



*4<sup>th</sup> International conference on Bioengineering  
and Polymer Science*

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**BOOK OF ABSTRACTS**

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## 4<sup>th</sup> International Conference on Bioengineering and Polymer Science - Board

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# PLENARY PRESENTATION

## Functional magnetic nanoparticles for biomedical applications

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Magnetic nanoparticles are fundamental building blocks in various applications, including high-density data storage, spintronics, and nanomedicine. The functional performance of these nanoparticles is dictated mainly by their magnetic anisotropies, which arise from surfaces, shapes, and interfacial interactions in hybrid structures. In this talk, I will introduce the key aspects of effective magnetic anisotropy and its characterization using RF transverse susceptibility measurements.

Tuning magnetic anisotropy is crucial for optimizing the performance of magnetic nanoparticles in biomedical applications, particularly in enhancing MRI contrast and improving magnetic hyperthermia for cancer therapy. A critical challenge in this field is the need to improve surface functionalization and increase the specific absorption rate (SAR) or heating efficiency of nanoparticles for effective cancer diagnostics and treatment. To address these challenges, strategies beyond conventional spherical nanoparticles—such as exchange-coupled core-shell structures, nanowires, and nanotubes—offer promising pathways to enhance saturation magnetization, effective anisotropy, and thermal efficiency in magnetic hyperthermia.

This talk will explore the fundamental physics underlying magnetic nanostructures and our latest research advancements in their application to cancer therapy and diagnostics in nanomedicine.

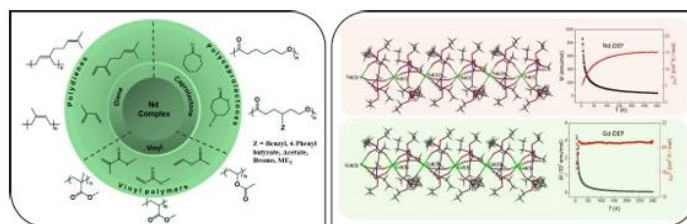
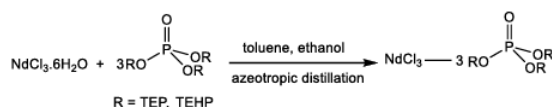
1. “Hybrid magnetic nanoparticles as efficient nanoheaters in biomedical applications” (mini-review) -G.C. Lavorato, R. Das, J. Alonso Masa, M.H. Phan and **H. Srikanth**, **Nanoscale Advances** **3**, 867 (2021)
2. “Competing magnetic interactions and field-induced metamagnetic transition in highly crystalline phase-tunable iron oxide nanorods” -S. Attanayake, A. Chanda, T. Hulse, R. Das, M.H. Phan and **H. Srikanth**, **Nanomaterials** **13**, 1340 (2023)
3. “Tailoring the magnetic and hyperthermic properties of biphasic iron oxide nanocubes through post-annealing” - S. Attanayake, A. Chanda, R Das, M.H. Phan and **H. Srikanth**, **Crystals** **14**, 519 (2024)

## Catalytic Activity and Magnetic Properties of Lanthanide Phosphates

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*Department of Chemistry and Biochemistry, The University of Texas at Dallas Lanthanide*

Ziegler-Natta (ZN) type catalysts are efficient catalysts for the stereospecific polymerization of dienes. However, these catalysts are poorly soluble in organic solvents and fail to polymerize polar vinyl monomers because these polar monomers deactivate them producing polymers with high polydispersity index (PDI). Therefore, neodymium-based ZN catalysts,  $[\text{Nd}(\mu\text{-DEP})_3]_x$  (DEP = diethyl phosphate),  $\text{NdCl}_3 \cdot 3\text{TEP}$  (TEP = triethyl phosphate), and (TEHP = tris(2-ethylhexyl) phosphate) were developed using the ligand exchange method during azeotropic distillation in a nitrogen environment for the polymerization of dienes as well as polar vinyl monomers such as methyl methacrylate (MMA), methyl acrylate (MA), and vinyl acetate (VA), in combination with triisobutylaluminum (TIBA) as a cocatalyst or in the presence of benzyl alcohol as an initiator. Adding an organic ligand and an electron donor improves the solubility of these catalysts while preserving stereospecificity, as  $\text{NdCl}_3 \cdot 3\text{TEP}$  showed 106 Kg polymer Nd-1 h-1 catalytic activity. Previous studies have shown that halide or pseudohalide, either from the catalyst directly or from an external source, is necessary for polymerization to proceed successfully in ZN catalytic systems. To the contrary,  $[\text{Nd}(\mu\text{-DEP})_3]_x$ , a novel halide-free Nd catalyst, polymerized bio-based  $\beta$ -myrcene with high stereospecificity (96% cis-1,4 content) and a narrow PDI (1.8). Additionally,  $\text{NdCl}_3 \cdot 3\text{TEP}$  was capable of homopolymerizing the dienes (isoprene and  $\beta$ -myrcene) with narrow PDI (1.6) and high cis stereospecificity (92% and 96%), respectively. Comparing anionic, cationic, and radical polymerization techniques, these catalytic systems were employed successfully to polymerize  $\epsilon$ -CL and  $\gamma$ -functionalized  $\epsilon$ -CL with decreased sensitivity to substituents in  $\epsilon$ -CL. Moreover, they performed better than the traditional catalysts:  $\text{Sn}(\text{Oct})_2$  and  $\text{Al}(\text{o-i-pr})_3$ , in the polymerization of functionalized  $\epsilon$ -CL with an ester linkage at the  $\gamma$ -position.  $\text{NdCl}_3 \cdot 3\text{TEP}$  and  $\text{NdCl}_3 \cdot 3\text{TEHP}$  synthesized both poly  $\epsilon$  caprolactone (PCL) and poly  $\gamma$ -functionalized  $\epsilon$ -CL with a variety of linkages at the  $\gamma$ -position, including benzyl, 4 phenyl butyrate, acetate, bromo, and  $\gamma$ -2-[2-(2-methoxyethoxy)ethoxy]ethoxy (ME3) with narrow PDI (1.22 - 1.84). According to kinetic studies, the TEP ligand reacted with  $\epsilon$ -CL at a lower rate than the bulkier TEHP ligand. Based on kinetic studies and the effective synthesis of block copolymers such as poly ( $\beta$ -myrcene)-b-poly (isoprene), poly ( $\beta$ -myrcene)-b-PMMA, PVL-b-PCL (PVL = polyvalerolactone), PBrCL-b-PCL, PME3CL-b-PCL, and P( $\epsilon$ -CL)-b-P(L-lactide), all our synthesized catalytic systems demonstrated the quasi-living behavior. Our research allowed us to show the capacity of a single catalytic system for the polymerization of lactones, both non-functionalized and functionalized lactones, in addition to dienes and vinyl monomers. Furthermore, after analyzing the crystal structure of  $[\text{Nd}(\mu\text{-DEP})_3]_n$  obtained from single crystal XRD, that showed the bridging phosphate groups with distorted tetrahedrons connect the neighboring 6-coordinated  $\text{Ln}^{3+}$  metal ions, eventually forming one-dimensional polymeric chains. The neighboring  $\text{Ln}^{3+}$  ions are spaced widely in the same polymeric chain that may act as separate magnetic domains. Thus, two isomorphous lanthanide organophosphate coordination polymers (LOCPs) using Nd and Gd metal ions:  $[\text{Nd}(\mu\text{-DEP})_3]_n$ , and  $[\text{Gd}(\mu\text{-DEP})_3]_n$  were synthesized using the azeotropic distillation method, and were investigated for the magnetic properties using SQUID magnetometer. The magnetic studies showed superparamagnetic behavior in these compounds with a magnetic moment and cluster density for  $[\text{Nd}(\mu\text{-DEP})_3]_n$  are 2.66  $\mu\text{B}$  and  $3.28 \times 10^{20} / \text{cm}^3$ , and that for  $[\text{Gd}(\mu\text{-DEP})_3]_n$  compounds are 8.12  $\mu\text{B}$  and  $1.71 \times 10^{21} / \text{cm}^3$  respectively estimated from the Langevin fitting, which closely agrees with the theoretical values. The study shows these compounds are very promising to be used as Single Chain Magnets and might be helpful for high-density data storage devices in the future.



## The Do's and Don'ts of 3D Printed AUP Meniscal Substitutes

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### INTRODUCTION

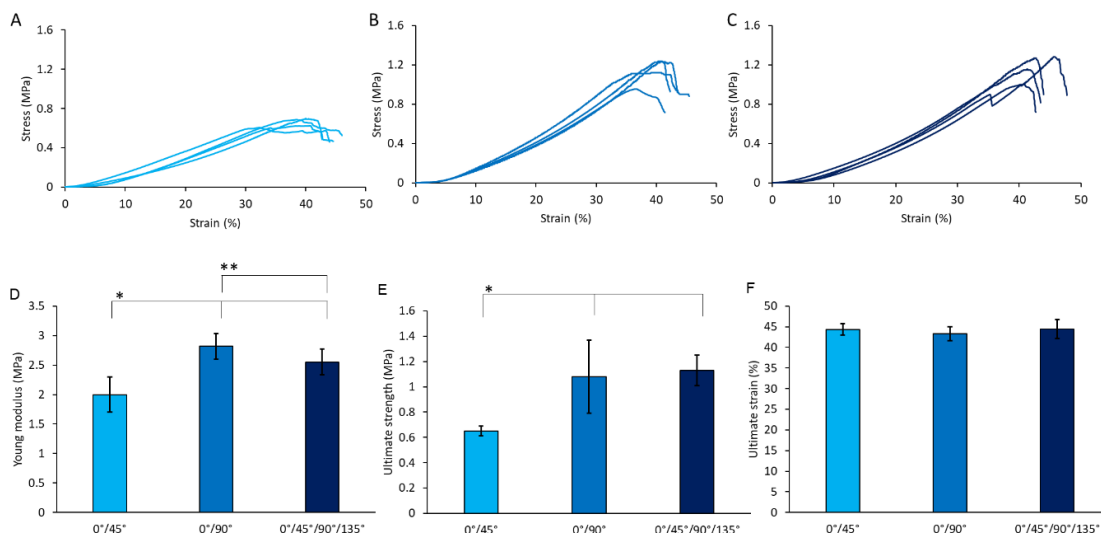
Hydrogels are widely applied in the biomedical field. Several years ago, our laboratory developed a novel type of hydrogel building blocks (the so-called AUP) that elegantly combine (1) biocompatibility, (2) solid state crosslinking behavior, (3) processing capabilities and (4) adaptability of the physico-chemical properties.<sup>1</sup> In the present work, we apply this material as potential meniscal implant.

### EXPERIMENTAL METHODS

The AUPs applied in our work were synthesized by reaction of PEG with a molar mass of 1000-8000 g/mol with two equivalents of IPDI, followed by the reaction with two equivalents of a monoacrylated oligo(ethylene glycol) spacer. Starting from these hydrogel building blocks, scaffolds were 3D printed by extrusion of the polymer melts. Scaffolds with varying printing patterns, pore sizes and strut diameters were developed. Scaffold visualisation was realized using optical microscopy and micro-CT analysis. The compressive properties were evaluated on water-swollen AUP scaffolds.

### RESULTS AND DISCUSSION

As an example, the effect of the printing pattern on the compressive properties of 3D printed AUP4K scaffolds are shown in the below figure.



### CONCLUSION

AUP can be successfully 3D printed and the scaffolds reveal mechanical properties that match those of the human meniscus while not (yet) meeting the required fatigue resistance.

### REFERENCE

1. WO2017005613A1, Novel urethane-based materials, derivatives, methods of their preparation and uses.

### ACKNOWLEDGMENT

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## Additive Manufacturing of Biodegradable Polymers for Tissue Engineering and *In Vitro* Cancer Modelling

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The biomedical community's great interest in additive manufacturing (AM) is a result of its versatility in terms of processing approach, materials selection, and customization of the resulting device. Specifically, the unmatched ability to control structural and compositional characteristics at the macro- and microscale is making AM the technology of choice for fabricating biodegradable medical devices. This contribution aims to provide an overview of recent research activities on AM of biodegradable polymers for application in the biomedical field.<sup>1</sup> The main AM techniques that have been applied to biodegradable polymers will be presented by discussing the necessary requirements for materials processing. The presentation will cover the different classes of biodegradable polymers that have been studied for AM, including proteins, polysaccharides, and aliphatic polyesters. Case studies that demonstrate how material-extrusion AM can be used to process synthetic and microbial aliphatic polyesters, e.g., poly( $\epsilon$ -caprolactone) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate), as well as polysaccharides from marine sources, e.g., chitosan and alginate, will be discussed. Moreover, significant emphasis will be given to recent research on innovative AM strategies for loading polymeric scaffolds with osteoinductive ceramics or natural anti-inflammatory/antimicrobial agents. Tailored experimental activities will be described to highlight the potential of the developed bioactive devices for advanced biomedical applications, such as bone regeneration, wound treatment, and *in vitro* cancer modeling.<sup>2-5</sup> Ongoing research on photocrosslinkable macromolecules of natural origin for bioprinting strategies will also be presented.

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## **Points of view in bioengineering: molecular diagnostic, transdermal drug delivery and nanomedicine**

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The presentation consists of two parts. The *first part* will be dedicated to a new approach in paper-based microfluidics: heat-driven evaporation. Paper-based microfluidic devices are attractive diagnostic platforms for nucleic acids testing (NAT) due to their low-cost and ease-of-use. However, paper-based microfluidic platforms are relatively less sensitive and require a substantial amount of nucleic acids (NA) for detection. Therefore, sensitive and specific detection of the (NA) using paper-based microfluidics requires filtration, NA concentration, and amplification to be incorporated and developed on the same device. The current work proposes a paper-based microfluidic platform that uses thermophoresis and driven-evaporation for nucleic acids concentration. The sample concentration was achieved by overlapping a “hot spot” (required to generate thermal gradients) with the “spot” designed as evaporative concentrator. The proposed paper-based microfluidic platform presents a sandwich structure with four components: a rigid plastic support to hold a filtration paper, a nitrocellulose paper to drive the NAs through the collection point and a plastic tape, with a small opening defining the evaporation area, to seal the structure, and a Vivid filtre paper. Through vertical flow the sample is collected into a nitrocellulose paper, while a lateral flow is used to concentrate the sample. Simulation results showed that, once the device comes in contact with a hot surface, the structure assures a “hot spot” around the evaporation area. The focalization process using evaporative concentrator and thermophoresis was visualized first with colour die. The positive Soret coefficient of the DNA allowed its concentration on the hot area, while the evaporative concentrator enhance it. The method was tested also using spiking salmon DNA in blood. The *second part* will be focused on the current research spotlights in the eBio-hub Centre: the molecular diagnostic, transdermal drug delivery and nanomedicine.

## Multi-Omics Data Integration: Advancing Community-Oriented Tools to Meet Tomorrow's Needs

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With recent advancements in biomedical research, we have reached a turning point where system-level characterization of biological systems has transitioned from being merely desirable to becoming standard practice. In cancer research, tumor profiling is now increasingly conducted alongside microenvironment analysis for a more comprehensive understanding of the system as a whole. Reducing the level of complexity to individual cellular phenotypes, omics profiling, which assesses the abundances of cellular components such as proteins and transcripts, has proven invaluable for characterizing key functions within biological systems. However, due to the complex nature of these systems, analyzing a single omics profile often falls short of capturing the most important molecular traits and interactions<sup>1-3</sup>. The latest breakthroughs in sequencing technologies have made it possible to measure multiple molecular components simultaneously, allowing cells to be viewed as dynamic systems composed of interacting omics layers.

Measuring multiple omics profiles for the same cellular phenotypic state generates an abundance of complementary and unique information about key features and signaling pathways that govern molecular functions, driving the need for various computational tools to integrate multi-omics datasets<sup>4</sup>. During the lecture, we will explore some of these tools that employ either data-driven approaches, relying solely on measured data to infer relationships, or knowledge-based tools, leveraging previously validated interactions to enhance biological interpretability<sup>5</sup>. While data-driven methods can be applied to almost any omics dataset and rely entirely on mathematical frameworks, knowledge-based integrative approaches leverage the continuous expansion of mapped biological interactions, enabling greater interpretability by relying on existing knowledge rather than inferred relationships. However, current knowledge-based multi-omics platforms often employ inference frameworks that are not specifically tailored for biological applications and impose limitations on both the number of datasets that can be integrated and the selection of underlying interaction databases.

