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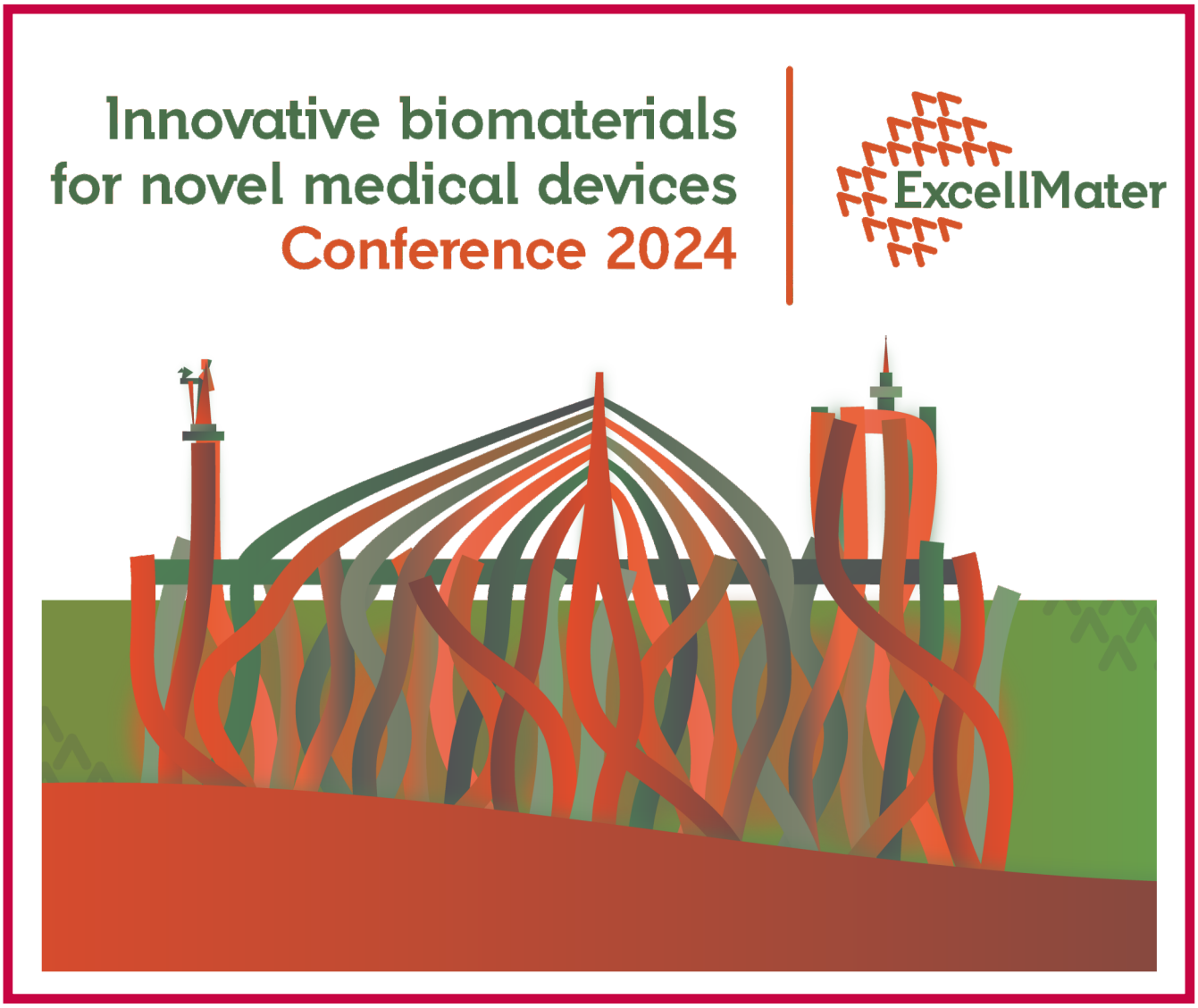

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ExcellMater Conference 2024: Innovative biomaterials for novel medical devices

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Keywords: Biomaterials engineering; tissue engineering; tumor engineering; antimicrobial biomaterials; orthopedic biomaterials; Twinning projects

EDITORIAL

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The ExcellMater Conference 2024: Innovative Biomaterials for Novel Medical Devices is organized in **Belgrade, Serbia, on April 10-12, 2024** [1] **as** the final event within the Horizon 2020 project: *Twinning to excel materials engineering for medical devices – ExcellMater* (GA 952033) [2]. The conference gathered 77 abstracts from 14 countries aiming to cover breakthroughs in biomaterials science, translation of basic research to potential clinical applications, new medical devices, and commercial possibilities for utilization of novel biomaterials. In specific, several broad topics were addressed: Tissue engineering and *in vitro* tissue and organ culture models, Cancer research, Polymer gels and composites for biomedical applications, Biomaterials for orthopedic and dental applications, Antimicrobial biomaterials and strategies as well as Various applications of novel materials. Each of the topics is introduced by a keynote lecture providing an overview of recent achievements in the field and future trends, given by internationally renowned scientists: Gordana Vunjak-Novakovic (Columbia University, US), Ivan Martin (University of Basel, Switzerland), Tiziano Serra (AO Research Institute Davos, Switzerland), Meriem Lamghari (University of Porto, Portugal), Artemis Stamboulis (University of Birmingham, UK) and Albena Daskalova (Bulgarian Academy of Science, Bulgaria). Then, a variety of oral and poster presentations described particular research studies focusing on specific problems or gave a perspective on a particular topic such as bioreactors for cartilage tissue engineering addressed by Mauro Alini and current regulations for biomaterials aimed for medical devices presented by Michael Gasik. Part of the conference was devoted to presentations of the results obtained in the ExcellMater project as well as in two other Horizon 2020 projects that is Premurosa (GA 860462) and AIMed (GA 861138). In addition, a Twinning session was organized as an occasion to showcase several Twinning projects and exchange experiences and practices in management of such projects. A significant number of conference participants were early-stage researchers who had the opportunity to present their research results, connect with fellow researchers as well as to meet senior scientists and discuss their scientific ideas and career potentials.

This supplementary issue of the journal *Hemijska industrija* presents the ExcellMater Conference 2024 Abstracts aiming to bring at least a touch of the event atmosphere to the readers.

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[1] https://www.excellmater2024.tmf.bg.ac.rs Accessed March 28, 2024
[2] https://excellmater.tmf.bg.ac.rs Accessed March 28, 2024

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Translational studies of engineered human tissues

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Keywords: Organ engineering; organs on chip; heart; lung; bone; vasculature

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INTRODUCTION: This talk will discuss the applications of tissue engineering in two areas of medicine: regenerative engineering of whole organs at the clinical scale [1,2], and modelling of diseases and therapeutic modalities using micro-sized "organs on chip" platforms [3,4,5].

EXPERIMENTAL: Engineering of human tissues involves the use of human stem cells (in most cases derived from a sample of blood) and two engineering components that are "instructing" the cells how to form a specific tissue. Th first component is a tissue specific biomaterial scaffold that serves as a structural and logistic template for tissue formation. The second component is the bioreactor designed to provide homeostasis and tissue-specific molecular and physical regulatory factors, as well as sensing and imaging modalities necessary to monitor and measure tissue development and function. In all cases, we aim to recapitulate the cell niches, using bioengineering tools.

RESULTS AND DISCUSSION: To illustrate the state of the art in the field and reflect on the current challenges and opportunities, this talk will discuss representative examples of whole organ engineering and organs-on chip models of injury, disease and regeneration. At the clinical scale, the goal is to achieve regeneration by treating the whole organ damaged by injury of disease. We will discuss regenerative engineering of a joint (that can be engineered de novo), and the lung and heart (that are being regenerated with the use of therapeutic cells). In organs-on-chip platforms, micro-sized human tissues are matured to adult-like phenotypes and functionally linked by vascular perfusion containing circulating cells and factors.

At the micro-scale, we will focus on organs on chip models of cancer metastasis, radiation damage, and side effects of some common drugs. Finally, we will discuss the key challenges the field is currently facing.

CONCLUSIONS: Tissue engineering is increasingly successful in recapitulating human physiology in health and disease, in patient-specific settings, supporting the development of high-fidelity research models and treatment modalities tailored to the specific patient.

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Regenerative engineering: designing grafts, processes and signals

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Keywords: Regenerative surgery; osteoinduction; cartilage repa

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Cellular grafts for the regeneration of cartilage and bone have been engineered using a variety of cell sources, scaffolds, and manufacturing systems. Clinical implementation of some of these approaches by the own group has led to promising outcome results (1-4) but is still associated with manufacturing and standardization challenges. In order to gain repeatability and robustness, alternative strategies have been conceived, inspired by recapitulation of developmental processes, with proofs of principle in the context of bone and cartilage regeneration (5-6). Along this line, it was identified that signals inducing regeneration processes may not require living cells to be efficiently delivered but could be encoded in cell-laid and subsequently devitalized extracellular matrices (ECM) (7-8). The resulting off-the-shelf biomaterials contain a combination of multiple cytokines and morphogens, presented to the recipient site through physiological sets of ECM molecules, which synergistically potentiate their effects. Such materials could be generated based on highly standardized processes, thanks to the use of cell lines and bioreactor-based systems, and at the same time enriched in defined factors to address specific disease stages and patient profiles, in a perspective of personalized medicine. They would not function primarily as tissue replacements, but rather as "germs" for *de novo* tissue development. Recent pre-clinical data substantiate that grafts in this new class exhibit unprecedented osteoinductive properties, unmatched by synthetic matrices or by living engineered tissues (8).

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Cartilage bioreactors: where we are and where we are going!

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Keywords: Mechanical loading; ex-vivo system; slaiding; lubricin; osteochondral unit

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Advanced biomaterials and tissue engineered constructs have been developed to improve tissue repair; nevertheless, their clinical translation has been hampered, also by the lack of reliable *in vitro* models suitable for pre-clinical screening of new implants and compounds mimicking the *in vivo* situation.

Tissue regeneration is strongly influence by the mechanical properties and behavior of biomaterials, which can be completely different when tested in "isolation" or in a biological context. Therefore, it is important to evaluate the performance of such advance biomaterials in *in vitro* models, which reproduce closely the *in vivo* tissue status.

To such end, we have developed several complex organ models (here, cartilage) which include, not only the tissue part, but the tissue is cultured within a bioreactor, reproducing loading patterns similar to the *in vivo* microenvironment. Here, we will focus on bioreactor systems that transmit a mechanical stimulus, as this is a key parameter in the homeostasis of various musculoskeletal tissues, such as bone, cartilage, tendon, and intervertebral disc. By testing regenerative therapies under conditions that are closer to the ones encountered *in vivo,* bioreactors can provide a useful screening tool and standardization opportunities for the evaluation of various biomaterials, but as well as cell types, drugs, or tissue engineered products. This will allow to reduce the number of samples for the final *in vivo* evaluation, allowing the 3R philosophy approach to be implemented.

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Ex vivo testing of biomaterials for intervertebral disc repair using organ culture bioreactors

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Keywords: Nucleus pulposus; annulus fibrosus; hydrogel; scaffold; mechanical load

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INTRODUCTION: The intervertebral disc (IVD) functions to distribute mechanical loads acting on the spine and to enable flexibility of the spine in multiple degrees of freedom. Degeneration of the IVD is a multifactorial condition that can lead to chronic low back pain and impaired mobility. Degeneration is characterized by a breakdown of the extracellular matrix (ECM) within the nucleus pulposus (NP) and the annulus fibrosus (AF) of the IVD. Natural and synthetic biomaterials hold great promise for IVD repair. Hydrogels are particularly suitable for the NP, which is a highly hydrated tissue, while fibrous scaffolds may be suitable for closure of the AF. Organ culture bioreactors are instrumental for preclinical testing of the biomaterials' performance, bridging the gap between in vitro and in vivo studies. Here the requirements for NP and AF repair are discussed, and examples of bioreactor-controlled ex vivo studies are demonstrated.

EXPERIMENTAL: IVDs used for organ culture are harvested from bovine tails that are obtained from the slaughterhouse. To induce breakdown and loss of the ECM, either enzymatic or mechanical damage is applied. For NP repair, a hydrogel is injected into the center of the IVD. For AF repair, suture, or adhesive material is required to avoid dislocation under mechanical load. The IVDs are then cultured and loaded in the bioreactor under physiological loading conditions (*i.e.* axial compression, 0.2 MPa, 0.2 Hz) for 2 h /day. Disc height changes, cell viability, ECM content, and gene expression are assessed after 1-4 weeks of culture.

RESULTS AND DISCUSSION: For NP repair, the properties of the hydrogel were optimized to meet the mechanical requirements, while preserving the phenotype of embedded cells. We found that a hyaluronic acid-based interpenetrating network hydrogel was suitable as a cell carrier for NP repair [1]. When implanted in an ex vivo IVD organ culture model, the hydrogel supported cell viability, phenotype expression of encapsulated NP cells and IVD matrix production over four weeks under physiological loading. In another, enzymatic IVD degeneration model [2], a synthetic NP repair hydrogel showed potential to retain the disc height and integrate into the native tissue after two weeks of culture in a bioreactor under physiological loading. For AF repair, a biomaterial strategy comprising of electrospun polycaprolactone scaffold and fibrin-genipin adhesive was optimized and tested in an AF delamination organ culture model [3]. The repair material created a tight seal on the damage and restored the mechanical properties, while showing minimal cytotoxicity. This outcome was achieved after one week of culture under physiological uniaxial loading conditions, while the persistence under multiaxial loading will need to be confirmed.

CONCLUSIONS: These examples show the suitability of IVD organ culture bioreactors to test the feasibility of a range of biomaterials for NP and AF repair. Depending on the research question, different models can be created, and different biological and biomechanical parameters can be evaluated. Ex vivo loaded organ culture models are in line with the 3Rs principles of in vivo testing.

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Engineering of multicellular systems by hydrodynamic waves

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Keywords: Biofabrication; *in vitro* models; sound-based assembly

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In morphogenesis, ensembles of cells are gently orchestrated and perfectly arranged through chemical gradients, structural anisotropies, and hierarchical compositions.

Traditional approaches in tissue engineering involve the development of physiologically relevant living microenvironments, by combining materials, cells, and biochemical factors to direct the generation of functional tissues.

Over the last few years, the application of extrinsic fields is opening to exciting new perspectives to better control and reproduce the structural complexity of tissue organization toward the *in vitro* engineering of clinically relevant constructs. Within this recent trend, acoustic, magnetic, hydrodynamic fluids, and optical fields have shown time-effective, gentle, and contactless strategies to organize cells, materials, and biochemical factors toward morphogenesis and morphologically relevant tissue fabrication.

In this talk, I will focus on our research activities investigating the use of hydrodynamic waves to biofabricate multi-tissue/organs for regeneration and modelling (1-3).

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Sound based assembly of spatially organized porous constructs

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Keywords: Bioassembly; pattern; standing waves; gelatine beads; fibrin hydrogel

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INTRODUCTION: An emerging contactless method for creating biologically relevant constructs is acoustic bioassembly. This method induces the assembly of particulate systems through fluid patterns (*e.g.* pressure fields, surface instabilities, waves). These fluid patterns produce hydrodynamic forces that are spatially specific and control the arrangement of micron-sized particles [1]. The frequency and amplitude of the chamber vibrations directly regulate these forces. We decided to exploit this novel technique in combination with cell-laden gelatine beads. We then patterned the beads to generate spatially orchestrated porous constructs where cells can easily invade and proliferate.

EXPERIMENTAL (or Materials and Methods): Gelatine beads (15 % weight / volume) and cell encapsulation were obtained by emulsion process in dextran solution (20 % weight / volume). Adapting the protocol from [2]. A stirring speed of 100 rpm was used to obtain gelatine beads with an average radius of 70 µm and therefore a suitable size for cell encapsulation. Subsequently the beads were spatially organized, in a layer of fibrin solution, through sound-based bioassembly. This final procedure was repeated optimizing a specific set of parameters such as: chamber thickness, chamber dimensions, and frequency applied. Using a circular chamber, we obtained concentric circles as patterns.

RESULTS AND DISCUSSION: The obtained population of beads shown a radius between 50 and 100 µm, an adequate size for encapsulation of cells (d = 10 µm). The encapsulation efficiency obtained was indeed 90 %. Cell viability assay was then performed immediately after encapsulation, showing a promising result of biocompatibility. Furthermore, analysis of the obtained patterns shown a direct correlation between the frequency applied to the system and the complexity of the obtained pattern (number of concentric circles).

CONCLUSIONS: Overall, this could be a suitable method to generate spatially orchestrated porous constructs. In this specific case, we show a proof of concept of cell encapsulation and gelatine beads assembly within fibrin hydrogel. By opportunely loading our sacrificial beads with different cell populations and tuning pattern shape and size, this approach will open the way to create reproducible, shape-defined multicellular systems for biological modelling.

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Development of an in vitro branched vasculature using bioprinting technique in combination with sacrificial materials

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Keywords: 3D bioprinting; angiogenesis, pre-vascularization

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INTRODUCTION: Successful vascularization represents a bottleneck in the production of functional, engineered tissue constructs. The current methods of vascularization either rely on cells self-organization into the capillary network, or predesigned, biofabricated vessels that are limited in size and do not allow the replication of complex vascular networks. Proposed strategies for solving this problem include combinations of natural and synthetic hydrogels with different gelation properties, 3D bioprinting and single or multiple cell cultures with endothelial cells [1]. However, producing defined, capillary-sized hollow channels remains to be beyond reach [2].

EXPERIMENTAL: The objective of this work was to establish a procedure for obtaining a network of interconnected vascular-like channels of varying size, lined with human umbilical vein endothelial cells (HUVECs), using a bioprinting process in combination with sacrificial materials. In addition, the aim was to make the channels hollow with size comparable to the size of capillaries in vivo and to embed them into a hydrogel matrix that could be modified by cells. The method combined extrusion based 3D printing technology and double sacrificial materials- Pluronic F-127 and gelatin type A to produce a sacrificial template for the network. The produced gelatin-cell templates were embedded into a extracellular matrix (ECM)-like hydrogel composed of collagen and fibrin. After the gelatin was removed, remaining hollow structures lined with HUVECs were cultivated statically and dynamically and HUVECs state was further examined.

RESULTS AND DISCUSSION: The size of the network channels ranged from about 1000 µm to less than 20 µm. The ECM hydrogel composed of collagen and fibrin was able to support the stability of the microchannels. The exposure of cells to the inlet flow led to cell sprouting and expression of VE-Cadherin.

CONCLUSIONS: The results indicate that the developed method holds potential for the production of networks, that mimic both structural and functional characteristics of physiological capillaries. Therefore, the principle of using double sacrificial materials for fabrication of hollow vascular channels would enable downsizing of the channels achievable with 3D printing of single sacrificial materials, and that it could be used in the future to produce functional networks with predesigned structure.

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Unraveling the transcriptome profile of pulsed electromagnetic field stimulation in bone regeneration using an *in vitro* investigation platform

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Keywords: Pulsed electromagnetic field stimulations, biophysical stimuli, bone-like tissues, perfusion bioreactors, bone regeneration

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INTRODUCTION: Perfusion bioreactors are currently a good tool for applications in regenerative medicine and bone tissue engineering [1]. In the realm of bone and cartilage regeneration, pulsed electromagnetic field (PEMF) stimulation has become widely utilized in clinical practice [2]. Numerous signaling pathways involved in its osteogenic, chondrogenic, and anti-inflammatory effects have been identified, but the majority of the pathways are yet unknown [3]. Using a novel *in vitro* investigation platform, this study aimed to identify the signaling pathways altered by PEMF by exposing3D bone-like models to physiological-like perfusion and PEMF stimulation.

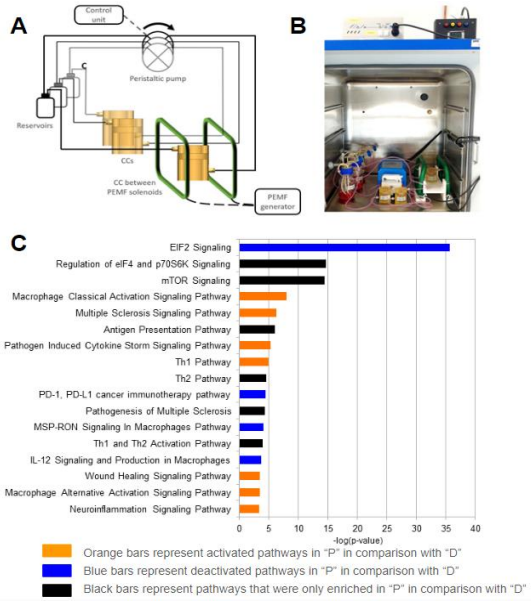

***Figure 1**: A - Schematic representation; B - the platform; C - selected pathways in the comparison of P vs. D*

EXPERIMENTAL: *Bioreactor* -An automated perfusion bioreactor with tunable perfusion (0.006-24 mL/min) and a PEMF generator (1.5 mT, 75 Hz) (Fig. 1A & 1B). *Scaffolds* - 3D-printed polylactic acid (PLA) scaffolds resembling trabecular bone microarchitecture. *Biological evaluations* - Scaffolds were seeded with human mesenchymal stem cells (hMSCs) and exposed to perfusion (0.3 mL/min) with ("P") and without ("D") PEMF stimulation (4 h/day) for 21 days in basal or osteogenic medium. Samples were evaluated in triplicates (n = 3). Static cultures served as control ("S"). RNA sequencing (RNA-Seq) and real-time qPCR were conducted to detect the signaling pathways elicited by PEMF.

RESULTS AND DISCUSSION: In the absence of biochemical, and according to RNA-Seq analysis PEMF stimulation in basal medium addresses the four stages of bone regeneration: inflammatory, fibrovascular, bone formation, and bone remodeling stages, even in the absence of a pathological state (Figure 1C).

CONCLUSIONS: The suggested *in vitro* research platform represents a novel tool for studying bone biology.

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Whey protein isolate: a versatile dairy-derived hydrogel for bone and vascular tissue engineering and antimicrobial applications

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Keywords: Antibacterial; carrier; scaffold; osteoblast

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INTRODUCTION: Whey Protein Isolate (WPI) is an inexpensive by-product of the dairy industry, available in large quantities and used as a dietary supplement. WPI is over 97% protein; three-quarters is beta-lactoglobulin (β-LG) [1]. WPI in cell culture medium promoted the proliferation and differentiation of bone-forming cells [2]. Solutions of WPI can be heated to form hydrogels, which withstand sterilization by autoclaving; an important practical advantage. Denaturation of β-LG leads to increased hydrophobic interactions and disulphide bond formation and thus crosslinking to form the polymer hydrogel network [3]. We have studied WPI hydrogels as scaffolds for bone-forming cells and carriers of hydrophobic substances.

EXPERIMENTAL: WPI hydrogels 15% to 40% (w/v) have been made [4-5]; inorganic particles, like bioactive glasses, alpha-tricalcium phosphate, aragonite and hydroxyapatite (HA) can easily be added during hydrogel formation [6-9]. Hydrophobic molecules such as phloroglucinol (PG), the fundamental subunit of marine polyphenols, and poly-gamma-glutamic acid (PGGA), can be incorporated during hydrogel formation.

RESULTS AND DISCUSSION: WPI hydrogels support the adhesion and growth of a range of bone-forming cells, including MG-63 osteoblast-like cells, normal human foetal osteoblasts (hFOB) normal mouse calvarial preosteoblasts (MC3T3-E1) and dental pulp stem cells [6-10], as well as human umbilical vascular endothelial cells (HUVEC) [5]. Addition of aragonite [8] and PGGA [11] promoted osteoblastic differentiation, while incorporation of PG endowed antimicrobial activity towards a wide range of microbes including methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis while maintaining cytocompatibility [11].

CONCLUSIONS: WPI hydrogels are both promising scaffolds for bone cells and hydrophobic drug carriers.

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Innervation of the musculoskeletal system in physiological and pathological conditions: Insights from organ-on-a-chip models

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Dysregulation of musculoskeletal tissues innervation can have profound effects on the locomotor system, contributing to the emergence of chronic pain and disability, thereby imposing a heightened burden on individuals and society. Such is the case with conditions like rheumatoid arthritis (RA), osteoarthritis (OA), implant aseptic loosening (AL), bone metastases, fractures, and various rare bone disorders. This talk will specifically concentrate on elucidating how bone tissue reciprocally influences the growth and guidance of peripheral nerve terminals, both in physiological and pathological contexts. Notably, we will explore the intricate interplay between bone and nerve dynamics, shedding light on their reciprocal modulation. Furthermore, a focal point of discussion will be the utilization of micro-physiological systems as invaluable tools for delineating the intricacies of this crosstalk. By employing these sophisticated systems, we aim to unravel the nuanced interactions between bone and peripheral nerves, providing insights into the underlying mechanisms that shape the pattern of innervation. This comprehensive examination seeks to enhance our understanding of the bidirectional communication between the skeletal and nervous systems, with potential implications for therapeutic interventions targeting conditions characterized by disrupted bone-nerve dynamics.

