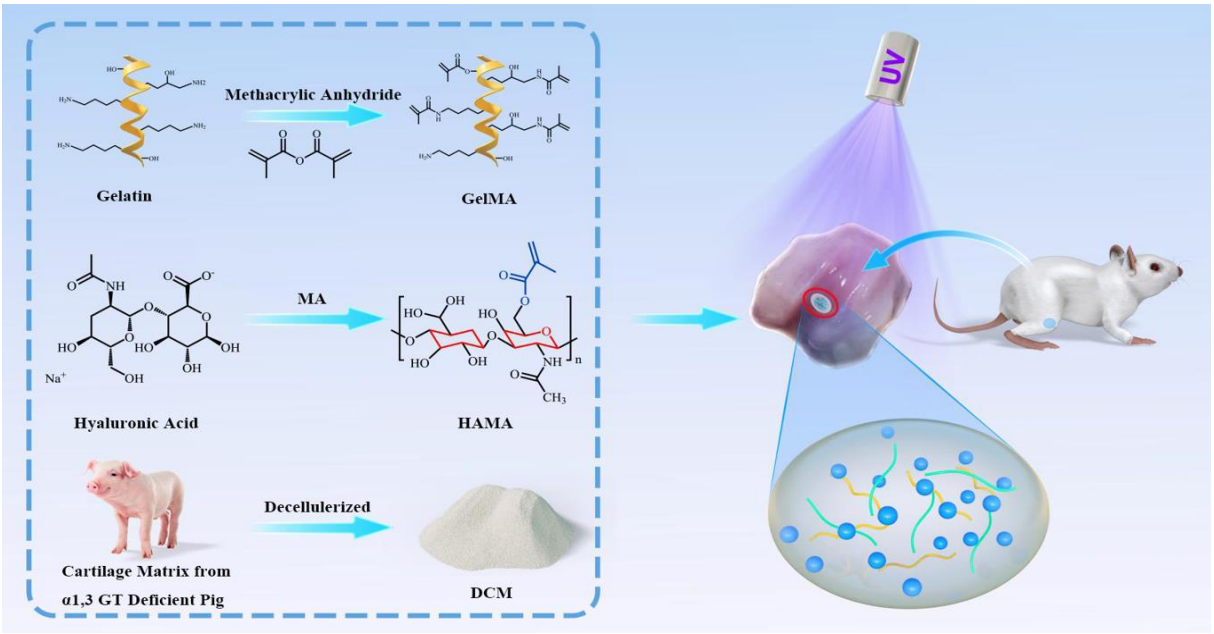

GTKO pigs
Decellularized
Bone （DCB）
Cryomilled
GelMA
Or
PCL
DCB-PCL
Composite
Injectable
DCB-GelMA
Composite
Bone Tissue
Reconstruction
3D Printing Scaffold

Fig. 2


Methacrylic Anhydride
Gelatin
GelMA
MA
Hyaluronic Acid
HAMA
Decellulerized
Cartilage Matrix from
α1,3 GT Deficient Pig
DCM
UV

O 43

Bacteriophage delivery from collagen-based materials to treat bacterial infections in bone

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Currently, antibiotics are used to treat bacterial infections. However, antibiotic resistances are increasing and only few new antibiotics are developed. Alternatively, bacteriophages, bacteria-specific viruses only infect their host bacterium without harming mammalian or plant cells [1,2], could be a promising approach. However, little is known regarding the optimal application route, frequency, and dosage. Therefore, the release and efficacy of *Escherichia coli* (*E. coli*) specific phages from different biomaterials was analyzed.

Mesoporous bioactive glass (MBG) as well as mineralized collagen (MColl) with and without MBG addition was loaded with *E. coli* specific T4-phages (MBG: 10^10 PFU/mL, MColl: 10^6 PFU/mL) and incubated for up to 44 days at 37 °C. Supernatants were taken at different time points and analyzed using bacterial growth curve and spot plaque assay to detect active phages. Using MBG, active phages were released over 44 days and decreased bacterial growth after a 12 h incubation period by 56-86 %. While MColl scaffolds without MBG addition released active phages over 34 days, MBG-modified scaffolds showed a prolonged release for up to 37 days. For both scaffold variants, bacterial growth was reduced by 20-40 % after 12 h.

A sustained release of active phages was shown for all biomaterials and was dependent on the initial phage titer used for loading the biomaterials. For MColl the delivery of phages could be improved by addition of MBG.

[1] Ferry et al., 2021, DOI: 10.3390/v13122414
[2] Moriarty et al., 2022, DOI: 10.1038/s41572-022-00396-0

O 44

Macrophage-based *in vitro* model for compatibility testing of biomaterials

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Biomaterial biocompatibility is mainly influenced by interaction with the immune system. Although there are different *in vitro* models for testing implant material reactions, animal models are still considered as gold standard. However, animal studies require enormous financial and time investments.

This study aims to develop an improved *in vitro* cell model to test material candidates at an early stage and thus avoid unnecessary animal testing. Macrophage-based models are particularly suitable for this purpose, as macrophages play a key role in the foreign body reaction, as they are among the first cells to migrate to the biomaterial and are activated to be pro-inflammatory. After activation, they secrete characteristic pro-inflammatory cytokines and which allows an immune response analysis to the biomaterial.

THP-1 cells were differentiated into M0 macrophages. Subsequently, the biomaterial was added to investigate a potential differentiation of M0 macrophages into activated M1 macrophages. Chemically induced M1 macrophages served as a positive control. The differentiation status of the cells was checked by expression of typical cell surface markers (e.g. CD11b). Characterization of material biocompatibility was performed by analyzing the secretion of pro-inflammatory cytokines (IL-1 β, IL-6) at different time points.

The study showed, that the established *in vitro* macrophage model can be used to characterize implant materials with regard to their biocompatibility. Known typically well tolerated materials, such as platinum or titanium, did not lead to increased secretion of pro-inflammatory cytokines. In contrast, the usage of incompatible materials (e.g. thermanox) greatly increased their secretion.

In conclusion, this macrophage-based *in vitro* model for the analysis of the pro-inflammatory M1 macrophage response to implant materials was established and could be successfully used for biomaterial tests and thus offers an alternative to animal models.

O 45

Inhibition of the macrophage response to *Staphylococcus aureus* infection after exposure to arthroprosthetic metal ions

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Peri-implant tissues can be exposed to CoCrMo wear particles and ions. Exposure associated adverse local tissue reactions (ALTR) are characterized by macrophage-induced inflammation. ALTRs have been linked to an increased risk of periprosthetic joint infection (PJI) after revision arthroplasty. However, the underlying cellular mechanisms leading an increased PJI risk remain unknown.

The aims of this study were to analyze local Co and Cr exposure and macrophage influx in peri-implant tissue samples of patients who underwent revision of non-MoM arthroplasty implants and to analyze the impact of Co- and Cr-ions on human primary macrophages including the ability of exposed cells to respond to *S. aureus* infection.

Local exposure analyses and wear product characterization were realized by synchrotron-based µCt and µ-XRF. CLSM was performed to evaluate macrophage infiltration in these samples. Human monocytes were isolated from whole blood and differentiated to macrophages. Cells were exposed to clinically relevant concentrations of metal ions and infected with *S. aureus* strain 6005. Flow cytometry analyses were performed to analyze the cells" phenotype. The number of phagocytosed bacteria was counted by plating lysed cells on blood agar plates.

Synchrotron imaging indicated that peri-implant tissues are exposed to Co and Cr and CSLM imaging revealed macrophage infiltration. There was no macrophage activation observed in response to *S. aureus* infection following exposure to Co(II) and Cr(III). The expression of PD-L1 was not upregulated by infected cells exposed to Co(II). HLA-DR expression was decreased after exposure to Co(II) either in infected or non-infected cells. The phagocytic activity of macrophages was decreased following exposure to Co(II).

Co- and Cr-ions inhibit essential immune functions of phagocytes. These data indicate possible mechanisms of an increased risk for PJI due to ALTR and support the understanding of metal-induced (immuno)toxicity.

O 46

In Vitro Effects of Metal Ions on iPSC-derived Cardiomyocytes – Chrome and Cobalt Ions Representing Typical Metal Implant Corrosion Products

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Metal-on-metal (MoM) implants, typically consisting of chromium-cobalt alloys, have been the main option for treating severe osteoarthritis. However, significantly higher failure rates have been reported. Further investigations revealed increased metal ion serum levels observed in patients with failed MoM implants, presumably due to corrosion and abrasion of metal implant components. Furthermore, systemic complications were observed, involving inflammatory responses and oxidative stress, that are attributed to elevated chrome and cobalt ion levels. According to the literature, particularly cobalt ions are considered leading to systemic effects, especially on the cardiovascular system. However, detailed knowledge regarding cardiotoxic effects due to chrome and cobalt ions is lacking. The present study investigates the effects of both chrome and cobalt ions on human iPSC-derived cardiomyocytes (hiPSC-CM) in vitro. Therefore, the cells were treated with different concentrations of metal ions over three weeks. During the treatment, various cellular parameters were analyzed, including cytotoxicity. The results indicated that cobalt leads to cytotoxic effects, especially at high concentrations. Moreover, the examination of contraction frequencies using MEA and Image J´s Myocyter revealed dose-dependent effects for both metal ions, which also indicated impacts resulting from prolonged exposure. In search for potential causes that lead to altered contraction frequencies induced by metal ions, the sarcomere structure was examined using Super-Resolution-Fluorescence-Microscopy. Particularly the length and content of sarcomeres were analyzed, whereby only the number of sarcomeres within the cells appeared to be affected. Overall, the outcomes indicate that chrome and cobalt ions have an effect on hiPSC-CM´s functionality and viability in vitro, the exact causes and pathways need further investigation.

O 47

Integrative Evaluation of Corrosion Characteristics of Orthopedic Implants: A Novel Approach Combining Potentiostatic Measurements and Microscopic Analysis

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Introduction: The biomaterial-tissue-interface plays a pivotal role in the long-term success of implants, influencing factors such as biocompatibility, mechanical stability, and corrosion resistance. Corrosion, in particular, poses a significant risk, potentially leading to implant failure and adverse tissue reactions. Traditional methods for evaluating corrosion properties of implant surfaces, while informative, often fall short in replicating the complex in vivo conditions, in particular within the crevices between individual implant components.

Objectives: Using surface-modified TiAlV-samples, this study introduces a novel approach combining active and passive corrosion evaluation methods and an array of microscopic analytical techniques, (SEM, Raman, optical microscopy). This multifaceted method aims to provide a more comprehensive and accurate assessment of the corrosion characteristics at the biomaterial-tissue interface.

Materials & methods: Potentiostatic measurements in a three-electrode-setup were conducted in simulated body fluid based on PBS with addition of HCl and H2O2. The open circuit potential in the equilibrium state was assessed, followed by a linear sweep voltammetry. Furthermore, a setup for simulation of crevice corrosion acc. To ISO 18070 was adapted and used to simulate this geometry for two weeks using a fluid that simulates inflammatory in vivo conditions using HCl and H2O2.

Results: The parameters determined by the Tafel analysis enable a quantitative and comprehensive comparison of corrosion properties. The resulting curve also allows conclusions about the integrity and strength of the passive layer of the material. The qualitative analysis of pre- and post-crevice-test samples allows conclusions about the performance under worst-case conditions.