Additionally, I will introduce during the talk NOODAI<sup>6</sup>, a software platform developed to integrate the outcomes of distinct omics analyses into a joint framework by merging the differentially expressed elements obtained from each omics profile into protein-protein interaction networks that are analysed collectively. The user-friendly webtool allows for the identification of the most important proteins within the joint integrative network by considering a wide range of network centrality metrics. Besides identifying top central proteins, network neighbourhoods are obtained using the MONET<sup>7</sup> tool dependency. The identified clusters are associated with specific signalling pathways that collectively characterize the samples under study, considering simultaneously all input omics profiles. As one of the few tools that allow researchers to jointly analyse and interpret different omics profiles, NOODAI facilitates the identification of robust molecular traits of the systems under study.

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## Tomorrow's Healthcare: Current Trends and Directions in Bioengineering and Biomaterials Science

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Current healthcare and diagnostics have many constraints today and there is a great demand to improve the current healthcare facilities by improving the existing methods and development new approaches. Bioengineering is one of the most viable options which has a potential to improve the existing healthcare scenario. Continuing research in the field of bioengineering has placed considerable demand on the versatility, variety and quality of available of biomaterials, reflected in the biomaterial industry's value.

Biomaterials play a fundamental role in the development of implants designed to replace or repair damaged tissues and offer innovative solutions in the fields of tissue engineering, regenerative medicine, artificial organs, and drug delivery systems.<sup>1</sup>

Biomaterials science has advanced from pioneering practices when engineers, chemists, and physicists made the rules on this field, to the present time when bioengineers are key players into the interdisciplinary teams.

This contribution explores key facts in biomaterials, their types along with their examples, advantages, disadvantages and their applications and what are the factors that we should keep in our mind before choosing any biomaterial. This systematic analysis offers a comprehensive overview of biomaterials, highlighting various types of materials used in clinical research, and bring a contribution to a deeper understanding of biomaterial applications in clinical settings. Emerging biomaterials, such as biodegradable metals, are at the forefront of biomedical research, promising transformative advances in health care treatments.<sup>2</sup>

The development of engineered biomaterials into clinically translated therapeutics relies heavily on effective clinical trials to demonstrate safety and efficacy, as well as to determine appropriate dosages and other parameters. Rigorous evaluation of these materials contributes substantially to advancements in patient care and treatment modalities.<sup>3</sup>

However, there is still a huge gap between applied basic research on biomaterials and their translational products - medical devices. Then based on clinical needs, market analysis, and relevant regulations, some ideas are proposed to integrate the two different mindsets to guide applied basic research and translation of biomaterial-based products, from the material and technical perspectives.

Whichever the nature of the materials used (synthetic or biological), for sure future biomaterial-based therapeutic approaches will be addressed towards the so-called "personalized medicine". It overcomes the traditional "one-size-fits-all" approach and considers each patient as an individual, tailoring the required therapy on the basis of the specific needs. This will be a real revolution in medical care, and it requires advances in biomaterials research that enable innovative biomaterials design to diagnose and treat patients' diseases. This approach looks to be more feasible in clinical practice than the idea of biological renaissance by "tissue and organ banks" that are available to any patient. Despite great advances in science and technology, there is still need a lot of interdisciplinary research work ahead, and numerous efforts are currently underway to improve the gap between research results and clinically successful trials of some commercial products.

Furthermore, the lecture will provide a comprehensive perspective on the future of bioengineering from biomaterials science perspective, offering information about new trends in biomaterials and strategic directions about biomaterials for clinical translation of medical devices.

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# Graphene-based materials for (bio)applications: the need & emerging role of Artificial Intelligence

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Last year, we celebrated the 20th anniversary of the discovery of graphene, the atomic monolayer of carbon atoms forming a honeycomb lattice in two dimensions. After two decades of intense efforts to bring the wonder material to real life applications, supported by the European Commission and member states through the Graphene Flagship Instrument, a variety of scientific knowledge has been acquired and novel technologies successfully developed and commercialized [1]. In this talk, I will overview the milestones and key achievements of the field, presenting how morphological specificities of this material (low dimensionality, transparency, flexibility, record electronic and thermal mobilities, chemical reactivity, biocompatibility and so on) have enabled for instance the design of innovative (bio)devices (such as ultrasensitive and selective biosensors, brain implants and scaffold for neuronal growth and activity monitoring). The possibilities for further disruptive advances in health and biotech ecosystems afforded by the emergence of Artificial Intelligence (AI) tools will be also discussed.

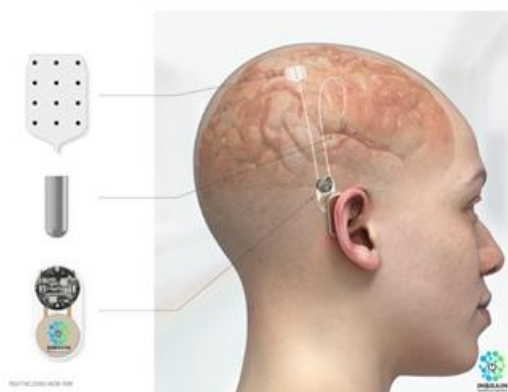


Figure 1: Schematic of a brain implants made from graphene-based composites and developed by ICN2 spinoff InBrainNeuroelectronics (<https://inbrain-neuroelectronics.com/>)

1. Reference [1] Science (special issue), Volume 386, Issue 6718 (11 Oct 2024)

## **Shaping the Future of Global Leaders: Insights and Directions in Bioengineering and Polymer Science**

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As the fields of bioengineering and polymer science continue to evolve, they play a crucial role in shaping the future of global leaders in research, industry, and innovation. This contribution explores key insights and emerging directions in these disciplines, highlighting advancements in biomaterials and sustainable biopolymer development and uses based on recent research efforts from our B4 group.

By addressing current challenges and technological breakthroughs, we examine how interdisciplinary collaboration and leadership in these fields can drive scientific progress and industrial transformation. Furthermore, the lecture will provide a comprehensive perspective on the future of bioengineering and polymer science, offering strategic directions for researchers, educators, and policymakers.

## Functionalized clay-based hydrogel composites for agricultural applications

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In agriculture, two key factors essential for plant growth are an adequate supply of water and fertilizer. Therefore, enhancing the efficiency of water and nutrient fertilizer use is crucial. A key goal in agronomic management is improving fertilizer use efficiency by reducing its environmental loss. One approach to achieving this is the use of slow or controlled release fertilizer (SRF) systems, which help minimize fertilizer losses and boost its efficiency. First, we will discuss the formation of composite hydrogels, which are prepared through in situ polymerization of acrylic acid and acrylamide (using an organic co-crosslinker), and incorporate either a clay-urea intercalate or separate clay and urea into the reaction medium. We will see that clay-urea intercalates can be prepared quickly (within 15 minutes) through mechanochemical grinding using a planetary ball mill, with minimal amorphization of the clay<sup>1</sup>. The two preparation methods for the hydrogel composites resulted in noticeable differences in swelling behavior, water retention, and urea release kinetics. Composites made with clay-urea intercalates exhibited superior water retention compared to those made by simple mixing, primarily due to the more uniform dispersion of kaolinite, which created a more tortuous path for water molecules. Additionally, urea release from hydrogel composites with clay-urea intercalates was slower than from those prepared by the one step method. In the second part of the presentation, we will explore how it is possible to functionalize clay to facilitate the polymerization of the acrylic monomer on its surface, resulting in a gel-like structure at room temperature without the need for an additional organic co-crosslinker. The hydrogels obtained in this way are both flexible and tough, with good elastic recovery properties, making them suitable for withstanding a large number of swelling-deswelling cycles.

<sup>1</sup>Y Elhadj, FX Perrin, Appl Clay Sci, 213, 106250, 2021.

# KEYNOTE PRESENTATIONS

## Sturgeon collagen - new source for fabrication of medical devices

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A promising solution for soft tissue regeneration is tissue engineering, a multidisciplinary field of research which involves the use of biomaterials, growth factors, and stem cells to repair, replace, or regenerate tissues and organs damaged by injury or disease. The success of tissue engineering depends on the composition and microstructure of the used scaffolds. Ideally, scaffolds should be similar to natural tissues. Collagen is the major component of the extracellular matrix of most soft tissues. As a natural molecule, collagen possesses a major advantage, being biodegradable, biocompatible, presenting low antigenicity, easily available and highly versatile. Nowadays, the main source for collagen biomaterials are the bovine, porcine and cadaver skin. Due to religious constraints and bovine spongiform encephalopathy (BSE), the attention for new sources of collagen is absolutely requested. On the other side, a huge amount of wastes (about 50-70%) of original raw materials is generated by fish processing factories, causing serious environment pollution with offensive odour. Therefore, the use of such wastes (skin) in the production of value-added products is a very promising solution from environmental and economical point of view, being part of the circular economy.

Fish skin, scales, and bones have been reported in many studies as source of collagen, used especially in food industry. Collagen extraction from different marine origin skin and its physical, biochemical, structural and biological characteristics were reported so far. Very few studies gained their attention on biomedical applications. The big constrain for using collagen fish in biomedical field is its mechanical strength, thermodynamic stability and high biodegradability.

In the present research, collagen gels from sturgeon skin were obtained and characterized by physical chemical, structural and rheological properties. The collagen sturgeon gels were freeze-dried, and corresponding sponges were evaluated by FT-IR, SEM, water uptake, enzymatic degradation, antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, proliferation of human fibroblasts on sturgeon collagen sponge at 24, 48 and 72 h, and by *in vivo* tests of sturgeon collagen sponge on Wistar rats with induced burn during 16 days. The results showed sturgeon collagen sponges as a promising biomaterial for wound healing.

Starting from these promising and excellent results, we selected a three factors-two levels factorial design as a first step in fabrication of sturgeon collagen sponges. The independent variables were collagen gel concentrations, crosslinking agent concentrations and freeze-drying temperatures during freeze-drying process, while the dependent variables were swelling degree, weight loss and pore sizes. Based on the optimization technique, three combinations of formulation and process factors were selected. The human dermal fibroblasts cultivated for 3 weeks evidenced *de novo* collagen deposition on the scaffolds. The best and final formulation in term of porosity, absorption, biodegradability and biocompatibility was chosen to be the one with 0.6 % collagen, -10°C and 0.5% crosslinking agent. The process of fabrication will follow the Medical Device Regulation – MDR 2017/745, ensuring conformity with medical device standards. There is no any risk for TSE or BSE and the presented biomaterial is ready to be medical device very soon. However, even if the results were excellent, the biomaterials cannot be used by humans until they become medical devices.

### ACKNOWLEDGEMENT

This work was supported by a grant from the Ministry of Education and Research, Romania, project NUCLEU code 01 01 23 26. The tests were performed in many laboratories such as Innovative Therapeutic Structures Research and Development Center (InnoTher), “Carol Davila” University of Medicine and Pharmacy, Bucharest; Cell and Tissue Engineering Department, “Nicolae Simionescu” Institute of Cellular Biology and Pathology, Bucharest; Advanced Polymer Materials Group, National University of Science and Technology POLITEHNICA Bucharest. Many thanks to be part of this research.

## Circular Solutions for Additive Manufacturing: Bio-Based Composites with Catalytic Potential

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**INTRODUCTION:** Additive manufacturing (AM) is an advanced fabrication technique that enables the creation of complex polymeric, ceramic, metallic, and composite structures through a layer-by-layer deposition process [1]. By building components directly from CAD data, AM offers significant advantages in terms of design flexibility, material efficiency, and rapid prototyping, making it a transformative approach across various engineering and biomedical applications. The development of bio-derived materials as alternatives to conventional petroleum-based feedstocks for various 3D printing technologies opens new avenues toward achieving a truly sustainable and circular economy. This shift not only reduces environmental impact but also promotes material recyclability, biodegradability, and resource efficiency in additive manufacturing processes [1-2]. In this study, we present the preparation of chitosan–polylactic composite blends utilizing two chitosan samples extracted in-house from shrimp waste and compare their performance with that of commercially available low and medium molecular weight chitosan.

**EXPERIMENTAL METHODS:** Composite blends of chitosan including commercial sorts of low and medium molecular weight, as well as laboratory-extracted chitosan derived from shrimp head and shell waste were prepared with polylactic acid (PLA) using extrusion molding. Comprehensive characterization of the extruded filaments was performed to assess the influence of chitosan molecular weight and loading on their physico-mechanical and thermal properties. Melt flow index measurements, tensile testing, dynamic mechanical analysis, and differential scanning calorimetry were employed to evaluate flow behavior, mechanical performance, and thermal transitions. The filament morphology was examined using scanning electron microscopy (SEM) to investigate the dispersion and interfacial compatibility of the chitosan phase within the PLA matrix. Furthermore, the potential for incorporating high metal content (Nickel) into the composite filaments was explored, with particular attention to maintaining adequate printability and structural integrity for additive manufacturing applications.

**RESULTS AND DISCUSSION:** The findings revealed that specific formulations of chitosan-PLA composite filaments allow for the successful incorporation and retention of nickel particles, thereby demonstrating their suitability as novel catalyst support materials. The composite filaments were processed via fused deposition modelling (FDM), a thermoplastic extrusion-based 3D printing technique, to fabricate test specimens with controlled geometries. Composite filaments were 3D-printed into geometries with a high surface area-to-volume ratio, tailored to enhance mass transfer and active site accessibility, thereby targeting potential applications as catalytic supports in heterogeneous reaction systems. Post-printing, the internal architecture, homogeneity, and potential porosity of the specimens were analyzed using micro-computed tomography (micro-CT), enabling non-destructive evaluation of the structural integrity and distribution of the embedded metal phase.

**CONCLUSION:** This study shows the innovative use of FDM for the fabrication of catalytic supports, offering enhanced design flexibility, high geometric precision, and the ability to tailor surface architecture for improved catalytic performance. It exemplifies a sustainable approach to material design by valorizing seafood industry wastes specifically chitosan extracted from shrimp shells and heads as a functional component in advanced composite systems. It contributes to the development of circular economy strategies by converting biowaste into high-performance materials with potential applications in heterogeneous catalysis and other environmentally relevant technologies.

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## **3D printed and microfibrillar nanostructured blue hydrogels for bio interfaces with potential in tissue regeneration**

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Extrusion 3D printing represents a versatile fabrication technology that uses inks to print three dimensional (3D) scaffolds promising personalised tissue regeneration by controlling architecture and functionalities. Electrospinning generates microfibrillar structures presenting porosity through fibers entanglement, beneficial for bone, cartilage engineering, or skin regeneration [1].

Tissue loss, caused by infection, resective therapy, or trauma, represents a significant challenge both clinically and societally. Bone is a highly complex tissue and the fabrication of bone grafts to regenerate large bone defects remains challenging. Scaffolds for bone regeneration are often inspired by the bone extracellular matrix (bECM). Natural origin hydrogel matrices are typically combined with a mineral phase [1;2]. Sustainable and blue biomaterials hold the promise to successfully assist tissue regeneration. Blue biotechnology uses marine bioresources for food and drug discovery, called blue materials, they include biopolymers such as fish collagen & gelatin. Moreover, to resemble filamentous proteins from bECM, such formulations are electrospun while to mimic the porosity of trabecular bone they are 3D printed. Nanoadditives are often used to reinforce or mimic the nanocomposite nature of bone. Typically, the effect of individual nanospecies is dogmatically investigated as rheological additive for improved printability, for bulk mechanical features and for overall cell-interactions. Interesting nanomaterial-cell interactions are reported but insufficiently explored or understood.