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Extracellular vesicles derived from mesenchymal stem/stromal cells derived from dental pulp of exfoliated teeth induce osteogenic differentiation

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Keywords: Large bone defects; bone regeneration; extracellular vesicles; internalization

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INTRODUCTION: Large bone defects in the field of orthopedic surgery remains challenging, primarily due to restricted regenerative capacity of bone tissue. The biomimetic bone implants could improve the osteoregeneration process, but the optimal composition for biomimetics remains unknown. Considering the great potential of extracellular vesicles (EVs) derived from mesenchymal stem/stromal cells (MSCs) in regenerative medicine, our aim was to investigate the osteoinductive potential of EVs derived from MSCs from the dental pulp of human exfoliated deciduous teeth (SHEDs) either undifferentiated or those undergone differentiation into osteoblasts (osteoSHED-EVs), for potential use in biomimetic bone implants.

EXPERIMENTAL: SHEDs were isolated from the dental pulp of 5 different donors of deciduous teeth and subjected to characterization by Flow cytometry (FCM). EVs-SHED and EVs-osteoSHED were isolated by sequential ultracentrifugation, and analysed by Western blot, NTA, and electron microscopy. Uptake of PKH67-labelled EVs by SHED was analysed by confocal microscopy and FCM. The effects of EVs on osteogenic differentiation of SHED cells were analysed by monitoring RUNX2 and BMP2 expression by RT-PCR.

RESULTS AND DISCUSSION: SHEDs displayed typical MSC morphology and phenotype, as well as potential to differentiate into osteoblasts as confirmed by FCM, alkaline phosphatase activity, Alizarin red S staining, and gene expression analysis. Both types of EVs were internalized by SHED, as shown by the confocal microscopy and FCM after 4h and 24h incubation, and no significant differences were observed between the two EVs types. Importantly, the osteogenic differentiation of SHEDs cultivated in basal medium was significantly improved in the presence of osteoSHED-EVs, as shown by the upregulation of early osteogenic genes RUNX2 and BMP2.

CONCLUSIONS: These findings significantly improved our understanding on the potential of EVs derived from SHEDs in osteoinduction, laying the groundwork for developing targeted methods that utilize EVs from SHED as biomimetics for effective repair of bone defects.

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Examination of the effects of X-ray phase contrast imaging dose on DNA in mesenchymal stem cells by comet assay

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Keywords: DNA damage; monitoring

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INTRODUCTION: Imaging techniques based on X-ray phase-contrast (XPC) have shown tremendous promise for applications involving biomaterials and soft tissue formation [1,2]. XPC imaging can be applied at higher energy offering the potential for lower dose imaging. Essential to the development of this technique and its routine use is an understanding of the potential damage of X-ray dose on cells and tissues.

EXPERIMENTAL: In this study the comet assay, a sensitive assay for DNA damage, was used to evaluate DNA damage on mesenchymal stem cells (MSCs) exposed to X-ray irradiation. We examined the effects of early (immediately following irradiation) and delayed (24 h post-irradiation) X-ray effects caused by low (15 mGy) and intermediate (150 mGy and 1.5 Gy) exposure on MSCs during a monitoring period of 4 weeks (five irradiations, one weekly). Cells were submitted to a polychromatic X-ray source (Thermo Fisher PXS10 conditions: voltage 45 kV, source current 160 μA, source power 7.2 W, source spot size 9 um, photon flux on the sample 7.66 10^6 photons s^{-1} mm^{-2} irradiation).

Statistical analysis was performed by using Two-way analysis of variance (ANOVA) with Tukey's multiple comparisons posttest in GraphPad Prism 5.0.A difference at $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION: Results of the DNA comet assay indicated that early effects of low- and intermediate-dose of XPC induced an increase in the number of cells with DNA damage after each irradiation, where intermediate-dose (150 mGy and 1.5 Gy) produced significantly higher damage relative to controls. DNA damage induced by low and intermediate doses returned to the control value 24 h after the irradiation exposure, suggesting a strong protection of MSCs at the tested doses of XPC irradiation.

CONCLUSIONS: The data presented in this study shows that 24 h after the last of five weekly low and intermediate doses XPC irradiation, the harmful effects on DNA in MSCs were not detected. The current study reinforces the need of investigating consequences of low and intermediate doses of X-ray PC irradiation in the field of tissue engineering and provide new basis for MSCs using in the clinics.

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β-glucan-enriched fraction from mosaic puffball induces inflammation in an in vitro 3D bovine chondrocytes model

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Keywords: Mushrooms; Bovistella utriformis; osteoarthritis; cartilage

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INTRODUCTION: Fungal β-glucans are well-known for their immunomodulatory activity. They act as pathogen-associated molecular patterns (PAMPs) and can bind to a number of pattern-recognition receptors (PRRs). Activation of PRRs leads to inflammatory response and, although these receptors are primarily found in immune cells, chondrocytes express certain types of PRRs as well (toll-like receptors –TLRs). Although β-glucans are primarily considered immune-stimulatory agents, recent research found that they may have beneficial effects in some inflammatory conditions (hence the term "immunomodulators"), in a complex way that is yet to be uncovered. The aim of this study was to investigate if the mushroom β-glucans could induce any changes in metabolic activity and phenotype of bovine chondrocytes, using a 3D cell culture model. For this purpose, glucan-enriched extract of mosaic puffball fruiting bodies, containing up to 70% (1→6)(1→3)β-D-glucan-protein complex was used.

EXPERIMENTAL: Bovine chondrocyte pellets were incubated with the extract at a concentration of 100 µg/mL for 7 days, with regular medium changes. During incubation, nitric oxide (NO) and glycosaminoglycans (GAGs) concentrations were monitored in the medium, using photometric assays [1]. At the end of the incubation, GAGs and total DNA content were determined in the pellets [1]. The gene expression of aggrecan (ACAN), collagen type 1 (COL1), collagen type 2 (COL2), interleukin-6 (IL-6), matrix metallopeptidase 3 (MMP3) and matrix metallopeptidase 13 (MMP13) was determined by real-time RT-PCR. All measurements were done in triplicate and one-way ANOVA was used for statistical analysis of the results.

RESULTS AND DISCUSSION: The treatment of the pellets with the extract caused an initial increase in the release of GAGs (by 157% on the 2nd day, compared to the control), which was followed by a steady decline and a decrease of GAG content both in the medium (by 55 %) and in the pellets (by 82 %), compared to the control at the end of the incubation period. This is consistent with the 80% decrease in ACAN expression compared to the control. The expression of COL1 and COL2 also decreased by 71 and 93 %, respectively, indicating a strong suppression of both aggrecan and collagen synthesis. The production of matrix-degrading metalloproteinases was on the other hand greatly enhanced, as expression of MMP3 and MMP13 increased by 248-fold and 217-fold, compared to the control, respectively. The IL-6 expression was also upregulated (increase by 1718-foldcompared to the control). Initially, NO production was enhanced (by 17-fold on the 2nd day of the incubation) but declined during the incubation and was 73 % lower than that in the control on the 7th day. There was no significant difference in total DNA content between the control and treated pellets, indicating that the treatment did not cause cytotoxicity.

CONCLUSIONS: In a 3D chondrocyte monoculture, the β-glucan-enriched mosaic puffball fraction exhibited strong pro-inflammatory and catabolic activity during a short-term treatment. Further research in more physiologically relevant models is needed to better understand the way β-glucan products could affect cartilage homeostasis, as well as to assess the influence of their structure, concentration, and route of administration on their potential activity.

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Composite based on resveratrol and selenium as an antioxidative component in tissue engineering

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Keywords: Polyphenols; selenium nanoparticles; bioactivity; biomaterial

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INTRODUCTION: Inflammation and oxidative stress are common problems in biomaterial science. Therefore, antioxidative components, especially those of natural origin, can be of great value in improving biomaterial formulations for medical devices [1]. It is already well known that the most notable exogenous antioxidants are ascorbic acid, polyphenolic compounds, and minerals such as selenium and zinc [2]. In our research, we prepared a composite based on polyphenolic resveratrol nanobelts and selenium nanoparticles and explored its' antioxidative potential.

EXPERIMENTAL: Resveratrol and selenium-based composite (ResSeNPs) was made by combining pre-synthesized resveratrol nanobelt-like particles (ResNPs) [3] and selenium nanoparticles (SeNPs) [4], using high-speed homogenization. The suspension was characterized by using OPTICA B-500MET light microscope (Optica SRL, Italy) and Nicolet iS10 FT-IR Spectrometer (Thermo Fisher, USA). The antioxidative effect was assessed by DPPH reduction assay, Ferric cyanide (Fe^{3+}) reducing antioxidant power assay (FRAP) for measuring of iron ion reduction, and thiobarbituric acid assay for assessment of inhibition of lipid peroxidation. All experiments were done in triplicate and average values calculated, followed by student's t-test for statistical significance compared to the controls, with threshold being set to $*p<0.05$.

RESULTS AND DISCUSSION: The obtained ResSeNPs composite was bright orange, homogenous, and stable. Agglomerates of SeNPs were seen around ResNPs on the optical microscope. FTIR spectroscopy showed the appearance of new hydrogen bonds, most possibly formed between ResNPs and the surfactant component of SeNPs, which was bovine serum albumin. ResSeNPs exhibited significant free radical reduction (over 80 % reduction at all tested concentrations), up to 80 % inhibition of lipid peroxidation, and in FRAP assay it reduced iron up to significantly high $A_{700} = 6.63$, at the highest tested concentration (1.5 vol.% in the solution).

CONCLUSIONS: ResSeNPs composite, consisting of ResNPs and SeNPs bonded by hydrogen bonds, exhibited notable antioxidative activity by various mechanisms of action - radical scavenging, lipid peroxidation-inhibitory effect, and reduction of ferric ions, even at very low concentrations. These findings highlighted the significant potential of ResSeNPs as an antioxidative component in further material design.

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Chemotherapy and novel proton radiotherapy in spatially advanced multicellular models of pancreatic cancer: On the design of platform for enabling low cost animal free preclinical treatment testing

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Keywords: 3D cancer models; pancreatic cancer

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INTRODUCTION: With a 5-year of only 11 % pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest diseases. This is partly attributed to the tumour's resistance to currently available treatment, resulting from a complex and highly heterogeneous tumour microenvironment (TME). A key challenge in cancer tissue engineering is to mimic the different key features of the TME. In this work we have developed robust PDAC biomimetic models for *in vitro* therapeutic assessment.

EXPERIMENTAL: We have advanced our previously developed 3D polyurethane (PU) based polymeric scaffold PDAC model [1,2] by incorporating biological complexity (multiple cell types: pancreatic cancer, pancreatic activated stellate and endothelial cells) [3], spatial complexity (scaffold compartmentalization) and fluid flow (perfusion). Chemotherapy (with Gemcitabine-GEM) [4] as well as proton therapy were carried out within our models. Imaging of cellular proliferation/spatial organization, apoptosis of the different cell types and ECM secretion was carried out along with assessment of biomarkers linked to chemo-resistance.

RESULTS AND DISCUSSION: For chemotherapy treatment, within our static models, we observed that the dual scaffold showed a higher resistance to GEM in comparison to the single scaffold [4]. Our results highlight that the spatial arrangement of the cells, within a 3D model, affect the response to chemotherapy. For proton therapy treatment, pancreatic cancer was more susceptible to proton beam therapy as opposed to photon therapy, the latter resulting in a higher cell viability and lower expression of apoptotic markers post-treatment. Furthermore, the introduction of dynamic flow affected the cell spatial organization, and biomarker expression involved with EMT, matrix remodeling highlighting the importance of fluid flow in PDAC's evolution and response to chemotherapy.

CONCLUSIONS: Our work highlights the importance of spatio-temporal cellular arrangement and interstitial fluid flow for accurate *in vitro* studies of the chemoradiotherapy resistance for PDAC.

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Bioengineering for creating biomimetic microenvironments: bioreactors and biomaterials

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Keywords: 3D cultures; soft tissues; bone tissue; tumor engineering; drug screening

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INTRODUCTION: Millions of patients are still awaiting new therapies as the traditional models rely on monolayer cell (2D) cultures and *in vivo* studies, which have numerous limitations resulting in misleading conclusions. Consequently, there is a burning need for the development of alternative 3D models able to accurately mimic the complexity of human diseases. This research aim is to create microenvironments based on biomimetic bioreactors and alginate hydrogels as cell carriers for reliable disease research and drug screening.

EXPERIMENTAL: Alginate cell carriers in forms of microfibers and microbeads with immobilized different cells were obtained by extrusion techniques [1], while macroporous cell carriers for imitating bone tissue based on alginate or gellan gum and bioactive inorganic particles (hydroxyapatite, ß-tricalcium phosphate, bioactive glass) were prepared by a simple controlled gelation and freeze-drying method [2] followed by manual seeding of cells onto the partially rehydrated scaffolds. The obtained carriers with cells were cultivated in perfusion bioreactors ("3D Perfuse", Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia) under continuous medium flow (superficial velocity: 15-100 µm s^{-1}) for up to 7 days. To evaluate these cell carriers for drug screening, microfibers with different cancer cells were treated with cisplatin or doxorubicin, while 2D cultures served as control. The cells were assessed regarding the metabolic activity (viability) by MTT, morphology, and distribution within carriers by scanning electron microscopy and histology (H&E stain).

RESULTS AND DISCUSSION: Cell immobilization in alginate microbeads (diameter: ~300 µm) and microfibers (diameter in the range 300-500 µm) resulted in uniform distribution, while the macroporous scaffolds with open and connected pores (porosity: ~60 %) provided cell adherence as individual cells and in aggregates (seeding efficiency: above 80 %).The majority of cells stayed viable and metabolically active, while retrieved cells from alginate carriers retained their morphology, viability, and ability to proliferate under 2D conditions. Alginate carriers with cervical carcinoma, glioblastoma, and osteosarcoma cells were further cultivated in perfusion bioreactors. After cultivation under biomimetic conditions the cells retained viability and proliferative capacity, spontaneously formed spheroid-like structures, and exhibited higher metabolic activity as compared to static controls. The obtained results imply the positive effects of medium flow on cells due to providing efficient mass transport and controlled levels of hydrodynamic shear stresses. Evaluation of these models for drug screening has shown that the immobilized different cancer cells exhibited up to 10-fold higher half-maximal inhibitory concentration than the cells in 2D cultures.

CONCLUSIONS: The overall results have shown potentials of the applied approach based on 3D models comprising biomimetic bioreactors and alginate-based scaffolds for disease research and drug screening.

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A 3D *in vitro* cell culture model based on perfused bone-like scaffolds for healthy and pathological bone research

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Keywords: Alginate composite scaffolds; perfusion bioreactor; osteosarcoma; tumor engineering; mesenchymal stem cells

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INTRODUCTION: Comprehensive research, particularly in evaluating drug efficacy**,** still heavily relies on the results obtained by the utilization of cell monolayers and animals. However, the inherent limitations of these models such as their physiological disparities from humans pose significant obstacles to acquiring reliable results thus impeding further scientific progression. To address this challenge, 3D *in vitro* cell culture models emerged as physiologically relevant models having the potential to enhance research and drug discovery. Our study aimed to develop a 3D *in vitro* cell culture model based on bone-like scaffolds in conjunction with a perfusion bioreactor ("3D Perfuse", Innovation Center FTM, Belgrade, Serbia) for studying both physiological and pathological (i.e. tumors) bone conditions.

EXPERIMENTAL: Bone-like scaffolds were obtained by cross-linking the mixture of Na-alginate solution (2 wt.%) and hydroxyapatite (2 wt.%) with calcium ions followed by slow freezing and lyophilization. Scaffold porosity and pore sizes were determined by using optical microscopy. To model osteosarcoma tumor**,** scaffolds were seeded with murine K7M2-wt osteosarcoma cells**,** whereas for mimicking bone physiological conditions either human bone marrow-derived mesenchymal stem cell line (hBMSCs) or primary mesenchymal stem cells were used. Each cell type was cultivated for 7 days in a perfusion bioreactor with medium flow rate of 0.27 cm^3/min, corresponding to the medium superficial velocity of 40 μm/s. Static cell cultures served as controls. Cell behavior was assessed by cell metabolic activity assays (MTT or resazurin), histological and immunocytochemical analysis, phalloidin/DAPI staining, and gene expression analysis (qPCR). Shear stresses were calculated from histological sections using a cylindrical pore model.

RESULTS AND DISCUSSION: Obtained scaffolds had an initial porosity of 60 % and contained a variety of pore sizes with a predominant presence of macropores, mimicking in that manner trabecular bone structure. All cell types adhered to the scaffolds indicated by cell seeding efficiency exceeding 80 %. In perfusion culture, osteosarcoma cells exhibited characteristics corresponding to *in vivo* tumor cell behavior: high cell metabolic activity, spontaneous assembly into compact spheroid-like structures, secretion of extracellular matrix and expression of pluripotency-associated genes. Furthermore, immunocytochemical staining revealed an increased presence of α-tubulin as compared to the control. Regarding the healthy bone model, both types of mesenchymal stem cells retained their intrinsic cellular shape and self-organized into aligned structures which was strongly induced by perfusion conditions. The overall positive influence of perfusion conditions could be attributed not only to improved mass transport but also to adequate values of hydrodynamic shear stresses calculated to be up to 5 mPa.

CONCLUSIONS: Our 3D *in vitro* model can support cultures of different bone cell types and shows potential for further adjustment and utilization in the fields of tumor and tissue engineering.

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Doxorubicin and quercetin combined effect on SAOS-2 cells grown in 2D and 3D model systems

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Keywords: Osteosarcoma, alginate microbeads, anticancer treatment

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INTRODUCTION: Osteosarcoma (OS) is a highly aggressive primary malignant bone tumor that most commonly affects children, adolescents, and young adults. The standard treatment for OS consists of surgical resection and chemotherapy, whereas radiation therapy is recommended for the unresectable tumor. Due to its easy metastasis and recurrence, the 5-year overall survival rate is only 66.5 % [1]. Thus, there is a critical need to recognize the molecular mechanisms underlying OS development and pathogenesis. Traditionally, two-dimensional (2D) cells are widely used in cancer biology and pre-clinical studies. However, 2D models are unable to mimic cell–cell and cell-extracellular matrix interactions which are crucial for adequate cellular function. Three-dimensional (3D) model systems are able to recapitulate key features of human cancer and are recognized as a promising platform for fundamental and translational research [2]. In the present work, we established an osteosarcoma 3D model based on alginate microbeads and studied the effect of combined treatment with doxorubicin (Doxo), widely used chemotherapeutic, and quercetin (Quer), a plant pigment with anticancer properties, on OS model systems.

EXPERIMENTAL: In our research, human permanent cell lines derived from osteosarcoma, SAOS-2 (ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS (fetal bovine serum) and 1 % AA (antibiotic-antimycotic) at 37°C with 10 % CO_2. Cells were treated with doxorubicin (Ebewe), quercetin (Sigma), and their combination. The cells were immobilized in 1.5 wt.% alginate in the form of microbeads by manual extrusion followed by cultivation up to 21 days. Cell viability was determined using the MTT test, and viability rates were compared using Student's t-test with Graphpad Prism software. The experiment was performed in at least 3 technical replicates.

RESULTS AND DISCUSSION: Cells were successfully immobilized in alginate microbeads (diameter: ~1230 μm) and their viability significantly increased during the cultivation up to 21 days. Literature data have shown that quercetin could enhance chemotherapeutic effect of doxorubicin on cancer cells. Therefore, osteosarcoma cells were treated with both Doxo and Quer. Experimental results have shown that the combination of 1 μg/ml Doxo and 5 μM Quer significantly decreased the viability of SAOS-2 cells cultured in 2D conditions compared to cells treated with 1 μg/ml Doxo. On the other hand, viability of the cells cultured in 3D conditions treated with the same combination of Quer and Doxo did not show any statistically significant effect on cell viability. We can hypothesize that microenvironment-based mechanisms modulate doxorubicin sensitivity and increase resistance to treatment of osteosarcoma cells cultured in 3D conditions [2].

CONCLUSION: Collectively, quercetin sensitized osteosarcoma cells to doxorubicin in 2D model. However, in an *in-vivo* like 3D model system, the effect on of the combined treatment was not observed. Further research is needed to investigate the possible role of quercetin in tumor treatment.

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Adaptable alginate-based microfibers for 3D *in vitro* cultures of cancer cells: an anticancer drug testing model

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Keywords: Hydrogel; 3D cancer model; hydroxyapatite; preclinical drug screening

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INTRODUCTION: The slow advance in anticancer drug development can be attributed to the limitations of conventional models, predominantly monolayer cell (2D) cultures and animal models, which inadequately recapitulate the complex nature of human malignant tumors. Three-dimensional (3D) *in vitro* models are invaluable tools in drug screening; however, creating a universal model for all cancer types poses challenges due to the diverse nature of cancers. The aim of this work was to develop a single, versatile model using alginate microfibers to accommodate cultivation of various cancer cells.

EXPERIMENTAL: Two cancer cell types were used: osteosarcoma (human HOS and U2OS, and murine K7M2-wt cell lines) and non-small cell lung carcinoma (NCI-H460 human cell line). Cells were suspended (4×10^6 cells cm^{-3}) in alginate solution (2 or 2.8 wt.%) or in a solution containing 2 wt. % alginate and 2 wt. % commercial hydroxyapatite (HAP) powder. To obtain microfibers, the suspensions were manually extruded through a 25 or 26-gauge needle into the gelling bath containing 0.18 M Ca^{2+} or 0.045 M Ba^{2+}. The obtained microfibers were washed and transferred into culture flasks and then cultured up to 21 days. The 3D cultures were validated in anticancer drug testing: 3 cm of microfibers per well in a 96-well plate were treated with 0.25-20 µM doxorubicin (K7M2-wt) or 0.5-50 µM cisplatin (NCI-H460). Treated cells in monolayer served as a control. The viability and distribution of the cells were examined using live/dead assay and histology (H&E staining). The half-maximal inhibitory concentration (IC_{50}) was determined by the MTT assay.

RESULTS AND DISCUSSION: The obtained results of osteosarcoma cells immobilized in Ca-alginate microfibers with and without HAP, and lung cancer cells immobilized in Ba-alginate microfibers have shown that the microfibers supported cell viability, metabolic activity, and formation of cellular aggregates [1]. The results of anticancer drug testing have shown that IC_{50} values for K7M2-wt cells immobilized in alginate microfibers with and without HAP, as well as for the 3D cultures of NCI-H460 cells were up to ~10-fold higher than the IC_{50} values of 2D cultures. These results align with the observed higher resistance to anticancer drugs in patients compared to traditional preclinical models.

CONCLUSIONS: These findings demonstrate the potentials of the developed 3D model for more reliable anticancer drug screening and enhancement of the preclinical platforms for drug discovery.

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Biomimetic tumor engineering to enhance drug discovery - BioengineeredTumor

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Keywords: 3D cell culture; cancer; perfusion bioreactor; carcinoma; osteosarcoma

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Development of novel, effective, and safe anti-tumor drugs is still a slow and cumbersome process, which is often attributed to weaknesses of current preclinical assays and low correlation of the preclinical *in vitro* and *in vivo* data with the results obtained in clinical trials. Consequently, there is a clear need for development of more reliable *in vitro* three dimensional (3D) tumor models, which will capture key features of the *in vivo* tumor cell microenvironment and provide drug testing results relevant for human patients. The aim of the project "Biomimetic tumor engineering to enhance drug discovery – BioengineeredTumor" funded by the Science Fund of the Republic of Serbia is to develop 2 novel, simple and robust 3D models for cultures of carcinoma and osteosarcoma cells by applying systematic and integrated methodology to comprehensively define the key model components. In specific, the aim is to use different human and animal cancer cell lines in conjunction with alginate-based biomaterials as artificial extracellular matrices imitating tumor environments and to cultivate the obtained constructs in perfusion bioreactors providing enhanced transport of nutrients, gases and biochemical signals to the cells as well as adequate levels of hydrodynamic shear stresses. Thus, the strategic goal is to establish an adaptable platform suited to the use by scientists without technical expertise for long-term *in vitro* studies of cancer cells for applications in anti-cancer drug discovery and validation, development of personalized medical treatments, and cancer research.

The project is structured based on 3 concept points: (*i*) development of a longer-term *in vitro* 3D tumor model relies on the use of biomimetic scaffolds and bioreactors providing adequate biochemical and physical signals, (*ii*) bottom-up approach starting form single well-defined components can yield controlled, reproducible and physiologically relevant 3D tumor models, and (*iii*) simple *in vitro* 3D tumor models capturing some of the key features of the tumor environment *in vivo* can present a useful and expandable platform for reliable anti-cancer drug testing and cancer research. The planned methodology is designed accordingly so that the project will comprise 3 phases: I) development of 3D tumor models, II) validation of the models, and III) utilization of the optimized 3D tumor models in short-term (up to 7 days) and longer-term (up to 28 days) studies of the effects of standard anti-cancer drugs (*e.g*. cisplatin, doxorubicin, 5-fluorouracil, or paclitaxel) on the cultured cells. In addition, the second phase of the project will include development or adaptation of analytical methods for comprehensive characterization of the cells in 3D cultures as well as mathematical modelling in order to assess the effects of culture conditions on cell viability, morphology, apoptosis and cytokine profiles. Such a systematic experimental and analytical approach will provide significant insights in cancer cell biology regarding 3D environment and guidelines for further optimization of 3D tumor models in general.