Conclusion: The new test strategy provides deeper insight into the different corrosion mechanisms present in the human body, thereby facilitating a prediction of corrosion processes in vivo.

O 48

3D bioprinted glioblastoma models for preclinical studies

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[2]University of Rostock, Chair of Microfluidics, Rostock, Germany
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[4]University of Rostock, Department Life, Light & Matter, Rostock, Germany
[5]University Medicine Greifswald, Department of Internal Medicine, Clinic III-Hematology, Oncology, Greifswald, Germany
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Introduction Glioblastoma is one of the most aggressive malignant cancers, with a poor 5-year survival rate. The complex three-dimensional structure, consisting of complex cell-cell and cell-matrix assemblies of tumor and stromal cells, and extracellular matrix, results in a highly dynamic microsystem that is extremely resilient to current treatments.
Objectives: Here, we present 3D-bioprinted glioblastoma models using patient-derived cell lines mimicking the natural tumor microenvironment.

Materials & methods: Different compositions of alginate and gelatin in various concentrations were investigated for their rheological properties and printability. In the final experiments, patient-derived iRFP-680-tranduced glioblastoma cells were suspended in a hydrogel composition consisting of 3% (w/v) and 15% (w/v) gelatin, which was robotically printed into scaffolds. Tumor cell growth and viability within the scaffolds was monitored over a 28-day period using iRFP-680 fluorescence microscopy. In addition, scanning electron microscopy of scaffolds was done for structural analysis.

Results: The investigated hydrogel compositions showed shear thinning properties and good printability in an extrusion-based system. The final composition was successfully printed into scaffolds. The first results showed constant growth over a time period of 21 days and steady state until day 28. Scanning electron microscopy confirmed cellular integrity within the scaffold and formation of small cell clusters, with large pores within the matrix (Figure 1).

Conclusion: Overall, a preliminary 3D glioblastoma model using patient-derived cells was established. The model proved to be a very promising platform with good cell-material interaction for nutrient and substance exchange. Current efforts focus on improving biomechanics, bioink composition, and culturing under dynamic cell culture conditions for preclinical drug screening.

Fig. 1

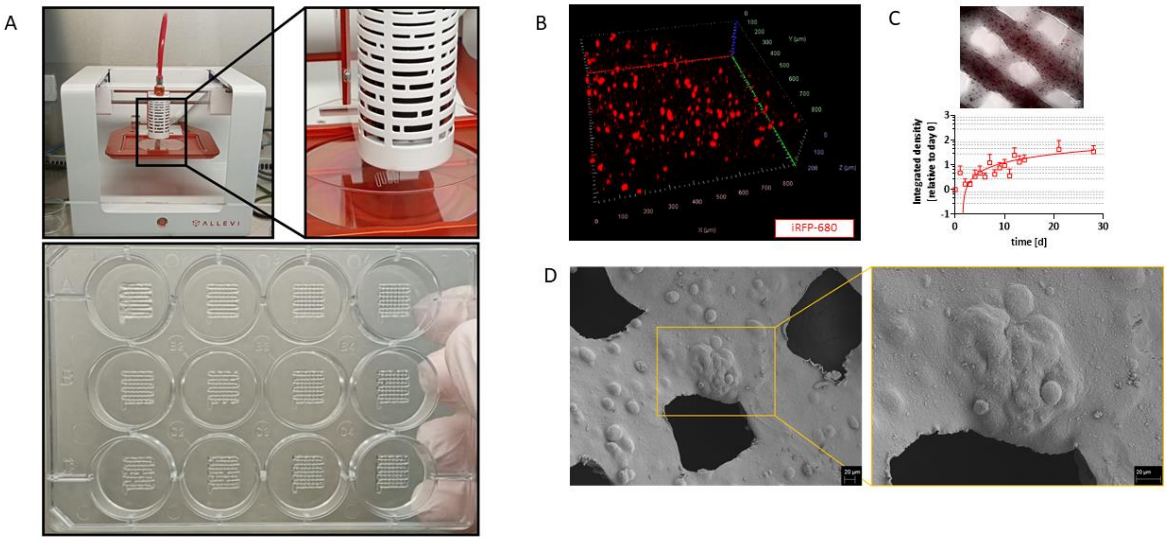

Figure 1: 3D-bioprinting of glioblastoma cells. (A) Extrusion-based printer and scaffolds after printing into 12-well plates; (B) Confocal image of 3D-structure showing spatial distribution of iRFP-viable glioblastoma cells; (C) Scaffold with iRFP-viable glioblastoma cells and growth curve analysis; (D) Scanning electron images showing integrity of the scaffold.

RP 1

3D-Printed Zinc Alloys as Biodegradable Implant Materials

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[2]Forschungsinstitut Edelmetalle + Metallchemie, Schwäbisch Gmünd, Germany

Additive manufacturing by selective laser melting (SLM) offers the potential for producing customized implants with intricate geometries and desired bulk and surface properties. Zinc, with its ease of biodegradation and low cytotoxicity, stands out as a promising material for implants, ideally dissolving to facilitate concurrent new bone formation.

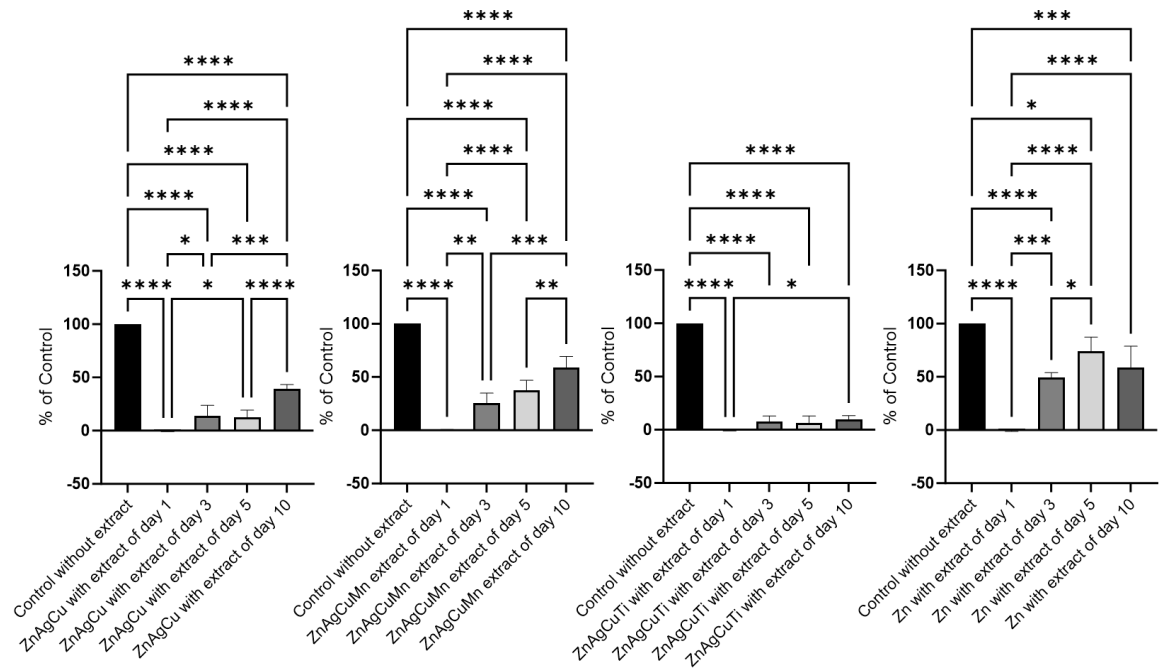
This study aimed to assess the cytotoxicity of 3 SLM-printed zinc alloys to determine the suitability of these manufactured samples as implant materials.

SLM-manufactured zinc alloys (ZnAgCu, ZnAgCuMn, ZnAgCuTi) platelets were tested alongside zinc (Zn), copper (Cu), and titanium (Ti) sheets. The cytotoxicity of the different metals was assessed according to ISO 10993-5: 2009 using indirect and direct contact tests. Extracts were collected over 10 days and incubated with the human osteoblast-like cell line SAOS-2 for 24 h. Cell proliferation was analyzed via BrdU assay, direct sample contact through microscopy.

Undiluted extracts from SLM-produced sample platelets exhibited cytotoxic effects on SAOS-2 cells. The cytotoxicity decreased with prolonged collection time, with ZnAgCuMn exhibiting the lowest among the newly SLM-printed Zn alloys (Figure 1). The microscopic analysis of the direct contact test revealed minimal cell growth directly on the sample and in the immediate vicinity of the samples. The cell layer became denser with increasing distance from the platelets (Figure 2).

The findings indicate that the three SLM-produced zinc alloys, without subsequent surface treatment, are too cytotoxic to SAOS-2 cells for direct use as implant material. Further investigations are necessary to determine whether cytotoxicity is primarily influenced by surface enlargement during manufacturing, corrosion rates, alloy composition, or oxidation states of the samples. Subsequent experiments should also explore potential surface treatment processes to reduce cytotoxicity.

Fig. 1


% of Control
150
100
50
0
-50
Control without extract
ZnAgCu with extract of day 1
ZnAgCu with extract of day 3
ZnAgCu with extract of day 5
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ZnAgCuMn extract of day 1
ZnAgCuMn extract of day 3
ZnAgCuMn extract of day 5
ZnAgCuMn extract of day 10
ZnAgCuTi with extract of day 1
ZnAgCuTi with extract of day 3
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Zn with extract of day 1
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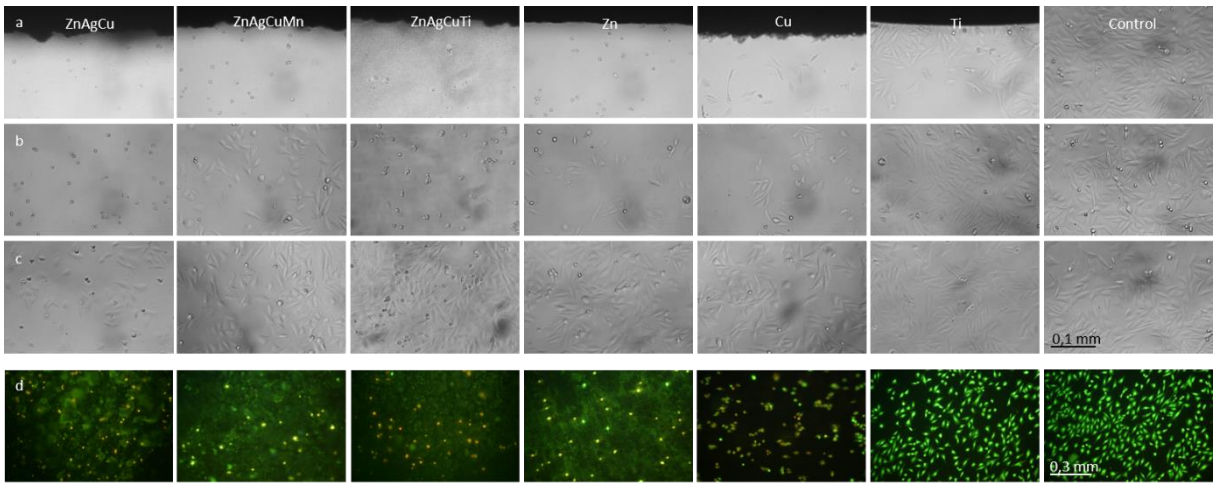
Zn with extract of day 5
Zn with extract of day 10

Fig. 2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ZnAgCuMn
ZnAgCuTi
Zn
Cu
Ti
Control
b
c
0,1 mm
d
0,3 mm

RP 2

Response of subtoxic concentrations of various metal ions on adipose-derived mesenychmal stem/stromal cells

O. Hahn[1], K. Ekat[2], O. T. Waheed[1], B. Müller-Hilke[3], A. Jonitz-Heincke[4], R. Bader[4], T. Fiedler[2], K. Peters[1]

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[2]University Medical Center, Institute for Medical Microbiology, Virology and Hygiene, Rostock, Germany
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One of the complications of joint arthroplasty is aseptic implant loosening due to adverse biological reactions. Despite major advances the development of implants, they can release metal ions and particles, such as nickel, cobalt, and chromium, which can affect the surrounding tissue. The aim of this study was to investigate the low-threshold, "adapted" cellular responses to non-cytotoxic concentrations of the divalent metal ions Ni2+ (tested as NiCl2) and Co2+ (tested as CoCl2) and the trivalent Cr3+ (tested as CrCl3) in mesenchymal stem/stromal cells in vitro. The analyses focused on the effects on proliferation, inflammation, cell stress, and energy metabolism.