For the next bone fillers, it is important to tackle the potential of locally nanostructured microfibrillar structures to stimulate osteoblast response. The overall goal of this study is to develop platforms for bone regeneration including for the subchondral compartment, by converging 3D printing and electrospinning technologies, biomimetic nanostructured hydrogels, and bioactivity to replicating the complexity, specificity and function of native tissue. ECM-mimetic hydrogels, composite platforms, coatings and 3D printing inks have been widely engineered to match tissue properties and promote enhanced regeneration. We recently reported that calcium carbonate and biosilica two blue biomaterials, significantly enhance osteogenic response when enriching gelatin 3D printed and fibrillar structures [1;2]. In this study, a blue gelatin obtained from fish skin, is chosen as organic matrix for the incorporation of nanostructured blue mineral phase and for double-nanostructuring. The highly porous trabecular bone, including for the subchondral compartment will be extrusion 3D printed using biopolymers incorporating bioactive nanofilers (calcium carbonate and biosilica), and silver nanoparticles ensure antimicrobial activity. We developed bECM-inspired formulations for both electrospinning and 3D printing and then explored the effect of the nanoadditive content on the physico-chemical properties of the scaffolds and on the cellular response. The study aimed to support tissue growth and osteogenic differentiation. The physicochemical and mechanical properties of the scaffolds and the potential of the nanocomposites to support osteogenesis has been examined, including advanced microstructural analysis.

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## Thyroid Cancer Diagnosis and Treatment Challenges - The Place for AI Improvement

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Thyroid cancer is one of the most rapid growing forms of endocrine neoplasia. Genetic predispositions augmented by environmental factors such as high radiation exposure, Hashimoto's thyroiditis, endocrine disruptors and improvement in detection technology contribute to the growing rates in diagnosis of these cancer types. Thyroid imaging studies, fine needle aspiration biopsies and histological biomarkers availability for assessing cancer cell proliferation have lately emerged as useful tools in guiding diagnosis and treatment.

However, diagnosis work-up and personalized effective course of treatment for each patient and cancer subtype are time and resource consuming. The utilization of computer-aided diagnosis systems as radionics used in conjunction with ultrasonography imaging for analyzing thyroid images has seen a significant increase in use recently. These systems, have enhanced diagnostic accuracy and reduced operator-dependent eye-based image recognition of ultrasonography imaging, becoming cost-effective and practical diagnostic method in clinical practice.

Classifying tumors based on images and biomolecular data sets can train machine learning for improvement of the accuracy of thyroid carcinoma classification. Machine learning (ML) and deep learning (DL) may improve automating the classification of thyroid nodules in applications such as US, fine-needle aspiration (FNA) aiding in early detection and more effective treatment planning. (1).

AI can analyze complex medical data at a scale and precision beyond human capacity, improving early disease detection, accurate diagnoses, and personalized treatment planning in patients inflicted by thyroid cancer (2).

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## Crosslinked Possibilities: The Expanding World of Poly(2-isopropenyl-2-oxazoline)-Based Hydrogels

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Soft materials are ubiquitous in our daily life and are key players in many industries, such as electronics, automobiles, environment, and robot engineering. Hard and heavy materials used 50 years ago have been replaced by soft and light materials such as polymers, liquid crystals, gels or colloids. Moreover, a high percentage of our body is made of soft materials having complex structural and dynamic properties. Thus, much scientific effort has been devoted to developing highly functional soft materials. Among soft materials, hydrogels are a promising material platform, especially in view of their biomedical applications such as drug delivery, tissue engineering, ocular devices, and tissue and organ replacement. [1] Hydrogels can be synthesized from both natural and synthetic polymers via physical or chemical crosslinking, offering a broad design space. While a multitude of synthetic hydrogel materials strategies have been reported, they all come hand in hand with shortcomings that can limit their applicability. [2]

The rising interest in smart and adaptable materials has prompted extensive exploration of new polymer systems capable of enabling the design of advanced hydrogels. A major focus in this pursuit is the discovery of polymers that offer flexible functionalization, reliable crosslinking options, and tunable degradability, features that conventional hydrogel precursors often lack or possess only to a limited degree.

In this respect, poly(2-isopropenyl-2-oxazoline) (PiPOx), has attracted increased scientific attention as a reactive polymer for the synthesis of advanced functional materials. [3] PiPOx is a versatile polymer soluble in water and various organic solvents, can be prepared with well-defined characteristics, and exhibits high thermal and good hydrolytic stability. [4] Furthermore, PiPOx was shown to be biocompatible, rendering it suitable for medical and pharmaceutical applications. [5,6] The pendent 2-oxazoline group can be transformed in an efficient, mild, and selective manner, providing an extremely valuable toolbox for the synthesis of advanced materials. The post-polymerization modification reaction with (di)carboxylic acids enabled access to a wide variety of structures with defined and controlled properties. The versatility of this modification method allows the synthesis of a wide variety of functional polymers with tunable properties from soft to hard materials. [7-9]

Recent developments from our research group that illustrate the potential of PiPOx as smart (bio)materials will be discussed in this lecture, ranging from fundamental studies on ring opening addition of PiPOx with carboxylic acids to emerging applications of these polymers as biomaterials, nanosensors, and drug delivery vehicles.

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## **Nano-Structured Conducting Polymers and Their Nanocomposites: From Synthesis to Applications**

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Conducting polymers have demonstrated their important applications in electronics, energy conversion and storage, sensors and medical devices. In recent years, 2D-organic materials such as graphene even received great attractions among both industrial and science communities with their unique properties including electrical, mechanical, and thermal properties. However, the synthesis of 2D and 3D structures of conducting polymers is a still challenging issue from technical point. In addition, 2D and 3D structures of conducting polymers are expected to improve the device performance including polymer solar cell, batteries, supercapacitor, sensor, and medical applications. Therefore, in this research, we report a novel fabrication method of 2D-, and 3D- conducting polymers, and their nanocomposite, which is a cost-effective, environmental-benign and scalable synthesis technique. This method uses a bicontinuous emulsion reactor to produce 2D and 3D conducting polymer via interfacial polymerization technique. By changing reactor conditions we can modify morphology thus the physical properties of polymer. In this work we show effect of oxidant, solvent systems, and temperature on the polymer morphology. Various analytical tools including SEM, BET, XRD are used to characterize these changes. Both electrical and thermal conductivity properties will be reported.

## Advances in Polymeric Membrane Materials Synthesis for Hemodialysis and Osseointegration

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Since the beginning of their use in selective separation processes, polymeric membranes have proven their effectiveness in a wide range of applications. They became extensively used and studied after the Second World War, in Germany, where the newly founded Millipore company, through the Marshall Plan of the United States, transformed huge quantities of nitrocellulose (used as an explosive powder for bomb production) into microfiltration and ultrafiltration membranes [1]. Although the largest volume of membranes currently produced is dedicated to water filtration, the applications of these materials extend to the food, electronics, chemical and petrochemical industry. Because of these materials' remarkable properties, namely, selectivity, membranes are also used in a wide range of biomedical applications that require separations. Considering the fact that most organs (apart from the heart and brain) have separation processes associated with the physiological function (kidneys, lungs, intestines, stomach, etc.), technological solutions have been developed to replace the function of these organs with the help of polymer membranes [2].

Two membrane processes are essential at present, not for development, but for everyday life – desalination and hemodialysis. Another growing biomedical field for polymeric membranes is related to osseointegration – membranes that are usually used at the interface between bone and implant with primary role to facilitate the integration of implant into the bone. This presentation is focused on the latest developments in the field of membrane materials for hemodialysis and improved osseointegration. A short introduction to the field of membrane materials will open this fascinating journey, the main subject being the synthesis and applications of these materials in hemodialysis and osseointegration, as well as these processes combined with controlled drug delivery. Surface treatment or preparation of composite polymeric membranes, in vitro and in vivo tests, controlled release of antibiotics, anti-inflammatory or cytostatic drugs will be presented and discussed. Some future trends and actual scientific projects will end this presentation.

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# From Material Design to Biofabrication: Advancing Light-Based 3D Printing for Tissue engineering

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## INTRODUCTION

Tissue engineering increasingly relies on the rational design of biomaterials combined with advanced 3D printing techniques. Light-based strategies, including digital light processing (DLP) and volumetric additive manufacturing (VAM), enable the fabrication of complex scaffolds with tunable mechanical and biological properties. This work presents the development and processing of synthetic (acrylate-endcapped urethane-based precursors (AUPs)) and natural polymers (thiolated gelatin-gelatin norbornene (GelSH-GelNB)) using these techniques to create functional constructs serving tissue engineering.

## EXPERIMENTAL METHODS

AUPs were developed by varying the polymer backbone (poly(ethylene glycol) (PEG) versus poly(propylene glycol) (PPG)) and endcap chemistry (di- vs. hexa-acrylate), yielding UPEG2, UPEG6, UPPG2, and UPPG6. Digital light processing parameters were optimized for each material to fabricate tubular and porous structures for cartilage and vascular construct applications.

For biofabrication, photo-crosslinkable gelatin-based hydrogels (10% (w/v), GelSH-GelNB, degree of substitution ~60%) were used as bioinks in VAM, containing MSCs ( $1 \cdot 10^6$  cells·mL<sup>-1</sup>). Lineage-specific differentiation was assessed after 21 days using Alizarin Red S (osteogenesis), Alcian Blue (chondrogenesis), and Oil Red O (adipogenesis).

## RESULTS AND DISCUSSION

DLP-printed AUP scaffolds showed tunable properties via backbone and acrylate variation.<sup>1</sup> Increased acrylate content lowered swelling (to 18%) and raised stiffness (to 5.3 MPa). PPG-based formulations offered superior stability and printing accuracy. Tubular constructs for vascular applications reached elastic moduli between 45 and 259 kPa, closely matching those of native vessels.<sup>2</sup> Cubic, porous constructs demonstrated mechanical profiles suitable for cartilage TE, highlighting the versatility of these materials.

VAM-printed gelatin hydrogels showed storage moduli from 206 Pa to 12.5 kPa, depending on the formulation. Constructs printed with 10% (w/v) GelNB-GelSH had compressive strengths up to 508 kPa and moduli around 21 kPa. Biofabricated scaffolds supported MSC proliferation over 21 days. Stiffer VAM constructs favored osteogenesis, while softer hydrogels promoted chondrogenic and adipogenic differentiation, confirming the ability of scaffold mechanics to guide MSC fate.<sup>3</sup>

## CONCLUSION

Combining material design with advanced light-based fabrication allows the creation of complex scaffolds tailored for tissue engineering. Our results show that synthetic AUPs and natural gelatin hydrogels can be processed via DLP and VAM to meet mechanical, architectural, and biological demands. Volumetric bioprinting, in particular, represents a transformative step toward rapid, cell-friendly manufacturing for regenerative medicine.

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## Over two decades of graphene as biomaterial

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Graphene is regarded as one of the most debatable nanomaterials in modern scientific research, particular when it comes to biomedical applications due to the concerns arisen in regard to the graphene potential toxicity, particularly at higher concentrations (ranges of 50-300 mg/L). Conversely, understanding the potential relevance to risk of the graphene is much more complex particular in the hypothesis that engineered nanoparticles are dangerous until shown otherwise. One of the furthestmost challenges to use graphene in the biomedical field being impossibility to prove through research that is utterly safe.

Contemporary scientific discoveries demonstrated that graphene has a wide array of potential applications due to its unique properties in both technology and medicine i.e. drug delivery, biosensors, tissue engineering, antimicrobial coatings or conductive inks. Each of the aforementioned applications is exploiting different features of this outstanding nanomaterial demonstrating its versatility and potential to revolutionize multiple fields.

The high mechanical strength have made graphene also a promising material for scaffolds in bone tissue engineering and regenerative medicine. Later findings demonstrated that graphene improves the mechanical properties to an extent never seen before and also can induce well-defined new functionalities i.e. osteoconductive and osteoinductive properties which are well beyond the initial anticipation.

Later discovery of 3D printing fabrication technique open new avenues also in the field of bone tissue engineering however the development of inks capable of producing scaffold with high biocompatibility, mineralization capabilities, and adequate mechanical properties remains a significant challenge.

Once again graphene demonstrated to be an effective solution for the development of functional formulations for bone regeneration. Graphene based formulations are foreseen as very promising and able to help to go beyond the limitations of the 3D printing technique i.e. resolution or dimensional accuracy and thus related to bone regeneration i.e. need for osteoconductive and osteoinductive features.

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## **Disruption of the self-assembly pathway of hepatitis B virus capsid by antivirals**

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The hepatitis B virus (HBV) is a major health problem worldwide. Despite the existence of a safe and effective vaccine, more than 292 million people are chronically infected by HBV, mainly in Africa and South Asia, due to a poor vaccination rate. Capsid assembly modulators (CAM) are antiviral molecules that disrupt the formation of HBV capsids, some of them being currently in clinical trial. I will first review the self-assembly and disassembly pathways of HBV capsids under various ionic conditions investigated by time-resolved X-ray scattering and cryoelectron microscopy. Then, I will detail our recent findings about the effects of various CAMs on the morphology and assembly kinetics of HBV capsids. In particular, I will show that CAMs alter the elastic properties of capsids leading to either slightly elongated or aberrant shapes. Elucidating the mechanisms by which antivirals disrupt the capsid self-assembly pathways is not only essential to combat viral infection, but it can help design bio-inspired nanocapsules with controlled morphology for delivery applications.

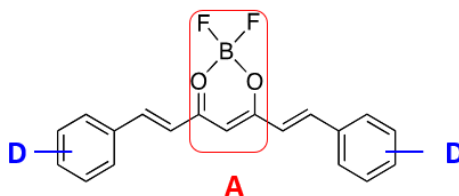
## New Dyes for Optoelectronics

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Borondifluoride complexes of 1,7-bis(aryl)-1,6-heptadiene-3,5-dione derivatives or curcuminoids are donor-acceptor-donor (D-A-D) dyes which show great promise in organic electronics. An appropriate functionalization of their phenyl groups leads to near infrared fluorescence emission in solution and in the solid state.<sup>i,ii</sup> We prepared BF<sub>2</sub>-curcuminoids with a large series of aromatic donor cycles, allowing efficient modulation of optical and electronical properties in solution.<sup>iii,iv</sup> These molecules have also been studied in the solid state (nanoparticles, single crystals, thin films) and high values of near-infrared fluorescence quantum yields were obtained.<sup>v</sup>

The advantage of these materials lies in their simple synthesis and low cost. When used as a donor material in combination with PC<sub>61</sub>BM in solution-processed bulk heterojunction organic solar cells they showed a remarkable photovoltaic performance considering the simplicity of the synthesis. The open circuit voltage over 1.0 V was achieved with moderated photovoltaic yield of 4 %.<sup>vi</sup>

Device properties were shown to depend on chromophore aggregation. In order to understand the properties of this class of materials in the solid state we synthesized a series of curcuminoid dimers covalently linked by a flexible chain. The results are discussed in the light of the data obtained for the D-A-D dyes in the solid state.<sup>vii</sup>



**Fig. 1** Chemical structure of the curcuminoid-BF<sub>2</sub> dyes featuring lateral electron donor (D) groups and the electron acceptor (A) dioxaborinine unit.

# ORAL PRESENTATIONS

## Efficient ASO extraction using graphene-based platforms for designing bioinspired 3D-printable scaffolds employed in Bone Tissue Engineering

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Bone tissue engineering (BTE) has emerged as a pivotal field in regenerative medicine, particularly for addressing bone defects and damage caused by diseases such as multiple myeloma, where traditional therapies often fail to fully restore the complex structure and function of damaged bone tissue. Recent advances in 3D printing technology have greatly impacted BTE by enabling the creation of custom scaffolds that replicate the natural structure and function of bone, providing a platform for cellular interactions that promote tissue growth and healing. Integrating antisense oligonucleotide (ASOs) into these scaffolds offers an exciting opportunity to further enhance bone regeneration, as ASOs, such as single-stranded deoxyribonucleic acid (ssDNA), play an important role in gene regulation, biomarker identification, and cellular signalling. However, the small size and susceptibility of oligonucleotides to degradation present challenges for their effective use.