Overall, the project BioengineeredTumor is addressing an urgent clinical problem, aiming to provide important fundamental insights in cancer cell biology as well as usable products and methods for advancements in pharmaceutical and healthcare sectors.

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Human amniotic membrane homogenate: A novel biomaterial-based strategy to impede migration and invasion of bladder cancer cells

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Keywords: Human amniotic membrane; bladder neoplasm; papilloma; urothelium, focal adhesion kinase; anticancer mechanism

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INTRODUCTION: Bladder cancer ranks among the top ten most frequently diagnosed cancers globally, with approximately 25% of diagnosed cases presenting as initially aggressive muscle-invasive tumours, marked by poor prognosis and frequent metastasis [1]. Human amniotic membrane (hAM) is an extraembryonic membrane with anti-inflammatory, antifibrotic and antimicrobial properties [2], however, despite the growing number of studies, the cellular mechanisms underpinning its anticancer activity remain poorly elucidated. In our investigation, we delved into the mechanisms by which hAM homogenate influences bladder cancer and normal urothelial cells.

MATERIALS AND METHODS: The hAM homogenate was prepared from placentas collected from healthy donors after elective caesarean section according to the protocol described in [3]. Wound healing assay [4] was used to evaluate the influence of hAM homogenate on migration of normal porcine urothelial NPU cells, human papillary cancer urothelial RT4 cells and human muscle-invasive bladder cancer T24 cells. For the invasion of cancer cells, invasion assay [4] was performed using transwell chambers with membranes of 8 µm pore size coated with diluted Matrigel matrix. The effect of hAM homogenate on the migration pattern of T24 cells stably transfected with enhanced green fluorescent protein (eGFP) was examined using time-lapse confocal microscopy. Protein expression levels of molecules involved in cell migration were measured by Western blot analysis, while gene expression was quantified by RT-qPCR [3, 4]. To compare statistical differences between at two experimental groups, parametric unpaired two-tailed Student's t-test or the non-parametric Mann–Whitney test was used.

RESULTS AND DISCUSSION: Our study revealed that hAM homogenate impedes the migration rate of bladder cancer RT4 and T24 cells, but not of normal NPU cells. In addition to cell migration, hAM homogenate significantly decreased the invasion rate of muscle-invasive bladder cancer T24 cells. Furthermore, this antimigratory effect was associated with the downregulation of FAK and Rho GTPases expression—crucial proteins in actin cytoskeleton reorganization.

CONCLUSIONS: In summary, the findings highlight the significant potential of hAM homogenate as a valuable biomaterial in the field of medicine, particularly as a complementary component in the treatment of bladder cancer.

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Designing biopolymer scaffolds and oral mucoadhesive films for controlled drug delivery

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Keywords: Electrospinning; 3D-printing; buccal films; tissue engineering

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INTRODUCTION: There is a great potential to use electrospun nanofibers and 3D-printed structures as drug carriers for biomedical applications, due to the possibility of delivering drugs at a controlled rate over some time at the site of action.

EXPERIMENTAL: Poly(ε-caprolactone) (PCL), polyvinylpyrrolidone (PVP), gelatin (GE), and organic solvents were supplied by Sigma-Aldrich. The antibiotic Cefazolin and the waste yarrow were received from Pharmanova and the Institute of Medicinal Plant Research "Dr Josif Pančić," Belgrade, Serbia, respectively. Propranolol hydrochloride (PRH) and Ibuprofen (IBU) were obtained from Galenika a.d., Belgrade, Serbia while alendronate sodium trihydrate (ALN) was provided from Hemofarm. A.D. Vrsac, Belgrade. Cefazolin or yarrow-loaded PCL nanofiber mats (PCL/CEF and PCL/YAR, respectively) were produced from PCL solutions by using the blend electrospinning method (vertical electrospinning setup CH-01, Linari Engineering, Italy) [1,2]. Commercially available silicone and rubber urinary catheters were coated with the nanofiber mats by using adhesive n-butyl-2-cyanoacrylate. Semi-solid extrusion 3D printer (Ultimaker 2+ (Ultimaker B.V., Utrecht, Netherlands) was used to obtain gelatin-based mucoadhesive films and scaffolds using PRH, ALN, and IBU as model drugs [3,4].

RESULTS AND DISCUSSION: Under the conditions that simulate catheterization *in-vitro*, it has been shown that coated catheters can prevent bacterial growth and the formation of biofilm, which is a source of infection in real conditions. Yarrow powder and cefazolin retained their biological activity during the fabrication process, as confirmed by the antioxidant and antibacterial activity of these nanofiber scaffolds. Both in vitro release studies and in silico simulations indicated that processed oral films could provide effective drug transport through the buccal mucosa. Gelatin-based scaffold with IBU, enabling a synergic effect of tissue regeneration and controlled drug delivery.

CONCLUSIONS: The newly developed biomaterials show high potential for further practical utilization in biomedicine. PCL nanofiber mats with CEF or YAR could be used as a relevant drug scaffold with pronounced antibacterial activity, while 3D-printed gelatin-based films and scaffolds could be used for buccal applications and osteoporosis treatments.

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Evaluation of crosslinked gelatin-polyvinylpyrrolidone scaffold for application in drug delivery and tissue engineering

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Keywords: 3D printing; semi-solid extrusion; genipin; mechanical testing: cytotoxicity

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INTRODUCTION: The objective of this study was to process and evaluate a suitable scaffold matrix system for drug delivery and tissue regeneration. A bioinspired approach was applied. The scaffold based on natural polymer gelatin, blended with polyvinylpyrrolidone, and crosslinked by genipin, was 3D printed by semi-solid extrusion (SSE). This 3D printing technique does not require high temperature or UV curing, so it allows the use of thermo- and UV-sensitive drugs, cells, or other biological components. The influence of genipin, a natural crosslinking agent, and its content on the mechanical properties and the cytotoxicity of obtained scaffolds were investigated.

EXPERIMENTAL: Type A gelatin from porcine skin (~300 g Bloom) (GA), polyvinylpyrrolidone (K30) (PVP), and genipin (G) were purchased from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA); glycerol 85%, used for good printability, was purchased from Zorka Pharma (Zorka Pharma HEMIJA d.o.o., Sabac, Serbia). 3D printing was performed on the Ultimaker 2+ printer (Ultimaker B.V., Utrecht, Netherlands) adapted with Discov3ry paste extruder (Structur3d Printing, Kitchener-Waterloo, ON, Canada). The solutions of gelatin/PVP (1:1) and glycerol were prepared for 3D printing as in previous research [1]. For the crosslinking, G was added to polymer solutions 30 min before printing. Three series of samples were processed: pure polymer blend, with 0.5% w/w G and with 1.0 % w/w G. Characterization of obtained scaffolds was performed by FTIR analysis, SEM analysis, and mechanical testing (tensile test and micro-indentation) using the same equipment as in previous research [1]. Cytotoxicity analysis was performed according to ISO 10993-5 standard.

RESULTS AND DISCUSSION: The FTIR analysis revealed the reaction between ester groups of G and primary amine groups of gelatin. As a consequence of this reaction, the characteristic dark blue colour was achieved [2]. The improvement in mechanical properties (tensile strength, modulus of elasticity, hardness, and reduced modulus of elasticity) of crosslinked scaffolds was observed with the increase in G content. FESEM images have shown good morphology of scaffolds. Qualitative and quantitative cytotoxicity assessment in a direct test indicated the absence of cytotoxicity in tested preparations. The cytotoxicity index based on the metabolic activity of cells in an indirect test showed up to 20% reduction of viability compared to the control, indicating the absence of cytotoxicity.

CONCLUSIONS: The obtained scaffold has appropriate mechanical strength; it is biodegradable and without cytotoxicity. Due to these properties, and by applying SSE 3D printing of this material loaded with drugs or other components, it is possible to obtain a scaffold for delivering drugs, cells, or genetic materials into the surrounding tissue and promote regeneration.

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Electrospun poly(ε-caprolactone) nanofiber mats with gallic acid and glucosamine sulfate for cartilage repair

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Keywords: Electrospinning; nanofibers; anti-inflammatory drugs; bovine chondrocytes

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INTRODUCTION: Cartilage defects are a common clinical problem, and tissue engineering has become a promising approach for cartilage regeneration because of its high efficiency [1]. The goal of this research was the development of poly(ε-caprolactone) (PCL) nanofiber mats with incorporated active substances gallic acid (GA) and glucosamine sulfate (GAS) as an unexplored alternative for cartilage repair.

EXPERIMENTAL: PCL granules, GA powder, dimethylformamide (anhydrous, 99.8 %, DMF), and dichloromethane (DCM) were obtained from Sigma-Aldrich. The GAS was received from Goodwill Pharma, Serbia. PCL was dissolved in the mixture of DMF/DCM (1:4) solvents to make 10 wt.% PCL solution followed by the addition of surfactant Span 80 (1 % v/v). The aqueous solution of GA and GAS was slowly dripped into PCL solution (1 % of GA and GAS relative to the weight of polymer), to form w/o emulsions. For comparison, neat PCL nanofibers without incorporated drugs were also produced. The emulsion electrospinning method (vertical electrospinning setup CH-01, Linari Engineering, Italy) was applied to produce nanofiber mats where the process parameters were as follows: the flow rate of 3 cm^3h^{-1} and the distance of 10 cm from the collector for both neat PCL and PCL/GA/GAS solutions. At the same time, the voltage was adjusted to 18 kV and 14.5 kV for the neat PCL and PCL/GA/GAS solutions, respectively. Bovine chondrocytes were seeded onto PCL-based nanofiber mats and were cultured in a chondropermissive medium for 7 days, with an added inflammatory factor (Interleukin IL-1β) to induce an inflammatory process in the system. In the pure drug groups GA and GAS, the chondrocytes were seeded in well plates, with the addition of the drug in the medium. After that, the DNA content within the scaffold-cell constructs was measured by Hoechst 33258 dye assay using calf thymus DNA (Sigma-Aldrich) as a standard. Glycosaminoglycan (GAG) content within the scaffold-cell constructs was measured with dimethylmethylene blue dye binding assay using bovine chondroitin sulfate (both Sigma-Aldrich) as the standard. Nitric oxide (NO) production was measured with a Griess kit. All tests were performed in triplicates from one donor.

RESULTS AND DISCUSSION: After seven days of cell culture, pure GA and GAS drugs retained their biological activity, and their incorporation into PCL nanofibers decreased the inflammatory process, as shown by a reduction of NO release, compared to neat PCL. The neat PCL group had the greatest extracellular matrix (ECM) production. The neat PCL can promote chondrocytes proliferation, as reported in several previous studies, and chondrocytes seeded into PCL scaffolds showed the synthesis of cartilage-specific ECM proteins [2].

CONCLUSIONS: Based on the obtained results, PCL nanofiber mats with GA and GAS could be used as a relevant drug scaffold with pronounced anti-inflammatory effects which may be a treatment method for inflammatory cartilage defects.

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Environmentally friendly hydrogels for medical and pharmaceutical applications

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Keywords: Environment-responsive; aerogels; polysaccharides; poly(methacrylic acid); plant extracts

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The Polymer group of the Faculty of Technology and Metallurgy University of Belgrade deals with environment-sensitive hydrogel as carriers for biomedical applications, including drug delivery, wound healing, and regenerative medicine. The first hydrogel generation synthesized in our labs was discs made of polyacrylamide and its derivatives. The synthesis was at higher temperatures (50 °C), with traditional crosslinker, N, N'-methylenebisacrylamide, and persulphate/pyrosulphate as initiator.

Due to the growing demands for environmental sustainability, the general approach changed to reduce petrochemical raw materials and prepare eco-friendly materials focusing on 1) renewable polymers, initiators, and crosslinkers and 2) the application of simple, cost-effective, and eco-friendly approaches in hydrogel synthesis.

To obtain the second hydrogel generation, we use renewable polymers: polysaccharides, proteins, and polyhydroxyalkanoates [1]. Still, the mechanical strength was weak. Hence, the application of carboxylic acids, methacrylic and itaconic, and N-isopropyl acrylamide improves mechanical properties and enhances the environmental stimuli of the carriers. Instead of traditional crosslinkers - organic molecules, we gave the advantage to plant extracts like genipin, sodium tripolyphosphate, citric acid, and calcium chloride. We made beads, microgels, aerogels, discs, films, and cylinders sensitive to pH, temperature, magnetic field, or specific molecules such as glucose. Facing an everyday challenge, improving drug delivery routes, especially for poorly water-soluble drugs, and finding an alternative to traditional antibiotics, encapsulation, and controlled release remain a challenge. Therefore, we used a mild condition (e.g., deep coating) to encapsulate/release traditional water-soluble and poorly water-soluble drugs, proteins, phenolic compounds, or supercritical carbon dioxide ($scCO_2$) for thymol, carvacrol, and eugenol, a promising alternative for traditional antibiotics [2]. Furthermore, we reduced the hydrogel production temperature to ambient conditions and made a simple and cost-effective production process that doesn't require special equipment.

The promising results we got pushed us further. So, the current research focuses on the encapsulation/controlled release of antioxidant phenolic compounds extracted from orange peel waste by applying ultrasonic-assisted extraction and deep eutectic solvent (DES) based on glycerol: urea: water. We use a new initiator system based on vitamin C and H_2O_2 to polymerize a methacrylic acid [3] that could be useful for other monomers. As a concluding remark, we achieved significant progress in hydrogels encouraging us to continue further.

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Biocharacterization of hydrogels based on poly(methacrylic acid) prepared by eco-friendly method

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Keywords: pH-sensitive polymers; enzyme; gene expression; pro-inflammatory mediators; anti-inflammatory effect

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INTRODUCTION: Inflammation process in human body can lead to many serious inflammation-related diseases. Hence, there are urge to find better solution for the treatment of the inflammation processes. Solution can be found in application of poly(methacrylic acid) hydrogels which have potential for targeted delivery and controlled release of drugs. These pH-sensitive hydrogels can swell at the pH values between 5 and 8, and release drug in the process. So, taking into account that pH value at the inflammation site is around 6, these hydrogels are materials of choice.

It is very important that the system for controlled release be prepared through mild and non-toxic conditions in order to preserve bioactivity of the drug and keep good impact on environment. Enzymes are good candidates for eco-friendly preparation of hydrogels, because these green substances can initiate polymerisation of various monomers.

EXPERIMENTAL (or Materials and Methods): In this study, hydrogels based on poly(methacrylic acid) were prepared through eco-friendly method by using enzyme/hydrogen peroxide (HP)/ascorbic acid (AA) as initiator. Two groups of the samples were prepared: in the first group peroxidase isolated from potato peel waste (with activity of 0.8 IU) was used in the initiation system, whereas in the second group peroxidase isolated from soya bean coats (with activity of 0.8 IU) was employed in the initiation system. The amounts of HP and AA in both series were 40 µL and 10 mg, respectively. Anti-inflammatory drugs, dexamethasone (5 mg/mL) and diclofenac (4.5 mg/mL) were encapsulated in the first and the second group of the PMAA samples, respectively. Anti-inflammatory effect of the PMAA hydrogels with encapsulated drugs were tested on the Bovine Chondrocytes cells.

RESULTS AND DISCUSSION: Results showed that the level of pro-inflammatory mediators NO and IL-8 decreased. The PCR analysis showed that the proinflammatory TNα, IL-6 genes expression level decreased, the matrix catabolism MMP1 and MMP3 genes expression level decreased and the fibrotic COL2 and ACAN genes expression level increased.

CONCLUSIONS: The study showed that the PMAA hydrogels have anti-inflammatory effect and have potential for treatment of the inflammation processes.

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Citric acid-crosslinked gelatin/hydroxypropyl methylcellulose hydrogels for biomedical applications

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Keywords: Mechanical properties; degradation; drug delivery

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INTRODUCTION: Hydrogels, characterized by their three-dimensional hydrophilic polymer networks capable of retaining substantial amounts of water or biological fluids, hold significant promise for biomedical and pharmaceutical applications. While numerous polymers have been explored for hydrogel development, those derived from natural sources possess inherent advantages due to their abundance, affordability, biocompatibility, and biodegradability. Gelatin, a widely used natural polymer in biomedicine, stands out for its cost-effectiveness, compatibility with biological systems, and degradability. Hydroxypropyl methylcellulose (HPMC), a cellulose derivative, exhibits hydrophilicity, biodegradability, and biocompatibility. However, natural polymer-based hydrogels often exhibit low mechanical strength and solubility in physiological conditions, necessitating innovative cross-linking strategies to enhance their functionality. Citric acid (CA) emerges as a promising crosslinking agent owing to its affordability and non-toxic nature.

EXPERIMENTAL: HPMC-G hydrogels were synthesized by dissolving gelatin (0.05 g) and HPMC (0.1 g) with specified amounts of CA and sodium hypophosphite in 1 ml of distilled water in a reaction vessel. The mixture was homogenized, poured into a Teflon mold, and frozen at -20°C for 24 hours. After lyophilization, the hydrogels underwent crosslinking at 160°C for 7 minutes. By varying the CA content from 0% to 40% (w/w) while maintaining constant temperature and time, the optimal CA/HPMC ratio was determined. Subsequently, various heat treatments (140–180°C, 3-12 minutes) were applied to explore the optimal curing conditions.

RESULTS AND DISCUSSION: The investigation elucidates how the CA/HPMC ratio and curing conditions impact the physicochemical and mechanical properties of HPMC-G hydrogels. Swelling tests and compressive mechanical property evaluations reveal that the incorporation of citric acid, along with increases in curing temperature and time, enhances compressive modulus and degradation stability while reducing equilibrium swelling. Scanning electron microscopy analysis reveals a highly porous microstructure in the resulting hydrogels, while differential scanning calorimetry curves indicate the formation of strong interactions between gelatin and HPMC.

CONCLUSIONS: The utilization of these materials not only contributes to environmental conservation efforts but also drives the advancement of eco-friendly technology, aligning with the principles of the circular economy. Moreover, it offers promising avenues for innovative solutions in potential biomedical applications.

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Development of cornstarch aerogels with high porosity and their impregnation with natural bioactive compounds

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Keywords: Biomaterials; supercritical carbon dioxide; supercritical drying; supercritical impregnation

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Aerogels are materials with high specific surface area and high porosity, which can be produced from polysaccharides such as starch [1,2]. Starch is an abundant and low-cost polymer with versatility in processing. Aerogel properties are influenced by the process parameters including starch-to-water ratio, gelatinization temperature (T), selection of non-solvent for water replacement in hydrogels, selection of drying method, drying pressure (P) and T, *etc.* [1,2]. Supercritical drying, which employs supercritical carbon dioxide ($scCO_2$), is an environmentally friendly process that allows relatively fast production of aerogels. Despite the superior properties of starch aerogels, they do not express biological activity. This can be overcome by the incorporation of bioactive compounds (BCs) into aerogels using the supercritical impregnation (SCI) process [3,4]. SCI implies the dissolution of BCs in $scCO_2$, diffusion of BC-$scCO_2$ solution into a polymer matrix, possible chemical or physical interaction of BC with polymer, and complete removal of $scCO_2$ from the BC-polymer after a decrease of P and T to atmospheric. This process allows one-step production of solvent-free added value materials at relatively low T and the incorporation of high amounts of BC with various biological activities. Aerogels can be impregnated with pharmaceutical drugs but also with natural BCs (single or mixture) such as plant extracts [3,4]. To produce aerogels, 10 g of cornstarch (amylose content 20–30%, HeMoss, Serbia) was mixed with 100 mL of distilled water, gelatinization T was changed from 70 to 100 °C, water contained in hydrogels was replaced with acetone or ethanol during 1 or 5 days, drying P was varied from 8 to 20 MPa while drying T was 35 or 40 °C. Drying of gels was performed in a 25 mL high-pressure view cell while impregnation of aerogels with BCs was performed in a 280 mL high-pressure unit (Eurotechnica GmbH, Germany). Developed aerogels were tested as possible carriers of hemp and bilberry extracts as well as carriers of neat components such as carvacrol and citronellol. Produced cornstarch aerogels showed high liquid absorption capacity (ca. 400%). Due to high specific surface area, low density, high porosity, and biocompatibility, obtained aerogels present promising candidates for the development of biomaterials that will release BCs in a controlled manner and enable antimicrobial activity. The SCI processes enabled the impregnation of a high amount of tested natural BCs achieving loadings up to 33%. By controlling preparation process parameters, which leads to tuning of pore size and pore size distribution, tailoring of aerogel properties for special purposes is possible.

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Peculiarities of alginate gellation triggered by calcium ions in the presence of hydroxyapatite particles

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Keywords: Rheology; sol-gel; bone; tissue engineering; ceramics; scaffold

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INTRODUCTION: Hydroxyapatite (HAP), a crucial constituent of natural bone mineral, significantly enhances the osteoconductivity of alginate hydrogels (AH) in biodegradable composites mimicking the mineral composition, porosity, and mechanical properties of bone tissue. Ionic crosslinking of sodium alginate hydrogels with Ca^{+} ions result in the rapid formation of egg-box structures, leading to enthalpy-driven hydration and dehydration mechanisms observable through swelling and subsequent shrinkage of the crosslinked aerogel. The fast shrinkage induces non-linear sizing of water-containing pockets, impacting the unilinear pore size distribution of freeze-dried aerogels, thereby influencing the porosity and mechanical properties of the final scaffolds.

EXPERIMENTAL: Kinetics of gel-sol phase transformations of 2 % AH gel with fixed amount of mixture of hydroxyapatite (HAP) in 2 % $CaCl_2$ water bath with mass ratios of HAP to $CaCl_2$ (100:0, 35:75, 50:50, 75:35 and 0:100 respectively) was observed on a plate-plate Anton Paar 302 rheometer. Viscoelastic measurements were conducted in linear viscoelastic region (LVE) with constant strain (γ = 0.1 %) and varying angular frequencies (ω= 0.1, 0.5 and 1 rad. s^{-1} that simulated different mixing rates. Once that viscoelastic parameters (Transient complex viscosity (η^{*+}), Storage modulus (G') and Loss modulus (G'')) reached their plateau values, samples were removed from rheometer were freeze dried for morphological observation with Scanning electron microscope (SEM) Hitachi S-4700, (Chiyoda City, Tokyo, Japan).

RESULTS AND DISCUSSION: Kinetic of Ca+ ions induced crosslinking of alginate hydrogels is a complex, non-linear process governed by colloidal forces and change in system enthalpy, dependent on ions concentration and mixing frequency. Stepwise process of adsorption of Ca^{2+} ions onto HAP particles and their release into sodium alginate hydrogel results in nonlinear time-dependent crosslinking kinetic dependent on suspension ions concentration and mixing rate followed by reversable hydration and dehydration. Formation of smaller egg-box structures that contain interlocked water is observable through change in rheological parameters and in structure of freeze-dried aerogels.

CONCULSIONS: Step-wise adsoption of Ca^{+} ions ono HAP particles in $CaCl_2$ suspension, enables their controlled release once the suspension is poured into sodium alginate hydrogel and formation of uniform pores size distribution.

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Regulatory science for biomaterials: are we doing right things right?

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Keywords: Regulations; medical devices; testing; biomaterials; assessment; compliance

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INTRODUCTION: The aim of this work is to highlight gaps and drawbacks in modern development of biomaterials which are intended themselves alone or in combination to be deployed in medical devices, especially for Class III under the EU MDR 2017/745. More tight requirements for biomaterials safety and efficacy demand proper testing and assessment which might be not always aligned with the R&D process [1].

MATERIALS AND METHODS: We analyse here several examples related to the assessment of properties several biomaterials (silicone, PLA, titanium, zirconia) and align these with the objectives of the regulations, which in general do not state specific methods or outcomes to be reached for orthopaedic and dental applications. We show in particular how biomechanical and physical-chemical properties should be evaluated in the *in vitro* conditions to mimic as close as possible their intended purpose [1,2].

RESULTS AND DISCUSSION: Results of the testing show that the same biomaterial exhibit functionalities which are different for varied applications, and hence known standard methods are lagging in providing information relevant to the clinical purposes. In particular, known ISO 10993 for biocompatibility assessment in lacking essential stimuli needed for orthopaedic and dental uses, and is insufficient for the regulatory authorities to prove biomaterials usability. This requires a deeper knowledge of the target tissues and locations where these biomaterials are intended to be applied, considering i.a. biomechanical compliance, proper hemodynamic and neurologic stress windows, foreign body response, inflammatory corrosion resistance under physiological loads, *etc*.

CONCLUSIONS: Most of existing standard testing protocols require a substantial update and detailing for biomaterials to take into account those biomaterials specific purpose, and not in general. We suggest that biomaterials tuning should always include a specific purpose which will be complemented with a tailored test protocol. This would allow early detection of unsuitable solutions before seeing failures in clinical trials or on the market [1,3].