Exposure to the different metal ions at non-cytotoxic concentrations did not lead to obvious changes in cell morphology and cell number. The exposure of stem cells to the metal ions Ni2+ and Co2+ led to a slight decrease in the release of the pro-inflammatory factors interleukin-6 and -8, but without detectable effects on NF-kB localization. Divalent metal ions are known to influence the gene expression of a variety of signaling molecules related to tissue regeneration by stabilizing the transcription factor hypoxia-inducible factor (HIF)-1α. Thus, a typical HIF-1α-signaling dependent factor, vascular endothelial growth factor (VEGF), was shown to be increased by exposure to Ni2+ and Co2+, although it was not possible to detect a significant stabilization of HIF-1α. Furthermore, Ni2+ and Co2+ caused a clear shift in energy metabolism towards a glycolytic metabolism. Both aspects triggered by the divalent ions support the induction of oxygen deficiency signals. In contrast, the trivalent Cr3+ did not trigger any effects.

This study shows that even non-cytotoxic concentrations of metal ions can have significant effects on cell behavior. The complex mechanisms of the biological reactions triggered by metal ions remain to be fully elucidated in order to prevent or treat metal implant failure.

RP 3

Influence of metallic particles and TNF-α on the NLRP3 inflammasome in human osteoblasts

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Following total joint arthroplasty, released metal particles and their corrosion products induce cellular inflammation, which can lead to osteolysis and implant failure. Interleukin (IL)-1β is a critical factor that promotes osteolysis by enhancing the inflammatory response through the induced release of IL-6 and IL-8. The release of mature IL-1β is tightly regulated by the NLRP3 inflammasome, which requires two signals for activation: an NF-κB-activating priming signal and an inflammasome-activating signal.

This experimental study aimed to investigate the extent to which TNF-α and metal particles can initiate the priming signal, activate the inflammasome, and amplify the inflammatory response in human osteoblasts (hOBs).

For this purpose, hOBs were exposed to metallic particles made of cobalt-chromium (CoCr; 10 µg/mL) and TNF-α (0.5 µg/mL) alone or in combination for incubation times ranging from one hour to three days. Temporal gene expression profiles of TLRs, related pathways, and inflammatory markers were examined.

Initial changes in gene expression were observed after four hours of particle exposure, with induction of genes associated with particle recognition and inflammasome priming signal. Increased levels of proinflammatory cytokines were detected after eight hours of stimulation. The combined treatment with CoCr+TNF-α led to an increase in the expression of TLRs and associated genes, as well as increased levels of *proIL1B*. A significant increase in IL-6 release was also shown after TNF-α treatment alone.

HOBs appear to respond directly to metallic particles via TLRs. However, the particles alone did not show increased activity of the NLRP3 inflammasome, whereas the combination with TNF-α, which can prime the inflammasome, led to the induction of IL-1β, IL-6, and IL-8 production. This demonstrates that the wear-induced inflammatory response in hOBs is strongly induced by signaling molecules in the environment.

RP 4

Chitosan-Quercetin Complex: Osteogenic Induction in Mouse MSCs and Fracture Healing in a Zebrafish Osteoporosis Model

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Introduction: Fractures are frequently occurring and significant traumatic injuries. The process of fracture healing is complex, with osteogenesis and recovery time being impacted by multiple factors like blood supply, stability, and inflammation. Approximately 5–10% of fractures do not successfully undergo the healing process (Gao et al., 2023). Recent scientific studies have focused on exploring the effectiveness of mesenchymal stem cell (MSC)–based therapeutic techniques in both clinical and preclinical settings for treating fractures within the field of regenerative medicine (Smolinska et al., 2023).

Objective: To Investigate the therapeutic potential of a chitosan-quercetin bio-conjugate as an osteogenic agent.

Materials and Methods: In this study, we utilized a zebrafish model, employing the widely accepted dexamethasone-induced osteoporosis model to study bone regeneration. In-vivo toxicity profiling using zebrafish larvae demonstrated the biocompatible dose of the chitosan-quercetin complex.

Results: The in-vitro assessment in mouse MSCs showed that chitosan-quercetin significantly enhanced the expression of key osteoblast markers—including Runx2, ALP, type1 collagen, osteocalcin, and osteonectin—and promoted mineralization more effectively than either chitosan or quercetin alone. In the osteoporosis-induced zebrafish models, the bio-conjugate exhibited remarkable potential for bone regeneration, stimulating bone calcification, callus formation, and bone healing. The study also revealed the bio-conjugate's inhibitory effect on osteoclastic activity through decreased TRAP activity and reduced hydroxyproline release, confirming its potential for mitigating bone resorption.

Conclusion: This research provides evidence of the chitosan-quercetin bio-conjugate's osteogenic potential, offering promising prospects in the fields of bone tissue engineering, regenerative medicine, and the treatment of conditions like osteoporosis.

RP 5

Nanodiamond-functionalized titanium surfaces for the formation of a vital oral soft tissue structure

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Introduction: Dental implants are a popular treatment for replacing natural teeth, but their increasing number is leading to complications like peri-implantitis, which causes bone resorption and implant loss. Bacterial biofilms on oral surfaces are the primary cause. Nanodiamond (ND)-based coatings appear promising for implants due to their high biocompatibility, adjustable size distribution and versatile functionalization strategies.

Objectives: The study aims to investigate the impact of topography, surface energy, and -chemistry on the interaction with oral cells and biofilm formation, by integrating functionalized NDs into titanium oxide layers.

Materials & Methods: Commercially available NDs were functionalized with hydroxyl, carboxyl or APTES groups, deagglomerated and fractionated. NDs were partially embedded in Ti surfaces, tested for salivary adhesion, biofilm formation and human gingival fibroblast adhesion under static conditions.

Results: APTES and hydroxyl modifications of the NDs resulted in a positive net charge, raw and carboxy-functionalized NDs were negatively charged. Cluster sizes ranged from 50 nm to 190 nm. Stable fixation of NDs on Ti was done by electrochemical anodization. Salivary adhesion showed no difference on these surfaces compared to cpTi surfaces. Biofilm formation of S*. intermedius* was reduced on ND surfaces, live/dead imaging showed altered biofilm structures. Gingival fibroblasts adhered to all ND surfaces, especially with higher nano-roughness. Increased cell numbers were observed on APTES- and hydroxyl-NDs.

Conclusion: The study reveals that nano-roughness and surface chemistry influence fibroblast cell adhesion and biofilm formation. Differences in cell adhesion indicate that the altered surface topography may affect adhesion of soft tissue. The formation of biofilms and their removal is currently being tested, and further modification of the surface chemistry of ND is currently being investigated.

RP 6

Implementation of 3D perfusion bioreactors for stem cell colonization of bone scaffolds for chairside applications

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Introduction: There is a significant need for new regenerative strategies to treat craniofacial bone deficiencies. Vertical and voluminous augmentations are a challenge. Therefore, oversized ceramic bone substitutes should be seeded with endogenous patient-derived stem cells.

Objectives: In this study, 3D printable perfusion bioreactors are designed for chairside applications with a specific emphasis on improving the speed and uniformity of stem cell distribution in bone substitute materials.

Material and Methods: This study combines mathematical modeling, 3D printing, perfusion experiments with adipose-derived stem cells, advanced imaging techniques (micro-PET/CT), scanning electron microscopy, and immunohistochemistry to comprehensively investigate and visualize the processes of stem cell attachment, spreading, and osteogenic differentiation within various 3D-printed bioreactor geometries.

Results: We have shown that mathematical models determine the homogeneity and efficiency of sowing depending on defined parameters such as the bioreactor geometry, the perfusion rate, and the cell density of the perfusate. The combination of the conical inlet shape with a tailored silicone sheath resulted in significant, homogeneous cell distribution using unidirectional perfusion. When using oscillating perfusion, the homogeneity of cell distribution can be increased. This bioreactor is suitable for the homogeneous colonization of individual scaffolds with stem cells in less than an hour. In the present studies, the following four parameters were crucial for efficient cell colonization of the scaffold: 1. cell concentrations of 0.5 to 1.0 × 10^6 cells/ml, 2. a low perfusion rate of 0.5 - 1 ml/min, 3. Scaffold coating with a silicone sleeve and 4. an oscillating perfusion mode.

Conclusion: This newly designed bioreactor may be considered as a prototype for chairside application.

RP 7

Electroactive oxidized Alginate-Gelatin-PEDOT hydrogels for 3D Printing

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[2]University of Rostock, Chair of Microfluidics, Rostock, Germany

Introduction: Electrically conductive hydrogels (ECH) are an attractive material choice for tissue engineering due to their mimicry of electrically conductive tissue and the promising effect of electrical stimuli on cell proliferation and differentiation. One strategy to produce ECHs is the functionalization of natural hydrogels with electrically conductive polymers.
Objectives: Here, we present a novel composite hydrogel composed of an alginate-dialdehyde-gelatin (ADA-GEL) matrix modified with synthesized Poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) particles to produce 3D-printable ECHs for tissue engineering applications.

Materials & methods: The ADA-GEL hydrogel was modified by the addition of 0.5, 1.0, 2.5, and 5.0 w/v% PEDOT:PSS particles. The resulting hydrogels were characterized in terms of electrical and mechanical properties, 3D-printability, and cell-material interactions using NIH-3T3 fibroblasts.

Results: The investigated hydrogels showed tailorable properties depending on the PEDOT:PSS particle concentration. The results indicated an increased electrical conductivity (EC) with increasing particle concentration. In contrast, no significant change in mechanical properties was observed, thus allowing to study the effect of EC on cells exclusively. Furthermore, all composite hydrogels were 3D-printable and presented favorable cell-material interactions up to a particle concentration of 1.0 w/v%.