To address these challenges, a novel approach was developed for effective oligonucleotide extraction, using a commercially available nitrocellulose (NC) membrane non-covalently modified with a combination of single-walled carbon nanotubes (SWCNTs) and polyethylene glycol (PEG)-aminated reduced graphene oxide (GA). The platform was evaluated for the extraction of a fluorescent labelled ssDNA, with fewer than 30 nucleotides, from complex solutions containing various ionic species ( $\text{MnCl}_2$ ,  $\text{MgCl}_2$ , and  $\text{MnCl}_2/\text{MgCl}_2$ ). Characterization techniques, including Fourier Transform Infrared Spectroscopy (FTIR), confirmed the successful modification, revealing characteristic peaks of NC, SWCNT, and GA. Raman Spectroscopy and X-ray Photoelectron Spectroscopy (XPS) showed distinctive changes after the membrane's interaction with divalent cations and ssDNA. Scanning Electron Microscopy (SEM) revealed morphological changes in the SWCNTs/GA-NC hybrid membrane, displaying a smoother surface compared to the porous structure of the unmodified NC membrane. Wettability assays indicated hydrophobic properties for the SWCNT/GA-NC hybrid membrane, with a water contact angle exceeding  $110^\circ$ , in contrast to the hydrophilic nature of the NC membrane. The membrane demonstrated optimal performance in ssDNA extraction in the presence of  $\text{MgCl}_2$ , achieving a recovery rate of 781 pg (approximately 16%) of total ssDNA, in contrast to the unmodified NC membrane. This demonstrates the superior efficacy of graphene-based platforms in enhancing ssDNA adsorption, highlighting their potential for adsorbing and delivering ASOs.

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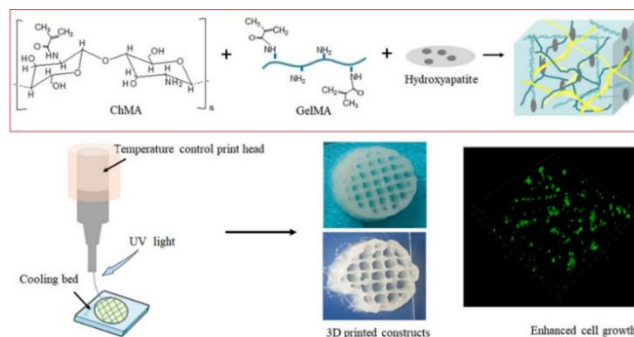
## 3D-Printable Thermo/Photo-Crosslinked Methacrylated Chitosan-Gelatin Hydrogel Composites for Tissue Engineering

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Biomimetic constructs imitating the functions, structures and compositions of normal tissues are of great importance for tissue repair and regeneration. Hence, there is an increase in the number of manufacturing technologies such as 3D bioprinting for rapid fabrication of biomimetic complex structured scaffolds. However, due to the lack of bioinks with suitable printability, high structural integrity, and biological compatibility, producing constructs that mimic the anisotropic 3D extracellular environments remains a challenge. Here we present a printable hydrogel ink based on methacrylate-modified chitosan (ChMA) and gelatin (GelMA) embedding nanohydroxyapatite (Hap). This polymer composite is first physically crosslinked by thermal gelation for post-printing structural stability, followed by covalent photo-crosslinking of ChMA and GelMA to form a long-term stable structure. The hydrogel inks showed sufficient shear thinning properties required for extrusion printing. By adjusting the content of GelMA, the swelling ratio of the printed constructs decreased, while the mechanical strength increased. Moreover, the formulated biomaterial inks exhibits biological characteristics that effectively support the spreading and proliferation of stem cells seeded on the scaffolds after 7 days of in vitro culture. Adding Hap has minor influences on the mechanical rigidity and cytocompatibility of the hydrogels compared with the group free of Hap. Together, 3D printed constructs represent promising candidates for tissue engineering application.



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## Metabolites Quantifiable by Magnetic Resonance in Spinal Cord Injury Implanted with Plasma-Synthesized Polymer in Vivo

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### INTRODUCTION

Traumatic spinal cord injury (TSCI) causes severe neurological disability, with major medical and socioeconomic repercussions.

The use of different treatments after SCI has been reported. Most therapeutic strategies after TSCI have not significantly recovered motor or sensory functions of the subjects.

We have reported that experimental subjects (ES) implanted with plasma synthesized materials containing amines in their composition (pP-NH), after TSCI, recover voluntary movements in their posterior joints.

In this work, pP-NH was implanted in an TSCI and ES were followed with magnetic resonance imaging (MRI) in vivo. Physicochemical changes were determined by in vivo quantification of metabolites in the evolution of motor recovery and MRI-derived DTI metrics.

### EXPERIMENTAL METHODS

The application of pP-NH in rats after SCI was analyzed by evaluating the preserved nervous tissue. Adult female Wistar rats with a body weight of 250-300 g, housed under standard conditions, were used as ES. All surgical and experimental procedures were carried out in accordance with the Regulations of the General Health Law for Research and Science, as well as with the Mexican Regulations for the Care and Management of Animals (NOM-062-ZOO-1999). All efforts have been made to minimize discomfort to the animals and reduce the number of animals used.

Twelve ES were used for this project. Of these, six were implanted with pP-NH particles in the lesion area, while the other six will be used as a control group.

### RESULTS AND DISCUSSION

DTI data were processed in DSI Studio. The FA, MD, AD and RD indices were obtained. The spectrum was processed in the scanner software, adjusting the curve for the detection of NAA, Cho and Cr.

DTI studies in the pP-NH group have shown that the anisotropy range is between 0.3 and 0.7. Thus, pP-NH could favor reconnection and axonal growth at the TSCI site. The AD values increased, which could favor tissue restructuring. And the RD values have remained stable, which could be favoring tissue remyelination. This could indicate that the fibers are aligned, and the correct regeneration of the nervous tissue is being favored.

The NAA was decreased in the spectrum, suggesting that after the recovery period of the TSCI, a significant loss of neurons or axons has occurred in the affected region. Cho, commonly associated with inflammatory or tissue repair processes, suggests that although there is neuronal loss, some cellular remodeling is occurring. The creatine (Cr) peak remains prominent. This suggests that the affected tissue still has a certain level of metabolic activity.

### CONCLUSION

Spectroscopy provides a useful tool to assess the metabolic status of nervous tissue affected by TSCI, allowing the identification of changes in markers such as NAA, Cho and Cr. An increase in the NAA/Cr ratio, together with a decrease in the Cho/NAA ratio, would indicate an improvement in neuronal integrity and a reduction in inflammation, suggesting that pP-NH implants are promoting neuronal regeneration and neuroprotection.

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## Tree-shelters made of recycled polypropylene: waste valorization and light transmission

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Proper agricultural plastic waste management is of utmost importance, due to the high quantities involved. Incorrect waste management leads to the accumulation of these plastic wastes in the environment, which results in environmental damage due to fires and the release of methane, microplastics and potentially toxic compounds derived from residues of additives and pesticides. In this context, mechanical recycling is the best option whenever possible, due to its simplicity, suitability to industrial scale, and low demand for raw materials and energy, which reduces the impact of plastic waste on the environment<sup>1</sup>. To promote the recycling of these plastic wastes, it is important to develop new applications. Herein this work it is proposed the use of polypropylene (PP) waste in the production of tree-shelters, commonly used in agriculture and reforestation. These tree-shelters increase the survival rate of seedlings by protecting against predators and generating a favourable microclimate. Nevertheless, most plastic waste shows unknown and diverse degradation and contamination, which affect negatively the quality of the final product<sup>2</sup>.

In this work, protective tubes containing various proportions of recycled polypropylene (rPP) (0, 50 and 100%) were manufactured at an industrial scale. A postindustrial clear rPP was used. A commercial additive, containing an antioxidant and a HALS, was used for UV stabilization. Light transmission was controlled by using a commercial brown dye. Manufactured shelters were used in plantations in four different locations in Spain. Polymer structure and degradation were characterized by using IR spectroscopy and Thermal Analysis. Light transmission of the shelters, both in the 400 to 700 nm wavelength range (Photosynthetically Active Radiation, PAR) and in the UV region, is a vital parameter for the growth and survival rates of the plants<sup>3</sup>. In this work, the light transmission was measured by using UV-Vis spectroscopy before and after accelerated aging tests and after 1-year outdoor exposure.

The results show that valorization of PP waste in tree-shelters is feasible since including this material in its production does not show a notorious effect on the material properties. The use of clear rPP does not reduce light transmission, which remains around 65 % in the PAR spectral region. By using dyes, light transmission in tubes made of clear rPP can be controlled, to adapt it to the needs of different plants in different climates. Aging and outdoor exposure tests evidenced UV-induced degradation of the tree-shelters, concentrated in the external face. Light transmission decreased during aging tests, but this effect is not more important in shelters made with rPP. Moreover, the decrease is moderate and aged tubes still show competitive transmission rates. Thus, PP waste can be used in high-performance tree-shelters for afforestation and agriculture.

### ACKNOWLEDGEMENTS

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## CBD-loaded deformable lipid vesicles for enhanced antimicrobial therapy against *S. epidermidis*

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### INTRODUCTION

*S. epidermidis*, traditionally considered a harmless skin commensal, is now recognized as an emerging opportunistic pathogen and a significant reservoir of antibiotic-resistance genes at the epithelial level. It is increasingly implicated in nosocomial infections, often displaying greater antibiotic resistance than *S. aureus* (1). Erythromycin, a macrolide antibiotic, is commonly used to treat *S. epidermidis* infections due to its ability to inhibit bacterial protein synthesis. However, rising resistance to erythromycin has become a concern, highlighting the need for alternative or adjunctive therapies. In this context, transdermal cannabidiol (CBD) delivery presents a promising antimicrobial strategy. CBD demonstrates broad-spectrum activity, including efficacy against resistant strains (2). Its potential to enhance the effects of existing antibiotics, such as erythromycin, and its suitability for topical or transdermal applications position CBD as a valuable option for combating *S. epidermidis*-related infections and antibiotic resistance.

### EXPERIMENTAL METHODS

Nanolipidic transthesosomes containing CBD and erythromycin (NT-CBD-E) were prepared using mechanical dispersion and size controlled through sonication and filtration. Characterization included size, polydispersity index, and zeta potential via DLS, with structural insights from TEM and X-ray diffraction. Entrapment efficiency and drug release were analyzed using UV-Vis and GC-MS. Biocompatibility was assessed on human dermal fibroblasts and keratinocytes through resazurin assays. Antimicrobial activity was determined by minimum inhibitory concentrations (MIC) and fractional inhibitory concentration indices (FICI), assessing synergism or antagonism. Flow cytometry was used to evaluate NT-CBD-E's mechanism of action.

### RESULTS AND DISCUSSION

The encapsulation efficiency of CBD-ERY exceeded 80%, indicating effective drug loading. TEM and XRD analysis confirmed the structure and crystalline nature of the NT-CBD-E system. The hydrodynamic diameter of the loaded NT was measured at  $255 \pm 15$  nm, with a PDI of 0.33, demonstrating an optimal polydispersed size distribution. The Zeta potential values were approximately -44 mV, suggesting good colloidal stability. Biocompatibility rates surpassed 90%, with minimal cytotoxic effects observed. Flow cytometry analysis suggested that the primary mechanisms of action involved cell wall permeabilization and inhibition of efflux pump activity, contributing to the antimicrobial efficacy of NT-CBD-E.

### CONCLUSIONS

A novel transdermal drug delivery system, NT-CBD-E, was successfully developed. The system showed strong antimicrobial and anti-inflammatory potential, with proposed mechanisms of action including bacterial cell wall permeabilization and efflux pump inhibition. These findings highlight its promise as an innovative strategy for treating wound infections while reducing antibiotic concentrations. The synergistic interaction between CBD and erythromycin enhances antimicrobial efficacy, potentially overcoming resistance and improving treatment outcomes. This approach offers a compelling alternative for managing *S. epidermidis*-associated infections and antibiotic resistance in clinical settings.

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## Development of gelatin bioconjugates with cell adhesive peptides

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The skin, the largest organ of the body, is constantly exposed to external factors, making it susceptible to diseases and damage. Due to this vulnerability, it is essential that scientific researchers focus on the protection and treatment of dermatological problems. Regarding the diagnosis, treatment and monitoring of skin conditions caused by epithelial tissue trauma (acne, chemical, thermal and electrical burns or wounds), significant progress has been made to improve people's quality of life and social engagement. The development of skin substitutes able to stimulate enhanced healing and tissue regeneration represent an appealing approach. In this context, researchers have focused on the use of cell adhesion molecules to improve the cellular response leading to enhanced tissue-graft interactions.

Gelatin has been investigated for a broad spectrum of biomedical applications, including use in wound dressings, drug delivery, cell culture, tissue engineering, and as an additive in pharmaceutical formulations. Due to its collagen-derived origins, this material is particularly relevant for tissue reconstruction and regeneration and has been extensively studied in its native form. Recent research is also investigating its potential when combined with or interacting with bioactive molecules, such as cell adhesion peptides containing the Arg-Gly-Asp (RGD) sequence<sup>1</sup>. Moreover, its methacrylamidated form, emerged as an appealing platform for tissue engineering and regeneration generating hydrogels through the polymerization of synthetic C=C bonds.

In this work, the main objective was to identify an efficient protocol for the bioconjugation of thiol-ended peptides with gelatin. Thus, we synthesized methacryloyl gelatin (GelMA), which was bioconjugated by the reaction of primary amine groups with the heterobifunctional conjugation reagent N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) that introduces thiol-reactive moieties into the macromolecular side chain. The reaction products were characterized in terms of substitution degree further available for subsequent coupling with terminal peptides containing thiol groups. The coupling efficiency of the gelatin-SPDP intermediate was preliminarily evaluated using glutathione (GSH) as a model peptide, as previously reported<sup>2</sup> and the conjugation was successfully achieved by thiol-disulfide exchange between the reactive moiety of the intermediate and the thiol group of glutathione.

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## **Supporting Cancer Research from Immuno-therapy to Nano drug delivery via hyperspectral microscopy**

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Advancing studies for cancer diagnosis or drug delivery requires that researchers can easily observe and measure nanoparticles interacting with in-vitro cells or ex-vivo tissue environments. Also, new cancer pathology research is focusing on label free imaging and spectral analysis of cells and tissue. CytoViva, Inc. provides enhanced darkfield hyperspectral microscopy system specifically designed for label free imaging and analysis of nanoparticles along in cells and tissue. This system includes patented enhanced darkfield optics that enable observation of nanoparticles as small as 10nm when isolated in solution and in cells and tissue. The hyperspectral imaging capability enables spectral characterization and spectral mapping of nanoparticles or their drug load in cells and tissue. Hyperspectral imaging can also differentiate between cancerous and healthy cells and tissue without any labeling or staining. This presentation will provide a detailed overview of this nanoscale optical imaging and spectral measurement capability. Specific illustrations of nanoparticles interacting with cells and tissue along with label-free cell and tissue characterization will be presented.

## Challenges in upscaling an immunomodulator formulation: from laboratory to pilot scale

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### INTRODUCTION

Scale-up processes from laboratory level to pilot level are vital to ensure effective knowledge and/or technology transfer. Several difficulties may be encountered during upscaling related to feasibility of technology transfer, product synthesis steps and product characteristics, quality control steps and shelf-life issues.

### RESULTS AND DISCUSSION

A polybacterial mixture from Gram-negative and Gram-positive strains was obtained, thermally inactivated, chemically lysed and nano-structured to allow sterilization via filtration. This study allowed the fermentation process upscaling to medium-volume InforsHT bioreactors, concentration step using a continuous in flux centrifuge, bacterial suspension processing in a high-volume microfluidizer, formulation of medium size batches and obtaining of final products via semi-automated means. The immunomodulatory effect was evaluated by in vitro tests on HEK293 cells transfected with Toll-like receptor 2 or 4 genes (TLR2, TLR4). DLS and FF-MALS methods were applied to characterize the bacterial suspension as such and after formulation. An organoleptic study was performed for the proposed formulation, together with formulation stability studies under normal and accelerated conditions.

### CONCLUSION

Within a multiannual project was possible to upscale to a pilot scale the formulation and fermentation processes of an immunomodulator. The information obtained is valuable considering on one hand the excessive use of antibiotics, antibiotic resistance and the need to stimulate the innate human system against diseases.