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3D printing of composites containing copper-incorporated mesoporous bioactive glass induce different cell responses depending on cell type and donor

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Keywords: Composite bioink, cell viability, osteoblastic differentiation

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INTRODUCTION: Bioactive glasses are used for dental and orthopaedic applications thanks to their osteoconductivity and bioactive properties. Mesoporous bioactive glasses (MBG) are special type of bioactive glasses with highly ordered channel structure and high specific surface area, which can be loaded with drugs and growth factors, making them suitable as delivery systems. Besides that, their structure can be modified by substitution with bioactive metal ions assessing desired therapeutic effect after release. With that in mind, ions showing antimicrobial effect would be appropriate solution for prevention and treatment of implant-related infections. However, certain ions such as Cu^{2+} can be cytotoxic at concentrations effective against bacteria. Ion release from MBG can be tuned and controlled by integrating MBG in established biomaterial inks. Our aim here was to investigate release of Cu^{2+} from 3D printed composite scaffolds containing MBG and to evaluate effects of release products on human pre-osteoblasts (hOB), primary and immortalized mesenchymal stem cells (hMSC).

EXPERIMENTAL: Calcium in MBG was partially substituted with 5 mol.% Cu^{2+} (5CuMBG) and completely with 15 mol% Cu^{2+} (15CuMBG), following already established protocol [1]. In order to make this particulate material extrudable, we integrated the different MBG variants in already established alginate-methylcellulose blend [2] to prepare composite biomaterial inks containing 2 and 7 wt.% MBG. Scaffolds were produced using extrusion 3D printing, crosslinked with 100 mM $CaCl_2$ and incubated in cell culture medium over 21 days. Ion release profiles were determined and the effect of release products on viability of hOB, primary and immortalized hMSC as well as on differentiation towards osteoblastic cells was investigated.

RESULTS AND DISCUSSION: Full substitution with Cu disturbed channel structure of the MBG, while it was maintained in 5CuMBG. Release of Cu^{2+} from all composite scaffolds was initially high, but it dropped over time. Initially released concentrations from all 15CuMBG-containing composites as well as from the ones containing 7 wt.% of 5CuMBG were highly cytotoxic towards all tested cell types. However, composites containing 2 wt.% of 5CuMBG showed different levels of cytotoxicity towards two different donors of hOB. Viability of both types of hMSC was not affected in the presence of release products of the same type of composite scaffolds, while specific ALP activity of osteogenically differentiated MSC was significantly increased.

CONCLUSIONS: Our findings show that the cytotoxic effect of CuMBG in composite scaffolds depends on cell type and is also donor-specific. Therefore, it seems that CuMBG can play a promising role in future patient-specific therapies.

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The effect of surface ion-doping on the bioactive glass cytocompatibility and antibacterial performance

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Keywords: Bioactive glass; silver; copper; ion exchange; cytocompatibility; antibacterial activity

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In the realm of orthopaedic infection treatments, biofilm prevention while fostering bone growth remains a critical challenge. Moreover, the pressing global threat of rapidly advancing antimicrobial resistance calls for alternatives to traditional antibiotics. Bioactive glasses doped with silver (Ag) or copper (Cu) ions emerge as promising candidates for addressing these challenges. However, concerns regarding the potential toxicity of these dopants must be carefully addressed, as their presence could pose cytotoxic risks.

This study investigates the cytocompatibility and antibacterial performance of two bioactive glasses, either doped with Ag- (Ag-SBA2) or Cu-ions (Cu-SBA3) via an ion exchange process in an aqueous solution. Human adipose stem cells (hASCs) were subjected to various culture conditions, including direct culture on glass discs with and without pre-incubation, as well as culture in a medium containing glass dissolution byproduct. In addition, the effect of protein adsorption on the cell response was studied by adsorbing fibronectin on the glass discs before direct culture with hASCs. The glasses' antibacterial properties against multidrug-resistant and Gram-positive bacteria, *Staphylococcus aureus,* were evaluated by following two different protocols: i) the International standard ISO 22196, and ii) the protocol published by authors from UPO university [1].

Ag-SBA2 and Cu-SBA3 initially inhibited the hASC viability in direct cell culture. However, viable cells with healthy morphology were maintained when cultured directly on pre-treated discs or indirectly with glass dissolution byproducts. This suggests that the cytotoxicity effect seems to arise from the contact toxicity between the cells and the material surface. Fibronectin adsorption significantly enhanced the cytocompatibility of Ag-SBA2, while Cu-SBA3 requires further optimization. Regarding antibacterial activity, Cu-SBA3 demonstrated a statistically significant reduction in metabolic activity and viable numbers of bacterial colonies adhered to the surface of Cu-SBA3 in comparison with the non-doped one after 24 hours, indicating its potential as a bioactive and antibacterial surface.

In conclusion, Ag-SBA2, through its contact toxicity, has the potential to treat early infection, without compromising long-term cytocompatibility and bioactivity. However, further optimization of the Cu-SBA3 glass is necessary due to its cytotoxicity towards hASCs.

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Multifunctional Sr,Mg doped mesoporous bioactive glass nanoparticles

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Keywords: Bone regeneration; drug delivery

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INTRODUCTION: Recently, mesoporous bioactive glass particles (MBGs) doped with therapeutical ions have emerged as a promising biomaterial with ability to simultaneously deliver drugs and ions, leading to synergistic outcomes. Sr and Mg ions are widely investigated in ion-doping approach of bioceramics and bioactive glasses due to their ability to stimulate osteogenesis and angiogenesis. The co-doping of MBGs with Sr and Mg might lead to accelerated bone regeneration, however the presence of dopant ions can also influence the mesoporosity of MBGNs, and thus affect bioactivity and drug release behaviour. The objective of this study was to investigate the influence of Sr,Mg co-doping on the textural properties, drug delivery, as well as pro-osteogenic and pro-angiogenic potential of MBGs.

EXPERIMENTAL: A modified micro-emulsion assisted sol-gel synthesis coupled with ultra-sonication was used to obtain Sr,Mg-MBGs. Mesoporosity was assessed by the N_2 adsorption (Micromeritics ASAP 2020, Norcross) and HR-TEM (FEI Talos F200X) analysis. Morphology of the particles and bioactivity was determined by FESEM (Tescan Mira 3 XMU). Ibuprofen was used as a model drug for analysis of drug delivery properties performed by the dialysis tubing diffusion method in PBS (pH 7.4). UV-VIS spectrophotometer (Shimadzu, UV-1800) and a simultaneous DSC-TGA instrument (SDT Q-600, TA Instruments) were employed to quantify amount of drug loaded and drug release rate.

Indirect assays were used to determine biocompatibility, and potential pro-osteogenic and pro-angiogenic properties of MBGs using human bone marrow-derived mesenchymal stem cells in 2D and endothelial cells EA.hy926 in 0.5 % collagen 3D models. Dissolution extracts were prepared by dispersing 1 mg/ml MBGs in DMEM without any supplements for 24 h. Resazurin reduction assay was used at days 1, 3 and 7 of cultivation for measuring cellular metabolic activity (n=3). To evaluate calcium deposition, and detect alkaline phosphatase (ALP) activity Alizarin red S (ARS) staining and colorimetric p-nitrophenyl phosphate assay were performed at day 7 of cultivation, respectively. Fluorescence staining with phalloidin and DAPI was done to visualize the morphology and spreading of EA.hy926 cells at day 3 and day 7.

RESULTS AND DISCUSSION: Results showed that ion-doping influenced the mesoporosity of MBGs changing the pore shape from worm-like to radial dendritic, which resulted in increased pore volume with smaller pore size. As a results, the drug-loading capacity of SrMg-MBGs was slightly reduced, while the drug release rate was somewhat increased. After 7 days of immersion in the SBF the surface of particles had rough appearance due to dissolution of glass and precipitation of nanocrystals on the surface, accompanied by formation of needle-like crystals characteristic of hydroxy-carbonate apatite. After 14 days, the surface of was completely covered in thick layer of the apatite crystals confirming the bioactive nature of the SrMg-MBGs. The biological evaluation assays showed that Sr,Mg-MBGNs has ability to slightly induce human bone marrow-derived mesenchymal stem cells osteogenic potential due to the improvement in ALP production and calcium deposition, and to stimulate the proliferation of endothelial cells in 3D models.

CONCLUSIONS: The study demonstrates that Sr,Mg-doped MBGs can be considered as a promising multifunctional biomaterial for application in biomedicine.

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Development of luminescent bioactive glass for multimodal diagnostic imaging

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Keywords: Bioglass; up-conversion; luminescence; bioimaging; rare earth

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INTRODUCTION: Bioglasss is glass-ceramic biocompatible material that contains silica, calcium, sodium, and phosphate, as main ingredients. It has excellent bioactivity and is widely used for scaffolds, implant devices and for repair of bone defects, among others [1]. Current research aims to develop luminescence bioglass, which will comprise different rare earth (RE) elements and open possibilities for its use in multimodal imaging diagnostics [2].

MATERIALS and METHODS: The materials used were calcium nitrate tetrahydrate ($Ca(NO_3)_2{\times}4H_2O$, Carlo Erba, ≥99 %), ethanol (EtOH, Sigma Aldrich, 96 %), hydrochloric acid (HCl, Macron, 35-38 %), ammonium hydroxide (NH_4OH, NRK Inženjering, 25 %), distilled water (H_2O), rare earth nitrates: $Yb(NO_3)_3{\times}5H_2O$, $Er(NO_3)_3{\times}5H_2O$, $Eu(NO_3)_3{\times}5H_2O$, $Gd(NO_3)_3{\times}6H_2O$ (all obtained from Sigma-Aldrich, 99.9 %), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4{\times}12H_2O$, Exôdo Científica, 99 %) and tetraethyl orthosilicate (TEOS, Sigma Aldrich, 98 %). Preparation of RE-doped SiO_2-CaO-Na_2O-P_2O_5 bioglass by sol-gel process relies on modified Stöber method [3], with the addition of corresponding rare earth nitrates. The obtained powders were characterized by X-ray powder diffraction (XRPD, Philips PW 1050 diffractometer), Fourier transform infrared spectroscopy (FTIR, Nicolet iS10 FT-IR Spectrometer), photoluminescent measurements (TE-Cooled CCD Fluorescence spectrometer, Glacier X, BWTEK, USA) and MTT assay.

RESULTS AND DISCUSSION: Analysis of crystal structure confirmed obtaining of glassy-amorphous system in undoped sample, while the RE-doped samples possess low crystallized RE-oxides and phosphates. FTIR spectroscopy revealed vibration modes of quaternary glass of desired composition, beside the bands of RE oxides. Photoluminescent measurements confirmed emission capability: up-conversion emission for Gd/Yb/Er doped sample and down-conversion emission for Gd/Eu sample. MTT tests implied that samples are not cytotoxic and can be used in medicine.

CONCLUSIONS: Applied sol-gel procedure resulted in formation of luminescent and biocompatible powders with promising use in multimodal bioimaging, cell labeling, and bone reconstruction.

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The current trend in innovative bioactive materials for dental and orthopedic applications

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Keywords: Bioceramic; biocomposite; scaffold; bioactive cement; dental insert

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INTRODUCTION: Bioactive materials for the repair and regeneration of human bone tissue, as well as for the restoration of teeth, are the focus of numerous studies in the field of biomaterials. Orthopaedic surgeons anticipate that bioactive materials have the potential to facilitate the formation of new apatite-like crystals upon contact with body fluids, promoting the development of new bone tissue under *in vivo* conditions. On the other hand, dentists expect that bioactive materials have the potential for remineralization of partially demineralized enamel and dentin. In the preceding years, the Bioceramic Materials Group, founded within the Department of Inorganic Chemical Technology at the Faculty of Technology and Metallurgy, University of Belgrade (FTM-UB), conducted extensive research on the advancement of bioactive and biocompatible materials with adequate mechanical properties, designed for application in dentistry, orthopaedics, maxillofacial surgery, and also bone tissue engineering (BTE).

EXPERIMENTAL: Different forms of bioactive materials were processed starting from nanostructured mesoporous calcium hydroxyapatite (HAp), synthesized by controlled precipitation and hydrothermal method, and mesoporous bioactive glasses (BAG), synthesized by sol-gel method, doped with various cations and anions. Calcium phosphate dental inserts and potential solo implants in maxillofacial region were obtained by isostatic pressing of HAp nano-powders, followed by sintering by different techniques. Calcination of multi-ion-doped calcium-deficient HAp powders enabled a phase transformation into α-tricalcium phosphate (α-TCp), known for its binding properties. Doped α-TCp, combined with polymeric materials and Ag and BN nanoparticles possessing antimicrobial properties, was the basis for the development of innovative bioactive dental cements. Three types of bioactive scaffolds were developed: bioceramic scaffolds obtained by the replica technique based on multi-ion doped HAp, β-TCP and BAG, additionally coated with polymers; polymer-based scaffolds created by combining various hydrogels with the BAG, β-TCP, and HAP as nano-fillers; metallic scaffolds modified through the deposition of biodegradable polymers, doped BAG, β-TCP, and HAP nanoparticles.

RESULTS AND DISCUSSION: Calcium phosphate dental inserts exhibited fracture toughness within the range of human dentin, as well as a strong bond with dental composites and adhesives, thereby ensuring the restoration of teeth with satisfactory mechanical properties. The dental cements demonstrated high bioactivity, biocompatibility, along with appropriate rheological, mechanical, and antimicrobial properties. Bioactive ceramic scaffolds for application in BTE with optimal macro-porosity and suitable biocompatibility were successfully fabricated, and biodegradable polymeric coatings significantly enhanced their mechanical properties. Macroporous hydrogels based on poly(methacrylic acid)/gelatine interpenetrating network exhibiting appropriate swelling behavior, while their mechanical properties were improved by the incorporation of bioactive nano-fillers. The greater antimicrobial properties and bioactivity of 3D-printed titanium scaffolds were achieved by the formation of multi-layered bioceramic coating composed of nanoparticles of doped HAp and ZnO.

CONCLUSIONS: Owing to the achieved mechanical and biological properties, the obtained bioactive materials show significant potential for application in dentistry, biomedicine, and bone tissue engineering.

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Effects of different doped hydroxyapatite-based materials on healing of critical size calvaria bone defects in rats

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Keywords: Biomedical application; Tricalcium phosphate; Bone regeneration; *in vivo*

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INTRODUCTION: Nanosized synthetic hydroxyapatite (HAp), closely resembling biological apatite found in human bones and teeth, is extensively studied for its potential in hard tissue repair. Ion-doping of HAp with therapeutic ions is emerging as a promising strategy to mimic biological apatite, promoting specific biological responses such as osteogenesis, angiogenesis, increased cell proliferation, and antimicrobial activity [1]. The aim of our study was to explore and compare the effects of Sr,Cu co-doped α tricalcium phosphate (αTCP) with/without Mg doped HAP on healing of critical size rat calvaria defects *in vivo*.

MATERIALS AND METHODS: Nanosized HAp powders, both single-ion doped with 5 mol.% Mg and co-doped with 3 mol.% Sr and 0.4 mol.% Cu ions , were synthesized using a modified hydrothermal method and α-TCP powder was obtained by calcination of the doped HAp at 1500°C for 2 h [1]. Six male Wistar Albino rats, 8 weeks old, were used to surgically induce two 5 mm bone defects on calvaria [2]. The defects were augmented with either Sr,Cu αTCP with/without Mg HAp material or with "gold standard" material, Bio-OSS (Geistlich Pharma, Wolhusen, Switzerland). Animals were sacrificed 8 weeks after augmentation. Collected tissues were further analyzed using CBCT, histology and qRT-PCR. Collected data were analyzed statistically.

RESULTS AND DISCUSSION: Results of CBCT analysis presented high defect closure using Bio-OSS and Sr,Cu αTCP, and median level of closure when using Sr,Cu αTCP/Mg HAp (p<0.05). This difference could be due to high levels of endothelial cell stimulation by magnesium ions [3] contributing to higher closure by fibrous tissue as presented in our histological analysis. However, using both Sr,Cu αTCP or Sr,Cu αTCP/Mg HAp resulted in stimulating mineralized tissue deposition. In our qRT-PCR analysis, Sr,Cu αTCP and Bio-OSS similarly (p>0.05) increased the expression of proinflammatory cytokine TNF-α, unlike using Sr,Cu αTCP/Mg HAp which showed lower levels (p<0.05). This could again be appointed to the aforementioned effect of magnesium ions [4]. Interestingly, both Sr,Cu αTCP and Sr,Cu αTCP/Mg HAp presented higher expression of TGFβ, a growth factor connected with better bone regeneration, compared to Bio-OSS. Additionally, all tested materials presented similarly high expression of ALP, known marker of osteogenic differentiation. However, Bio-OSS presented significantly higher RANKL/OPG ratio, a marker indicating osteoclastic activity, compared to Sr,Cu αTCP, while Sr,Cu αTCP/Mg HAp showed the lowest values.

CONCLUSIONS: This study demonstrates that Sr,Cu αTCP and Sr,Cu αTCP/Mg HAp exhibit promising bone healing outcomes of critical size calvaria defects, comparable to that of Bio-OSS.

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Sr/Mg – doped bioceramic scaffolds for biomedical application

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Keywords: Bone regeneration; mesoporous bioactive glass; tissue engineering

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INTRODUCTION: Bone is a mineralized connective tissue with remarkable self-healing capability. However, in the presence of large bone defects (≥ 2.5 cm), bone self-recovery is not efficient, necessitating surgical intervention and the introduction of a bone substitute. Hydroxyapatite (HAP) is a widely investigated material for bone tissue engineering (BTE) due to its similarity to the biological apatite found in bones and teeth. Mesoporous bioactive glasses (MBAGs), quickly bind to surrounding tissues and release ions promoting the formation of new bone [1]. The silica from glass enhances angiogenesis, which is a pivotal consideration given the high vascularization level of this tissue [2]. Ion-doping approach of both HAP and MBAG particles has gained great attention due to the ability of therapeutical ions to stimulate a certain cell response. The project aims to develop and characterize bioceramic scaffolds based on a combination of Sr/Mg-doped HAP and MBAG, thereby promoting osteogenesis and creating a favourable environment for the proliferation of endothelial cells.

MATERIALS AND METHODS: *Scaffolds fabrication*. Scaffolds were fabricated via sponge-replica technique sintered at 1400 ºC: pure HAP served as control; Sr, Mg-doped HAP = dHAP, combination of dHAP with 10% SrMg-MBAG = dBAG. *Mechanical properties.* A static compressive test has been used to verify the scaffolds strength. *Biological properties***.** The cytocompatibility of scaffolds towards human bone marrow-derived mesenchymal stem cells (hBMSCs) and human endothelial cells (Ea.hy926) has been verified with the Resazurin reduction assay after 1 and 7 days of cultivation. The viability of both cell lines was confirmed with the fluorescent assay Live/Dead. Scaffold suitability in sustaining Ea.hy926 adhesion and proliferation has been verified through FESEM and fluorescent microscope using phalloidin and DAPI after 14 days of cultivation. The pro-osteogenic behaviour of the scaffolds was assessed by culturing hBMSCs within the scaffolds for 21 days. The confirmation of osteogenic differentiation was achieved through a comparative analysis of the expression level of collagen I via real-time PCR. Furthermore, the correct differentiation toward an osteogenic lineage is verified with the collagen I/collagen II ratio. All samples were tested in biological triplicates (n=3).

RESULTS AND DISCUSSION: The results of the static compressive test indicate that the MBAG particles serve as sintering agent, leading to superior performance of dBAG. In contrast, the results of the Resazurin reduction assay didn't show any significant difference among the samples for both time points and cell lines in exam. Scaffolds' cytocompatibility is confirmed by the Live/Dead assay, highlighting not only cells' viability but also cells' distribution within the scaffolds. The morphological analysis revealed a better cell-cell and cell-material interaction in dHAP and dBAG scaffolds compared to HAP, demonstrating that these scaffolds provide an optimal environment for Ea.hy926 proliferation. The real-time PCR results suggest that all the scaffolds can sustain osteogenesis, however, dHAP and dBAG scaffolds show better performances. The collagen I/collagen II ratio in favour of collagen I demonstrate their differentiation toward an osteogenic lineage.

CONCLUSION: Bioceramic scaffolds based on Sr,Mg- doped HAP and MBAG present promising materials for bone tissue engineering.

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Characterisation of strontium-substituted hydroxyapatite as potential biomedical material

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Keywords: Apatite; strontium; bones; bioactivity

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INTRODUCTION: Owing to its similarity to the inorganic part of the natural bone, excellent bioactivity, biocompatibility, and ability to stimulate the osteoconductive process, synthetic hydroxyapatite (HAP) is very often the material of choice for biomedical applications. Diverse ions can be found as substitutes within natural bone structures, each playing a distinct and crucial role in the physiological processes governing the lifecycle of bones [1]. Among them, strontium ion has a very important role for the acceleration of osteogenesis and the inhibition of osteoclasts activity [2]. Current research aims to provide physico-chemical characterization of synthesized HAP and strontium substituted HAP (Sr-HAP) powders obtained by varying strontium concentration (10, 20 and 40 mol.%) in the starting solutions.

EXPERIMENTAL: HAP powder was synthesized by wet chemical precipitation, using aqueous solutions of $Ca(NO_3)_2$ $4H_2O$ (Merck, p.a.) and $(NH_4)_2HPO_4$ (Sigma-Aldrich, ≥99 %). By adding NH_4OH (CENTROHEM, *p.a.*), pH value was adjusted to 10. The obtained precipitate was heated up to 90 °C. The same procedure was followed for Sr-HAP powder syntheses, by adding $Sr(NO_3)_2$ (Sigma-Aldrich ≥99.0 %) and maintaining the (Ca + Sr)/P ratio at 1.67 in the mixed Ca^{2+}/Sr^{2+} solution. Synthesized powders were characterised by FTIR spectroscopy (Nicolet IS-10, Thermo Fisher Scientific), XRD analysis (Philips PW 1710, Philips, The Netherlands), TG analysis (Netzsch STA 449 F5 Jupiter instrument), and FE-SEM analysis (JSM-7001F, JEOL Ltd, Japan).

RESULTS AND DISCUSSION: FTIR spectra revealed the presence of carbonate-substituted hydroxyapatite in both pure and Sr-substituted HAP powders. The powders showed a granular, homogeneous morphology without the Sr separation. XRD analysis revealed that the amount of incorporated Sr in the HAP structure increased with increased Sr concentration in the starting solutions. Thermal stability of the Sr-HAP powders decreased with increased Sr concentration.

CONCLUSIONS: Physico-chemical characteristics of Sr-HAP powders are directly dependent on Sr ion concentration in powders.

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Novel enriched hydrogel nano-hap induced osteogenic differentiation of SCAP

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Keywords: Osteogenic regeneration, chondrogenic regeneration, chondroitin sulphate, sodium hyaluronate

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INTRODUCTION: Enriched nano-HAP hydrogels could serve as novel therapy material for osteogenic and chondrogenic regeneration. Here we investigate the biocompatibility and bioactivity of nano-HAP in the form of hydrogels, enriched by chondroitin sulphate, sodium hyaluronate, hydroxyl methyl cellulose, or propylene glycol.

MATERIALS AND METHODS: Stem cells from apical papilla (SCAPs), previously isolated and characterised, were used in the study. Biocompatibility was investigated by MTT test, in 96-well plates. Hydrogels were diluted in DMEM in various concentrations (0.125, 0.25, 0.5, and 1 %) and added to corresponding wells. After 24 h of culturing, MTT test was performed. Osteogenic and chondrogenic potential was investigated next. Cells were seeded in 24-well plates. Upon reaching confluency, osteogenic or chondrogenic mediums were added in corresponding wells. Hydrogels diluted to final concentration of 0.125 % were added in experimental groups. Cells were cultured for 7 days, and Alizarin red and Alcian blue staining were performed. Qualitative images were obtained, and afterwards the staining was further quantified. All experiments were performed in triplicate, in two separated experiments. Kolmogorov-Smirnov test was used for normality of data distribution, and One-way ANOVA was used for comparison between groups. GraphPad Prism software 9.0 was used.

RESULTS AND DISCUSSION: No cytotoxic effect was observed after 24 h of culturing. The concentration of 0.125 % for hydrogels shown to be the most stimulative for cell mitochondrial activity in most experimental groups, so this concentration was used for further bioactivity tests. All tested materials induced significant osteogenic differentiation after 7 days of culturing (p<0.0001). There was no difference between hydrogels, in regards to stimulation effect. On the other hand, no significant chondrogenic differentiation was observed in any experimental group, in comparison to the control.

CONCLUSIONS: Enriched nano-HAP hydrogels shown to induce significant osteogenic differentiation of SCAP during 7 days of culturing. Prolonged time period of chondrogenic induction, and various gel concentrations should be investigated in the future, to determine its' full effect on SCAPs in means of chondrogenic differentiation.