Conclusion: In conclusion, PEDOT:PSS-functionalized hydrogels with tunable properties were successfully fabricated and PEDOT:PSS composite hydrogels offer significant potential in 3D Printing. With further development of these biomaterials and the optimization of the 3D printing process, the investigated materials promise to create patient-specific scaffolds.

RP 8

Evaluation of nanogold and magnetic beads for immuno-labeling of cell surfaces in scanning electron microscopy (SEM)

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Introduction: Scanning electron microscopy (SEM) is valuable to analyze the interactions of cells and tissues with biomaterial supports over a wide range of scales. For a detailed characterization of cells the application of specific surface labeling is often desirable to expand ultrastructural analyses beyond of morphometric measurements. In SEM, this technique requires the use of electron-dense substrates.

Objectives: In our study we have compared the use of nanogold of different sizes (5-30 nm) and of magnetic microbeads for use in SEM cell surface labeling. We used non-adherent blood cells as well as co-cultured neurons and Schwann cells for labeling.

Materials & methods: Human lymphocytes were isolated from peripheral blood, glial and neuronal cell cultures were obtained from of dorsal root ganglia (DRG) of embryonic chicken (E12). Cell surface labeling was either performed with anti-CD45 or with anti-O4 (a glycosphingolipid marker) coated to magnetic microbeads for cell sorting or using gold-coupled secondary antibodies.

Results: Immuno-labeling of cell surfaces with gold-coupled antibodies can be successfully applied in SEM on isolated blood lymphocytes and on cultured neuronal cells. The appropriate choice of nanogold-size is dependent on labeling efficacy (small gold size) versus nanogold visibility at low magnifications (larger gold size). Cell surface marker quantification as well as combination with fluorescent labeling are possible. In contrast, magnetic microbeads frequently show clustered labeling which hampers marker quantification and despite their large size are difficult to image in the SEM.

Conclusion: We suggest nanogold-coupled antibodies with a diameter of 15 nm that offer a good compromise with respect to labeling efficiency and visibility using high resolution SEM. The application of magnetic microbeads for cell surface labeling in SEM is much less favorable in our models, despite their successful use in transmission electron microscopy.

RP 9

Proteomic Analysis of Bone Using Mass Spectrometry

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Introduction: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a widely employed technique in proteomics research for studying the proteome biology of various clinical samples in orthopedics and dentistry. Hard tissues, such as bone and teeth, are routinely preserved using synthetic poly(methyl methacrylate) (PMMA) embedding resins that enable histological, immunohistochemical and morphological examination. Yet, the suitability of PMMA-embedded hard tissues for proteome analyses remained unexplored.
Objective: This study aimed to be the first to report on the feasibility of PMMA-embedded bone for proteomic analysis using LC-MS/MS.

Materials & methods: Porcine bone was preserved either PMMA-embedded or fresh-frozen. For each preservation technique, the impact of decalcification was evaluated, resulting in an experimental design of four workflows, each performed in four replicates. Samples were processed for LC-MS/MS analysis using prefractionation strategies by high-pH reversed-phase liquid chromatography in combination with isobaric tandem mass tag labeling. State-of-the-art software was used for data analysis and statistical analysis.

Results: The advanced strategies employed in this study resulted in a proteome coverage exceeding 1000 protein identifications. Statistical analysis revealed less variance between replicates of the PMMA-embedded samples than those of the cryopreserved samples, which favors the use of PMMA embedding as a means to preserve bone for subsequent proteome analysis in high reproducibility.

Conclusion: The preservation of bone samples facilitates long-term storage at room temperature and thus the generation of sample cohorts. In these large-scale studies, proteome analyses can be used to advance our understanding of bone biology in health and disease.

RP 10

Application of voltage matrix from the cochlear implant electrode array to predict cochlear coverage

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Introduction: In cochlear implantations, previous research has demonstrated that broad coverage of the cochlear duct (CC) by the electrode array positively affects speech comprehension. However, during the surgical procedure, the surgeons view is limited to an external view at the cochlea only, lacking direct observation of the electrode arrays position within the cochlea. Postoperatively, radiological imaging is routinely applied to verify its placement.
The voltage matrix (VM) derived from the electrode array in patients with cochlear implants (CIs) mirrors the variations in tissue resistance within the scala tympani. Given apical increase in tissue resistance, it becomes conceivable to estimate the CC intraoperatively using the VM.

Objectives: This study aims to explore the correlation between CC and VM and to propose a model for intraoperative monitoring of the intracochlear electrode positioning.

Materials and methods: Patients undergoing a cochlear implant surgery MED-EL (Synchrony2, MED-EL, Innsbruck, Austria) between 2018 and 2022 were retrospectively enrolled in this study. The preoperative CT images were examined by OTOPLAN to model the cochlear duct length (CDL).
The CC was calculated taking the ratio of the length of the electrode array to the CDL. All the intraoperative voltage records were retrieved and analyzed to plot the voltage matrix.

Results: The voltage matrix showed a best correlation between the stimulating electrode 8 (E8) and measuring electrode E4. The voltage between E8 and E4 demonstrated a correlation with CC ($R = 51$, $p < 0.01$).
Conclusion: The voltage matrix can be utilized to offer the surgeon intraoperative real-time feedback on the cochlear coverage. This aids the surgeon in achieving optimal coverage to improve postoperative cochlear implant performance.

RP 11

Development of a 3D-bioreactor system for *in vitro* endothelialization analysis of cardiovascular stents

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(1) Endothelialization of cardiovascular stents represents a key factor regarding safety and clinical success in mid and long term. As a powerful tool bioreactor systems enable an evaluation of stent endothelialization behavior in artificial or native vessel models (scaffolds) under *in vitro* conditions. Bioreactor systems are able to provide valuable information about biomaterials or implant structure interaction with cells.

(2) Within the current work we developed a 3D-bioreactor system for *in vitro* endothelialization analysis of novel cardiovascular implants, particularly stents. A bioreactor system was developed technically and implemented into a biological laboratory setup. A proof of feasibility should be carried out.

(3) The bioreactor was designed and manufactured based on sterilizable polymers polymethylmethacrylat and polyetheretherketon. A silicone based scaffold was used. For homogeneous endothelial cell coverage of rotational symmetric implants, a rotation mechanism for the scaffold was implemented. The bioreactor system was implemented into an incubator under physiological conditions. After endothelialization of scaffolds morphological analysis was performed using immunofluorescence.

(4) The preliminary implementation of the bioreactor system as well as the first *in vitro* tests conducted were successful. Colonization experiments demonstrated a subconfluent monolayer of endothelial cells depending on the amount of initially seeded cells. Immunofluorescence evaluation of cells grown on the scaffold surface revealed a typical endothelial cell morphology.

(5) A 3D bioreactor system for *in vitro* endothelialization experiments was successfully developed and tested. Future expansions of the test setup such as a flow system for simulation of blood flow will further approximate the *in vivo* situation. The system will allow implantation of cardiovascular stents in order to investigate cell-stent interaction under physiological conditions in the future.

RP 12

The impact of quercetin-coated implants to promote osseointegration

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Introduction: Quercetin (QCT), a flavonoid present in many fruits and vegetables, is known by its anti-inflammatory and antioxidant properties. Recent research has suggested that QCT could enhance the integration of dental implants by mitigating inflammation and promoting bone growth. In addition, protein adsorption on biomaterials is influenced by its surface properties. Therefore, studying these protein adsorption patterns through proteomics can give us valuable information about the biological response to the material.

Objectives: The aim of this work is to develop coatings with increasing concentrations of QCT (0, 0.5, 1.5 and 2 in %wt) and analyze their *in vitro* immune response, cell adhesion and bone regeneration properties as well as its effect on protein adsorption patterns.

Materials & methods: Coatings for Ti surfaces were synthesized via sol-gel doped with increasing concentrations of QCT. Materials were physicochemical and biologically characterized on HOb and THP-1. Finally, the adsorption of human serum proteins onto the material surface was also evaluated through nLC-MS/MS.

Results: The addition of QCT to the sol-gel network resulted in HOb cell adhesion improvement. At low QCT concentrations, there was a decrease in the expression of pro-inflammatory genes such as MCP-1 and IL-1β. Simultaneously, proteomics analysis revealed that QCT coatings exhibit a greater affinity for proteins involved in cell adhesion including PROF-1 and COF-1 as well as those involved in oxidative stress management like GPX-3 and tissue regeneration such as COLA-1.

Conclusion: QCT-doped sol-gel coatings have demonstrated a remarkable immunomodulatory effect, enhanced cell adhesion, and the regulation of antioxidants. Moreover, a direct correlation was observed between the concentration of QCT and the modulation of immune response. Promoting cell adhesion and a controlled immune response, which could lead to an improvement in osseointegration.

Poster

P 1

Development of an advanced hen's egg chorioallantoic membrane system for biomaterial and pharmaceutical ingredient testing *in vivo*

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(1) The hen's egg chorioallantoic membrane (HET-CAM) test is an *in ovo* experimental organism test developed to replace rabbit irritation tests. The method is becoming increasingly popular also for the investigation of biomaterials, especially in view of the increased implementation of the 3R's (replacement, reduction, refinement) to avoid animal testing. The method is also interesting for analyzing injectable biomaterials such as nanoparticles in an *in vivo* system due to the blood vessels of the chicken embryo located directly under the CAM.

(2) Within the current study, an advanced HET-CAM system with optimized accessibility of blood vessels underneath the CAM and with an increased application area on the CAM was developed.

(3) For advanced HET-CAM testing, fertilized White Leghorn chicken eggs (day 3) were cracked under laminar flow and transferred into sterile weighing dishes. These were placed in glass Petri dishes previously filled with sterile water and transferred into an incubator at 37 °C with 60% relative humidity. The *ex ovo* developing embryos were monitored daily, measured and photodocumented until they had reached the developmental stage for a test application (day 11-14).

(4) The methodology of *ex ovo* incubation was improved and adapted within several successive experiments. As a result, the survival rate of the developing embryos was increased from 11% to 61%. Accessibility to the CAM and the vessels has been significantly improved, which makes intravenous injections much easier. Furthermore, the application area on the CAM was extended from approximately 7 cm^2 (*in ovo*) to 37 cm^2 (*ex ovo*).

(5) The HET-CAM *ex ovo* test was successfully established. It was possible to significantly increase the application area for biomaterials on the CAM and improve accessibility to the vessels, making injections much easier. The adapted examination method facilitates biomaterial testing *in vivo* and can help to reduce the number of animal experiments.

P 2

Incorporation of cobalt ions into polymer films to study ion release of metallic implants

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Introduction: Endoprostheses made of cobalt-chromium (CoCr) alloys may release particles and ions. Undesirable reactions in the surrounding tissue necessitating revision surgery, and also at distal sites may be caused. To simulate the wear particle release in vivo and gain a deeper insight into the cellular processes associated with systemically increased metal ions, a release systems is required. We have focused on the development of polymer release systems for the sustained release of incorporated Co ions.

Objectives: The aim is to demonstrate the manufacturability and the release assessment of incorporated Co ions in different polymers, especially in biodegradable poly(L-lactic acid) and biostable silicone (SIL) films. In an initial in vitro study, Co ions are extracted from the films and determined by HPLC.