### ACKNOWLEDGMENT

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## 3D Printing of Nanostructured Gellan/Gelatin Inks Reinforced with Graphene Derivatives for Bone Tissue Engineering

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Recent advances in fabrication technologies, with a particular emphasis on 3D printing, have enabled the development of bone tissue substitutes tailored for personalized patient solutions. Despite technological progress, printable formulations derived from natural polymers typically exhibit limitations in terms of mechanical properties, structural integrity, and stimulation of osteogenic activity.

To address these challenges, the present study describes the fabrication of innovative inks employing a gellan gum/gelatin polymeric mixture, enhanced with different reinforcement agents, including cellulose nanofibrils (CNF) and various functionalized graphene derivatives such as aminated reduced graphene oxide (NH<sub>2</sub>-rGO), carboxylate graphene oxide (COOH-GO), and a combination of both. Using the aforementioned formulations for 3D printing, scaffolds for bone tissue engineering were successfully created with high shape fidelity. Following 3D printing, all the structures were subjected to a double cross-linking procedure, initially in calcium chloride and then in a genipin solution. Then, samples were freeze-dried in order to be evaluated from a structural, morphological, mechanical and biological point of view.

Results indicated that scaffold reinforced with NH<sub>2</sub>-rGO present enhanced biological properties, while the ones reinforced with COOH-GO exhibit improved tensile and compressive strength. However, structures comprising both types of graphene derivatives (i.e. NH<sub>2</sub>-rGO and COOH-GO) demonstrated an overall superior performance with highest printability, optimal porosity, adequate degradation rate and swelling kinetics, improved compressive and tensile strength, biocompatibility and highest mineralization capacity. Our findings indicate that this formulation presents a synergistic effect, wherein COOH-GO can significantly enhance the mechanical strength, while NH<sub>2</sub>-rGO contributes to improved biocompatibility, therefore providing the most promising candidate for bone tissue engineering through 3D printing.

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## Foams with semi-interpenetrated networks of chitosan and organosilicate

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### INTRODUCTION

Clean water free of toxic chemicals and pathogens is essential to human health. Water also affects energy and food production, and the quality of our environment, affecting the economies of both developing and industrialized nations. Problems with water are expected to grow in the coming decades, even in regions currently considered water-rich. Addressing these problems calls for a tremendous amount of research to be conducted to identify robust new methods of purifying water at lower cost, while at the same time minimizing the use of chemicals and impact on the environment. So in the last decades, many researchers focused on developing new materials for water purification, by implementing natural polymers such as chitosan, alginate, etc. [1-2].

Chitosan is a natural polymer produced by chitin diacetylation. It has been used in foam preparation and has generated a lot of research attention due to its inexpensive cost, environmental friendliness, alkali stability, biodegradability, and high mechanical properties. According to some studies, chitosan has a major advantage over other materials due to its exceptional capacity to generate hydrogels/cryogels/xerogels in various forms, including foams [3-4]. A common method for producing artificial silicate networks is the sol-gel method, in which case tertaethoxy silane (TEOS) is the most used precursor. By combining the benefits of using a natural compound such as chitosan with sol-gel, semi-interpenetrated foams may be developed with outstanding properties in water purification [4-5].

### MATERIALS AND METHODS

For preparing the semi-interpenetrated network (semi-IPN) foams, commercial chitosan (CC) and two silane monomers (TEOS and mercaptopropyl trimethoxy silane (MPTMS)) were used. The sol-gel network was formed in acid catalysis using hydrochloric acid (0,1 N). Three series of semi-interpenetrated foams with various ratios of chitosan were prepared.

### RESULTS

Several characterization techniques (i.e., FTIR, SEM, stability in water/swelling and compression) were used to characterize the semi-IPNs. The FTIR spectra confirmed the formation of silanol bonds and the presence of chitosan in all foams. SEM images confirmed the formation of homogenous morphologies and porous networks. The swelling degrees (SDs) were studied at three different pH values (4, 7 and 9) to investigate the stability of semi-IPNs in various conditions, while compression tests provided significant information about the resistance of foams to pressure.

### CONCLUSIONS

New semi-IPN foams based on chitosan and silicate were developed. These materials show outstanding properties in terms of structure, morphology, swelling capacity, and compressive strength, making them potential candidates for future applications in water purification.

### ACKNOWLEDGEMENTS

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## **3D-Printed Gellan Gum/Cellulose Nanofibrils Scaffolds Reinforced with Chitosan/Polyethylene Oxide Electrospun Nanofibers for Bone Tissue Engineering**

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The increasing incidence of bone diseases in an aging population highlights the pressing need for novel and clinically applicable strategies for bone tissue engineering. Additive manufacturing technology has distinguished itself in this field by its potential to offer customized implants, specific to the needs of each patient. In this work, we propose a solution based on 3D-printed scaffolds using gellan gum (GG) and cellulose nanofibrils (CNF), reinforced with chitosan/polyethylene oxide electrospun nanofibers (ChNFs), to meet the structural integrity and biofunctionality required in bone regeneration. The printable formulation was optimized first by choosing the appropriate GG:CNF ratio between 2:1, 3:1, and 4:1 after characterizing the inks regarding their rheology and printability, and the resulted scaffolds in terms of structure and morphology by Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM), respectively. The optimal GG:CNF ratio was determined as 3:1 and this formulation was further reinforced with ChNFs to study the influence of concentration (0.5, 1, 2, and 4 %). Printability and rheological properties were determined for each ink and the subsequent scaffolds were analyzed by SEM and micro-computed tomography ( $\mu$ -CT) to characterize their surface and porosity, highlighting pore size and distribution, the homogeneity of the printed structures, and the interaction between materials. The stability and swelling behaviour of the scaffolds were also studied, with results indicating that the addition of ChNFs altered the internal architecture of the structures, improving the stability and swelling kinetics, especially with 1 % and 2 % ChNFs. In vitro analysis performed by MTT and LDH assays confirmed the biocompatibility of the scaffolds, while Alizarin Red S staining indicated enhanced mineralization potential. Based on the experimental results, the scaffolds fabricated by extrusion 3D printing with an optimized ink based on GG, CNF, and ChNFs, represent a promising solution for personalized implants in bone tissue engineering.

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## Scaling up the prototypes of sensors based on piezoresistance of filled rubber composites, origin of nonlinear signals.

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### INTRODUCTION

Traditionally, the electrical properties of rubber compounds have been investigated in the context of antistatic or insulating applications. However, interest has gradually shifted to, strain gauge sensors, sensors for supporting mobility, self-monitoring the behavior of filler network, fatigue life prediction [1] and the like, commonly based on piezoresistive effect in rubbers filled with various conductive fillers. Unfortunately, the possibilities of scaling up the developed prototypes as well as the corresponding nonlinearities in piezoresistive signal [2] were not explored.

### EXPERIMENTAL METHODS

The straining of the planar specimen always divides the sample into three regions: namely a region of pure shear (PS) and two symmetric regions of lateral contraction. (LC).

Therefore, in the PS region, the distances between the individual particles, aggregates and agglomerates composing the conductive fillers increase in the direction of the applied strain without any significant perpendicular movement. In the edge regions, the deformation consists of tension in the stretching direction and contraction in the perpendicular direction. With respect to such different deformation, where the size of the regions depends on scaling up preferences and can be unambiguously determined by Digital Image Correlation (DIC), a unique tool to analyze the electrical properties of the CB reinforced rubber through differential filler dynamics has been devised.

### RESULTS AND DISCUSSION

The piezoresistive response showed distinct samples size dependent nonlinear behaviors, where resistance surprisingly decreased during strain and increased during strain recovery. Resistance reversal occurred at strain stage, had lower amplitude for wider samples. During recovery, all samples exhibited the shoulder peak phenomenon (SPP), with lower peak values in larger samples. Additionally, the timing of the SPP peak shifted, highlighting differences in relaxation dynamics.

The ratio between LC and PS regions differ considerably for different samples sizes, and plays a crucial role in shaping nonlinear piezoresistive signals, with a wider LC region leading to higher amplitude responses. The results consistently demonstrated greater nonlinearity during recovery compared to stretching, suggesting distinct filler movement trajectories in each phase of deformation of samples, as confirmed by DIC technology.

### CONCLUSIONS

This study examined the scaling effects on the piezoresistive behavior of CB filled rubber, highlighting the influence of aspect ratio of samples on electric signal amplitude due to LC and PS region distribution.

Piezoresistance was influenced by two key size-dependent mechanisms:

- Filler dynamics evolving differently in LC and PS regions at the microlevel.
- At quantum-level resistance shifts due to CB aggregates responding to local hydrodynamic pressure.

These findings offer a comprehensive explanation for the nonlinear piezoresistive effects observed in the deformed samples.

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## Hydration and drug release mechanisms from 3D printed pharmaceutical dosage forms studied in situ using magnetic resonance imaging and relaxometry

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The future of medicine will likely turn to personalized medicine to tailor treatment to individual needs. The 3D-printing techniques seem to be a promising technology for manufacturing controlled-release drug delivery systems (printlets). Over the last few years, a rapid increase in interest in 3D printing in the pharmaceutical field has been observed<sup>1</sup>, especially in technologies such as selective laser sintering (SLS) and the 3D Vat Polymerization. The use of 3D-printing in pharmacies could enable the production of patient-tailored batches of dosage forms with different dosing and release characteristics<sup>2,3</sup>.

Pharmaceutical matrices containing various proportions of PEGDA/PEG and additives known from tableting with a low dose of ropinirole or metronidazole (Met) as the active substance were prepared by vat photopolymerization. High-dose tablets were prepared using the SLS method (with Met as API). Printlets were manufactured as a composite material consisting of elastic insoluble PA12 mesh filled with a high content (i.e. approx. 600 mg) of crystalline Met. Good mechanical properties were obtained. The printlets had hardness above 40 N comparable with compressed tablets.

The printlets were characterized using SEM, DSC and IR spectroscopy. Functional properties of the printlets were evaluated, i.e., drug release in USP 3 and USP 4 apparatus, together with floatation assessment (250 mL of 0.01 M HCl pH 2.0 was used as a dissolution medium). Magnetic resonance methods were used to assess the spatiotemporal characteristics of pharmaceutical matrices in situ.

Based on the magnetic resonance imaging (MRI) results, it is possible to determine the mobility of proton groups, and thus indirectly, the hardness of the sample or the way of binding water to the sample. The bidirectional mass transport of mobile phases in 3D-printed matrices was assessed. It was found that the observed phenomena are related to functional and pharmaceutical properties. It has been shown that using H<sub>2</sub>O and D<sub>2</sub>O as hydration (dissolution) media, it is possible to assess phenomena related to the evolution of the material originally contained in the matrix. The T<sub>1</sub>-T<sub>2</sub> maps obtained using LF TD NMR allows separation of various proton pools. The values of both T<sub>1</sub> and T<sub>2</sub> relaxation times give knowledge about the dynamics of nuclei and thus about the mobility of molecules containing these nuclei. Understanding the physicochemical processes occurring during the printing process and drug release from the pharmaceutical products can help to design them consciously. MRI methods allow for the assessment of mass transport phenomena at the molecular and macro levels, without disturbing the processes occurring inside the material<sup>4,5</sup>.

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## **Biocompatible microneedles patch for antiinflammatory transdermal drug delivery**

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It is acknowledged that oral drug administration even though straightforward suffers from low bioavailability (1), and that the human skin represents a selective and strong barrier to chemical permeation (2). Reducing the barrier-related limitations and the traditional hypodermic needles drawbacks (pain, bleeding and required professional assistance) was possible because of microneedles (MNs) as strong alternative (3). However, some are biohazardous (metal), must have their backing layers attached to the skin until complete drug release (dissolvable), have limited capacity, reduced skin perforation capability due to drug coating, or a risk of clogging and infection when repeatedly applied (coated microneedles) (4). In this work, we report a 3D printed drug coated Polyethylene Glycol diacrylate microneedles patch. Microscopic evaluation showed that (a) the patch of drug loaded sharp MN applied on the test skin (porcine) effectively pierced the stratum corneum, (b) the MNs support allowed intimate adherence to the uneven surface of the skin sample, while the MNs bodies remained inserted into the superficial epidermis for drug release. The materials the MNs were made of are biocompatible and do not cause any irritable side effects to the skin upon their application. This combination of drug loading could be one option for acute and slow drug release, thus reducing the need for repeated administrations. Therefore, it is expected to permit a new convenient, safe and self-administrated therapeutic modality with controlled release, rapid and slow, for treating various local and systemic medical conditions. The method showed simple, safe, user friendly and cost efficient.

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# Microbiome-Based Liquid Biopsy and Tumor-Associated Biomarkers in Colorectal Cancer: A Metagenomic and Functional Pathway Analysis

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## INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide, and early detection remains critical for improving patient outcomes. Emerging evidence suggests that the gut microbiome plays a significant role in CRC pathogenesis, with specific microbial taxa serving as potential biomarkers for disease detection and prognosis. Microbiome-based liquid biopsy approaches offer a promising non-invasive strategy for CRC screening, leveraging microbial signatures present in stool or circulating microbial DNA (cmDNA).

## METHODS

In this study, we performed 16S rRNA-based microbiome profiling on tumor and normal tissue samples from CRC patients. Taxa abundance analysis identified differentially enriched microbial species, while KEGG pathway enrichment analysis provided insights into functional metabolic alterations. Statistical comparisons, including log2 fold change analysis and hierarchical clustering, were used to highlight microbial biomarkers and their associated metabolic pathways.

## RESULTS

Fusobacteriaceae, a well-known CRC-associated taxon, was significantly enriched in tumor samples (Log2FC = 1.22) and linked to biofilm formation, tumor immune evasion, and inflammatory responses. Sphingobacteriaceae and Sutterella were found to be elevated in tumors, suggesting potential involvement in intestinal barrier dysfunction and lipid metabolism, key factors in CRC progression. Clostridiales Family XI, which participates in secondary bile acid metabolism, was increased in tumors, reinforcing its association with carcinogenic activity in the gut microbiome. In contrast, beneficial SCFA-producing taxa (*Eubacterium*, *Roseburia*, *Ruminococcus*) were reduced in tumors, indicating potential microbiome dysbiosis and metabolic imbalance. These findings highlight the potential of gut microbiome signatures as non-invasive biomarkers for CRC detection through liquid biopsy. The presence of circulating microbial DNA (cmDNA) from *Fusobacterium*, *Sutterella*, and *Clostridiales* Family XI in blood or stool samples may serve as diagnostic indicators, complementing conventional CRC screening methods. Furthermore, the functional shift from SCFA production to pro-inflammatory and tumor-associated metabolic pathways suggests a mechanistic link between gut dysbiosis and colorectal carcinogenesis.

## CONCLUSIONS

This study underscores the diagnostic and prognostic potential of microbiome-based liquid biopsy approaches in colorectal cancer. The identification of tumor-specific microbial biomarkers and pathway alterations provides a foundation for developing non-invasive CRC screening tests and potential therapeutic interventions targeting the gut microbiota. Future research should focus on validating these microbial signatures in large-scale clinical cohorts and integrating microbiome data with host genetic and metabolic markers for enhanced precision oncology applications.

## ACKNOWLEDGEMENTS

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## Electrical Bacterial Lysis: Impedimetric and Thermal Control

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Cell lysis is a crucial step in microbiology and molecular biology, enabling access to intracellular components such as DNA, RNA, and proteins while maintaining their structural integrity for downstream applications. Various lysis techniques have been developed over time, including chemical, mechanical, enzymatic, thermal, and electrical methods, each with specific advantages and limitations (Wang et al., 2007). The increasing demand for rapid, reagent-free, and scalable lysis methods has driven the development of alternative techniques, particularly for Point-of-Care (PoC) diagnostic applications, where sample preparation must be simplified and automated (Lange et al., 2016).

In this study, we introduce an on-chip bacterial lysis device that utilizes an electrical field with impedimetric and thermal control to achieve efficient, high-quality bacterial lysis. The device consists of Cr/Au interdigitated electrodes microfabricated on a glass substrate, with a 3D-printed polymeric chamber securely attached to create a confined lysis environment. The electrical field interacts with the negatively charged bacterial membrane, altering the transmembrane potential, destabilizing the cell envelope, and inducing the formation of transient pores. This process ultimately leads to complete cell disruption, releasing intracellular components into the surrounding medium.