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Anodization/anaphoretic deposition of composite zein/hydroxyapatite coatings on titanium substrate

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Keywords: in situ synthesis; biomedical application; cytotoxicity; adhesion

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INTRODUCTION: Hydroxyapatite, the main inorganic component of human bone, is a widely used bioceramic for bone implant coatings due to its chemical and structural similarity with bone minerals and approved biocompatibility. Nowadays, the surface properties of implants are also modified by the addition of different biopolymers. Features such as biocompatibility, tuned biodegradability, and non-cytotoxicity make these materials excellent candidates for these applications. Zein is one of the natural polymers that has gained great interest for biomedical applications due to its natural renewable resource, biodegradability, biocompatibility and potential antibacterial activity.

EXPERIMENTAL: In this study, zein/titanium dioxide (zein/TiO_2), hydroxyapatite/zein/titanium dioxide (HAp/zein/TiO_2) and strontium-doped hydroxyapatite/zein/titanium dioxide (Sr-HAp/zein/TiO_2) coatings were obtained on a titanium substrate by *in situ* anodization/anaphoretic deposition method at a constant voltage of 60 V and deposition time of 1 min. For the fabrication of HAp/zein/TiO_2 and Sr-HAp/zein/TiO_2 composite coatings, HAp and Sr-HAp nano-sized powders obtained by a modified chemical precipitation method were used. The microstructure and morphology of all coatings were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Adhesion strength was measured according to the ASTM D3359-02 standard. Morphology and cell adhesion were analyzed by SEM.

RESULTS AND DISCUSSION: The advantage of this methodological approach compared to cataphoretic deposition is reflected in the simultaneous performance of several processes. The first is the anodization of the substrate surface, whereby a passive oxide layer (TiO_2) is formed on the surface, which changes the structure and increases the surface roughness in a controlled manner. Another parallel process is the deposition of the HAp based coatings on the substrate. This way of coating formation shows better results than cataphoretic deposition in terms of better coating adhesion to the substrate. Adhesion strength was significantly improved compared to coatings obtained by cataphoretic processes, without the need for subsequent treatment. Cytotoxicity tests showed that there was no significant decrease in the survival of healthy human lung fibroblasts (MRC-5) cells exposed to the obtained coatings.

CONCLUSIONS: Altogether, zein/TiO_2, HAp/zein/TiO_2 and Sr-HAp/zein/TiO_2 could be promising non-toxic biomaterials for orthopedic and dental applications.

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Influence of bone substitute PerOssal® on bone marrrow mesenchymal stem cells

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Keywords: Mesenchymal stem cells; bone regeneration, cell migration, osteogenesis

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INTRODUCTION: PerOssal® is biologically degradable and osteoconductive bone substitute consisting of nanocrystalline hydroxyapatite (51.5 %) and calcium sulfate (48.5 %) [1]. It is used for bone defect treatments as a synthetic carrier for antibiotics suitable for infected areas. Since the effect of PerOssal® has not been investigated so far in combination with mesenchymal stem cells (MSC) for bone tissue regeneration, we aimed to explore in vitro whether this material can support growth and osteogenesis of MSC from bone marrow (BM-MSC).

Materials and Methods: BM-MSC were isolated from BM mononuclear cell fraction of human healthy donors and cultivated in standard conditions. One pellet of PerOssal® was dissolved in 10 ml of Phosphate Buffered Saline and added to cells in different ratios. Viability of BM-MSC was assessed by MTT test [2] after one, five and seven days. Migration capacity of BM-MSC was followed by both scratch assay and transwell system [3], while osteogenic potential was investigated by histochemical staining after induced osteogenic differentiation [4].

RESULTS AND DISCUSSION: Initial experiments showed that BM-MSC adhere to PerOssal® surface. Considering that culture media induced decomposition of PerOssal® which aggravated functional evaluation of attached BM-MSC , our further in vitro studies were directed on testing the effects of different PerOssal® dilutions on BM-MSC functional properties. Our results demonstrated that at all dilutions tested PerOssal® didn't affect viability of BM-MSC in a short-term treatment, while in higher doses it decreased cell viability at day 7. At the same time point, morphology of BM-MSC was changed with high dose of PerOssal®. On the other side, at lower doses PerOssal® stimulated migration of BM-MSC as demonstrated by both scratch and transwell assays. As for the analyses of BM-MSC osteogenic differentiation, stimulatory effect on early osteogenesis was noticed for lower PerOssal® doses, opposite to decreased BM-MSC osteogenesis observed when higher doses were applied.

CONCLUSIONS: These data imply that osteoinductive effect of PerOssal® related to BM-MSC recruitment likely considers later phases of material resorption corresponding to its lower concentrations. For potential use as a biomaterial for cell therapy in tissue engineering, PerOssal® should be applied in lower doses.

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Electrochemical behavior of metallic implants in inflammatory conditions

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Keywords: Titanium; tantalum; corrosion behaviour; simulated inflammation; protective coatings

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INTRODUCTION: This extensive study explores the significant impact of inflammation on the electrochemical corrosion of metallic implants, which can compromise their integrity and function, often leading to foreign body reactions. To mitigate these issues, the research introduces the promising Ti-Nb-Zr-Si (TNZS) alloy, 3D patterned Ti Gr2 and Ti Gr23 layers, alginate coating on 3D patterned Ti biomaterials, and Mn_3O_4 coating on anodized Ta.

EXPERIMENTAL: The TNZS alloy was synthesized by introducing Nb, Zr, and Si elements into titanium, while commercially purchased Ti2 and Ti23 alloys were utilized. Patterned layers were prepared on the flat counterparts of Ti2 and Ti23 using the LPBF by respective commercial powders. Alginate coatings with and without octacalcium phosphate (OCP) were developed on the patterned Ti layers. Electrophoretic deposition (EPD) was employed to apply Mn_3O_4 nanoparticles onto anodized tantalum. Electrochemical tests were conducted in a three-electrode system. Specimens were exposed to phosphate-buffered saline solutions, mimicking normal conditions or inflammatory environments by introducing H_2O_2, HCl, bovine serum albumin, and calcium L-lactate hydrate. Various electrochemical techniques, including open circuit potential, potentiodynamic polarization, and electrochemical impedance spectroscopy, were employed to assess corrosion behavior and passive layer stability.

RESULTS AND DISCUSSION: In this study, the newly developed TNZS alloy demonstrated superior corrosion resistance compared to Ti 2 and Ti23 under normal, inflammatory, and severe inflammatory conditions. The high corrosion resistance of TNZS was attributed to the presence of silicide phases in its microstructure, known for their exceptional stability in acidic environments containing H_2O_2 [1]. Furthermore, the study demonstrated that patterned Ti layers exhibit enhanced corrosion resistance in comparison to untreated flat Ti with a similar composition, owing to modifications in surface topography and wettability [2]. The application of alginate hydrogels, both with and without octacalcium phosphate (OCP), onto patterned Ti groups demonstrated enhanced corrosion resistance, particularly in biological media, with notable improvement when OCP was included. Despite a decrease in corrosion resistance during inflammatory conditions, the hydrogel coatings effectively shifted the corrosion potential to nobler values, reducing corrosion susceptibility in all conditions. The inclusion of OCP particles additionally enhanced electrical charge transfer resistance at the substrate-coating interface [3]. The findings further confirmed that the anodic/EPD coating displayed a denser microstructure and superior bond strength compared to the anodic coating, providing effective protection for tantalum against corrosion in acidic inflammatory conditions. Additionally, the Mn_3O_4 coating safeguarded Ta from H_2O_2 oxidant-mediated damage by preserving catalytic activity and suppressing reactive oxygen species ROS generation in an acidic pH environment [4].

CONCLUSIONS: In conclusion, the study emphasizes the importance of understanding the electrochemical behavior and corrosion resistance of biomaterials in inflammatory conditions, showcasing promising potential for advanced orthopedic implant materials.

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Determination of metal ion levels in circulation in patients with joint replacement

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Keywords: Orthopaedics; implant; titanium; ion release; ICP-MS

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INTRODUCTION: In the past century, total joint arthroplasty has been recognized as one of the most successful and effective orthopaedic procedures. This approach, which treats mostly hip and knee joint disorders, may reduce pain, correct deformity, and improve the patient's quality of life. Materials of choice for orthopaedic implants include stainless steel, cobalt-chromium alloys, and titanium alloys. [1] When using a metal implant, some degree of material degradation is unavoidable, which can cause issues for the patient. Various factors leading to implant deterioration are expected to result in the release of nanoparticles or ions, which can cause loosening and osteolysis. The release of oxides and metal ions can be detectable within the first few days after the alloy implantation. The tissue surrounding the implants can collect dissolved metal ions, or the ions can be discharged into bloodstream and accumulate in distant organs. The present study is focused on monitoring metal ions released from implanted material into circulation.

EXPERIMENTAL: Patients indicated for total joint replacement who have signed an informed consent and agreed to enter the study were included in the project. Blood samples of 100 patients were drawn before implantation, 24 hours after implantation, and in 6 to 12 months' follow-up. Concentrations of Ti, V, Co, and Ni in samples of whole blood were determined by inductively coupled plasma mass spectrometry (Agilent 8900 ICP-MS/MS, Agilent technologies) after 10× dilution of samples by solution containing deionized water, a nonionic surfactant Triton X-100 (0,04 %), ammonia (1 %), butanol (2 %), EDTA (0,04 %), and the appropriate internal standards for ICP-MS/MS (20 ng/ml of Sc, Ge, In, Lu and Bi). The method was validated by analysis of samples spiked with known amount of analytes and by analysis of following certified reference materials (SERO AS, Norway): Seronorm™ Trace Elements Whole Blood Level 1 and Seronorm™ Trace Elements Whole Blood Level 2.

RESULTS AND DISCUSSION: Concentrations of vanadium ions were elevated immediately after implantation, irrespective of patient characteristics. In some cases, higher concentrations of cobalt and nickel were measured, especially in the case of implants with a CoCr/polyethylene articulating surface. An increase in Ti ion concentration was observed in blood drawn 6–12 months after surgery. All cases of significant increases involved uncemented implants of several specific types.

CONCLUSIONS: The results showed that the use of cement in implants affects the release of metal ions, especially titanium. The presence of metal ions in human body, as well as their influence on tissues and cells, raises questions regarding their safety and toxicity over time.

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Corrosion behavior of PEO coatings with Mn_3O_4 on Mg-Zn-Ca alloys in inflammatory conditions

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Keywords: Magnesium; biodegradable implants; composite coatings; Mn-based additives; electrochemical resistance; H_2O_2-containing solutions

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INTRODUCTION: The inflammatory response triggered by orthopedic devices results in the generation of reactive oxygen species (ROS) and a decrease in pH, accelerating the corrosion rate of Mg implants. To address corrosion challenges, various strategies are explored, including alloying with zinc and calcium elements and surface modifications. Plasma electrolytic oxidation (PEO) emerges as a promising technology, forming porous MgO coatings on Mg surfaces [1]. The electrolyte composition and the incorporation of additives not only affect coating characteristics but also influence the thickness and porosity of PEO coatings, collectively playing crucial roles in determining and preventing corrosion [2]. This study underscores the potential use of additives with ROS-scavenging properties, such as manganese-based additives in the PEO electrolyte, and the synthesis of MgO-Mn_3O_4 coatings on Mg-Zn-Ca alloy, as a means to mitigate corrosion rates, especially in inflammatory conditions.

EXPERIMENTAL: In this study, PEO coatings incorporating Mn_3O_4 were fabricated on Mg-Zn-Ca substrate using two distinct methods: the introduction of $KMnO_4$ salt and the inclusion of Mn_3O_4 nanoparticles in the electrolyte composition. In the first approach, composite coatings were chemically synthesized within the plasma microdischarge area, while the second route involved physical processes through electrophoretic adsorption. The electrochemical and immersion corrosion tests were conducted under simulated normal conditions using a PBS solution (pH 7) and under inflammatory conditions, achieved by introducing H_2O_2 and HCl (pH 5.2) into the PBS solution.

RESULTS AND DISCUSSION: The experimental results showed that the inclusion of $KMnO_4$ into electrolyte led to a reduction in voltages, while Mn_3O_4 resulted in an elevation in process voltages, directly impacting the structural characteristics of the coatings. Importantly, incorporating these additives decreases surface porosity and increases PEO coating thickness. Electrochemical and immersion corrosion tests, conducted under both simulated normal and inflammatory conditions, underscored the vulnerability of uncoated Mg-Zn-Ca alloy to corrosion, particularly in inflammatory settings (with corrosion rates increasing from approximately 2 to 16 $mm \cdot y^{-1}$). Notably, the composite PEO coatings, incorporating Mn3O4 nanoparticles, displayed superior corrosion performance. This superiority manifested as a significant decrease in corrosion current density and an increase in total impedance resistance compared to basic PEO coatings. For instance, potentiodynamic polarization results indicated a substantial reduction in corrosion current density, decreasing from 73.9 $\mu A \cdot cm^{-2}$ for basic PEO coatings to 5.5 $\mu A \cdot cm^{-2}$ for Mn_3O_4-incorporated PEO coatings. The enhanced performance was attributed to the catalytic activity of Mn3O4 in scavenging H_2O_2 in simulated inflammatory conditions, as well as the greater thickness and lower porosity of the composite coatings compared to basic PEO. Collectively, these features hindered the penetration of corrosive agents to the substrate. Moreover, the coatings showed a controlled release of Mn ions into the surrounding environment within a safe concentration range for the human body.

CONCLUSIONS: These findings suggest that PEO coatings incorporating Mn_3O_4 present promising protective solutions for Mg implants, showcasing improved corrosion behavior associated with inflammation.

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Two faces of biodegradable molybdenum nanoparticles regarding oxidative stress and biomedical applications

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Keywords: Molybdenum; nanoparticles; macrophages; cytotoxicity; ROS

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INTRODUCTION: Recently, biodegradable metal materials have drawn a significant scientific interest owing to their attractiveness for biomedical purposes. In fact, if it were not for the presence of non-degradable metals in a human body, the risk of inflammation, infection and implant malfunction would be reduced. Molybdenum (Mo) is one of the promising materials with high potential for use in load-bearing orthopaedic and cardiovascular implants [1]. However, upon contact with tissues and body fluids, a cascade of reactions leading to the formation of MoO_3 (nano)particles (MoO_3NPs) is triggered. As structures foreign to the body, they are immediately recognized by phagocytic immune cells, especially macrophages [2]. In this work, we focused on the effects of MoO_3NPs on primary human macrophages with regard to oxidative stress.

EXPERIMENTAL: MoO_3NPs were purchased from Sigma-Aldrich Co., USA. To get closer to the real conditions during the immune response to the implant, blood samples from patients undergoing endoprosthesis surgery were used to isolate macrophages. A comprehensive cytotoxicity study of MoO_3NPs was conducted herein, by utilizing the MTT assay, and the analysis of cell morphology and behaviour through confocal microscopy. Macrophage polarization state was analysed using flow cytometry, where CD86+ and CD206+ were used to distinguish M1 and M2 macrophages, respectively. Furthermore, the ability of MoO_3NPs to induce reactive oxygen species (ROS) in macrophages was tested by the nitro blue tetrazolium (NBT) assay and live cell imaging. In addition, the anti- or pro-oxidative properties of MoO_3NPs were evaluated using the DPPH test. All the experiments were performed at least in triplicates, and the results were evaluated using non-parametric statistical tests.

RESULTS AND DISCUSSION: We found that MoO_3NPs at low concentrations (below 0.1 mM) are well-tolerable by macrophages as they do not pose a threat to cell survival, and do not have an impact on morphology changes and natural ROS production. With an increasing concentration (to 0.3 mM), a more harmful effect of oxidative stress was observed. However, the concentration of 0.5 mM tends to trigger the intracellular ROS overproduction in macrophages, potentially leading to their death. Recently, the effect of a higher concentrations of Mo was described, with the greatest emphasis on cytotoxicity [3]. This study shows the crucial role of concentration-dependent effects of MoO_3NPs that alter macrophage polarization and ROS production.

CONCLUSION: Overall, molybdenum should be regarded as a potential material for biomedical applications, providing its cytotoxicity is mitigated.

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Assessing the biocompatibility of polyhydroxybutyrate scaffolds for dental stem cell applications

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Keywords: Polyhydroxybutyrate; dental stem cells; cell adhesion; cellular viability

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INTRODUCTION: Polyhydroxybutyrate (PHB), a promising biopolymer with significant potential in the dental field, is an immunologically compatible substance derived from natural and viable sources. Recognized as lipid acid polymers produced intracellularly by bacteria, PHB holds promise for various dental applications. If combined with dental stem cells (DPSCs), its unique properties could further enhance its suitability for innovative and biocompatible scaffold development.

MATERIALS AND METHODS: The production of PHB fibrillar membranes utilized the electrospinning method [1,2], employing an aluminum foil-covered copper plate as the collector. A solution of PHB at a concentration of 8% (w/v) was prepared by dissolving it in chloroform: 2-fluoroethanol (20:1 v/v) at 60°C using a heated magnetic stirrer. The resulting PHB solution was loaded into a 20 ml syringe connected to a syringe pump, set at a flow rate of 0.3 ml/hour. Electrospinning was performed on a copper plate with the power supply set to 10 kV. The isolation and characterization of dental pulp stem cells (DPSCs) were conducted following previously established protocols [3]. Cytotoxicity testing was carried out using the MTT assay, and cellular adhesion was assessed through immunocytochemical staining with DAPI.

RESULTS AND DISCUSSION: The MTT results demonstrated consistently high cell viability comparable to the control group. This indicates that the PHB membranes did not induce cytotoxic effects on DPSCs over the assessed time points (1, 3 and 7 days). The immunocytochemical images obtained after 1, 3, and 7 days of treatment exhibited robust cellular adhesion. This suggests that the PHB scaffolds provided a conducive environment for DPSC adhesion and growth.

This consistency in cellular response underscores the biocompatibility of the PHB scaffolds. The absence of adverse effects on cell viability and adhesion supports the potential of PHB as a suitable material for dental applications. The positive outcomes of this study suggest that PHB fibrillar membranes hold promise for use in dental applications, particularly in the context of DPSC interactions. Further investigations are warranted to explore the long-term effects, structural integrity, and specific molecular interactions between PHB and dental stem cells. These findings contribute valuable insights to the development of biocompatible materials for dental tissue engineering and regenerative medicine.

CONCLUSIONS: In summary, our study demonstrates the sustained viability, lack of cytotoxic effects, and robust cellular adhesion of dental pulp stem cells on PHB fibrillar membranes, affirming the promising biocompatibility of PHB for dental applications.

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Precision medicine for musculoskeletal regeneration, prosthetics and active ageing - PREMUROSA: a Marie Skłodowska-Curie Innovative Training Network

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Keywords: Precision medicine; tissue regeneration, tissue engineering, bone, cartilage, musculoskeletal tissue, new alternative methodology, biomaterials

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INTRODUCTION: Musculoskeletal diseases are a major burden on individuals, healthcare and welfare systems. Treatment of musculoskeletal disorders is currently based either on prosthetic or regenerative surgical procedures, often involving medical device implantation. In both cases, individual tissue healing and regeneration, together with the appropriateness of the implanted device, markedly affect the outcome. A great improvement could be achieved by precision medicine, specifically designed on patient's individual characteristics. This implies combining the personalized clinical approach with individual 'omic' characterization and proper choice of medical device. The concept is "To take care with care". This is the meaning of the Italian word "premurosa" and the ultimate goal of the Innovative Training Network PREMUROSA project, aimed to train a new generation of scientists with an integrated vision of the whole value chain in musculoskeletal regeneration technologies and able to boost the necessary innovations to achieve precision principles in developing innovative devices and optimized clinical applications.

MATERIALS and METHODS This aim have been achieved by a "triple i" (interdisciplinary, intersectoral, international) approach of thirteen Early Scientific Researchers (ESRs), who have had benefit from an excellent scientific environment, up-date technologies, and supervision by international leaders in the field.

RESULTS AND DISCUSSION: ESRs have investigated the links between physico-chemical properties of metallic (titanium and its alloys and ceramic (bioactive glasses and glass-ceramics) materials, including chemistry (chemical composition, surface functionalization), surface morphology (topography, roughness) and hierarchical porosity (at the macro-, meso-nanoscale), and cells (pro- and eukaryotic) and tissues functionality and they studied the impact of surface chemistry, charge and topography on model protein adsorptions and conformation, stem cells fate and extracellular vesicles release and composition. They clarify the role of the extracellular matrix composition as well as the role of the vascular, nervous, and immune system on musculoskeletal tissue regeneration and they developed ad hoc technologies to test safety and efficacy of biomaterials including bioreactors, cellular and computational methods. In addition, they learnt to integrate academic and industrial aspects and they sharpen their experimental and complementary skills in a well-designed and diversified and unprecedented training program.

More than 30 open access papers have been published on high impacted journal and posted on Zenodo.org, and more than 70 conference communications have been released.

CONCLUSIONS: PREMUROSA has contributed to develop personalized tools for the rational and appropriate application of the musculoskeletal regeneration technologies and to clarify the interplay between tissues, cells, and materials in view of regeneration technologies optimization.

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End-to-end multidisciplinary optimal design for improved personalized bioactive glass/ceramic bone substitute implants- ReBone: a Marie Skłodowska-Curie Doctoral Network

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Keywords: Bone scaffolds; glass-ceramic materials; 3D printing; optimal design; mechano-biology; mixed reality models

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INTRODUCTION: Common clinical problems frequently place a significant stress on the clinical system, and the musculoskeletal system is particularly susceptible to aging and traumatic occurrences. New solutions are required to address significant unmet needs for patients who require bone-substitute implants to treat critical-size bone defects, including personalized solutions for better clinical outcomes, material advancements to ensure higher mechanical reliability without sacrificing bioactive and bioresorbable properties, and optimized manufacturing techniques for materials and products of high reliability and quality. The four-year ReBone Doctoral Network, funded by the Europe Horizon Marie Sklodowska programme, aims to train a new generation of researchers in an innovative and multidisciplinary optimization process to provide technologies for customized bone-substitute implants based on bioactive ceramics and cutting-edge additive manufacturing techniques, to address the health and societal burdens of trauma and bone diseases.

MATERIALS and METHODS: A multidisciplinary network and training program have been planned in which ten doctoral candidates will jointly develop an innovative and integrated methodology to the design of personalized ceramic-based bone substitute implants. In order to achieve the purpose, a European network of partners and associated partners has been established encompassing diverse disciplines including biomechanics, clinics, materials engineering, mechano-biology, additive manufacturing technologies and mixed reality models for surgical planning simulations. Materials play a significant role in this project in terms of mechanical properties, bioactivity, biocompatibility, printing technology, and pertinent fidelity. Biologists, material engineers, bioengineers, and technology developers will collaborate to design and thoroughly characterize glass-ceramic based materials for the intended use. Four clinical cases of patients in the need of bone repair will be purposely selected with the aim to develop real-case scenarios of personalized design of bone-substitute implants. Clinical data will be used to create personalized multi-scale models of the implant at the organ level; concurrently, the design of device architecture, materials and parameters for manufacturing technology will be optimized to achieve improved implant outcome in terms of optimal mechanical performance in relationship to the shape and the anatomical location of the implant.

RESULTS AND DISCUSSION: As a primary result, a consortium of nine European countries has been constituted and an up-to-date multidisciplinary training program has been set. Furthermore, ten interdisciplinary doctoral research projects have been drawn; ten Doctoral Candidates will undertake the above-mentioned research program in a multidisciplinary environment.

Preliminary results achieved by the research institutes involved in the project in the area of ceramic materials development and characterization, additive manufacturing and biomechanics and a clinical research institute will constitute the solid background of the whole activity.

CONCLUSIONS: The Europe Horizon Marie Sklodowska program funds the ReBone Doctoral Network, which addresses issues with bone-substitute implants for critical-size bone lesions. A variety of research fields will be addressed by the multi-disccipliinary approach. Among these, technological and material development advancements are crucial and will serve as the foundation for the design of customized solutions.

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Novel hybrid biomimetic macroporous composites with tuned biodegradability, improved osteointegration and anticancer properties for bone tissue regeneration (HyBioComBone)

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Keywords: Large bone defects; interpenetrating network; hydrogels, bioglasses; calcium phosphates; extracellular vesicles

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INTRODUCTION: The treatment of large bone defects (LBDs) represents a major challenge in clinical orthopedics. Patients with LBDs caused by trauma, infections, or tissue resections due to cancer, often undergo multiple surgeries with long recovery times leading to deteriorated life quality and increased healthcare costs. The project HyBioComBone aims to develop novel biomimetic macroporous composites with multifunctional properties for bone repair, regeneration, and cancer treatment.

EXPERIMENTAL: The composites will be designed by combining the interpenetrating network (IPN) hydrogels, inorganic nanostructured bioglasses (BAG), and multi-ion doped calcium phosphates (CaP). Optimized composites will be further developed in three directions: a) as carriers for the extracellular vesicles (EVs) derived from adipose-derived stem cells (ADSCs); b) for loading and controlled delivery of commercial and newly synthesized antiproliferative drugs, and c) for loading and controlled delivery of antibiotics.