Materials and Methods: PLLA-Co films were cast from a mixture of PLLA dissolved in chloroform and cobalt chloride dissolved in acetone by a dip coating process. SIL-Co films were produced by mixing (a) cobalt chloride dissolved in acetone or (b) as salt with the SIL base elastomer. The curing agent was added according vendor instruction. Mixtures were degassed and poured into PTFE molds. Curing took place at 50°C for 48 h.
Co ions were extracted with water and determined as diethyldithiocarbamide chelate at l = 260 nm by HPLC.

Results: PLLA or SIL films with incorporated Co ions were produced. A final film thickness of 92 µm for PLLA-Co and 1.0 mm for SIL -Co films was achieved. HPLC measurements showed that Co ions were successfully incorporated and extracted from all tested polymer films.

Conclusion: In this study a method for the production of polymer films that release Co ions is presented. Individual release kinetics will be tested in future experiments. The methods generated here will be transferred to further polymers and metal ions in order to enable systematic studies on the toxicity of metal ions on different cell types and tissues.

Poster

P 3

Electrical Stimulation of Human Stem Cells: Optimization of Parameter Thresholds Using an Extended Experimental Setup with Alternating Current Monitoring

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Introduction: The complex field of electrical stimulation (ESTIM) of cells is a rapidly expanding area of tissue regeneration research. Despite numerous studies, no clear dose-response correlation has yet been established. This correlation is crucial for successfully implementing electrical stimulation in therapeutic applications. Electrical stimulation combined with an implant could be an alternative for treating critical bone defects. For this purpose, basic laboratory research must be carried out to investigate the effects of electrical stimulation on cells.

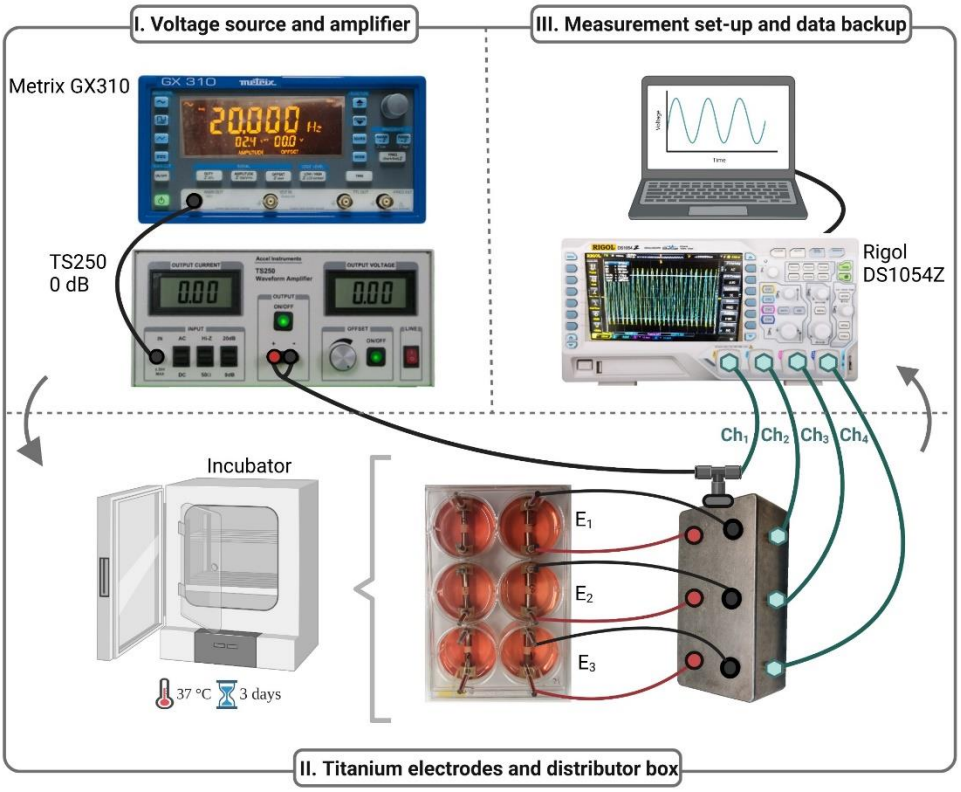
Objectives: In summary, our study on *in vitro* AC stimulation aimed to determine thresholds at the cellular level for human stem cells. This study addresses essential technical aspects and provides software solutions to improve the reproducibility and transparency of electrical stimulation experiments. These solutions support the development of an electrically active implant system.

Materials & methods: In this study, we expanded the experimental setup for *in vitro* electrical stimulation of stem cells via titanium electrodes. This setup, depicted in Figure 1, has an integrated measuring system. This enhancement allows real-time monitoring of applied voltage, current, impedance, and phase shift. Subsequent biological investigations focused on evaluating stimulated stem cells' relative metabolic activity and migration dynamics.

Figure 1: Experimental setup for *in vitro* stimulation.

Results & Conclusion: Although cell-specific parameter thresholds for AC stimulation have not been identified, the presented system can reliably and transparently perform electrical stimulation experiments and thus expands the field of electrical stimulation in an important way. Based on the electrical measurements during the stimulation experiments, a non-linearity of the current strength and impedance could be recognized. This proves the necessity of real-time monitoring during such experiments.

Fig. 1


I. Voltage source and amplifier
III. Measurement set-up and data backup
Metrix GX310
TS250
0 dB
Rigol
DS1054Z
Ch1 Ch2 Ch3 Ch4
Incubator
37 °C
3 days
E1
E2
E3
II. Titanium electrodes and distributor box

Poster

P 4

Enhancing Bone Cell Activities on Biomimetic Calcium Phosphate Substrates through Biophysical Stimuli: Insights into the Voltage-Frequency Relationship

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Introduction: Biomaterials and biophysical stimuli, such as electric fields (EF), offer complementary therapeutic strategies in bone healing, combining to mimic endogenous electrical potentials and currents promoting osseointegration [1].

Objectives: This study explores the synergistic effects of biomimetic calcium phosphate substrates and biophysical stimuli on bone cell activity, specifically investigating the relationship between voltage and frequency to optimize cellular responses [2].

Materials & methods: RAW 264.7 macrophages and MC3T3-E1 pre-osteoblasts were seeded on biomimetic calcium phosphate (BCP) coated Ti6Al4V substrates and stimulated with alternating electric fields at varying voltages (0.7, 2, and 5 Vrms) and frequencies (20, 500, and 1000 Hz). Cell viability, morphology, and gene expression were assessed after 7 and 14 days for RAW 264.7 and MC3T3-E1, respectively.

Results: Higher electrical voltages tended to reduce cell viability, while this effect could be counteracted with higher frequencies up to 1000 Hz. Examined osteoclastic gene markers Cd14 and cathepsin K were found to be significantly influenced by electrical voltage. Examined osteoblastic gene markers Runx2 and osteopontin were found to be significantly influenced by electrical voltage while the control factor frequency significantly influenced osteonectin and osteocalcin.

Conclusion: Our findings reveal a promising approach for advancing regenerative medicine, which highlights the complex interplay between material design and biophysical factors for enhanced bone cell functionality.

Acknowledgements: This research was funded by the DFG project DE 1924/4-1 and JO 1483/1-1 "Influence of electrical stimulation on bone remodelling process".

References:

[1] F. Sahm, et at., Front. Physiol. 13 (2022).
[2] T. Kreller, et al., Biomater. Adv. 146 (2023).

P 5

Application of an FSI-based model for the prediction of cell differentiation on a mechanically stimulated structured hydrogel scaffold

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Introduction: 3D hydrogel scaffolds have the potential to be utilized in the treatment of cartilage and bone defects. In the field of tissue engineering, the generation of cartilage and bone tissue can be specifically stimulated through the application of mechanical stimulation. **Objective:** The study aims to predict cell differentiation on a porous hydrogel scaffold due to mechanical compression stimulation using a fluid-structure interaction (FSI) model.

Methods: A structured scaffold was generated using CAD. The FE model consisted of three parts: piston, scaffold, and support. The piston had vertical movement to compress the scaffold. The material model of the scaffold was based on experimental tests with pure ADA-GEL. The setup of the CFD model was a laminar, Newtonian and incompressible flow. A transient, one-way co-simulation strategy was used for the FSI setup. Prendergast's modified mechanoregulation theory was then implemented in the numerical model to predict cell differentiation.

Results: Average octahedral shear strain (OSS_{avg}) and area-weighted averaged wall shear stress (WSS_{avg}) were calculated over a loading cycle for a range of compression amplitudes from 1 % to 10 %. The differentiation of bone cells decreased with an increase in the amplitude of compression. The differentiation of cartilage cells first increased within the range of 2 % to 7 % compression and thereafter decreased. The differentiation of fibrous cells was predicted from compression amplitudes of 5 % to 10 %.

Conclusion: The presented model predicts cell differentiation on the basis of both the mechanical deformation of the scaffold and the fluid flow induced by the compression during dynamic compressive stimulation. Thus, the model can be applied to analyze, for example, the effects of different scaffold designs and stimulation parameters on cell differentiation in mechanically stimulated 3D-structured scaffolds.
This study was funded by the Deutsche Forschungsgemeinschaft (German Research Foundation)– SFB 1270/2-299150580.

P 6

The influence of electrical stimulation on the membrane fluctuation of osteoblast-like cells

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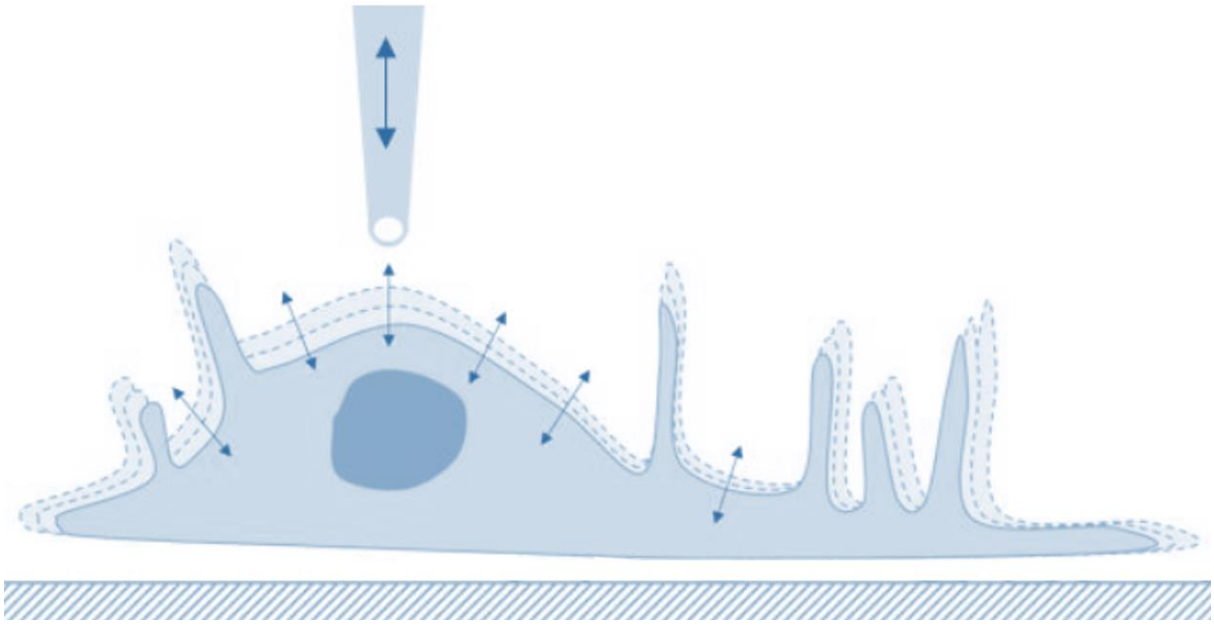
Electrostimulation can aid bone regeneration and adhesion of osteoblasts on model implant surfaces. Key factors for a better cell adhesion are the surface structure, electrical properties, and the stimulation parameters. Many parameters on a cell such as adhesion area on a surface, cell cycles or membrane fluctuation change upon stimulation, which makes it difficult to pinpoint a mechanism.