A key parameter in optimizing this process is the electrode spacing, which allows for bacterial lysis at relatively low voltages (1V). To evaluate lysis efficiency, we tested the device using mainly *E. coli*, a Gram-negative bacterium with a structurally complex outer membrane that typically requires efficient and non-destructive lysis methods. Optical and impedimetric measurements were conducted to assess membrane integrity before and after electrical stimulation, confirming successful lysis.

Beyond achieving efficient cell disruption, an essential aspect of our study was to preserve the quality of extracted DNA. To verify this, we measured the effect of thermal lysis that can occur when the electrodes are reaching a very high temperature that could otherwise accelerate nucleic acid denaturation.

The ability to perform efficient, reagent-free bacterial lysis while preserving DNA integrity has broad implications for molecular biology, clinical diagnostics, and PoC testing. This method eliminates the need for chemical reagents, reducing sample preparation time and complexity. Moreover, its compatibility with miniaturized, automated platforms makes it highly suitable for integration into lab-on-a-chip systems for rapid, on-site diagnostics. In conclusion, our study demonstrates that electrical bacterial lysis, coupled with impedimetric and thermal control, provides a rapid, non-invasive, and high-yield method for bacterial disruption. By ensuring the preservation of high-quality DNA, this approach enhances the reliability of nucleic acid-based detection methods, paving the way for improved molecular workflows in clinical, environmental, and research applications.

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## Next-Generation Chitosan-Based Materials for High-Efficiency Drinking Water Treatment

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Water is the most abundant and indispensable resource on Earth. However, as is already well known, the distribution of freshwater differs greatly from region to region. The demand for innovative materials with enhanced capabilities for advanced source water treatment is increasing globally, irrespective of socio-economic development status. In short, adsorption processes have come to the forefront lately, due to low processing and maintenance costs and high treatment efficiency, thus being one of the most widely used techniques for the removal of impurities from water. It is essential to use low environmental impact, economically viable adsorbents, with a focus on environmentally friendly, accessible and economical adsorbents such as clays, biopolymers obtained from algae (alginate) or crustacean waste (chitosan), sawdust, eggshells, etc [1,2].

Natural polymers are commonly used as adsorbents because they are biodegradable and biocompatible. Waste from other industries can be used to produce them, thus reducing production costs compared to synthetic polymers. One of the most widely used biopolymers as an adsorbent is chitosan because it has several specific characteristics such as biocompatibility, biodegradability, heavy metal retention capacity, antimicrobial properties, and antioxidant behavior. However, chitosan also has disadvantages such as low thermal and mechanical resistance. These limitations can be removed by adding inorganic fillers such as zeolites. The use of zeolite also increases the retention capacity due to its adsorbent properties [3,4].

The main aim of the study is to obtain a new generation of adsorbent materials based on chitosan and zeolite for advanced drinking water purification. A study was carried out to observe the influence of the amount of zeolite on the adsorption capacity of the materials. The obtained adsorbent materials were also characterized from several points of view to observe their behavior and adsorption capacity. The rheological behavior of the solutions and their ability to adsorb water was determined. From a physicochemical and morphological point of view, the materials were characterized using Fourier-Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) techniques. The materials were also tested to evaluate their capacity to retain contaminants in drinking water.

The study showed that the new-generation materials show promising results for the large-scale use of these materials for advanced drinking water treatment.

### ACKNOWLEDGEMENTS

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## Plasma treatment of polymers intended for 3D printing as green method to enhance surface adhesion

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### INTRODUCTION

Polymer surface modifications are important for biomedical applications because they increase adhesion, chemical properties, wetting behavior, and interactions with biological fluids and the body. Due to their flexibility, atmospheric pressure plasmas are frequently used in biomaterial processing [1-3].

### EXPERIMENTAL METHODS

This research employs electrical diagnosis and optical emission spectroscopy to characterize a dielectric barrier atmospheric pressure plasma jet source running in helium and argon. The discharge apparatus consists of a quartz tube and two copper tape electrodes, through which He or Ar was utilized at a flow rate of 2 standard liters per minute. A high-voltage alternating current power supply initiated the discharge. Three commercial polymeric filaments for 3D printing: polylactic acid, acrylonitrile butadiene styrene and polyethylene terephthalate, were plasma exposed for 180 s. Studies on alterations in surface characteristics after plasma treatment encompassed various techniques, such as atomic scale microscopy (AFM & SEM), spectroscopy (FTIR, XPS, BDS), diffraction (XRD), and static water contact angle (SWCA) assessments.

### RESULTS AND DISCUSSION

At around 48 kHz and up to 10 W mean power, the applied voltage on the discharge electrodes was roughly 10 kVpp, current intensity up to 15 mA, and an estimated average plasma energy of about 30 mJ. The global emission spectra, in the UV-Vis range contains He and Ar lines, along reactive O<sub>2</sub> and N<sub>2</sub> species (RONS), involved in surface modifications. After plasma treatment, the hydrophilic effect of the polymer surface went up by up to 50% for PLA and 70% for ABS. This means that the water adhesion work, Wa, rises up from 71 mN/m (the initial value) to 131 mN/m for plasma-treated PETG samples, from 91 mN/m to 127 mN/m for PLA, and from 68 mN/m to 139 mN/m for ABS. Also, morphology studies using AFM and SEM showed that plasma-treated polymers had a smoother surface with about 20% less root mean square roughness. The tensile strength of the polymers after being exposed to plasma before the 3D printing process showed that the plasma-treated polymers were under more strain than the untreated ones. XRD diffraction and ATR-FTIR spectroscopy were also used on polymer samples to show that plasma contact was helpful.

### CONCLUSION

A correlation of plasma parameters, treatment time, morphological and chemical modification of plasma exposed materials was performed. The electrical and optical diagnosis of the studied plasma sources reveals the favorable operational parameters for proper surface treatment. Following studies on the morphology of surfaces, as a result of plasma treatments, the surfaces are much smoothed, with evenly distributed nanometric formations. The experimental results highlight that the plasma treatment effectively modifies polymeric materials in the sense of improved surface adhesion, suggesting high interlayer bonding, supporting further possible applications in the field of biomedicine, such as coatings for prosthetic devices.

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## Lipid and Protein Nanocarriers for New Generation Vaccine Development

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### INTRODUCTION

As new innovative vaccines, such as mRNA vaccines or subunit vaccines, are being developed as a response to global pandemic threats, there is also a need for bioinspired efficient delivery systems. This work presents two promising technologies for new generation vaccine nanocarriers: charged lipid nanoparticles for mRNA delivery and temperature sensitive elastin-like polypeptide based nanoparticles for antigen delivery.

### EXPERIMENTAL METHODS

Lipid nanoparticles (LNPs) were prepared by mixing an aqueous phase containing the buffering system and surfactants with an oil phase containing squalene and ionizable lipids. The two phases were processed using a LM20 Microfluidizer Processor with a Y-type interaction chamber. After 25 passages at 30000 psi, the emulsion was sterile filtered and stored at 4°C. Particle size and zeta potential was measured using a Malvern Zetasizer Nano. These parameters were measured for nine months to assess emulsion stability. RNA uptake by prepared LNPs was assessed by monitoring size and zeta-potential upon addition of increasing amounts of RNA. Also, RNase protection assay was used to evaluate de capacity of lipid nanoparticles to protect bound RNA.

Elastin-like nanoparticles (ELP) were generated by means of recombinant protein expression in *E. coli*. Two ELPs, (VPGVG)<sub>96</sub> (V96), (VPGSG)<sub>48</sub>(VPGIG)<sub>48</sub> (S48I48), as well as their Green Fluorescent Protein (GFP) fusion partners variants, were produced in BL21(DE3)pLysS *E. coli* strain. Purification was performed using Inverse Transition Cycling (ITC). Presence of expressed proteins and successful purification was checked using SDS-PAGE. Purified proteins were subjected to differential scanning calorimetry (DSC), differential scanning fluorimetry (DSF) and particle size measurements.

### RESULTS AND DISCUSSION

Several LNP formulations were employed to explore the effect of different surfactants (Pluronic F68, Tween20, Sorbitan monostearate) and of different lipids (DOTAP, phosphatidylcholine, glyceryl trimyristate) on particle size, zeta—potential and stability. As a rule of thumb higher lipid concentration and Tween20 had a positive effect on emulsion stability as evaluated by measuring particle size and zeta-potential. Synthesized LNPs were able to bind RNA at a concentration of 50ug/mL, while RNA binding to LNPs hindered nuclease activity of RNase A.

ELPs and their GFP fusion counterparts were successfully produced in *E. coli*. Lysis of bacteria and several rounds of ITC yielded purified proteins without the need of laborious purification techniques. DSC showed phase transition temperatures at physiological temperatures in line with previous reports. Multiple heating/cooling scans showed the reversible nature of the phase transitions. DSF employing GFP fluorescence or SYPRO Orange stain further confirmed DSC results. Analyzing particle size variation with temperature for the four ELPs, allowed observation of an additional phase transitions for S48I48 and the two fusion proteins. In this particular cases we hypothesized that the phase transition triggered at physiological temperature corresponds to micelle formation due to large difference in hydrophobicity of the two blocks forming S48I48 or GFP-ELPs.

### CONCLUSION

LNPs with positively charged lipids were synthesized. Their stability, RNA binding and RNA shielding were assessed as criteria to be met for efficient mRNA delivery. Also, ELPs and GFP-ELPs were produced by recombinant expression in bacteria. Their ability to form nanoparticles upon reaching physiological temperatures was explored for potential nanoparticulate antigen delivery.

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# POSTER PRESENTATIONS

# Constructing Vascular Networks: Investigating WPMY-1 and HUVEC Interactions for Prostate Models

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## INTRODUCTION

Organ-on-a-chip technology presents an exciting, ethical alternative to animal models with precise control and reproducibility. While huge strides have been taken in modeling organs such as the lung, heart, brain, and gut, reproductive organs, particularly the prostate, are relatively less studied. This is in part due to the intricate physiological interactions these organs possess with their adjacent systems. The prostate, though not entirely reproductive, plays an important role in male fertility by its contribution to seminal fluid. Its intricate organization and dependency on vascular and stromal interactions have made it challenging to replicate in vitro. Thus, most existing prostate-on-a-chip models have focused on advanced-stage pathology such as metastasis, with little focus on early-stage development and pathology.

## EXPERIMENTAL METHODS

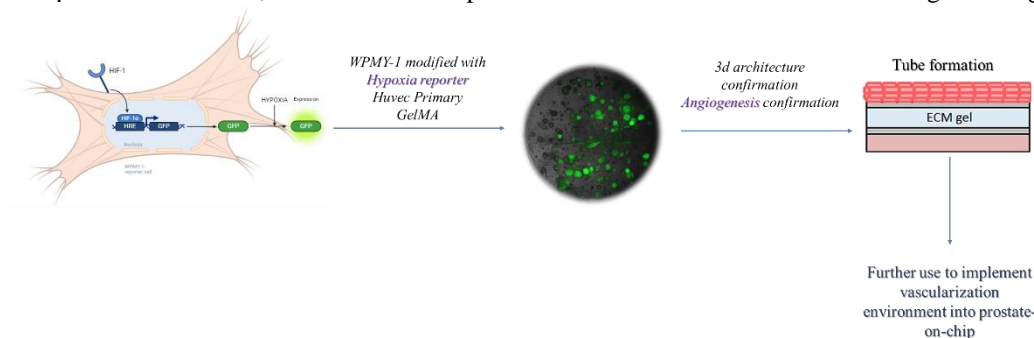
In order to examine stromal-endothelial interactions of relevance to prostate vascularization, techniques including flow cytometry, 2D and 3D microscopy, angiogenesis assays and qPCR were used. WPMY-1 prostate stromal cells and HUVEC primary endothelial cells were examined alone and in co-culture.

## RESULTS AND DISCUSSION

Results indicated WPMY-1 cells consistently being dominant over HUVEC cells at all densities that were tested, based primarily on persistent clustering. This suggests strong stromal regulation of endothelial function with implications for how blood vessel formation and remodeling take place in the prostate.

## CONCLUSION

Through targeting prostate angiogenesis in various concentration GelMA hydrogels, this study contributes to the creation of a more physiologically accurate prostate-on-a-chip platform. With its emphasis on early-stage endothelial and stromal component interactions, the model has the potential for more realistic disease modeling and drug screening purposes.



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## Nanocellulose-based biomaterials for water treatment applications

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Millions of people around the world are either forced to drink contaminated water or do not have access to clean drinking water. One of the most dangerous substances that can end up in water bodies as a result of human and natural activities are arsenic, lead and cadmium. Heavy metal removal technologies from wastewater, such as adsorption, membrane separation, precipitation/electrodeposition, ion exchange, coagulation-flocculation, and bioremediation, have advanced significantly during the past decade. As one of the most promising methods for handling wastewater, the adsorption method has been widely used since it is more economical and environmentally benign than the other approaches. Despite extensive research on a variety of adsorbents with carbon, silica, polymers, and natural adsorbents has been done, research is still ongoing on unique and extremely effective adsorbents for wastewater treatment.

In recent years, increasing attention has been paid to hydrogels derived from natural polymers because of their biodegradability and biocompatibility. Biopolymer-based hydrogels are generally characterized by poor mechanical properties that limit their ability to be regenerated and reused when saturated with contaminants. To overcome these challenges composite materials are being studied. One of the most important biopolymers in nature is cellulose. It has wide-ranging applications in fields as vast as tissue engineering, biomedicine, wearable devices, as well as environmental science and energy.<sup>1</sup> Using TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical) mediated oxidation and mild disintegration in water, native wood cellulose can be modified into individual nanofibers with a width of 3-4 nm and a length of a few microns, introducing also carboxyl groups (-COOH) on the cellulose surface to promote binding with heavy metals and other harmful contaminants.<sup>2</sup>

Due to their multiple benefits such as high mechanical properties, high specific surface area, customizable surface chemistry and their natural abundance, cellulose nanofibers (CNF) are receiving attention in numerous fields. They have recently proven to be a valuable candidate in wastewater treatment.<sup>3,4</sup>

This study aims to develop new composite materials with sodium alginate and CNFs crosslinked with two agents to ensure the best mechanical properties and high porosity to offer more adsorption and binding sites along the material. The synthesized material was analyzed for its morphology using FTIR, its thermal stability was investigated by TGA, and its porosity was assessed through micro-CT imaging.

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## **Stem cell niche formation as a driver of organogenesis in *Drosophila melanogaster***

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Adult organs rely on stem cell niches to maintain adult stem cells that replenish terminally differentiated cells lost to turnover. While organ structures are well-documented, the mechanisms of organogenesis—such as niche establishment, signaling crosstalk, and cellular dynamics—remain poorly understood, yet are crucial for stem cell biology and regenerative medicine. Organogenesis has fascinated scientists for centuries, as complex structures emerge from primordia through conserved patterns. Evolution favors stable configurations, driving tissues to fold and reorganize into functional organs, often initiated by stem cell niche formation—an early step that remains underexplored.

The *Drosophila* ovary is an ideal model to study this process. Our recent work revealed that multiple modes of Notch signaling activation specify the complex spatial cellular interactions needed for stem cell niche assembly in the developing ovary<sup>1,2</sup>. These studies showed that  $\sim 19 \pm 1$  germline stem cell (GSC) niches form, a number matching a mathematical model for optimal spatial efficiency, which we term the 'maximum compaction principle.' This suggests organs pack structures efficiently for consistent morphogenesis. To explore this further, we screened signaling pathways affecting ovarian organogenesis using RNAi transgenic mutations driven by somatic or germline drivers. This distinguished somatic versus germline effects on niche formation, targeting the proteostasis network (Insulin), cell growth regulators (Myc, Hippo), and cell fate determinants (TGF-beta, Notch). We identified novel signaling networks critical to niche assembly, with mutants exhibiting phenotypes from niche absence to doubling the control number. Disrupted niche formation arrested ovarian development, highlighting niches as organogenesis drivers.