RESULTS AND DISCUSSION: Biocompatible, bioresorbable, macroporous IPN hydrogels based on synthetic and natural polymers will be obtained. The network parameters of hydrogels will be tuned to obtain a broad range of materials with different biodegradability, mechanical and viscoelastic properties. Scaffolds, microgels, and 3D-printed hydrogels will be developed. Multi-ion doped bioactive inorganic particles (mesoporous BAG nanoparticles, rod-like HAp nanoparticles with morphology similar to the biological apatite, and highly bioactive calcinated CaP spherical microparticles) will be combined with the polymer matrix to improve bioactivity, provide therapeutic ions release and tune mechanical properties. The composites of desired properties will be loaded with commercially available antibiotics, to fight infection, and chemotherapy drug, for treatment of different types of cancer, but also with newly synthesized anticancer drugs. The encapsulation of ADSC-derived EVs in the composites with controlled biodegradation would enable the gradual release of EVs to the surrounding tissue and provide improved osteointegration and bone tissue regeneration.

CONCLUSIONS: New macroporous bioactive composites with adequate mechanical properties, biocompatibility, and tuned biodegradability for hard tissue restoration, coupled with either drugs for the prevention of osteosarcoma recurrence and/or osteomyelitis, or with adipose-derived EVs for the regeneration of large bone defects, will be developed through the project HyBioComBone.

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Antimicrobial coatings for orthopaedic applications

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Keywords: Antimicrobial peptides; titanium implants; surface functionalisation; plasma treatments

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A significant advance in modern medicine is the introduction of artificial medical devices, including dental and orthopaedic implants and prostheses. This has led to significant improvements in patient well-being and functionality. However, these 'foreign' devices can cause significant complications, of which bacterial infections remain the most common problem. AMR is the broader term for the loss of sensitivity in different microorganisms or viruses towards antibacterial, antiviral, antiparasitic and antifungal substances. It occurs when microorganisms such as bacteria, viruses, fungi, and parasites change in ways that render the treatment schemes used to cure the infections they cause ineffective [1]. The increase in infections by multi-antibiotic-resistant bacteria has become of great concern, especially in hospitals, after orthopaedic surgical procedures. The extra costs due to antibiotic resistance have been estimated at USD 1000 per infected patient [2]. The risk of reinfection during revision surgery is far greater than that of primary infection, which is estimated to fall around 2 %, depending on the type of the prosthetic implant, patient condition, clinical setting, and surgical procedure [2]. The overall infection rates associated with such surgery are approximately 5% for fracture-fixation devices, 2 % for primary joint replacements and 14 % for total hip and knee revisions [3]. Because of the risks (including fatalities) and costs associated with antibiotic-resistant bacterial infections, it is imperative to develop antimicrobial materials to be incorporated into implants that will not result in new types of bacterial resistance. Antimicrobial peptides (AMPs) are important components of the innate immune system of multicellular organisms and act as the first line of defense against exogenous pathogens, resulting in their death. They have shown broad-spectrum activity against a wide variety of pathogens (bacteria, fungi, protozoa, and viruses) with high potency, ability to modulate host immunity, low propensity to induce resistance and low toxicity to host cells. These properties make them emerging agents for the treatment of infections. Disadvantages in the production, properties, and efficacy of AMPs together with high manufacturing costs have contributed to slow the transfer from research to clinical practice and development of commercial products [4].

Acknowledgements: In this presentation, we will report recent advances on the use of antimicrobial peptides [5] developed in the MSCA Horizon 2020 ITN AIMed (grant agreement 861138) for the formation of antimicrobial coatings for titanium implants. We will report how we can achieve a stable immobilization of these antimicrobial peptides, and their antimicrobial effectiveness *in vitro* against orthopaedic pathogens. The work presented here is a contribution from the efforts of two research groups at the University of Birmingham and the University of Trieste and shows how an interdisciplinary team can advance research within the framework of a training network such as AIMed.

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Discouraging cellular and bacterial adhesion on surfaces for bone temporary devices through ZrO_2-Ag coatings

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Keywords: Silver; bone; temporary device; cytocompatibility; transcriptomics; proteomics

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INTRODUCTION: ideally bone temporary fixation devices must support the healing process but at the same time being surgically removed without hindering the newly formed tissue [1]. Moreover, they should prevent bacterial infection preserving the healing site. Accordingly, here we propose a surface coating of biomedical Ti6Al4V surface with inorganic zirconia matrix embedding silver nanoclusters (ZrO_2-Ag) where the Ag amount has been designed in order to prevent cells and bacteria adhesion avoiding cytotoxic side effect.

EXPERIMENTAL: ZrO_2-Ag coated specimens were produced introducing high (AH) or low (AL) amount of silver. Specimens were characterized for physical-chemical properties and then the biological assessment was performed by directly seeding human mesenchymal stem cells (hMSC) and the pathogen *Staphylococcus aureus* (*S. aureus*) to test the different surfaces in term of preventing adhesion. Finally, transcriptomics (RNAseq) and proteomics were applied to unravel the hMSC pathways regulated by the AH/AL-tuning.

RESULTS AND DISCUSSION: AL and AH specimens reported a different release of silver over time (Fig. 1a). The biological characterization suggested AH being toxic for both hMSC and *S. aureus* that were unable to colonize the specimens due to a contact-killing effect. On the opposite, AL specimens reported very promising results: cells and bacteria were unable to colonize AL surfaces too, but they were viable thus suggesting for a non-toxic anti-adhesion effect. In fact, both transcriptomics (Fig. 1b) and proteomics (Fig. 1c) studies of non-adherent hMSC revealed a strong down-regulation of genes and proteins related with pro-adhesion processes, whereas pro-apoptotic pathways were not up-regulated.

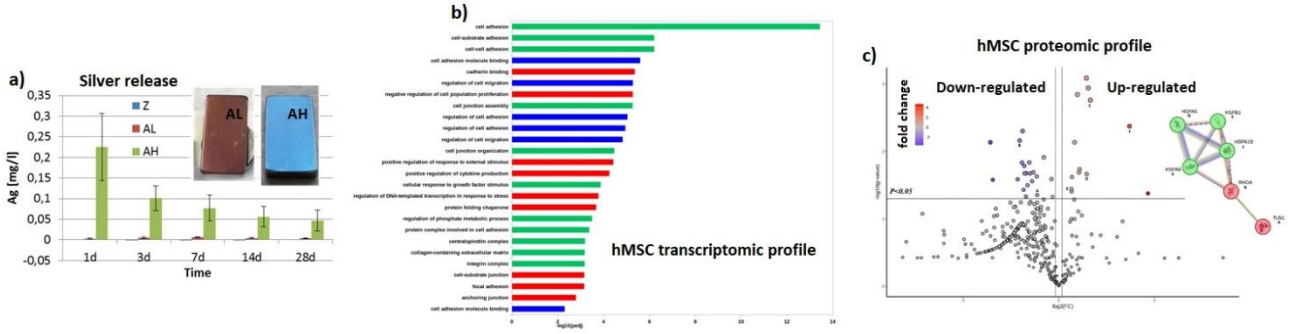

Figure 1. *a) silver release profile in function of time of AL and HL ZrO_2-Ag specimens; b) transcriptomics (RNAseq) studies of non-adhered hMSC cells onto AL specimens and c) their proteomics profile.*

CONCLUSIONS: the AL ZrO_2-Ag specimens reported a very promising solution for temporary fixation devices being able to prevent the surface colonization of both bacteria and cells by down-regulating the gene and proteins expression of several hMCS pro-adhesion pathways without cytotoxic effect.

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Nanostructured Ag- and Cu- doped ZnO antibacterial magnetron sputtered coatings for biomedical applications

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Keywords: Composite coatings, magnetron sputtering, silver, copper, zinc oxide, calcium phosphates

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INTRODUCTION: The formation of bacterial biofilms on the surfaces of medical implants and non-critical surfaces represents an increasing problem in medical practice. [1] Antibacterial modification of surfaces is considered a promising approach in preventing biofilm formation. Among different possible coatings, nanostructured magnetron sputtered metal oxide thin films are gaining attention as the physico-chemical properties can be controlled by simply changing the experimental conditions which consequently affects and modulates biological responses. In this study, for the first time, we compared the properties of magnetron sputtered ZnO thin films doped with Ag or Cu. In addition, the possibility of increasing the biocompatibility of the prepared coatings by biomimetic deposition of calcium phosphates on their surface was tested.

EXPERIMENTAL: Thin Ag- and Cu- doped ZnO films of different compositions were prepared by simultaneous magnetron deposition in a multi-source sputtering system. To increase their bioactivity, calcium phosphates were biomimetically deposited on their surface. The structure and composition of the obtained coatings were determined by grazing incidence small-angle X-ray scattering (GISAXS) and X-ray diffraction (XRD), while the morphology was observed by atomic force microscopy (AFM), helium-ion microscopy (HIM) and scanning electron microscopy (SEM). The content and distribution of elements was determined by energy dispersive spectroscopy (EDS), while ion release was determined by inductively coupled plasma mass spectrometry (ICP-MS). The viability of the human osteoblastic cell line MG-63 on thin films was determined by MTT cell test. The formation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms was determined as well.

RESULTS AND DISCUSSION: The results show formation of nanoparticles in all films, having composition-dependant size and shape properties. The obtained results indicated that increasing Ag or Cu amount in the thin films caused opposite effects on structure of the nanoparticles forming thin films, grain size and water contact angle. EDS confirmed that the grains are mixture of Ag and ZnO or Cu and ZnO, and XRD analysis suggested incorporation of Ag or Cu into ZnO structure. Calcium phosphates were successfully deposited onto the surfaces, resulting in slightly less elemental and released amount of Ag, Cu, and Zn. However, this deposition resulted in better biocompatibility, especially for Ag doped ZnO thin film, and better control of biofilms formation.

CONCLUSIONS: These results confirm that magnetron sputtering holds promising potential not only for coating materials for biomedical applications but also for a wide range of other applications because of its versatility and effectiveness in modifying surface properties.

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Polyelectrolyte multilayers with metal/metal oxide nanoparticles as antimicrobial solution for biomedical applications

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Keywords: Polyelectrolyte multilayers, silver nanoparticles, copper oxide nanoparticles, physico-chemical properties, cytotoxicity, antibacterial activity

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INTRODUCTION: The growing concern over implant-associated infections motivates the investigation of antibacterial coatings that can locally prevent microbial adhesion and proliferation [1]. Among different coatings, polyelectrolyte multilayer (PEM) films are attracting particular attention because of their ability to coat substrates of various size, composition, and topology, along with their promising biocompatibility [2]. Their antimicrobial properties can be enhanced by incorporating antimicrobial agents with low potential to cause antimicrobial resistance, such as metal/metal oxide nanoparticles [3]. In this study, the build-up of poly-L-lysine (PLL) and poly-L-glutamic acid (PGA) multilayers with embedded silver (AgNPs) or copper oxide nanoparticles (CuONPs) was investigated and the physico-chemical and biological properties of the two types of coatings were compared.

EXPERIMENTAL: The build-up of PEM with embedded NPs was analysed with quartz-crystal microbalance with dissipation monitoring (QCM-D) and atomic force microscopy (AFM). The elemental content and distribution of Ag and Cu within the PEM was determined by energy dispersive spectroscopy (EDS), while their total amount and release was determined by inductively coupled plasma mass spectrometry (ICP-MS). PEMs were tested for biocompatibility using the human osteoblastic cell line MG-63 by MTT test. The formation of *S. aureus* and *P. aeruginosa* biofilms was also tested on these samples.

RESULTS AND DISCUSSION: The NPs were embedded within PLL/PGA multilayer as an anionic component in the 3rd and 8th bilayer. QCM-D measurements indicated that the NP coverage was as a submonolayer with a rather low amount of adsorbed nanomaterial. SEM and EDS analysis confirmed this revealing individual or aggregated NPs distributed over the coated surface. The viability of MG-63 cells on all investigated PEM was greater than 70 %, which is the threshold value for non-cytotoxic materials. In addition, the absence of cytotoxic effect was confirmed by SEM images of the cells, which showed that the cells had a classical elongated shape with cytoplasmic extensions. In contrast, PEM containing AgNPs or CuONPs reduced the formation of biofilms of *S. aureus* and *P. aeruginosa*, with CuONPs embedded PEMs being more efficient.

CONCLUSIONS: The results obtained confirm the high potential of PEMs with metal/metal oxide NPs as antibacterial coatings for medical devices, but also for other applications.

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Zeta potential titration and Kelvin probe force microscopy as tools for the design of biomaterials

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Keywords: Surface charge; functionalization; coatings; peptoids; tocopheryl phosphat

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INTRODUCTION: The biomaterials' surface is the place where biomaterials, physiological fluids, proteins, and cells meet determining the host response. Surface features such as chemical composition, exposed functional groups, zeta and electric potential, wettability, topography, and roughness strongly affect this response. Surface functionalization and coating with biomolecules are strategic tools to modulate surface properties and control the biomaterial outcome. The design of a functionalization or coating process goes through the following main steps: selection of the biomolecule and process parameters, identification of the effective presence of the biomolecule on the surface and type of bond, and evaluation of the biological response. The role of advanced characterization techniques in these steps is here discussed.

EXPERIMENTAL: Ti6Al4V discs (10 mm in diameter-2 mm thick) were polished (MP), washed, chemically treated (CT), and irradiated with UV light. Alpha-tocopheryl phosphate (α-TP) was purchased (Sigma-Aldrich) while the GN2-Npm9 peptoid was synthesized at Roskilde University. For functionalization, a 100-µl drop of a solution of PBS and 1 mg/mL GN2.Npm9 was dropped onto the CT samples. The samples were left at 37 °C for 2 h, rinsed, and dried under a hood (CT_GN2-Npm9). Samples for coating were pre-soaked in a solution of $CaCl_2$, dried, soaked in a solution of α-TP in TRIS-HCL (5 mg/mL) for 3 h at 37 °C, dried, rinsed 3 times in ultrapure water, and dried again. An atomic force microscope (KPFM-Innova atomic force microscope, Bruker) equipped with a conductive tip (Sb-doped Si, frequency 75 kHz, SCM-PIT-V2, Bruker) was used. Surface zeta potential as a function of pH was measured by electrokinetic measurements (SurPASS, Anton Paar) equipped with an adjustable gap cell in an electrolyte solution of 0.001 M KCl.

RESULTS AND DISCUSSION: Two examples will be described: coating with tocopheryl phosphate [1] and functionalization with a peptoid for antibacterial purposes [2]. The role of Ca^{2+} in the chemisorption of the biomolecules and their surface distribution was evidenced by KPFM. The orientation and exposed functional groups of the biomolecules in solution and after adsorption on the CT surface were deduced from zeta potential titration curves. Biological tests evidenced different biological responses of the coated/functionalized surfaces according to different process parameters in terms of cytocompatibility (human mesenchymal stem cells) and antibacterial action (Staphylococcus Epidermidis).

CONCLUSIONS: Zeta potential titration curves as the function of pH and KPFM give significant information on the surface features of the substrate and functionalization solution, allowing the selection of process parameters and speculations on the mechanism of physical or chemical adsorption. Moreover, they allow detection and imaging of the adsorbed biomolecule on the functionalized surface. Peptoids and tocopheryl phosphate have a good potential as antibiofilm agents.

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Electrochemically synthesized biomaterials

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Keywords: Drug release; diffusion; cytotoxicity; antibacterial activity

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INTRODUCTION: Electrochemical methods were employed for synthesizing composites intended for applications in medicine as antibacterial coatings on titanium bone implants or as highly efficient antibacterial hydrogels for accelerated wound healing.

EXPERIMENTAL: Hydroxyapatite-based coatings were single-step electrophoretically deposited from four-component aqueous suspension containing 1 wt% hydroxyapatite powder (HAP, particles < 200 nm particle size, Sigma-Aldrich), 0.1 wt% poly(vinyl alcohol) (PVA, medium molecular weight, Sigma-Aldrich), 0.05 wt% chitosan powder (CS, medium molecular weight, Sigma-Aldrich), and aqueous gentamicin sulfate solution (Gent, concentration 50 mg/ml, Sigma-Aldrich) on titanium plates (Sigma-Aldrich). Antibacterial activity of HAP/PVA/CS/Gent coating was evaluated against *Staphylococcus aureus* TL (culture collection-FTM, University of Belgrade, Serbia) and *Escherichia coli* ATCC 25922. For poly(vinyl alcohol)/chitosan (PVA/CHI) hydrogel synthesis poly(vinyl alcohol) powder (PVA, Sigma Aldrich, USA), chitosan powder (CHI, Sigma Aldrich, USA), graphene (Graphene Supermarket, USA), glacial acetic acid (Beta Hem), silver nitrate (MP Hemija), potassium nitrate (Centrohem, Serbia) were used. For antibacterial properties evaluation, monobasic and dibasic (Sigma Aldrich, USA) potassium phosphates were used. Cell culture suspensions for cytotoxicity tests were prepared using MTT tetrazolium salt, EDTA, fetal calf serum and antibiotic-antimycotic solution (Sigma Aldrich, USA).

RESULTS AND DISCUSSION: Surface modification of titanium with innovative bioactive coatings enhances biomineralization and reduces post-operation pain and infection risk. Using powerful single-step electrophoretic deposition (EPD) at a constant voltage we have produced various hydroxyapatite-based composite bioceramic coatings in combination with polymers (lignin, chitosan), graphene, and antibacterial agents (silver, gentamicin).

Electrochemical methods enable *in situ* synthesis of AgNPs inside polymer matrices, with the main advantage being the complete absence of any chemical reducing agents that are often toxic and difficult to remove from the material. The electrochemical reduction of Ag^+ ions is achieved only using electrical current or pure hydrogen gas that is generated at the cathode in aqueous electrolytes, which allows for obtaining completely green and non-toxic product. Modifications in the synthesis process also enable the control of AgNPs properties, such as size and concentration.

CONCLUSIONS: Composite antibacterial coatings were obtained on titanium plates using EPD. Strong antibacterial activity against *E. coli and S. aureus* was found. Biocompatibility was confirmed using *in vitro* MTT testing since a non-cytotoxic effect was shown towards healthy human peripheral blood mononuclear cells PBMC, fibroblast cell lines MRC-5 and L929, suggesting high potential for bone tissue engineering and medical applications. Hydrogels composed of physically cross-linked poly(vinyl alcohol)/chitosan with embedded AgNPs were successfully developed for applications as anti-bacterial wound dressings due to their highly favorable properties of silver release, antibacterial activity, and non-toxicity.

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Innovative hydroxyapatite-based coatings for bone implants: A multifaceted approach

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Keywords: Composite coatings; hydroxyapatite; gentamicin; titanium

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INTRODUCTION: Tissue engineering strives for innovative solutions in addressing challenges associated with contemporary bone tissue implants. This study focuses on the electrophoretic deposition (EPD) of hydroxyapatite-based bioceramic composites containing antibacterial agents onto titanium surfaces. Two composite coatings, hydroxyapatite/chitosan (HAP/CS) and hydroxyapatite/chitosan/gentamicin (HAP/CS/Gent) were developed to combat issues such as poor adhesion, limited antibacterial potential, limited bioactivity, and potential toxicity of implant materials [1].

EXPERIMENTAL: EPD was performed at constant voltage (5 V, 12 min) on pure Ti plates from aqueous (HAP/CS and HAP/CS/Gent) suspensions. The uniformity and functionality of the deposited coatings were assessed through comprehensive physico-chemical characterization using X-ray diffraction (XRD) (*Philips PW 1710, Netherlands*) and scanning electron microscopy (SEM, Hitachi S-4700, J) equipped with energy dispersive X-Ray spectroscopy (EDS, X-Max, Oxford Instruments, UK). Antibacterial activity was evaluated against *Staphylococcus aureus* TL and *Escherichia coli* ATCC 25922 by quantitatively monitoring changes in the viable number of bacterial cells in suspension. Cytotoxicity against MRC-5 and L929 cell lines was investigated using trypan blue dye-exclusion test (DET) and MTT assay for assessing cell metabolic activity. Statistical significance was determined for antibacterial and cytotoxicity results by one-way analysis of variance (ANOVA), followed by multiple comparisons post-hoc test.

RESULTS AND DISCUSSION: XRD revealed broadened diffraction maximums corresponding to fine HAP crystallites. Porous surface with homogeneously distributed spherical HAP agglomerates embedded in wax-like polymers' matrix of CS was observed for both coatings by SEM. The addition of gentamicin significantly enhanced the antibacterial activity of the HAP/CS/Gent coating – complete reduction of *S. aureus* bacterial cells was achieved within 1 h of exposure. MTT and DET tests indicated low cytotoxicity against MRC-5 and L929 tested cell line for both samples. Slightly decreased cell percentage viability due to gentamicin presence was observed for HAP/CS/Gent.

CONCLUSIONS: Single-step EPD yielded antibacterial composite coatings with potential for biomedical applications. HAP/CS/Gent showed successful gentamicin loading, favorable crystalline structure, and strong antibacterial effects. The presence of fine HAP crystallites yielded a larger surface area, favorable for new bone growth and improved osseointegration. HAP/CS/Gent exhibited good antibacterial activity against both tested bacteria (especially pronounced against *S. aureus* – bactericidal effect), while preserving low cytotoxicity, indicating the high potential for biomedical applications.

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Bioactivity of gentamicin-loaded hydroxyapatite/poly(vinyl alcohol)/chitosan composite coatings aimed for orthopedic application

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Keywords: Antibacterial activity; electrophoretic deposition; alkaline phosphatase

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INTRODUCTION: Following orthopedic surgery, bacterial infection may lead to significant complications related to inflammation in the peri-implant region, including the potential for implant loosening. To address this concern, the surface of metallic implants was modified through the application of bioactive and antibacterial coatings, aiming to mitigate these issues.

EXPERIMENTAL: HAP/PVA/CS/Gent coatings was single-step electrophoretically deposited from four-component aqueous suspension containing 1 wt.% hydroxyapatite powder (HAP, particles < 200 nm particle size, Sigma-Aldrich), 0.1 wt.% poly(vinyl alcohol) (PVA, medium molecular weight 89 to 98 kDa, 99 % hydrolysed, Sigma-Aldrich), 0.05 wt% chitosan powder (CS, medium molecular weight, 190 to 310kDa with 75 to 85 % deacetylation degree, Sigma-Aldrich), and aqueous gentamicin sulfate solution (Gent, concentration 50 mg/ml, Sigma-Aldrich) on titanium plates (Sigma-Aldrich). Antibacterial activity of HAP/PVA/CS/Gent coating was evaluated against *Staphylococcus aureus* TL (culture collection-FTM, University of Belgrade, Serbia) and *Escherichia coli* ATCC 25922, while kinetics of antibacterial activity was monitored according to our previously published data [1]. In vitro cytotoxicity assay was performed towards two fibroblast cell lines of different origin-mouse origin cell line (L929 (ATCC CRL-6364)) and human lung origin cell line (MRC-5 (ATCC CCL-171)) [2]. To evaluate the statistical significance of the biological assay results (antibacterial activity, cytotoxicity and ALP activity), one-way analysis of variance (ANOVA) followed by a multiple comparisons posthoc test was used.

RESULTS AND DISCUSSION: HAP/PVA/CS/Gent coating exhibited strong antibacterial effect against both *S. aureus* and *E. coli*, especially pronounced against *S. aureus*, causing bactericidal effect. Cytotoxic effect of HAP/PVA/CS/Gent coating was not pronounced in investigated MRC-5 and L929 cell lines. MRC-5 fibroblast cells in contact with HAP/PVA/CS/Gent doubled alkaline phosphatase levels compared to their contact with the control samples (HAP/PVA/CS), indicating good osteogenic properties.

CONCLUSIONS: Electrophoretically deposited HAP/PVA/CS/Gent bioceramic coatings on titanium, demonstrated significant potential as implants in orthopedic practice, functioning as drug carriers. Not only do they possess antibacterial properties, but they also exhibit no adverse effects on living tissue.

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Towards laser based methods for improving surface properties of materials

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Traditional chemical modification techniques used to alter the surface properties of diverse biomaterials possess drawbacks, such as leaving additional chemical toxicity from the solvents used and altering the mechanical stability. As an alternative approach for surface treatment, ultra-short pulsed laser processing (Fig. 1) is a non-contact method that enables a unique route to manipulate diverse biomaterial surfaces without severe thermal damage leading to heat-affected zones. Application of ultra-short laser radiation induces precise surface modification of scaffolds and allows the creation of multifunctional geometries with the potential to affect the biomimetic and antimicrobial properties of the constructs.

By finely tuning the laser processing parameters (scanning velocity (V), laser fluence (F), and a number of applied laser pulses (N), it is possible to influence the surface roughness, thus altering the wettability of the materials without disrupting their chemical composition. The conducted research was performed on a number of materials and has demonstrated that surface topography has a great influence on the biomimetic and antimicrobial behavior [1,2] of materials used in biomedicine and in everyday life.