We aim to establish a viability assessment based on the membrane fluctuations of cells. Membrane fluctuations originate from thermal, metabolic, and quantum sources. The distribution of heights allows the extraction of mean fluctuation amplitudes. Frequency response analysis, using power spectral density, can reveal scaling exponents, which may reveal insights in certain physical excitation and damping mechanisms.
We investigate the influence of electrical stimulation via scanning ion conductance microscopy (SICM) on the membrane fluctuation of individual osteoblast-like cells (MG-63). We measure the membrane fluctuation over one point of the cell after electrical stimulation. We record and analyze time series data after 96h of adhesion and with a sinus-shaped AC voltage (110Hz, 5Vrms,30 min-daily). Thin rod-shaped Pt electrodes are placed in the electrostimulation chamber.

We find fluctuation amplitudes in the regime of a few 10nm for the stimulated and unstimulated cell ensemble. The Scaling exponents are in the regime of -1.7 (below f~2Hz) and -2.7 (beyond f~2Hz).

In initial experiments on membrane fluctuation, a low response to electrical stimulation in the low frequency range was observed. So far, the amplitude of the cells has not been affected by the electrical stimulation. More data is needed for verification. In the future, we will investigate the use of substrate electrodes based on polyelectrolyte films to reduce possible electrode corrosion from metallic electrodes that could affect the cells.
The research is funded by the DFG via SFB ELAINE 1270/1,2 - 299150580.

Fig. 1



P 7

Biomaterials Engineering Assisted by Low-energy Non-thermal Electron Beam Technology

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Innovative medical and healthcare technologies will improve the quality of life for people worldwide. The demand for the advanced manufacturing of personalised and efficient health care solutions is growing. Enhanced control of the biological response of interfacing biomaterials can be achieved by surface modification technologies. Particularly, low-energy, non-thermal electron beam technology (e-beam) represents a multifunctional tool for surface and material engineering. By low-energy e-beam processes adjusted and scalable modification effects can be realized at accelerating voltages up to 300keV. This sustainable e-beam technique is particularly gentle without altering the original bulk material properties. By the specific selection of the e-beam process parameters, surface as well as material engineering with customised characteristics can be ensured. Recently, low-energy accelerated electrons are successfully applied to covalently immobilize defined biobased and biocompatible substances to textile or foil surfaces as durable thin layers. Exemplary, this low-energy, non-thermal e-beam grafting technology can be applied to create hydrophilic and anti-adhesive surface properties. Complementary surface characterization methods like ATR-FTIR spectroscopy, AFM or wettability measurements were used to prove the successful thin layer immobilization and evaluate the hydrophilicity increase by a significant reduction of the static water contact angle. The biological response was investigated via cytotoxicity and cell adhesion tests. Furthermore, the low-energy e-beam technology can be additionally applied for biological tissue preparation. A newly developed process, called SULEEI, makes it possible to sterilize (S) and preserve decellularized tissue like pericardium by means of photo-initiated ultraviolet (U) crosslinking with low-energy electron irradiation (LEEI). The SULEEI procedure is a unique multi-component procedure increasing the biocompatibility of prepared biological tissues. Applying the SULEEI process eliminates the need for any subsequent tissue sterilization and is opening up new options for extending the functional lives of tissue-based prostheses.

P 8

Amino Groups in Biomaterial Coatings Influence the Cell Response

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Rising life expectancy in industrialized countries leads to an increased incidence of osteoporosis and the need for bioactive bone implants. The integration of implants can be improved by chemical surface modification. It has been recognized that amino group coatings improve cell adhesion and intracellular signaling. This work aimed to determine the role of amino group density in this positive cell behavior by developing controlled amino-rich nanocoatings.

Nanocoatings with different amino group densities were prepared on (i) titanium (Ti) by INNOVENT, e.g., trimethoxysilylpropyl modified poly(ethyleneimine) (TMS-PEI), or on (ii) silicon (Si) by IS2M, e.g., self-assembled monolayer (SAM). The wettability, surface charges, and element composition were characterized. Human MG-63 osteoblasts were cultured in DMEM with 10% FCS. PKH-stained MG-63s were observed via a confocal laser scanning microscope for cell spreading (30 min). Differential gene expression was analyzed with microarrays (24 h).

We could show a correlation between amino group density and the spreading of MG-63s. The positively-charged Ti-TMS-PEI, which mostly improved cell area after 30 min, possessed the highest amino group density with an N/C ratio of 32%. The mRNA microarray data showed a premature transition of the MG-63s into the beginning differentiation phase after 24 h [Seemann S. *et al.,* Molecules. 28 (2023):6505].

The negatively-charged Si-SAM with 75% NH_2 indicated enhanced spreading, improved Ca^{2+} mobilization, and an increased number of differentially expressed genes.
In conclusion, the cell spreading on amine-based nanocoatings correlated well with the amino group density (N/C). The content of amino groups on biomaterial surfaces seems to be essential in improving cell contact and cell response at the interface.

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P 9

Plasma deposited thin film coatings with improved biocompatibility and antibacterial properties

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Many implants must fulfill their function in the body for many years and this requires that the implant surface has to interfere with bone-, cartilage-, or connective tissue cells over a long period of time. Besides structural requirements, especially the chemical properties of the implant surface are very important for a fast and successful integration of the implant and to prevent undesirable effects like infection and inflammatory or allergic reactions. Plasma is already widely used in medical device manufacturing for surface activation or coating via plasma-enhanced chemical vapor deposition (PECVD) of polymer and metal surfaces for improved bonding, sealing, or printing and could well be used to improve the surface for an optimized implant-tissue-interface.

We used PECVD coating to deposit functional groups, such as hydroxyl- or amino groups, or antimicrobial compounds on implant materials, leading to a shift in surface energy to more hydrophilic properties, increased surface roughness and antimicrobial properties.
Low pressure- and atmospheric pressure plasma was used for various PECVD coatings on common implant materials like stainless steel, titanium, and polymers, like polyetheretherketone (PEEK). Coatings were physicochemical characterized and analyzed for biocompatibility, antibacterial properties and for potential cytotoxic effects.

Various coatings were generated and investigated and showed no cytotoxic effects, but enhanced cell attachment and highly improved viability of bone cells, osteoblasts in cell culture. This kind of thin film coating would be beneficial for a rapid implant osseointegration. Other coatings showed strong antibacterial effects and thus, might prevent infections.
PECVD processes can be used to generate bioactive thin film coatings on common implant material surfaces. The functionalized surfaces are non-toxic, have improved biocompatibility or have antibacterial properties.

Fig. 1

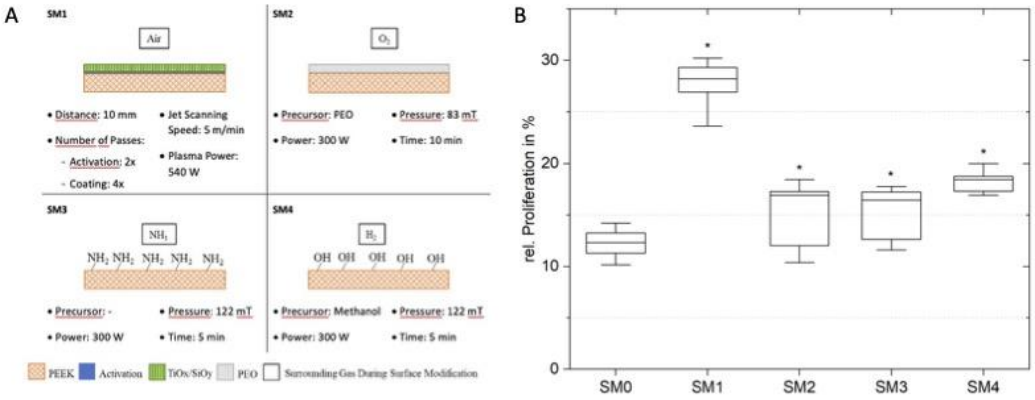

Figure 1: **PECVD coatings for improved biocompatibility.**
A: PECVD coatings functionalized surfaces SM1 to SM4 using atmospheric pressure plasma (SM1) and low pressure plasma (SM 2 to 4).
B: Proliferation of cultured osteoblast after after 7 days in culture on test surfaces SM1 to SM4 in relation to uncoated polymer surface, SM0. Proliferation rates were determined relatively to confluent cells grown in a common multi well plate after 7 days of incubation (positive control = 100 %), * represent statistical significance ($p < 0.05$); L. Strauss et al. ***Plasma Process. Polym.*** 2023, e230082.

P 10

Effects of intraoperative cochlear implant electrode conditioning on impedances and electrically evoked compound action potentials

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Introduction: The cochlear implant (CI) functions as an electrical sensory prosthesis by directly stimulating the auditory nerve, thereby restoring hearing for individuals who have profound hearing impairment. An essential step in the CI fitting process involves a surgical procedure carried out under anesthesia. This procedure is crucial, as it requires careful verification of the device's functionality through objective measurements.

Objective: The present study seeks to investigate whether conditioning the electrode array through the application of electrical stimulation within a saline solution during CI surgery can have a positive impact on subsequent intra-operative measurements. The hypothesis suggests that, drawing on previous research, conditioning will result in lower impedance values and enhanced reproducibility of Electrically Evoked Compound Action Potentials (ECAPs).

Methods: Half of the electrode contacts underwent conditioning within a saline solution prior to electrode insertion, employing 12 channel CIs (Synchrony 2, MED-EL, Innsbruck, Austria). Impedance measurements were taken at five distinct time points for both the conditioned and non-conditioned groups. Repeated assessments of Electrically Evoked Compound Action Potentials (ECAPs) were conducted and compared between the two groups.

Results: 23 cochlea implantations were included. Conditioning in saline reduced electrode impedance by 31%, but post-implantation showed no significant differences. The hypothesis of improved reproducibility wasn't confirmed. In the saline solution, 44% of electrode contacts had air bubbles, limiting conditioning effectiveness. Conditioning's effect on reference electrode stimulation accounted for approximately 16% of the total impedance reduction.

Conclusion: Our data does not support the clinical necessity of intraoperative electrode conditioning. Additionally, an in-vivo ECAP recording can be considered as a method for conditioning the electrode contacts.