In controls, the  $\sim 19$  niches follow a stereotypical pattern, aligning with theoretical packing models (e.g., congruent circles in a larger circle). Mutants deviating from this number showed reduced packing efficiency and underdeveloped ovaries. Using a Random Forest model, we predicted ovary size in controls based on niche dynamics, confirming a homeostatic optimum at  $\sim 19$ , though accuracy dropped in mutants, suggesting deviations disrupt organ balance. These findings build on our earlier work<sup>1,2</sup>, reinforcing that maximum compaction, dependent on somatic-germline interplay, is a key principle of ovarian organogenesis.

This principle's broader applicability requires testing across systems. By integrating developmental biology, mathematical modeling, and machine learning, our research—rooted in studies of Notch signaling<sup>1,2</sup>—offers a predictive framework for organogenesis. Understanding how signaling and cellular interactions establish optimally packed niches provides insights into organ formation, with implications for tissue engineering and regenerative therapies.

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## The potential of lignin-based adsorbent materials for water purification

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Although water is vital for sustaining life on Earth, a significant portion of the global population experiences limited access to clean drinking water. Rapid development, population growth, industry, and urbanization have resulted in serious soil and water contamination. Toxic contaminants discharged into wastewaters have the potential to seriously harm human, animal, and plant health. Numerous contaminants, mostly of human origin, can be found in waters. These contaminants include, but are not limited to, pesticides, dyes, poisonous metals and metalloids, medicines, and surfactants [1]. Heavy metals exhibit extreme toxicity, significant carcinogenic potential, and are non-degradable, which leads to their accumulation in the human body over time [2]. Toxic metals can be removed from waterways and wastewaters using a variety of treatment techniques, such as membrane filtration, ion exchange, bioremediation, chemical precipitation, chemical coagulation and flocculation, electrochemical techniques and adsorption. Among these technologies, adsorption is widely recognized as one of the most effective and sustainable methods for water treatment, due to its high removal efficiency, cost-effectiveness and ease of operation [3].

This study aims to develop new adsorbents for the removal of heavy metal (Pb, Cd or As) that are totally biobased and meet a number of criteria, such as a high and fast sorption rate, regeneration and reuse. One of the most prevalent amorphous biopolymers in the world is lignin. Its macromolecular structure includes several active functional groups, such as hydroxyl (-OH), carboxyl (-COOH), and phenolic groups, capable of interacting with contaminants through various mechanisms like hydrogen bonding, electrostatic interactions, as well as  $\pi$ - $\pi$  stacking interactions. However, lignin alone demonstrates limited efficiency in removing heavy metals from water sources [4]. To address this limitation, cellulose nanofibrils (CNF) with 25% lignin content are introduced. The unique properties of CNF, such as their high specific surface area, chemical versatility and the ability to create interconnected porous structures, significantly enhance their interaction with pollutants, providing a more robust and efficient adsorption capacity for contaminant removal [5].

Furthermore, cellulose nanofibers with 25% lignin content was integrated into a biopolymer matrix consisting of carboxymethyl chitosan (CMC) and sodium alginate (SA), which provide additional binding sites for heavy metal ions. The adsorbents were synthesized as hydrogel beads by crosslinking with two different agents, namely calcium chloride (CaCl<sub>2</sub>) and citric acid. The optimization of parameters, including the concentrations of CMC, SA, and crosslinking agents, as well as the distance between the syringe tip and surface of the crosslinking bath solution, needle size, the drip rate and the crosslinking time, was carried out to control the size, shape, morphology, mechanical properties, and stability of the hydrogel beads. Subsequently, the chemical composition of the beads was analyzed using FT-IR spectroscopy, their thermal stability was assessed through thermogravimetric analysis (TGA), and the total porosity along with pore size distribution was determined via micro-CT imaging.

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## Alginate beads for deep-wound care: an optimization study

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Skin penetration due to injuries, burns or chronic diseases might lead to infection, which might have a serious impact on a patient's quality of life. Wound healing is a dynamic process that begins with local inflammation, followed by tissue degradation and reassembly<sup>1</sup>. Numerous efforts have been made to elaborate materials that keep the wound safe from infections while allowing scar-free reconstruction. Hydrogels are intensively studied for wound healing applications due to their ability to uptake the exudate while maintaining a moist microenvironment and ability to easily be loaded with various active molecules<sup>2</sup>. In the case of deep wounds, the matter of porosity is also paramount since this characteristic has major influences in moisture retention and tissue reconstruction.

The present study focuses on creating innovative wound dressings incorporating alginate beads within a polymeric matrix. These beads serve dual functions: as porogen agents to enhance the matrix's porosity and as carriers for the local delivery of lidocaine, a common analgesic. The evaluation of the antimicrobial properties of these materials due to the innate antimicrobial character of the used materials was also aimed.

The alginate beads were fabricated using four different crosslinking agents: calcium chloride, barium acetate, copper sulfate, and zinc sulphate. The shape of the beads and their porosity was investigated through micro-computed tomography. Beads' dimension and polydispersity were assessed through laser diffraction measurements. In addition, the ability of the beads to pass through a narrow nozzle while hydrated was assessed through injectability tests. Finally, the stability of all four types of alginate beads was monitored for a period of 24 hours in PBS.

Preliminary results indicate that the type of crosslinking agent significantly affects the structural integrity and drug release behavior of the alginate beads. Calcium and zinc -crosslinked beads demonstrated optimal porosity and sustained release of lidocaine, making them particularly suitable for deep wound applications.

This study highlights the potential of alginate bead-integrated polymeric dressings as multifunctional wound care solutions, combining enhanced structural properties with effective pain management. The results of the study are encouraging, and further tests will be performed to evaluate the antimicrobial effect of the synthesized materials and their ability to promote cell adhesion and proliferation.

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## Alginate Beads for His-Tagged Protein Immobilization and Delivery

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**INTRODUCTION.** Recombinant protein based vaccines are gaining more interest as advances in biotechnology allow for superior yields at lower costs and purification strategies are becoming easier to scale. Nonetheless, there are still challenges to overcome, such as poor folding of proteins resulted from heterologous expression in bacteria. More often than not, these proteins accumulate in insoluble aggregates known as inclusion bodies and the process of extraction of soluble protein involves the use of chemicals that block further folding towards biological active conformation. This study describes immobilization of such proteins from urea solubilized inclusion bodies by means of a protein tag (His-Tag) using alginate beads reticulated with  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$ . Upon addition of protein loaded alginate beads to physiological solutions, the beads decompose and release immobilized biological active proteins.

**EXPERIMENTAL METHODS.** Alginate beads were prepared by dripping addition of a 1% aqueous solution of alginate (medium viscosity) into 2.5% aqueous solutions of different divalent cations ( $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ ) under continuous stirring. To test for specific HisTAG mediated binding, alginate beads were incubated with Enhanced Green Fluorescent Protein (EGFP) and EGFP-HisTAG and after multiple washes, beads were scanned with a high resolution fluorescence scanner (Typhoon FLA2000).

Recombinant interleukin-2 (IL-2-HisTAG) was expressed in BL21(DE3) E. coli strain cultivated in LB media. Bacterial culture was incubated at 37°C under continuous agitation. At optical density of 0.6, 1mM IPTG was added to trigger protein production. After 3h, bacterial mass was harvested and lysed using ultrasonication. Insoluble fraction was washed and solubilized in urea. Soluble IL-2 was purified by ion exchange chromatography and desalted to remove  $\text{Na}^+$  ions. Purified urea solubilized IL-2-HisTAG was immobilized on previously prepared alginate beads. Beads were washed and added to HEK-Blue IL-2 cells cultured in adequate cell culture media in order to test binding of IL-2 to corresponding receptor expressed by HEK-Blue IL-2 cells. Binding was assessed using qPCR to quantify SEAP transcripts.

**RESULTS AND DISCUSSION.** Although reticulation of alginate beads was achieved with all the divalent cations used in this study, only  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Ca}^{2+}$  alginate beads both maintained integrity and bound EGFP-HisTAG. Although  $\text{Ni}^{2+}$  is the traditional divalent cation used for specific interaction with HisTAG, in our setup, addition of protein to  $\text{Ni}^{2+}$  reticulated bead induced dissolution of alginate. EGFP alone also bound to alginate beads, most probably due non-specific binding or diffusion into beads' pores.  $\text{Ca}^{2+}$  beads bound (or trapped) EGFP (tagged or non-tagged) to some degree, probably due to beads' porosity.

IL-2-HisTAG was expressed in BL21(DE3) in insoluble fraction as inclusion bodies. Inclusion bodies were solubilized with 8M urea. Purification yielded high purity IL-2-HisTAG in 8M urea. IL-2-HisTAG was immobilized on prepared beads and urea was washed. Upon urea removal we hypothesize that immobilized IL-2-HisTAG may proceed rapid folding, while spatial confinement do not allow aggregation. After IL-2-HisTAG loaded beads were added to adhered HEK-Blue IL-2 cells, beads disintegrated and released IL-2-HisTAG. Specific IL-2 to IL-2 receptor binding was observed by quantifying SEAP RNA. IL-2-HisTAG released by alginate beads interacted with IL-2 receptor of HEK-Blue IL-2 cells, while IL-2-HisTAG alone (in 8M urea) did not.

**CONCLUSION.** We provide a solution to benefit from poorly folded recombinant proteins expressed in prokaryotic hosts. We also believe that this that can be potentially used for vaccine formulation. Alginate beads reticulated with  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$  were able to bind a His-tagged protein, to promote its folding and to release it in its biological active form upon contact with  $\text{Na}^+$  medium (cell culture medium).

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## Additive Manufacturing of Metal-Loaded Structures for Applications in Catalysis

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### INTRODUCTION

Three-dimensional (3D) printing enables the fabrication of complex catalytic structures using various materials, including polymers, metals, and ceramics <sup>1</sup>. Among the most promising 3D-printed architectures are monoliths and triply periodic minimal surface (TPMS) structures, with the latter being particularly valued for their high surface area, low pressure drop, and mechanical strength <sup>2</sup>. Although direct ink writing (DIW) and fused deposition modeling (FDM) are commonly used for 3D printing structures with metal components, stereolithography (SLA) provides higher resolution and greater design flexibility <sup>3</sup>. However, achieving uniform metal distribution within resins remains challenging. To address this challenge, this study introduces functional monomers into a commercial resin to improve the dispersion of nickel ions through nitrogen and sulfur coordination. The resulting formulations are used to fabricate highly porous TPMS structures via SLA, intended as efficient supports for catalytic applications.

### EXPERIMENTAL METHODS

The experimental approach involves modifying a commercial resin by gradually incorporating the selected monomers, followed by the addition of the metal source. Rheological measurements are performed to evaluate the viscosity of the modified resin and to assess the effect of monomer and metal incorporation on printability. Once an optimal formulation is identified, the mixture is loaded into the SLA printer's resin tank and used to fabricate TPMS structures. The resulting 3D-printed structures undergo comprehensive characterization, including scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), micro-computed tomography (micro-CT), and contact angle measurement, to evaluate surface morphology, elemental composition, internal architecture, and wettability, which are key parameters for assessing their performance in aqueous catalytic applications.

### RESULTS AND DISCUSSION

The modified resin successfully enabled SLA printing of TPMS structures with uniform morphology and good fidelity to the digital model. The resin was successfully modified by incorporating functional monomers and a metal source, enabling SLA printing of highly porous structures. Preliminary observations suggest that the modified resin achieved the intended homogeneity and printability. While rheology, micro-CT, and XPS tests are pending, the results thus far suggest that the resin is suitable for producing the desired catalytic supports.

### CONCLUSION

This study demonstrates the novel application of SLA for 3D printing catalytic supports, providing high precision and design flexibility. The TPMS structure, known for its excellent surface area and mechanical properties, shows great potential as a catalyst support. Further tests will confirm its effectiveness in catalytic applications. This approach sets a solid foundation for developing high-performance catalytic systems based on SLA-printed TPMS structures.

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## Core-shell electrospun nanofibers loaded with CS-DNA complexes

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### INTRODUCTION

Electrospinning is a versatile fiber production technique that uses high-voltage electrostatic fields to produce nano-fibers from polymer solutions<sup>1</sup>. The high surface-area-to-volume ratio and interconnected porous structure of electrospun fibers facilitate efficient loading and controlled release of various bioactive molecules, including genes<sup>2</sup>.

### EXPERIMENTAL METHODS

In this study, core-shell fibers were fabricated using electrospinning, where the core consists of polyethylene oxide (PEO) and the shell is made of gelatin. Chitosan/DNA (CS/DNA) complexes were incorporated within the core. The complexes prior to their incorporation were characterized by DLS. The structure of the electrospun fibers were investigated by FTIR spectroscopy and their morphology by SEM imaging, finally, degradation and water uptake tests were performed to assess the stability and hydrophilic behavior of the fibers.

### RESULTS AND DISCUSSION

It was found that at mass ratio of 3 and 5, the complexes had a mean diameter of 129 and 136 nm and a polydispersity < 0.35. Additionally, FTIR spectrum displayed several key absorption peaks such as the band at 3300 cm<sup>-1</sup> corresponding to O-H and N-H stretching vibration from gelatin, C-O-C Stretching at 1100 cm<sup>-1</sup> of PEO. Furthermore, The SEM imaging shows that the electrospun fibers exhibit a smooth, uniform structure, typical of core-shell fibers. The fibers appear to have a consistent diameter and to be randomly oriented. The SEM images also reveal the presence of CS/DNA particles embedded within the core fibers. The particles appear as small, aggregates or granules in the core SEM images. After crosslinking the images indicate a slight increase in fiber thickness. This may be attributed to the swelling effect induced by the cross-linking reaction of gelatin. The fibers exhibited a notable swelling behavior in aqueous environments where the samples had a degree of swelling of 1000% after 2 hours followed by a progressive decrease that can be linked to the samples degradation.

### CONCLUSION

In this work, CS-DNA particles were successfully incorporated within PEO/GEL core-shell electrospun fibers. FTIR spectra analysis of the fibers confirmed the presence of the different system components. The SEM images show well-defined electrospun fibers with uniform diameter, random orientation and consistent inter-fiber spacing. The fibers exhibited a notable swelling behaviour upon immersion in PBS followed by a progressive decrease that can be linked to the samples degradation.

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# A Comprehensive Study on the Curing and Properties of Sustainable Epoxy Thermosets from Eugenol

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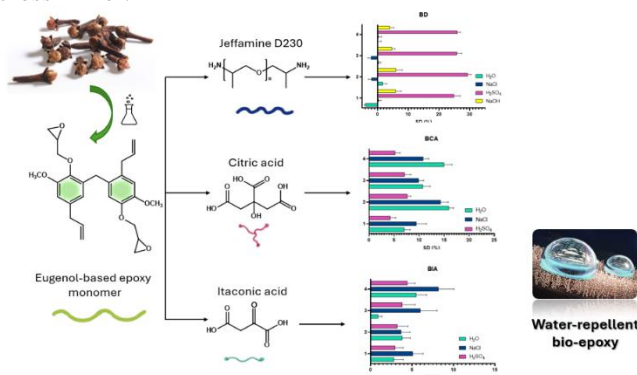
The need for novel and sustainable thermosets led to the development of green materials based on bio-based resources with balanced properties that can decrease the environmental impact [1].

Eugenol was successfully used as precursor for the synthesis of biobased epoxy monomer that was used alongside natural carboxylic acids and Jeffamine for the development of cured networks.

FTIR analysis confirmed the successful synthesis of the epoxide monomer derived from eugenol. The spectrum shows the absence of the -OH group signal, and the CH<sub>2</sub> vibrations of the epoxide ring which confirms the epoxidation. <sup>1</sup>H-NMR analysis reinforces these results, showing the signals corresponding to the epoxide rings and the methylene protons of the glycidyl ether in the regions 2.88-2.64 ppm and 4.21-3.81 ppm.

Curing kinetics and dynamics were evaluated through DSC and the corresponding results indicate a self-accelerating polymerization mechanism when CA and Jeffamine were used. BIA system cured at 100 °C show a different conduct suggesting that the dual functionality of the AI influenced polymerization behavior. As amine functionality gave a higher activation energy to the D230 cured systems due to the aliphatic nature of the crosslinker. Moreover, these results are consistent with the TGA analysis and a gradual transition showing two T<sub>g</sub> peaks in DMA for the BIA networks.