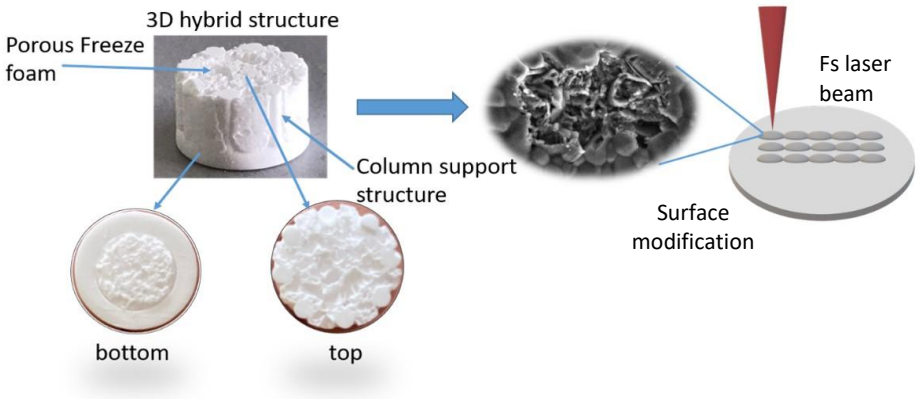

Fig. 1. Ultra-short laser surface modification of ceramics.

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A novel thermostable YtnP lactonase inhibits biofilm formation and induces decomposition of preformed *Pseudomonas aeruginosa* biofilms

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Keywords: Antivirulence; *Stenotrophomonas maltophilia*; quorum quenching; coating; implants; medical devices

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INTRODUCTION: Biofilm-associated infections are the main cause of biomaterial implant failure today. The increasing prevalence of antibiotic-resistant pathogens often results in the only solution of implant movement, with serious consequences for patients. Recently, various antimicrobial agents have been recognized as a promising strategy to prevent biofilm formation on implant surfaces [1]. Quorum sensing (QS) plays a central role in the control of bacterial virulence and biofilm formation. The use of quorum quenching (QQ) enzymes to target QS is therefore a promising innovative approach for the development of enzyme-based antivirulence therapeutics, which represent a potential solution to combat infections caused by multidrug-resistant pathogens. This study aimed to characterize the novel YtnP lactonase from the clinical isolate *Stenotrophomonas maltophilia* 6960 and to investigate its potential to combat the virulence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* MMA83.

EXPERIMENTAL: The biochemical analysis (pH and thermostability) of the purified recombinant YtnP lactonase (final concentration 50 µg/ml) was conducted with well-diffusion assay using *Chromobacterium subtsugae* CV026 as a biosensor strain with 10 µM *N*-octanoyl-L-Homoserine lactone (C8-HSL quorum sensing signaling molecule) as a substrate [2]. The pH stability of recombinant YtnP lactonase was analyzed in a pH range of 4 to 9 (pH interval of 0.5) using appropriate buffers. Recombinant YtnP lactonase was preincubated for 1h at temperatures ranging from 30 to 100 °C with an interval of 10 °C. Multiple alignments of amino acid sequences of YtnP lactonase with other close clustered functionally characterized lactonases were performed using ClustalW software (https://www.ebi.ac.uk/Tools/msa/clustalo/) and Espript 30 (http://espript.ibcp.fr). The effect of recombinant YtnP lactonase on *P. aeruginosa* MMA83 biofilm formation and decomposition was monitored using fluorescence microscopy [2].

RESULTS AND DISCUSSION: The recombinant YtnP lactonase retained its almost complete activity after exposure to temperatures ranging from 30 to 100 °C. The thermostability of YtnP lactonase can be explained by the absence of the N-terminal 63 amino acids found in both YtnP and thermostable lactonase from *Bacillus licheniformis*, and by the possible role of the N-terminal segment in disrupting the spherical organization of the proteins, possibly affecting their thermostability. YtnP lactonase exhibits broad pH stability, with optimal enzyme activity observed between pH 6 and 8 at 30°C, peaking at pH 7. In addition, significant enzyme activity was also maintained at lower pH values. Fluorescence microscopy shows the strong effect of YtnP lactonase in preventing the formation of *P. aeruginosa* MMA83 biofilms and initiating the decomposition of preformed biofilms [2].

CONCLUSIONS: Overall, advantageous biochemical properties, such as high temperature and pH stability makes YtnP lactonase a potential anti-biofilm agent that could be used for the coating of medical implants and for the design of innovative therapeutics to combat bacterial infections caused by MDR *P. aeruginosa***.**

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Novel alginate/activated-charcoal platform for local treatment of resistant pathogens in wounds

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Keywords: Antibiotic resistance, zinc ions, povidone iodine, antimicrobial activity, sustained release

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INTRODUCTION: Antibiotic resistance is one of the biggest threats to global health, food security, and development today [1] and new strategies to address this clinical problem are urgently needed. The aim of this work was to produce novel composites based on either Ca- or Zn-alginate *hydrogels* and activated charcoal (AC) particles that would, upon contact with physiological fluids, continuously release at least one bioactive agent directly into the wound area. In addition, AC particles served as carriers of other active substances such as povidone iodine (PVP-I), a very powerful antiseptic, which was used as a model substance.

EXPERIMENTAL: The composite Ca- and Zn alginate beads with incorporated AC particles impregnated with PVP-I were prepared as described previously [2,3]. The obtained beads were comprehensively investigated *in vitro* regarding its antimicrobial activity against wide range of wild resistant pathogens (MRSA, ESBL-*E. coli*, *P. aeruginosa*, *A. baumannii*, *P. mirabilis*, *E. faecalis*, *C. albicans*), all isolated from patients' wounds. Also, the beads were characterized regarding its textural parameters (ASAP 2020, Micromeritics, USA), morphology and iodine presence (MIRA 3 XMU Field Emission Scanning Electron Microscope, Tescan USA Inc., Cranberry Twp, PA). AC release profiles as well as the level of iodine adsorption and desorption from AC particles were determined by UV–visible spectrophotometer (UV-3100, Mapada Instruments, Shanghai, China) while flame atomic absorption spectrometer (Perkin Elmer, AAnalyst 300, USA) was used to determine Zn^{2+} release profiles. All experiments were carried out in triplicates.

RESULTS AND DISCUSSION: The obtained composites have exhibited excellent antimicrobial activity. Precisely, synergistic activity of AC particles and adsorbed iodine was shown to be crucial for excellent antibacterial activity while synergy of AC particles and Zn^{2+} ions showed equally strong antifungal effect. However, Zn^{2+} ions proved to be selectors of resistant strains of bacteria which could be of relevance in everyday life, since Zn compounds are widely used in ointments and skin preparations from a very early age. Also, it was shown that PVP-I is firmly adsorbed on AC particles and that its release in the surrounding medium is negligible which is very important in regards of preventing often reported systemic iodine absorption after its prolonged medical usage [4].

CONCLUSIONS: The presented strategy enables further development of efficient multifunctional wound dressings with sustained release of one or more potent bioactive agents *in situ* for prevention and topical treatment of resistant infections and thus, addresses antibiotic resistance, one of the most significant clinical problems today.

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Activated charcoal as a carrier of probiotics: A new approach for pathogen elimination in wounds

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Keywords: Antibiotic resistance; local delivery; topical therapy; biocomposites; biofilm; diabetes

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INTRODUCTION: Antibiotic resistance is one of the biggest threats to global health, food security and development today [1]. However, development of conventional anti-infective drugs is going slowly, so new innovative strategies and more research are urgently needed in identifying, developing, implementing and evaluating novel therapies for antibiotic-resistant infections. The two-year ProHealingAC project, funded by the Science Fund of the Republic of Serbia, aims to use beneficial properties of AC and probiotic microorganisms in developing a new strategy for prevention and local treatment of antibiotic-resistant infections in wounds. Previously, it has been shown that activated charcoal (AC) in conjunction with different active agents has an efficient antimicrobial activity [2,3]. The aim of this project is to develop biocomposites (BCs) based on AC fabric, as adsorptive component, and probiotics, as bioactive component in order to achieve their synergetic activity for efficient and sustained local delivery of bioactive agents directly into the wound area. Also, special attention has been given to the influence of glucose level (normo- and hyperglycemia) in the microenvironment of the wound.

METHODS: A multidisciplinary team will develop an efficient, simple and cost-effective BCs by combining the principles of engineering and life sciences (microbiology, molecular biology and medicine). Developed BCs will be comprehensively characterized *in vitro* regarding probiotic release profile, antimicrobial and antibiofilm activity, and modulation of macrophage, fibroblast and keratinocyte activity. Based on the obtained results, the best candidate will be selected for *in vivo* studies in wound model in diabetic and non-diabetic animals.

RESULTS: As a primary result, a consortium of five scientific research organizations has been constituted and detailed research plan has been set. Expertize in the area of biotechnology, microbiology, molecular biology and histopathology of the partners involved in the project will constitute the solid background of the whole activity.

CONCLUSIONS: The main goal of the ProHealingAC project is to develop novel non-conventional anti-infective BCs with sustained release of probiotics for prevention and local treatment of resistant infections with special attention to the influence of glucose level in the microenvironment of the wound. In addition, through efficient dissemination and communication of the results, ProHealingAC project will help raise people's awareness of the importance of the rational use of antibiotics in human and veterinary medicine as well as.

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Production technology and characterization of alginate-based impregnated gauze

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Keywords: Wound dressings; alginate hydrogel-based dressings; enhanced gauzes; moisture regulation; controlled release of active agents

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INTRODUCTION: Traditional cotton wound dressings, like gauze and bandages, remain popular in wound care due to their affordability. However, they have drawbacks: adhering to wounds, risking tissue damage and low absorbance of secretions, requiring multiple layers, and causing discomfort. Modern alternatives, such as alginate hydrogel dressings, target these issues. Designed for moderate to intense exudate wounds, they enhance comfort and treatment effectiveness. Yet, their higher cost limits accessibility. Moreover, neither alginate nor cotton gauze offer bioactivity, while mechanical strength of alginate hydrogel may be inadequate. This work aims to develop enhanced gauzes to overcome these challenges, offering improved functionality at affordable costs and superior wound care.

EXPERIMENTAL: The impregnated gauzes were prepared by a three-step process: pretreatment, impregnation, and gelling. Pretreatment involved passing cotton gauzes through a $Ca(NO_3)_2$ solution (5-15 wt.%), while impregnation was carried out using solutions containing alginate and glycerol (mass ratio 4:10) with a dynamic viscosity of 0.3 Pa s measured at a shear rate of 10 s^{-1} and at 25 °C. Two different gelling solutions were used: one based on 2 wt.% $Ca(NO_3)_2$ and the second based on 2.3 wt.% $Zn(NO_3)_2$, both supplemented with glycerol (5-20 wt.%) and polyethylene glycol (10-30 wt.%). All produced gauzes were dried at 40 °C overnight and the best impregnated gauze candidates were further characterized. Dressings produced following the same procedure, just without gauze, served as controls.

RESULTS AND DISCUSSION: The impregnated gauzes were evaluated for the polymer layer thickness, sorption capacity, adhesion, mechanical properties and active component release. While polymer retention was consistent (87.6 ± 3.3 g/m^2) regardless of the gelling agent ($Ca(NO_3)_2$ or $Zn(NO_3)_2$), differences were noted in polymer thickness and sorption capacity. Under $Ca(NO_3)_2$ gelling, the polymer thickness was 42.6 ± 7.7 µm, with a sorption capacity of 770 %. In contrast, $Zn(NO_3)_2$ gelling resulted in a thickness of 77.1 ± 10.5 µm and a sorption capacity of 700%, indicating stronger hydrogel formation with Ca ions. Peel-off tests showed low adhesion force (1.91 ± 1.25 N), making the dressings painless upon removal. In mechanical tests, impregnated gauze exhibited superior strength compared to the control films, with nearly double the tearing force and higher elongation at breakage (approximately 23 %). Zn-alginate dressings achieved complete release of Zn ions after 72 h, with 70-80 % release after 24 h, which is suitable for dressing changes every 1-3 days.

CONCLUSIONS: The advantages of the enhanced dressings obtained as a result of this study include: *i*) high absorption capacity indicating capacity for moisture regulation and absorption of excess fluids in the wound, *ii*) expected painless removal of the dressing after the treatment, without leaving any residue of cotton threads in the wound, *iii*) the possibility of incorporating various active agents (*e.g.* antimicrobial, immune-stimulating, *etc*.) and their controlled release for faster and more efficient wound healing (as confirmed by the release of zinc ions from the developed enhanced dressings). During the process, a final formulation has been developed to ensure good flexibility and toughness of the impregnated dressings, making their cutting, and shaping according to the needs of the wound treatment very simple and efficient.

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Characterization of *Vaccinium myrtillus* leaf extract-loaded liposomes

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Keywords: Bilberry; encapsulation efficiency; liposomal particles; polydispersity index; size; stability

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INTRODUCTION: *Vaccinium myrtillus* L. leaves contain bioactive components, such as polyphenols, stilbenes, iridoid glycosides, fatty acids, and fibers [1]. However, polyphenols possess low solubility, stability, and bioavailability, thus the encapsulation of the mentioned active principles in different carriers is necessary [2]. Liposomes are widely used as a carrier for the encapsulation, preservation, and controlled release of polyphenols in various products [3]. Therefore, the aims of the presented research are the development and characterization of *V. myrillus* leaf extract-loaded liposomes *via* the determination of encapsulation efficiency, particle size, polydispersity index (PDI), zeta potential, and mobility.

EXPERIMENTAL: The liposomes with encapsulated extract were prepared employing the proliposome procedure [3]. Encapsulation efficiency was indirectly calculated by the polyphenol concentration determined in the supernatant. Particle size, PDI, zeta potential, and mobility were measured by the photon correlation spectroscopy in Zetasizer. Every measurement was examined three times at 25°C.

RESULTS AND DISCUSSION: The encapsulation efficiency of polyphenols was >85 %. The liposomes contained only phospholipids resulting in a more rigid membrane [4] providing the prevention of the leakage of the encapsulated polyphenols, as well as a higher encapsulation efficiency. The diameter and PDI of the liposomes were 5408.7±56.4 and 0.249±0.049 nm, respectively confirming that higher liposomal vesicles possessed lower PDI values [4]. The zeta potential and mobility were -5.02±0.25 mV and -0.315±0.016 µmcm/Vs, respectively. Zeta potential possessed negative values that are related to the exposure of the phosphate group lying in an outer plane concerning the choline groups [4]. The mobility of liposomes represents a function of the size, zeta potential, and lipid composition. The liposomal vesicles with lower membrane fluidity also show low mobility. The changes in the mobility of the liposomes were attributed to the membrane fluidity and ability to deform. Additionally, when flavonoids (also presented in *V. myrtillus* extract) are adsorbed at the surface of the liposomes, it can decrease their mobility [5].

CONCLUSIONS: The beneficial effects of bioactive principles from *V. myrtillus* leaves on human health and their sensitivity highlight the application of liposomal particles as a carrier for *V. myrtillus* extract and their potential implementation in foods, functional foods, pharmaceutics, and cosmetics.

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Stability of liposomal particles with encapsulated coumarin derivate

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Keywords: Liposomes; mobility; polydispersity index; vesicle size; zeta potential

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INTRODUCTION: Coumarin derivates possess several biological effects, including anti-inflammatory, antioxidant, anticoagulant, antitumor, insecticidal, anthelminthic, hypnotic, antifungal, and HIV protease inhibition properties [1,2]. Due to their solubility in organic solvents and insolubility in water and body fluids, their bioavailability is significantly low. Thus, they can be encapsulated into liposomal particles with the aim of overcoming the mentioned disadvantage [3]. Hence, in the present study, coumarin derivate was encapsulated in phospholipid liposomes and their stability was monitored for 60 days in terms of vesicle size, polydispersity index (PDI), zeta potential, and mobility.

EXPERIMENTAL: Coumarin derivate-loaded liposomes were prepared by mixing 0.1 g of coumarin derivate, 1 mL of dimethyl sulfoxide, 2 mL ethanol, 1 g of phospholipids, and 7.5 mL of water in the proliposome technique [4]. Vesicle size, PDI, zeta potential, and mobility were determined during 60 days of storage at 4°C using the photon correlation spectroscopy and Zetasizer Nano Series, Nano ZS (Malvern Instruments, United Kingdom). Every measurement was performed in triplicates at room temperature. The statistical analysis was performed by using the analysis of variance and Duncan's *post hoc* test (STATISTICA 7.0). The differences were considered statistically significant at $p<0.05$, $n=3$.

RESULTS AND DISCUSSION: The vesicle size varied from 1669.0±55.1 (1st day) to 1583.5±78.8 nm (60th day). Due to a relatively high absolute value of zeta potential at the beginning, the absence of a significant change in the size was expected. PDI value, as a measure of the particle size distribution, significantly increased during the 60-day study, from 0.231±0.043 to 0.497±0.079 indicating the existence of a non-uniform system [5]. A single phospholipid provides the liposomal population with significantly lower PDI (better uniformity) in comparison to the commercial phospholipid mixture employed in the present study [3]. The zeta potential and mobility significantly decreased (absolute value), from -29.43±0.55 to -14.43±1.00 mV, and from -2.307±0.043 to -1.127±0.086 μmcm/Vs, respectively. After 60 days of storage, the liposomes had significantly higher PDI, but lower zeta potential and mobility, indicating their instability. However, even though the zeta potential value was low on the 60th day, there was no occurrence of fusion and fission confirmed by the absence of statistically significant changes in the diameter of liposomes.

CONCLUSIONS: Coumarin derivate-loaded liposomes were unstable during 60-day storage at 4°C, resulting in changes in PDI value, zeta potential, and mobility, therefore additional experiments for improving their stability should be performed.

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Luminescent fluoroapatite nano-biomaterial for labeling yeast cells as an innovative approach for identification, imaging and monitoring

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Keywords: Bioimaging; nano-apatite; contrast agent; *Saccharomyces cerevisiae*; cascade luminescence; monitoring

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INTRODUCTION: The ubiquity of pathogenic yeast species in the human body and the increasing number of immunocompromised people acquiring infections have drawn attention to fungal infections [1]. Improved diagnostic imaging techniques and tools to study infection are necessary due to the commensal nature of pathogens yeast and the severity of the diseases they cause. The ability to label non-pathogenic yeast cells, such as the budding yeast *Saccharomyces cerevisiae (S. cerevisiae)*, may facilitate the identification and monitoring of these microbes in different environments. The topic of interest in this research is the development of luminescent nano-biomaterials based on fluorapatite as a contrast agent for labeling and imaging *S. cerevisiae*.

EXPERIMENTAL (or Materials and Methods): The method used to manufacture fluorapatite nanopowder has been previously reported in research [2]. After being acquired locally, *S. cerevisiae* was suspended in saline. One milligram of the FAP sample was added to the yeast suspension. After mixing the resultant suspension, it was left to incubate at room temperature for one hour without being stirred. Following treatment, cells were taken out, preserved, and prepared in triplicate for microscopy. MIPAR software has been used to analyse the obtained images.

RESULTS AND DISCUSSION: Luminescent FAP nanoparticles were synthesized by precipitation and centrifugation at low temperature. The resulting single-phase nanomaterial exhibits cascade fluorescence in the violet and blue regions [2]. To investigate the performance of FAP nanoparticle fluorophores, cells of *S. cerevisiae* were labeled and observed with a Leica DMIL inverted fluorescence microscope. Nanofluoroapatite fluorophores were successfully labeled *S. cerevisiae* cells. MIPAR image analysis software extracted the luminescence of nano-biomaterials from yeast cells.

CONCLUSIONS: In this study, *S. cerevisiae* was used as a yeast model which, after labelling with a fluorapatite-based contrast agent, showed luminescent properties. The cascading nature of the agents' luminescence will allow us to monitor cellular uptake as well as monitoring cellular localization in future studies.

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GPT4 aided biomaterials research use case: stabilization of selenium nanoparticles with proteins

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Keywords: Material science; chemistry; prompt-engineering; artificial intelligence

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Recent advancements in LLMs based on various transformer architectures such as BERT and GPT family models [1], brought many new possibilities for application in scientific research. The specific architecture and broad knowledge of these models give them the ability to understand concepts, to plan and solve different kinds of problems, including various chemistry-related tasks [2,3]. In this work, we are evaluating a case of GPT4 performance for recommending proteins suitable for the stabilization of selenium nanoparticles (SeNPs). SeNPs exhibit diverse beneficial bioactivities, including antioxidant, antibacterial, and anticancer properties, and stabilization of SeNPs with suitable proteins may be an effective approach to improve their bioactivities.

Initially, we made a series of "zero-shot" tests to evaluate knowledge, problem-solving ability, and identify weaknesses of GPT4 on the research topic and subsequently, we optimized prompts if needed to get correct responses. Test questions in related domains of crystallography, colloidal chemistry, and biochemistry were selected from free sources online (e.g. sanfoundry.com) or created by us. Finally, we have evaluated model performances on the main task which is to suggest the best protein candidates for stabilization of SeNPs that we synthesized in laboratory experiments. The design of prompts was done according to proposed tactics for prompt engineering suggested by OpenAI and DAIR.AI [4]. For interaction with GPT4 model OpenAI, API and Python programming language were employed.

The model successfully completed all our benchmark tests with optimized prompts. It also demonstrated the ability to analyze procedures for the synthesis and stabilization of SeNPs. GPT4 exhibits the capability to recognize protein records by identifiers, correctly identify amino acid sequences, describe their properties and functions to some extent depending on data trained on, identify available binding sites for interaction with Se, and give, by our judgment, good proposals for SeNPs stabilizers.

The study demonstrates the successful application of advanced transformer architecture models like GPT4 in addressing relatively complex tasks in materials research. Despite GPT4 capabilities being largely dependent on the quality and size of training data, utilization of strategically designed and optimized prompts significantly improves it's performance.

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Novel micro- and nano- composite materials for water purification

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Keywords: Zeolite; hydroxyapatite; titanium dioxide; polyaniline; adsorption; photocatalysis

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Considering industrialization and the rising amounts of wastewater, effective water treatment has become a matter of great importance. Adsorption and photocatalysis show superior results compared to conventional water purification methods. Since they are economical and environmentally friendly, many recent studies are focused on developing efficient materials for application in adsorption and photocatalysis. Zeolite is a good sorbent for many metal ions due to its specific porous structure, but it shows low adsorption capacity towards Ni^{2+} and Cr^{3+} ions [1]. In order to improve its adsorption efficiency, zeolite and hydroxyapatite-based composite was synthesized in this study. Hydroxyapatite shows a significantly higher adsorption capacity for Ni^{2+} ions compared to natural zeolite. On the other hand, titanium dioxide is a widely used photocatalyst, but it suffers from high recombination rate and possess low efficiency under solar light. These limitations can easily be overcome by coupling TiO_2 with a conducting polymer [2]. Among many conducting polymers, one of the most promising is polyaniline (PANI) since it is stable, economical, and easy to synthesize. In this work, TiO_2/PANI composites with different amount of PANI (0, 1, 3, 5 wt.%) were synthesized.

Zeolite/hydroxyapatite (Zeo/HAp) composite was obtained by the hydrothermal crystallization of calcium hydroxyapatite in the presence of natural zeolite clinoptilolite at 160°C for 4 hours. TiO_2/PANI composites were obtained by physical mixing of hydrothermally synthesized TiO_2 and PANI, produced using the chemical oxidative polymerization. X-ray powder diffraction (XRPD) analysis was used to determine the phase composition of the obtained composites using an Ital Structure APD2000 diffractometer. Particle size distribution analysis was performed using Mastersizer 2000 (Malvern Panalytical) and Zetasizer Nano S (Malvern Panalytical). The adsorption property of the obtained Zeo/HAp sample was examined towards Ni^{2+} and Cr^{3+} ions. Photocatalytic properties of the prepared TiO_2/PANI composites were investigated towards degradation of toxic textile azo dye Reactive Orange 16 (RO16).

XRPD analysis showed that the composites were successfully prepared. The diffractogram of Zeo/HAp adsorbent confirmed the presence of clinoptilolite and hydroxyapatite in microcrystalline form. All four photo-catalysts crystalized in preserved anatase structure of TiO_2 with crystallites less than 50 nm, according to the XRPD analysis. Particle size analysis showed that all the prepared composites were stable in aquatic suspension without further tendency to agglomerate over time. Most of particles had size around 100 µm in the Zeo/HAp composite. Interestingly, it was revealed that the PANI had impact on particle size distribution in TiO_2/PANI composites: with an increase in PANI content, the average particle size increased from 213 nm for pure TiO_2 to 360 nm for TiO_2/5 % PANI. After 24 hours, Zeo/HAp composite adsorbed 42.4 % Ni^{2+} ions and 94.8% Cr^{3+} ions meaning that the adsorption capacity of Zeo/HAp composite is 2.7 times bigger for Ni^{2+} ions and 1.8 times bigger for Cr^{3+} ions compared to natural zeolite clinoptilolite. TiO_2/PANI composites demonstrated significantly better adsorption properties during process of photocatalysis comparing to pure TiO_2. The best photocatalytic activity was reached by TiO_2/1 % PANI which almost completely (99.8 %) degraded the dye after 120 min under simulated solar light.

In summary, micro-sized Zeo/HAp and nano-sized TiO_2/PANI composites were successfully obtained. Both, Zeo/HAp adsorbent and TiO_2/PANI photocatalysts showed better outcomes in water purification compared to the natural zeolite clinoptilolite and the pure TiO_2.