P 11

Modification of PEEK for biomedical application

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[3]Technical University of Liberec, Faculty of Health Studies, Liberec, Czech Republic

Polyetheretherketone (PEEK) is broadly used in biomedical applications, however, in its pristine form its properties regarding cell adhesion and cytocompatibility are still not optimal. This study focuses on plasma treatment of PEEK at elevated temperatures (temperature of human body and autoclaving temperature). Changes in surface properties of the samples were studied in relation to interactions with human osteoblastic cell line. Different temperature and different time of plasma treatment modulated the surface properties of PEEK and led to changes in its surface morphology, chemistry and wettability. The lowest water contact angle values determined by goniometry were observed immediately after modification, and then increased during the aging process. The XPS method showed that as the modification time increased, the atomic concentration of oxygen on the surface increased and the amount of carbon decreased. The surface roughness and morphology of the samples were also affected by the increasing treatment time.

Temperature and plasma treatment of PEEK significantly enhanced cell adhesion and metabolic activity of tested osteoblasts in comparison to pristine PEEK.

The surface modification of PEEK by plasma treatment at elevated temperature may allow its use in the clinics because its optimized surface properties do not change neither after heat sterilization nor in the body.

Poster

P 12

In vivo- evaluation of bone substitute scaffolds colonized with human stem cells using a 3D perfusion bioreactor

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Introduction: There is a significant need for new regenerative strategies to treat craniofacial bone deficiencies. Still the gold standard is autologous bone graft material, which also poses problems such as limited availability. The development of alternative regenerative substitute strategies is consequently of high clinical importance. The usage of bone replacement scaffolds combined with growth factors, and colonized with stem cells could be a putative regenerative strategy.

Objectives: In 3D-printed bioreactors bone scaffolds were colonized with human stem cells and evaluated in a rat model.

Material and Method: Scaffolds colonized with mesenchymal stem cells were implanted in a critical-size calvarial defect in rats. Four groups were set up: 1) scaffold without stem cells, 2) scaffold with stem cells. 3) scaffold with BMP2, 4) scaffold with stem cells + BMP2. Explantation took place after 6 weeks. New bone formation was calculated by histomorphometry and micro-CT analysis

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Results: Nearly no new bone formation was mentioned in group 1. Whereas the highest bone generation was calculated in groups 3 and 4. Histological and micro-CT analysis revealed new bone formation starting from the edges of the calvarial defect directly into the scaffold.

Conclusion: This model proves that stem cell colonization of bone substitute materials by 3D-perfusion bioreactors supplemented with BMP2 as an additional growth factor resulted in a faster and more homogeneous new bone formation.

P 13

Development of a 2.5D-mucosa model to study the dental implant/soft tissue interface

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Introduction: After implantation, an initial biofilm quickly forms on dental implant surfaces exposed to the oral cavity. Apart from cleaning procedures, a stable soft tissue sealing would provide protection against invading microorganisms. Therefore, particularly the influence of implant surface characteristics, i.e. topography and chemistry, on the soft tissue adhesion are of interest. Published 3D-mucosa models try to simulate the *in vivo* situation as closely as possible, however, there is one major drawback - the costly and time-consuming hard tissue preparation, that requires special equipment and extensive experience.

Objectives: Development of a 2.5D-model to investigate the interface between titanium and human gingival fibroblasts (HGF) & epithelial cells under static and dynamic conditions. For the latter, we use a self-constructed dynamic flow chamber (Fig. 1a) to study cell adhesion and migration.

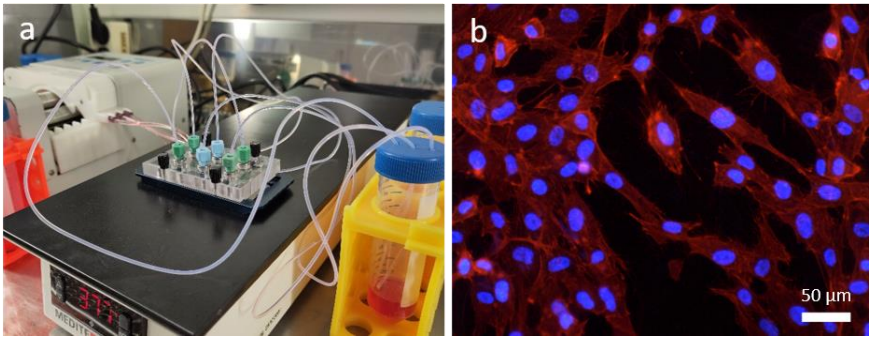
Materials and Methods: Initially, HGF are seeded statically, a special chamber allows the adhesion of cells to a restricted area. Subsequently, samples are transferred into the dynamic chamber and the adhesion and migration of the cells can be studied. Epithelial cells can be added once a stable fibroblast adhesion is reached.

Results: So far, mainly method establishment was performed (Fig. 1b). Among the different model parameters, the time of initial static adhesion and the flow rate have a great influence on the surface-cell interactions. In terms of surface properties, surface roughness in particular is anticipated to influence cell number and orientation of adherent cells, which will be also investigated.

Conclusion: Although such unidirectional flow is an artificial treatment, we believe that perturbing the cell-surface interaction by shear stress is capable of providing insight into the effect of various surface properties in a time-efficient manner.

Fig. 1 a) Dynamic flow chamber, b) HGF after 24 h of static adhesion, blue: nuclei, red: actin

Fig. 1



P 14

Biomimetic osteogenic differentiation in spheroid cultures to study biomaterial cytocompatibility

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[2]RWTH Aachen University, Institute of Structural Mechanics and Lightweight Design, Aachen, Germany
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Introduction: Cytocompatibility and osteoconductivity screenings of biomaterials still largely rely on inferior 2D *in vitro* characterization methods, while cells in 3D cultures generally behave quite differently, not only in drug screening assays.

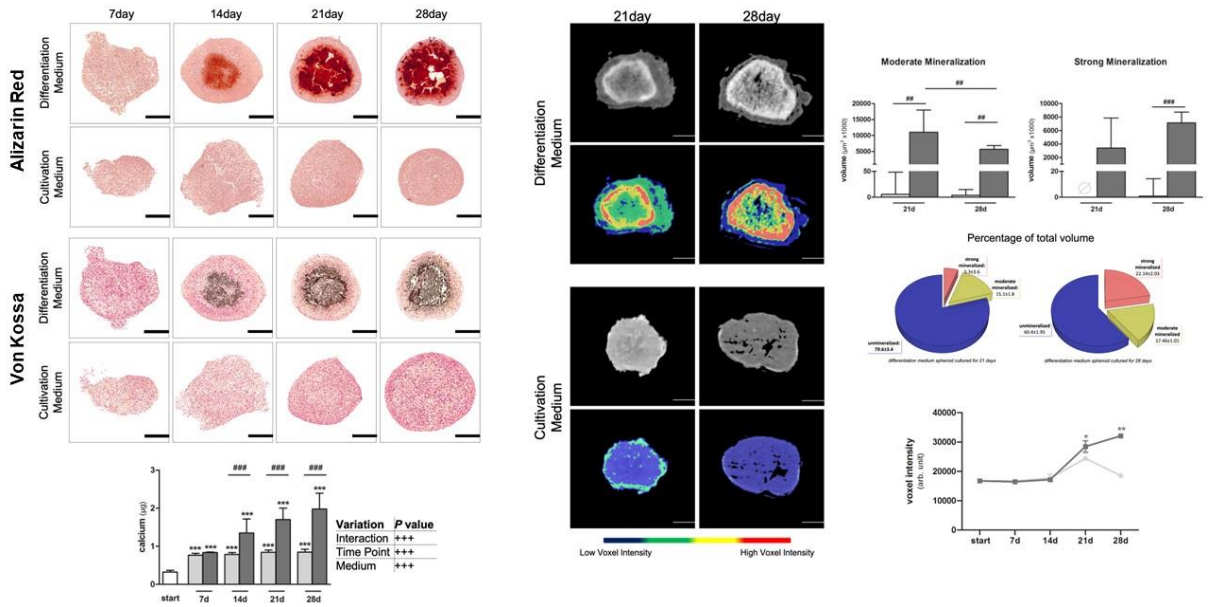
Objectives: For a more physiological cytocompatibility and osteoconductivity screening, we set out to develop a scaffold-free osteoprogenitor cell-based biomineralization model for such purposes.

Materials & methods: Murine MC3T3-E1 cells were cultured in non-adherent V-shaped plates at 37°C and 5% CO2 for up to 28 days, in α-MEM containing 10% FCS. Osteogenic differentiation was induced by 10 mM β-glycerophosphate and 50 µg/mL ascorbic acid. Mineralization stages were assessed through studying expression of osteogeneic marker genes by RT-qPCR, alkaline phosphatase activity, and calcium deposition by histochemistry. Furthermore, mineralization was evaluated qualitatively by Fourier transformed infrared (FTIR), scanning electron microscopic (SEM), and SEM-Energy-Dispersive X-ray (EDX) analyses as well as additionally quantified by micro-CT analyses.

Results: Upon brief forced gravitation, MC3T3-E1 cells formed uniform spheroids. Expression profiles of selected early- and late-stage osteoblast differentiation markers indicated a well-developed 3D biomineralization process with strongly upregulated collagen type I, osteocalcin, and alkaline phosphatase mRNA levels as well as collagen type I-, osteocalcin-, and osteopontin-positive immunohistochemistry. A dynamic biomineralization process with increasing mineral densities was observed during the second half of the culture period (days 14-28). EDX and FTIR ultimately confirmed a native bone-like hydroxyapatite mineral deposition ex vivo.

Conclusion: A robust and versatile 3D biomimetic osteogenic differentiation model with a bone-like mineralization process was established, which appears suitable for improved ex vivo biomaterial compatibility screenings.

Fig. 1


7day
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Differentiation Medium
Cultivation Medium
calcium (µg)
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7d
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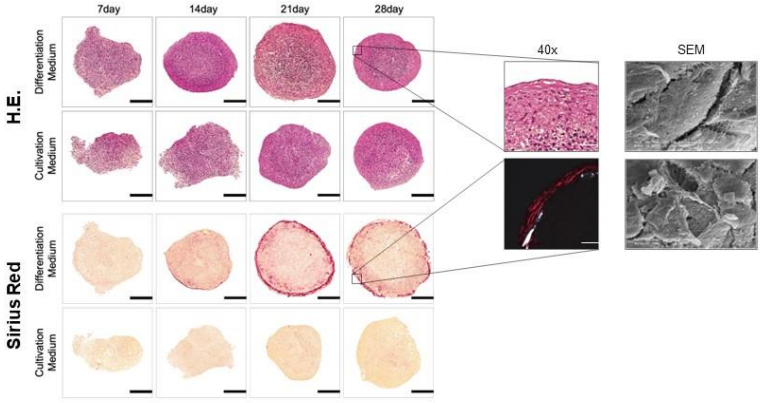
21d
28d
Variation
P value
Interaction +++
Time Point +++
Medium +++
21day
28day
Low Voxel Intensity
High Voxel Intensity
Moderate Mineralization
Strong Mineralization
Percentage of total volume
voxel intensity (arb. unit)

Fig. 2


7day
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Sirius Red
Differentiation Medium
Cultivation Medium
40x
SEM

P 15

The possible role of microphysiological systems in biomaterial testing

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In orthopaedics, pathologies and trauma of the joint can be treated by endoprostheses to restore mobility and quality of life. Orthopaedic implants are considered medical products meant to reside within the human body for a defined period of time. Within the EU, preclinical testing of implant materials is specified in the Medical Device Regulation (MDR). In order to ensure safety of their medical products, manufacturers have to follow the guidelines provided by the ISO 10993 [1]. While the respective *in vitro* and *in vivo* tests have helped to ensure the safety of medical devices in the past, they are limited in their predictive value since aspects like wear generation due to abrasion and corrosion are not considered. Aseptic implant loosening due to wear particle induced osteolysis is a leading cause of arthroplasty failure [2]. It is therefore proposed to supplement current testing strategies with complex *in vitro* models. Microphysiological bioreactors, also referred to as organ-on-a-chip, allow for the control over tissue-specific parameters such as mechanical forces and oxygen saturation [3]. In combination with 3D organoids, these systems can be utilized to provide physiological accurate organ- and tissue specific models. Therefore, organ-on-a-chip technology is considered to be implemented for biocompatibility testing of implant materials [4]. In this regard, bone-on-a-chip systems and their respective field of application and limitations will be discussed.