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Impact of different concentrations of alginate in alginate-yeast hydrogel biosorbent

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Keywords: Brilliant green; dye; biosorption; immobilization; wastewater

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INTRODUCTION: Dyeing industry wastewater is one of the major environmental problems. Biosorption technology is regarded to be inexpensive and ecologically beneficial. Spent brewery yeast used in this research is proposed as a promising adsorbent [1] but free cells are unsuitable due to separation problems which leads to immobilization as an important part of the practical application of biomass biosorption. Alginate is well known; widely used and inexpensive material and the extrusion technique is the economical and ecofriendly encapsulation technique for immobilization with alginate as a carrier.

EXPERIMENTAL: Brilliant green dye, yeast and hydrogel beads were prepared as described by Krunic *et al*. [2]. 3 types of biosorbent are made using extrusion technique: beads containing 6.0 % of yeast and 1.6, 1.2 and 1.0 % of alginate. Experiments were conducted in 3 Erlenmeyer flasks (500 mL) containing 200 mL of the dye (25 mg/L) and 40 or 60 g/L wet adsorbents. The concentration of the dye was analyzed using a spectrophotometer (UV/Vis spectrophotometer, Ultrospec 3300 pro, Amerischam Bioscienc) at 624 nm. The Fourier transform infrared (FTIR) allowed the identification interactions between dye and biosorbents. All the measurements were done using a Nicolet iS10 spectrometer (Thermo Scientific, Sweden).

RESULTS AND DISCUSSION: It was determined by light microscopy that the reduction of the amount of alginate in the biosorbent did not significantly affect the dimensions and sphericity of the particles. They were spherical beads with a diameter of about 3.3 mm. The biosorbent containing 1.2 % alginate showed the highest adsorption capacity, slightly lower capacity shows the biosorbent with 1 % alginate, while the biosorbent with the highest amount of alginate showed the lowest dye binding capacity. The addition of 40 g/L of wet biosorbent proved to be a more efficient way of purifying wastewater compared to the addition of 60 g/l hydrogel beads.

CONCLUSIONS: By combining alginate and yeast, it is possible to make an effective biosorbent that absorbs over 90 % of the dye from the aqueous solution for a short time. The biosorbent containing 1.2 % alginate and 6.0 % yeast showed the highest adsorption capacity. From the obtained results, it can be concluded that yeast has a greater capacity for dye binding than alginate and that increasing the concentration of alginate above 1.2 % does not contribute to increasing the capacity for binding dye to the biosorbent, while lower alginate concentration values of 1.2 % slightly decrease the capacity of the biosorbent.

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The effect of sulfuric acid treatment on physicochemical properties of g-C_3N_4 and its efficiency for photocatalytic reduction of Cr(VI)

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Keywords: Cr(VI) photoreduction, g-C3N4 modification, particle size distribution

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INTRODUCTION: A great deal of interest is directed towards g-C_3N_4 (CN) for the photocatalytic reduction of Cr(VI), due to its high stability in acidic conditions and medium band gap (~2,7 eV), but its practical application is limited because of the high recombination rate of electrons and holes. Sulfuric acid treatment is considered as one of the methods for optimizing properties of CN by certain surface and possibly structure modifications which would lead to an increased specific surface area (*S*p) and more active sites, anchoring electronegative groups to enhance charge separation, exfoliated bulk CN into the nanosheets, *etc*. The aim of this research was to investigate the influence of H_2SO_4 concentration and other experimental conditions (temperature and time) on physicochemical properties and photocatalytic efficiency of CN.

EXPERIMENTAL: For the synthesis of CN from urea, direct thermal polymerization (550 ℃, 4 h, 10 ℃/min) was applied. CN was modified with: a) 1M aqueous solution of H_2SO_4, by simple reflux method (2 h, 80 ℃) Sample CN-P), b) very diluted H_2SO_4, by impregnation and evaporation method (sample CN-S), and c) concentrated H_2SO_4, by mixing (2 h, 80 ℃) and pouring the mixture to cold water (sample CN-E) [1-3]. The properties of the samples were studied by FESEM, FTIR, BET, DRS and PL analysis, as well as by determination of particle size distribution, the point of zero charge (pH_{PZC}) and the number of acidic functional groups. Photocatalytic reduction of Cr(VI) was tested at pH=3, under the UV and simulated visible (Vis) irradiation, in the presence of citric acid as a hole scavenger.

RESULTS AND DISCUSSION: The typical g-C_3N_4 structure was maintained after the acid-treatment, with some decreased interlayer spacing for CN-E. The *S*p and mesopore volume decreased for all modified samples, while only CN-E had completely different morphology with respect to the pure CN. Particle size distribution revealed that most of the particles were micro-size and for the CN-E much bigger. All acid-treated samples exhibited blue shift in the absorption edge, corresponding to an increase in the bandgap (BG) energy, which was confirmed with both DRS and PL analysis. Compared with CN, modified samples had lower pH_{PZC} and higher content of surface acid groups. Apart from CN-E, the modified samples showed slightly improved photocatalytic reduction of Cr(VI) under Vis irradiation.

CONCLUSIONS: The main structure of g-C_3N_4 wasn't destroyed by the treatment, but the *S*p was decreased. Concentrated H_2SO_4 was probably responsible for the different morphology of CN-E, drastically decreased *S*p, as well as wider BG, hence reduced photocatalytic activity under Vis irradiation. Still, photocatalysis under UV irradiation was improved, meaning that widening of the BG was crucial parameter, not the rest of the changes achieved by H_2SO_4 treatment.

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Hybrid biobased composites with natural pyrophyllite

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Keywords: Wood; composites; WPC; compression molding; moisture absorption

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INTRODUCTION: Biocompatible wood-plastic composites (WPCs) typically consist of up to 80% wood fibres and particles, often sourced from industrial waste wood, along with a reduced amount of thermoplastic polymer, primarily polyethylene (PE). Hybrid WPCs, in addition to the main components, incorporate additional elements to enhance the overall properties of the final material. Incorporating biodegradable natural fibres into hybrid wood-plastic composites offers numerous benefits, including low density, high specific strength, excellent impact and flexural properties, eco-friendliness, and cost-effectiveness. Their environmentally conscious and resilient nature has led to widespread application in various industries, including the automotive sector, civil engineering, and interior and exterior design, successfully replacing inorganic fibres polymer composites. In this work influence of hybridization of matrix and introduction of natural pyrophyllite on the moisture resistance and mechanical properties of composites were investigated.

EXPERIMENTAL: Three series of samples were blended in a high-speed mixer: S1 (HDPE - 60 wt%, wood - 36 wt%, FB – 4 wt.%), S3 (HDPE - 60 wt.%, wood - 26 wt.%, FB - 4 wt.%, PHY - 10 wt.%) and S7 (HDPE - 30 wt.%, wood - 26 wt.%, FB – 4 wt.%, PHY - 10 wt.%, WP - 30 wt.%). The starting materials was: HDPE = High-density polyethylene (HIP Petrohemija, d.o.o., Pančevo, Serbia), Wood = Oak wood flour from sawmill, FB = Fusabond Du Pont WPC-576D, PHY = Pyrophyllite powder ($Al_2Si_4O_{10}(OH)_2$) supplied by AD Harbi B&H (www.adharbi.ba), WP = waste HDPE from HIP Petrohemija, d.o.o., Pančevo, Serbia dump site. The mixture was extruded through a screw extruder, forming composite bars, then granulated with a universal cutting machine. All sample series were processed consistently. The WPC composition was shaped using a steel mold under isostatic pressure. Half of the synthesized samples were immersed in water, while the other half underwent STA (DSC and TGA simultaneously (Perkin Elmer TGA7)) tests and DMA analysis (DMA 242 E Artemis (Netzsch Gerätebau GmbH))

RESULTS AND DISCUSSION: The study's findings illustrate how processing and usage conditions influence the results from moisture absorption resistance tests indicate that the addition of pyrophyllite powder leads to decreased water absorption and swelling. SEM analysis revealed that water absorption impacts the microstructure of composites due to their hydrophilic properties. Thermal analysis revealed that composite 3 has sown the highest Tg and Tm. According to DMA dependence of storage modulus (E`) on the strain showed that highest E` showed composite 3 with the lower strain, while with the higher strain composite 7 showed higher E`. Composite 7 has shown the highest value of tan δ which characterizes the mechanical damping and visco - elastic properties of material.

CONCLUSIONS: The comparative analysis presented in this study concludes that reinforcing WPC with PHY particles significantly enhances weather resistance thermal and mechanical properties. Samples containing PHY exhibited reduced water absorption, increased thermal stability and improved mechanical properties.

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The ExcellMater project: Advancing biomaterials engineering towards novel medical devices

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Keywords: Twinning project; biological biomaterial characterization; capacity building

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INTRODUCTION: The route of novel biomaterials from synthesis to utilization in medical devices and products is complex requiring comprehensive physico-chemical, biological and functional characterization of biomaterials followed by preclinical and clinical studies adhering to strict procedures and regulations. Faculty of Technology and Metallurgy (FTM) in Belgrade is one of the leading institutions in Serbia in the field of materials engineering including certain areas in biomaterials engineering. Various biomaterials with bioactive agents were synthesized by variety of methods and characterized in detail regarding composition, morphology, structure, and mechanical and thermal properties. However, the next phases towards translation to medical devices and products are lacking primarily due to the lack of knowledge in these fields. The aim of the project "Twinning to excel materials engineering for medical devices – ExcellMater" [1] funded by the European Union's Horizon 2020 research and innovation programme is to tackle this weakness by twinning the needed expertise to FTM from the international project partners: University of Eastern Piedmont, Italy, AO Research Institute Davos, Switzerland, and Aalto University, Finland.

METHODOLOGY: The ExcellMater project included various activities such as site expert visits (SEV), short-term staff exchanges (STSE), workshops, specialized seminars, and topic-focused schools, closing with the ExcellMater Conference 2024: Innovative Biomaterials for Novel Medical Devices.

RESULTS AND DISCUSSION: The ExcellMater project provided meetings of the involved research groups by 15 SEVs from FTM to the partners and 9 SEVs from the partners to FTM and acquaintance with the experiments, equipment and practices carried out at each institution. These visits also served to plan joint research specifically during the STSE missions, 17 in total, involving doctoral students and young scientists. Specialized seminars on biological characterization of biomaterials, medical imaging, regulatory aspects, and clinical utilization of biomaterials complemented with the workshop on the latter topic, gathered not only FTM scientists but also local partners working in the fields of life sciences, with the aim to build a viable local ecosystem for efficient translation of novel biomaterials to the clinical use. Two topic focused ExcellMater schools on biomaterials for dental and orthopaedic applications and on hydrogels aimed for wound treatments, respectively, targeted doctoral students in multidisciplinary fields and were accredited by FTM as PhD courses of 2 ECTS, each. Additionally, the project strengthened the capacity of FTM researchers in project proposal writing and project management by a dedicated seminar followed by a hands-on workshop. The ExcellMater Conference 2024 organized in Belgrade as a final project event gathered internationally renowned experts in the field and provided possibilities to present scientific results obtained in the ExcellMater project as well as in two related H2020 projects.

CONCLUSIONS: Various intensive and dynamic activities performed within the ExcellMater project accompanied with efficient dissemination and communication significantly increased the scientific and technological capacity of FTM researchers in the field of biomaterials engineering and established fruitful and sustainable collaboration among the project partners as well as within the local scientific community.

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Twinning for intensified enzymatic processes for production of prebiotic-containing functional food and bioactive cosmetics (TwinPrebioEnz)

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Keywords: Human microbiota, biochemical engineering, prebiotic technology, enzyme modification

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INTRODUCTION: The primary aim of TwinPrebioEnz project is to enhance the research and innovation capabilities of the Faculty of Technology and Metallurgy in the field of prebiotics technology through collaboration with leading institutions possessing complementary expertise.

METHODOLOGY: The project consortium comprises Maastricht University and Radboud University Medical Center, due to their expertise in the analysis of human microbiota that will be applied to determine the activity of potential gut and skin prebiotics. Additionally, the Spanish National Research Council contributes expertise in protein engineering, facilitating the enhancement of catalytic properties of enzymes used in prebiotic production. In addition to transferring knowledge within these two areas of expertise, specific objectives is also to strengthen management capacities of the FTM. While the main focus is on advancing FTM, the project encompasses various objectives that benefit the entire consortium. For instance, specific work packages are dedicated to unlocking the potential of young scientists, establishing a network within the scientific community and potential industrial partners in prebiotic production and research. Joint research activities are intended to result in collaborative scientific publications, elevating the research profile of the project consortium and extending the interdisciplinary development of new prebiotic products. Dissemination and communication strategies target diverse audiences to achieve broader economic, technological, and societal impacts.

RESULTS AND DISCUSSION: Up to this point, FTM has conducted training sessions for young scientists, including Ph.D. students and early career researchers, within EU institutions. Methods and techniques for in vitro analysis of skin microbiota, chemical modification of enzymes, and structural characterization of prebiotics (oligosaccharide and polyphenols) were successfully transferred to FTM facilities. Additionally, an international workshop involving the participation of students and young scientists has been arranged at the FTM to facilitate broader knowledge transfer. Further dissemination of knowledge is planned through the organization of a summer school. Noteworthy advancements in project management skills were achieved at FTM through dedicated seminars and workshops, focusing on the transfer of both pre- and post-award management skills and knowledge. Furthermore, collaborative research within the project consortium has yielded joint publications in open-access journals. Notably, there has been a significant enhancement in the impact of FTM's publications in the field of prebiotic technology, evident in an increase of the average impact factor by more than 50%. The international conference for young scientists in the field of Biochemical Engineering and Biotechnology, organized by early career scientists in Belgrade, facilitated the dissemination of project results and objectives within the broader scientific community.

CONCLUSIONS: Activities during the first half of TwinPrebioEnz project timeline enabled significant improvement of FTM's research potential and management capabilities and laid the groundwork for future networking opportunities among institutions within project consortium and young scientists in this specific scientific domain.

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Knowledge and skills transfer for the application of nanotechnology in biosensors for foodborne pathogens

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Keywords: Nanomaterials, biosensors, field-effect transistors, microfluidics, pathogen detection, technology transfer

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The Know4Nano project aims to unlock synergetic research and innovation potential between EU-partner institutions and Biosense Institute (BIOS) to enhance the research and management capacity of BIOS staff in the field of bionanotechnology (BnT), project management and technology transfer to perform research activities toward the development of innovative and user-friendly personalized point-of-care diagnostic tests for food safety applications along the farm-to-fork food chain. Good practice, innovation, and scientific excellence from the leading European research institutions in the field of BnT: Catalan Institute of Nanoscience and Nanotechnology, Barcelona, Spain, with expertise in nanotechnology and nanoscience-based biosensors, and microfluidics, National Research Council, Rome, Italy, an expert in molecular biology, optical biosensors, biosensors testing and validation, and food safety, University of Chemistry and Technology, Prague, Czech Republic, an expert in materials science, materials synthesis, functionalization, and characterization of materials, will be transferred to BIOS by establishing a knowledge transfer platform based on carefully designed mobilities, trainings and mutual collaboration. Know4Nano will enable researchers from BIOS to acquire essential expertise, competencies, and skills in the field of nanomaterials, biosensors, microfluidics, and food safety, and enhance BIOS research management capacities and administrative skills for further commercialization, exploitation.

The complementary expertise of the team members will be combined with the purpose of developing highly sensitive portable biosensors for rapid quantitative detection of the most common foodborne pathogenic bacteria. The R&I activities will be focused on developing, testing and validation of low-cost sensing device comprised of an area of a field-effect transistor (FET)-based biosensors integrated inside the MF disposable cartridges for in-field PoC diagnostic of multiple pathogens. Certain novelties rely on the following: 1) Synthesis of various types of nanomaterials with superior charge transfer and high specific surface properties as A) metaloxide semiconductor (MOS) nanostructures, and B) 2D nanostructures (transition metal dichalcogenides (TMDs) and MXenes) and their composites. 2) Designing complex material heterostructures to explore their synergistic effects in devising innovative highly sensitive, selective, and reliable FET-based biosensors. 3) Design and fabrication of low-cost biosensors for PoC testing, 4) Developing novel protocols for biomolecules immobilization (enzymes, aptamers, and antibodies) on new nanomaterials; 5) Development of the procedures for biosensors testing and validation; 6) Integration of the biosensors into MF platform for multiple pathogens detection simultaneously.

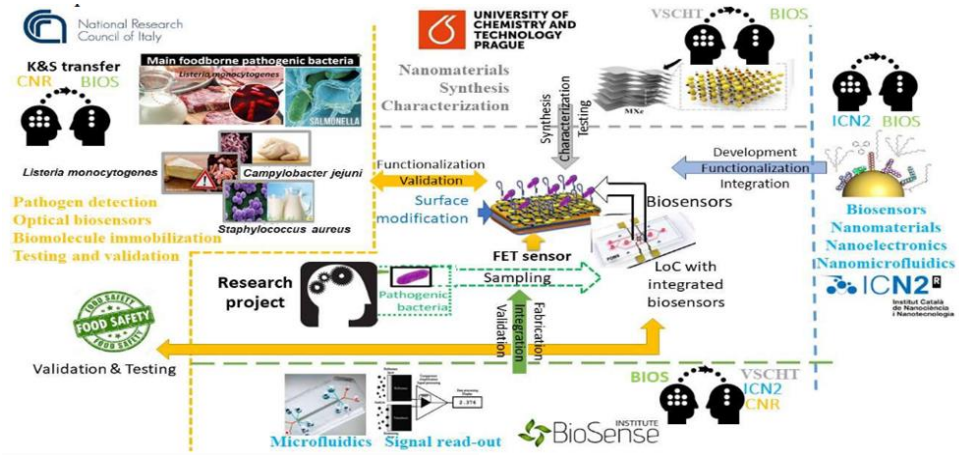

Fig. 1. The overall concept of Know4Nano project

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Experiences from BiH: H2020 Twinning project SMARTWATER

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Keywords: Cooperation, irrigation, ESR, sustainability, maize

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In January 2021, the new Horizon 2020 project "*Promoting SMART agricultural WATER management in Bosnia and Herzegovina*" (SMARTWATER) was launched. It was the first time that an academic institution from Bosnia and Herzegovina implemented a Horizon 2020 project as Coordinator. The main objective of SMARTWATER is to reinforce networking, research and S&T cooperation capacities of the University of Banja Luka (UNI-BL), the University of Sarajevo (UNSA) and other connected national institutions, in the field of sustainable agricultural water management and to increase their competency and fund-raising skills for a successful participation in the European Union Research Programs. Main project topics include: 1) cloud-based smart technologies, 2) new generation of satellite remote sensing data, 3) water-energy-food nexus and 4) climate change impact on agriculture. At two locations in BiH (Aleksandrovac and Butmir) 3-year field experiments on maize (*Zea mays* L.), hybrid BL 43 (from FAO 400 group) were completed. The Randomized Complete Block design included two factors, irrigation (3 irrigation regimes) and fertilization (2 nitrogen levels). During the project implementation, our scientific teams published several academic papers in peer-reviewed international Journals and Proceedings and these documents are available in open access on Zenodo platform [1]. The project consortium is preparing additional scientific papers. The project outputs are: 3 advanced training courses, 3 summer schools, joint research activities (experiments) at 2 locations in BiH, 3 stakeholders' meetings (roundtables), 3 post-graduate MSc courses, 13 mutual staff exchanges, 3 hands-on workshops on R&I, the development of 2 smart water management tools and the organization of an international conference in BiH at the end of the project. So far (period 2021-2023) most of these activities were finished. All project reports were prepared and sent to the EC. All info about the project is being disseminated, on a regular basis, and for this purpose social media profiles were used: Facebook, Twitter/X, LinkedIn and YouTube as well as the SMARTWATER website [2]. The dissemination of SMARTWATER achievements is an ongoing process. SMARTWATER project officially ends in June 2024. The remaining activities in 2024 include the organization of academic exchanges in Portugal and Italy, the 3rd stakeholders' meeting, the completion of the scientific publishing and the organization of an international conference in Trebinje (BiH) in May. We ask all interested stakeholders to visit our sites, to attend our events and to join the SMARTWATER network.

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Boosting Institute of Chemistry, Technology and Metallurgy in water biomonitoring - BIOLAWEB

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Keywords: Twinning project; lakes monitoring; biodiversity; freshwater ecology; metabarcoding

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Project "Boosting Institute of Chemistry, Technology and Metallurgy in Water Biomonitoring" (BIOLAWEB) aims to strengthen the research and innovation capacity of the Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, University of Belgrade (UB-ICTM) in biodiversity assessment and biomonitoring. UB-ICTM researchers made a noticeable contribution to the study of biodiversity, community ecology, and conservation of water bodies in South-Eastern Europe. However, a knowledge on index development and intercalibration following the EU standards for lakes and watercourse monitoring is still lacking in this geographic region. Similarly, there is a knowledge gap in DNA-based ecological status assessment in SEE. Overcoming these gaps and achieving the objectives of the BIOLAWEB is realized through networking with international institutions with a strong expertise in metabarcoding approach and in biological indices development: the French National Research Institute for Agriculture, Food and Environment (INRAE) and the Norwegian Institute for Water Research (NIVA). Staff exchange, common field, and laboratory work, and a variety of courses are used for an effective knowledge transfer from the partnering institutions to UB-ICTM. In order to strengthen research and innovation capacity, through BIOLAWEB project the International Cooperation and Project Office was established at UB-ICTM to support project application, management, and reporting at the international level.

The implementation of the BIOLAWEB results will raise the research profile of the coordinator and partner institutions and contribute to UB-ICTM's vision of becoming a lighthouse for attracting the best talents and tackling the burning issues of environmental assessment.

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STREAMLINE HUB: a high capacity hub for research of neurodevelopmental disorders in the Western Balkan region

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Keywords: iPSCs; WGS; transcriptome; drug testing; networking; neural differentiation

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Neurodevelopmental disorders (NDDs) are caused by alterations in early brain development. They are a group of geographically dispersed, complex and heterogeneous disorders that give rise to the psychiatric conditions such as autism spectrum disorders, intellectual disability, schizophrenia and bipolar disorder. In order to build global research activity for study of NDDs, the main goals of the Twinning project STREAMLINE are to enhanced strategic networking and reinforce research and innovation potential of the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade (IMGGE) in order to develop IMGGE as a high capacity hub for research of NDDs in the Western Balkans. This will be achieved by twinning IMGGE with three top-class research institutions in Europe (Cardiff University, University of Maastricht and Centre for Research and Technology Hellas) with an exceptional expertise in the stem cells based research of NDDs, -OMICS technologies, bioinformatics data analysis and drug testing and through staff exchanges, training, and organization of summer schools, Industry Open Days, symposia and workshops. In order to increase research and innovation capacities and empower research staff of IMGGE, short-term staff exchanges of IMGGE researchers to partner institutions are planned: Cell reprogramming, Generation and analysis of brain organoids, Analysis of electrophysiology of the neurons, RNA-seq, Bioinformatics data analysis and formation of a digitalized repository and High-throughput cell-based assays for *in vitro* drug testing. In conclusions, formation of the STREAMLINE hub will position IMGGE as a leader in NDDs research in Western Balkan region with equivalent research capacity and codes of practice as similar centres in Europe.

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Twinning for graphene-based composites in EMI shielding

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Keywords: Graphene; graphene oxide; electromagnetic shielding; polymers; nanomaterials; nanotechnology

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In the era of intensive development of microelectronics, energy, and car industries along with Radio-Frequency (RF) telecommunications, the pollution caused by Electromagnetic Waves (EWs) is ever-present. EW interferences (Electromagnetic Interference - EMI) exhibit perturbation and negative impact on devices and systems including those used in everyday life as well as on the specialized, sensitive, and sophisticated instruments used in research laboratories. EMI could cause untrusted signals and RF noise. To prevent these issues, materials able to block or absorb the radiated EWs are urgently required. The GrInShield project is focused on developing new graphene-based shielding nano-materials and increasing researchers' expertise in EMI shielding measuring, protective materials, and possibilities to bring these new products to the market.

INTRODUCTION: The GrInShield project uses graphene oxide (GO) obtained by Hummers' reaction and electro-chemical exfoliation of graphite [1,2]. We have analysed the factors that affect the shielding efficiency of materials [3] and studied the reaction conditions that lead to obtaining graphene with different sizes and oxygen content [4].

RESULTS AND DISCUSSION: The GrInShield project aims to produce composites of GO with silver nanowires (AgNWs) to develop GO-AgNW composites for EMI shielding applications. To achieve these goals, the project gathers experts from the chemistry of nanomaterials, and polymer processing, along with specialists for near-field microscopy tools and radiofrequency (RF) characterization of materials.

CONCLUSIONS: The GrInShield project is developing new nanomaterials for EMI shielding based on carbon nanomaterials, metallic nanomaterials, and polymers. The fabrication of low-cost, sustainable, eco-friendly, durable EMI shielding material should be achieved.

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