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Poster

P 16

Functionalization of biocompatible metallic glasses through surface micro-/nano-patterning

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Introduction: Bulk metallic glasses (BMGs) are emerging materials ideally suited to small, mechanically-challenging applications such as bone implants [1]. Due to their amorphous structure, the BMGs exhibit remarkable mechanical properties and unusual temperature-dependent mechanical behavior, which enables plastics-like processability, which does not exist for crystalline metals. Thermoplastic forming (TPF) of BMGs is uniquely suited to generate nano-scale surface topographies leading to a stronger cell-material interaction.

Objectives: The goal of this work is to investigate the TPF ability for micro/nano-scale surface patterning of biocompatible BMGs, which may result in good biomechanical performances combined with bioactive surfaces.

Materials & methods: Two BMGs ($Ti_{40}Zr_{10}Cu_{34}Pd_{14}Ga_2$ and $Zr_{48}Cu_{36}Al_8Ag_8$) were produced by mold casting. The surface patterning was carried out by TPF into etched cavities of silicon chips. Parameters such as temperature and time for optimized pressing conditions while retaining the fully glassy state are determined by thermo-physical &-mechanical measurements.

Results: The correlation between the filling depths of alloys is best described by the formability criterion. The highest material flow occurs when the temperatures for the Zr- and Ti-BMGs are selected as 748 K and 693 K, respectively, while retaining a pressure of 40 kN and a time of ~3 min constant. An order of magnitude difference between the viscosities of alloys and the variation of the maximum applied load during TPF are reflected in the final feature height and geometry [2].

Conclusions: The practicality of the TPF with high-resolution surface patterning capability, together with their intrinsic properties make the BMGs potential candidates for implant applications.

Financial support through the EC (H2020-BIOREMIA, GA 861046) is acknowledged.

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P 17

Biomechanically Stable and Piezoelectric Ti6Al4V-Barium Titanate Scaffolds for Bone Tissue Engineering

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Introduction: Addressing critical-size bone defects in load-bearing areas is a significant challenge in orthopaedic surgery. While titanium alloy (Ti6Al4V) scaffolds offer excellent biomechanical stability, their limited electrical activity hinders broader therapeutic applications. This study aims to develop Ti6Al4V-barium titanate bulk composite scaffolds, merging Ti6Al4V's biomechanical stability with enhanced electrical activity through barium titanate [1].

Objectives: The primary goal is to integrate electrical activity into load-bearing Ti6Al4V scaffolds, addressing critical-size bone defects by combining Ti6Al4V's mechanical stability with the piezoelectric properties of barium titanate.

Materials & Methods: A hollow cylindrical Ti6Al4V structure is additively manufactured via electron beam melting and combined with barium titanate powder for joint processing in field-assisted sintering. Comprehensive analysis, including mechanical and piezoelectric property assessments, is conducted on the manufactured specimens.

Results: Scanning electron microscope images of the Ti6Al4V-barium titanate composite scaffold interface show a well-bonded structure post-sintering, with the Ti6Al4V lattice integrating with the barium titanate matrix. The Ti6Al4V-barium titanate scaffold exhibits initial average piezoelectric constants of (0.63±0.12)pC/N post-sintering, increasing to (4.92±0.75)pC/N after corona poling. Nanoindentation values indicate that Ti6Al4V is the harder and stiffer component within the composite scaffold.

Conclusion: The fabricated Ti6Al4V-barium titanate scaffold holds promise for treating critical-size bone defects in load-bearing areas, providing both biomechanical stability and piezoelectric stimulation for tissue regeneration.

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P 18

On the stability and physico-chemical properties of nitrogen-rich hydrocarbon-based plasma polymer coatings for the improvement of cell-adherent surface properties

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Introduction: Physico-chemical surface properties, like hydrophilicity, surface energy, and chemical composition, determine, i.a., the cellular acceptance of materials. Plasma processes are a well-established tool for surface modification. Plasma-polymerized nitrogen-rich hydrocarbon (ppCHN) coatings allow surfaces with cell-adhesive properties. Their long-term stability is essential for the possible use of implants.

Objectives: Studies on the change in surface properties were carried out to identify differences in surface chemistry due to contact with ambient air and storage under vacuum. Furthermore, these investigations were correlated to initial cell biological experiments.

Materials & methods: Planar silicon-titanium (1x1 cm; TU Chemnitz) and pure titanium alloy (Ti6Al-4V, Ø 1 cm) samples were used as substrates. 50–100 nm thick films were deposited in a low-pressure microwave discharge. Their properties were analyzed over by XPS, FT-IR, water contact angle (WCA), zeta potential and surface energy measurements. The initial surface occupation by osteoblastic cells (MG-63) was studied after 30 min (LSM).

Results: The physico-chemical analyzes verified the stability of ppCHN films. No significant change in WCA was detected. The XPS element ratios changed in the first 90d due to storage in ambient air. Contact with water accelerates this ageing process. But, amino group densities stayed in the range of 1…3% in all measurements. Cell biological experiments with MG-63 cells demonstrated an enhanced adhesion and spreading on ppCHN samples, for all storage times. The cell-promoting properties are highly significant over one year.

Conclusion: The favourable properties of ppCHN layers are stable over long storage times, as confirmed by physico-chemical and biological results.

Acknowledgements: We thank U.Kellner (Greifswald) for excellent technical assistance.
The cell biological experiments were financially supported by ANR-DFG (DFG NE560/19-1, project 446225522).

P 19

Osteogenic potential and mechanical behaviour of functionally graded gyroid structures

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Introduction: Novel geometries for metallic implant surfaces offer promising applications in medical technology due to their optimised mechanical properties. Additively manufactured and functionally graded gyroid structures based on triple periodic minimal surfaces (TPMS) enable innovative fields of application. Due to their unique features, such as open porosity, large surface area and surface curvature, they are believed to have bone-like properties and remarkable osteogenic potential [1].

Objective: With a continuous gradient design, the scaffolds were designed with low to high porosity in different directions to mimic the complex internal structure of human trabecular bone [3]. For functionally graded gyroid structures fabricated from a biocompatible titanium alloy Ti6Al4V by electron beam melting (EBM), no experimental studies are available on whether a modulus of elasticity comparable to natural cortical bone can be realised. In addition, cell biological in vitro experiments have rarely been performed with continuous gradient scaffolds.

Materials and Methods: Functionally graded Ti6Al4V scaffolds based on gyroid structures were fabricated by EBM. Mechanical stability was evaluated using axial compression tests. Cell biological tests were performed with human osteoblastic cells in long term (7-21d) culture.

Results: The ultimate limit load behavior as well as the fatigue strength were determined in dependence of the grading. The colonisation of the 3D scaffold by cells was demonstrated using electron and fluorescence microscopy and the proliferation and differentiation of human osteoblasts were investigated.

Conclusion: Gyroid scaffolds based on triple periodic minimal surfaces with gradient design offer great potential for innovation of osseointegrative implants.

This research was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – SFB 1270/1,2 – 299150580.

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P 20

Surface modification of drug delivery catheters by laser structuring

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Introduction: Drug-coated Balloon (DCB) catheters improve the performance of balloon catheters by delivering additional therapeutic effects to the treated vessels. The problem of poor integration drug on the surface and subsequent loss during insertion procedure itself limit its application in modern medical practice.

Objectives: This work introduces a laser surface micro-structuring technique to modify the catheter surface with drug carrying protective cavities.

Materials & methods: Catheter tubes which are made up of different medical polymers (TPU, PVC, PE and PEBAX) were used for micro-structuring. A nanosecond Nd:YAG laser source of wavelength 355 nm and frequency of 15 kHz is selected for micro-structuring.

Results: The laser source with various power irradiations and number of scans have structured drug cavities of depth from 23 µm to 25 µm which was measured by light microscopy. The smooth, uniform structure which was observed by scanning electron microscopy (SEM) helps to improve the efficiency of drug storage and delivery of drug coated catheters.

Conclusion: In conclusion, Nd: YAG laser source (355 nm, 15 kHz) can make good quality micro-structures on different catheter surface efficiently. The amount of material removal and quality of structures depends on material properties as well as various laser ablation conditions.

P 21

Implant-associated metal ions affect biofilm formation and antibiotic susceptibility of *Staphylococcus epidermidis* and *Staphylococcus aureus* strains

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Introduction: Corrosion and wear products can cause a strong inflammatory reaction in the tissue surrounding metal implants. Implant failure is often associate with infections caused by coagulase-negative staphylococci and *Staphylococcus aureus* that form biofilms on the implant. It is unclear to what extent wear and corrosion products affect antimicrobial activity of cells in the implant periphery and biofilm formation and antibiotic susceptibility of implant-infecting bacteria.

Objectives: To assess whether Corrosion and wear products increase the risk of implant infection, growth, formation of biofilms, and antibiotic susceptibility of *S. epidermidis* and *S. aureus* upon exposure to wear and corrosion products of metal implants was analyzed. Furthermore, it was investigated whether and how metallic corrosion and wear products can directly or indirectly influence the antimicrobioal activity of phagocytes towards *S. aureus* and *S. epidermidis.*

Materials & methods: Growth, biofilm formation and metabolic activity of *S. aureus* and *S. epidermidis* with varying concentrations of Ni^{2+} or Co^{2+} was measured via optical densities, bacterial counts, crystal violet staining, and MTS assays. Antibiotic susceptibility upon Co2+ exposure was determined in MIC assays. Quantitative phagocytosis assays were done with both species and primary neutrophils exposed to Ni^{2+} or Co^{2+}.

Results: *S. aureus* and *S. epidermidis* confer high tolerance towards Co^{2+} and Ni^{2+}. Growth is unaffected by up to 2 mM of both metal ions and adaptation to even higher concentrations can be observed. Both metal ions hamper biofilm formation of *S. epidermidis* and viable counts in biofilms of *S. aureus*. Adaptation to cobalt-ions can reduce the susceptibility of *S. epidermidis* to levofloxacin and ampicillin. Phagocytic activity of neutrophils is affected by both Co^{2+} and Ni^{2+}.

Conclusion: Corrosion and wear products seem to facilitate infections of metal implants by *S. epidermidis* and *S. aureus*.

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