Considering the coating applications of the sustainable eugenol-based epoxies, the surface characteristics were critically analyzed showing a high surface free energy for D230 systems, while the CA-cured sample showed a more hydrophilic behavior, similar to conventional epoxy resins [2]. The reduced surface free energy of the BI system suggests a better diffusion of the material as a coating in accordance with the antibacterial activity tested against *Escherichia coli*. The degradation of bio-based epoxy coatings in corrosive environments revealed high resistance in acidic environments for BCA and BIA materials, with limited swelling. XPS analysis confirmed that the degradation mechanism varies according to the nature of the cross-linker.



Graphical abstract

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## Antioxidant phyto-hybrids with high therapeutic potential

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### INTRODUCTION

Phyto-derived hybrids represent innovative structures with wide applicability in nanomedicine due to their interaction properties at the cellular level [1, 2].

### EXPERIMENTAL METHODS

The present work presents the development of biohybrid systems based on phytosomes (lecithin – curcumin vesicles) and Geranium-derived silver nanoparticles. The formation of biohybrids was monitored by UV-Vis (Jasco, Japan V-570) and FTIR (Bruker, Alpha System, Germany) spectroscopy. The hydrodynamic size of the biohybrids was evaluated by Dynamic Light Scattering (DLS), and their physical stability by Zeta potential measurements. Biohybrids were evaluated in terms of antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) methods.

### RESULTS AND DISCUSSION

A SPR band appears in the UV-Vis absorption spectrum at 437 nm, confirming the phytosynthesis of AgNPs. The UV-Vis absorption spectra showed the spectral signatures of the components in the biohybrids. FTIR spectra revealed the main functional groups responsible for the development of phyto-derived hybrids. DLS results showed that the particle size of biohybrids varied in the range of 100-390 nm. The zeta potential measurements highlighted that the obtained bio-entities have moderate and good stability. DPPH and ABTS tests demonstrated the antioxidant properties of phyto-derived biohybrids.

### CONCLUSION

By integrating inorganic components into biomimetic platforms, bio-based hybrids with enhanced bioactivities can be developed. The samples showed component-specific spectral fingerprints and the spectral analyses demonstrated the formation of biohybrids. The obtained biohybrids presented a higher antioxidant activity value than the individual components.

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# Flexible Hydrogel-Based Sensors: Toward Next-Generation Wearable and Bio-Integrated Electronics

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The rapid evolution of wearable technology, soft robotics, and human-machine interfaces has created an urgent demand for materials that are not only mechanically flexible and biocompatible but also capable of accurate sensing under dynamic and complex conditions [1]. Traditional rigid sensors are often limited by their inability to conform to soft, curvilinear surfaces, making them less suitable for applications that involve direct skin contact or continuous mechanical deformation.

Flexible sensors, particularly those made from hydrogels, offer an attractive alternative. Hydrogels possess high water content, tunable mechanical properties, and intrinsic softness that closely mimics biological tissues [2]. This makes them ideal candidates for continuous physiological monitoring, gesture recognition, and smart prosthetics. Moreover, their potential for stretchability, transparency, and even biodegradability opens new frontiers in eco-friendly and imperceptible electronics. The relevance of flexible hydrogel-based sensors extends into healthcare, where non-invasive, real-time monitoring of vital signs like heart rate, respiration, or joint movement is becoming central to personalized medicine. In the realm of soft robotics, such sensors can provide feedback necessary for adaptive movement and object manipulation [3]. Their applications further span areas like athletic performance tracking, environmental sensing, and brain-computer interfaces, where dynamic, high-resolution, and durable sensing systems are essential.

In this study, we developed a multifunctional hydrogel sensor through the strategic copolymerization of methacrylic acid (AM), dopamine methacrylamide (DOPA-MA) and vanillin methacrylate (VA-MA). Each component was selected for its unique chemical functionality, contributing to the hydrogel's overall performance in terms of mechanical integrity, conductivity, adhesion, and self-healing. Methacrylic acid forms the foundation of the hydrogel's polymeric network, providing a robust backbone and introducing carboxylic groups that promote hydrogen bonding and pH responsiveness—key features for adaptable sensing environments. The incorporation of dopamine methacrylamide introduces catechol groups that emulate the adhesive capabilities of mussel proteins, enabling strong interfacial interactions with diverse substrates and enhancing electrical conductivity through redox-active sites. Vanillin methacrylate, inspired by the aromatic aldehyde structure of natural vanillin, introduces dynamic aldehyde functionalities into the network. These facilitate reversible imine (Schiff base) bonding when combined with amine-rich components, contributing both to autonomous self-healing behavior and increased mechanical toughness through additional  $\pi$ - $\pi$  stacking interactions.

The final hydrogel demonstrates exceptional stretchability, with the ability to sustain significant deformations while maintaining structural integrity. It also exhibits rapid self-repair following physical damage, attributed to the reversible nature of its internal bonding mechanisms. Electrical performance tests confirm a high gauge factor, excellent signal stability, and repeatable responsiveness under cyclic strain, establishing the hydrogel as a robust candidate for motion sensing and wearable electronics. Notably, its intrinsic adhesiveness allows it to bond directly to skin or other soft surfaces without auxiliary adhesives, significantly improving user comfort and long-term stability in practical applications. This molecularly engineered hydrogel presents a versatile and customizable platform for the development of flexible, bio-integrated sensors, offering promising solutions for applications in soft robotics, personalized healthcare, human-machine interaction, and real-time physiological monitoring.

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## UV-Stabilized Zein Inks for Biopolymer-Based 3D Printable Structures

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### INTRODUCTION

As 3D printing technologies continue to advance and find applications across industries, the demand for sustainable, tunable materials capable of meeting specific performance and processing requirements is increasing. Zein, a biodegradable biopolymer derived from corn, has emerged as a promising alternative to synthetic polymers due to its renewability, biocompatibility, and suitability for material formulation <sup>1,2</sup>. This research explores the development of a UV-curable, zein-based ink tailored for pneumatically driven extrusion 3D printing. For this purpose, two methacrylation strategies are compared to functionalize zein and obtain a UV-crosslinkable ink formulation.

### EXPERIMENTAL METHODS

The experimental work focuses on two approaches to chemically modify zein for use in UV-curable 3D printing inks. The first method follows a two-step process: esterification to introduce hydroxyl groups and enhance zein's reactivity, followed by methacrylation with methacrylic acid. The second method simplifies this by using a one-step methacrylation with 2-hydroxypropyl methacrylate. The ink formulation is being optimized through iterative adjustments, including blending methacrylated zein with other biopolymers, comparing two different photocrosslinkers, and testing various photoinitiators to fine-tune its properties. The two strategies are being evaluated based on methacrylation degree, determined by Fourier Transform Infrared (FTIR) and Proton Nuclear Magnetic Resonance (<sup>1</sup>H-NMR) spectroscopy, as well as rheological behavior, 3D printability assessed through statistical analysis, and photocrosslinking performance.

### RESULTS AND DISCUSSION

The two methacrylation strategies produced zein derivatives with distinct characteristics. Preliminary FTIR and <sup>1</sup>H-NMR analysis revealed differences in the degree of methacrylation. Rheological testing indicated that both formulations have suitable flow properties for pneumatic extrusion, with some variation in their performance. Both methods enabled photocrosslinking, but further testing is needed to assess mechanical stability and curing efficiency. Blending with polyvinyl alcohol improved viscosity and printability, enhancing ink consistency for 3D printing. Ongoing optimization will determine which methacrylation method offers the best balance of reactivity, printability, and photocrosslinking efficiency.

### CONCLUSION

This study contributes to the limited research on using zein in ink development by exploring two distinct methacrylation strategies to create UV-curable ink formulations. By optimizing the ink's rheological properties, printability, and photocrosslinking efficiency, this work expands the potential applications of zein in sustainable 3D printing.

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## Encapsulation of 2-(Morpholin-4-yl)ethoxy Substituted Zinc Phthalocyanine in pH-Responsive Polymersome and its Photodynamic Activity *In Vitro*

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### INTRODUCTION

Photodynamic therapy (PDT) is a promising cancer treatment alternative to conventional approaches such as radiotherapy and chemotherapy. It is based on applying a photosensitizer (PS) that in combination with light and oxygen leads to cell death. However, PDT has limitations, including poor selectivity, low solubility, and rapid degradation of PS. To address these challenges, many studies explore nanosystems to enhance the delivery of PS into cells, as well as the use of derivatives of existing photosensitizers<sup>1</sup>. One promising strategy involves the use of polymersomes—polymeric vesicles that mimic the membrane-like structures<sup>2</sup>. Here, we present biophysical and in vitro characteristics of a 2-(morpholin-4-yl)ethoxy-substituted zinc phthalocyanine (TG3)<sup>3</sup> encapsulated in a pH-responsive polymersome D<sup>4</sup>.

### EXPERIMENTAL METHODS

Polymersome D (Psome D) was synthesized using an amphiphilic block copolymer called BCP-D. Subsequently, TG3 was encapsulated within Psome D. The hydrodynamic diameter was measured to control the formation of the Psomes D and to assess their responsiveness to pH changes. Singlet oxygen generation was evaluated in an aqueous medium. Additionally, we measured cellular uptake, phototoxicity, dark toxicity, and the production of reactive oxygen species in A2780 cells.

### RESULTS AND DISCUSSION

The encapsulation of TG3 within Psome D was effective, achieving more than 82% efficiency. This process increased the ratio of monomers to dimers in the PS. Psome D-TG3 exhibited enhanced generation of singlet oxygen (<sup>1</sup>O<sub>2</sub>). Cellular uptake in A2780 cells was 15 to 17% higher for Psome D-TG3 compared to free TG3. Neither free TG3 nor Psome D showed any cytotoxic effects in the dark, whereas Psome D-TG3 resulted in decreased cell viability below 80%. After irradiation, free TG3 was not cytotoxic, likely due to aggregation and reduced cellular uptake. In contrast, Psome D-TG3 caused significant phototoxic effects, reducing cell viability to below 10% at TG3 concentration of 0.5 μM. To confirm the photodynamic mechanism, reactive oxygen species (ROS) levels were measured immediately after irradiation, showing higher ROS production with Psome D-TG3.

### CONCLUSION

Polymersome D is a promising nanocarrier for photosensitizers that are characterized with low solubility in water and tendency to aggregate and a good candidate for further studies on its cellular transport paths and mechanisms of action.

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## Gravimetric and FTIR study about the water absorption of PLA-Chitosan blends

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Poly(lactic acid) (PLA) and chitosan (CH) are biopolymers obtained from renewable sources and widely used. Plastics made from blends and mixtures of both polymers have good properties that could justify their use in applications such as food packaging, since PLA provides good mechanical properties, and CH improves the moderate O<sub>2</sub> barrier of PLA and provides antimicrobial character<sup>1</sup>. In this study, we have analyzed how the presence of chitosan affects the material's water vapor absorption behavior, a factor that also plays an important role in food preservation.

Films of virgin PLA, recycled PLA (rPLA), chitosan and blends of PLA and chitosan, with a thickness between 10-20 microns, were prepared by casting. The films have been kept during different times at  $21 \pm 1$  °C or  $40 \pm 1$  °C, at a relative humidity of  $97 \pm 2$  %. The water vapor absorption has been monitored by gravimetry and Fourier transform infrared spectroscopy (FTIR).

The gravimetric study shows that the water absorption of chitosan follows a Fickian behavior, reaching a water absorption of around 80% after 99 hours in an atmosphere with 97% RH, due to its hydrophilic character. Similar Fickian behavior is observed in the water absorption curve of the PLA mixture with 20 wt.% of chitosan (PLA20CH). Water absorption in PLA is low as expected, due to its hydrophobic character. Recycled PLA absorbs slightly more water than virgin PLA but deviates from Fickian behavior, which is probably due to the degradation experienced by the biopolymer over time.

Water absorption can be followed by using IR spectroscopy. Absorption in chitosan is observed in its spectrum in a simple way, with very intense bands that indicate a strong water absorption. In PLA films the amount of absorbed water is very low, but it can still be studied by IR spectroscopy using the characteristic bands of the absorbed water, corresponding to the two O-H stretching modes of absorbed water, that of O-H bound by hydrogen bonding and that of free O-H. For times greater than 99 hours, another absorption band appears centered at  $3450\text{ cm}^{-1}$ , which corresponds to larger clusters of water molecules and is sometimes called free water<sup>2</sup>.

The material with 80% PLA and 20% CH has a spectrum where the bands of water absorbed by PLA and those of water absorbed by chitosan can be differentiated, thus showing that IR spectroscopy allows differentiating the absorption of water by the different components of the mixture. In this material the rule of mixtures is not fulfilled since the measured water absorption is 10 times lower than expected. This result may reveal that PLA interacts with CH, reducing its absorption capacity. These results indicate that it is possible to better understand and control the water absorption of mixtures of different polymers.

### ACKNOWLEDGMENT

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# Dopamine and Sericin as Cell-Adhesive Agents in Multifunctional Hydrogels for Wound Healing: A Comparative Study

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## INTRODUCTION

Chronic diabetic wounds remain a significant healthcare concern, necessitating the implementation of novel therapeutic strategies. Hydrogels are an emerging treatment for diabetic wound recovery and skin tissue regeneration owing to their inherent hydrophilic and porous framework, which promotes moisture retention, fluid absorption, gas exchange, and regulation of water evaporation. Furthermore, the similarity to the extracellular matrix enables hydrogels to effectively replicate its architecture and functions, hence facilitating cell migration, attachment, and development [1][2]. To overcome the limitations of existing therapies, we sought to create multifunctional biomimetic hydrogels with comprehensive therapeutic properties that can ensure antibacterial, adhesive, anti-inflammatory activity while promoting dermal regeneration. Taking inspiration from marine mussels' ability to form strong interactions with different types of substrates due to the secretion of dopamine-containing proteins we grafted dopamine on the backbone of gelatin in order to increase the hydrogels' ability to adhere to tissues and to promote the adhesion and proliferation of cells [3]. Alternatively, we also considered the inclusion of sericin (Ser), another natural based adhesive molecule obtained from silkworms that proved to significantly increase cell adhesion and proliferation [4].

## EXPERIMENTAL

The current research study is focused on pursuing the potential in dermal regeneration of chronic wounds associated with diabetes of gelatin-based hydrogels that can be enzymatically cured *in situ* with transglutaminase at physiological temperature. Gelatin was functionalized with dopamine via EDC/NHS coupling chemistry, resulting in Gel-Dopa, and FTIR, UV-Vis Spectroscopy, and NMR were employed to confirm the chemical change. One of the two adhesion components, Gel-Dopa or Ser, was combined with a matrix consisting of gelatin and chitosan, enzymatically crosslinked hydrogels being obtained thereafter. The resultant materials were structurally investigated by FTIR, with their rehydration, stability, morphology, and mechanical properties examined prior to *in vitro* biological experiments.

## RESULTS AND DISCUSSION

Rehydration studies revealed that both Gel-Dopa and Ser containing hydrogels possess similar and elevated ability to absorb fluid which is essential for wound healing. All proposed formulations present suitable internal porous microstructure that facilitates cell penetration and nutrient diffusion, which is further proved by *in vitro* cytocompatibility studies that revealed excellent biocompatibility, proving the hydrogels' potential in guiding dermal regeneration.

## CONCLUSION

The effective grafting of dopamine onto gelatin is demonstrated herein, and the resulting multifunctional hydrogels containing either Gel-Dopa or Ser exhibit appropriate physicochemical and mechanical properties to be used in tissue engineering. Moreover, the *in vitro* cellular investigations indicated that cell proliferation significantly improves with the incorporation of the adhesive component (Gel-Dopa / Ser), suggesting that the proposed formulations hold considerable potential for diabetic wound healing and skin regeneration.

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## Hybrid Nanocomposite Membranes for Efficient Heavy Metal Retention

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Polysulfone (PSf) is a thermoplastic polymer widely used in membrane fabrication due to its excellent thermal stability, mechanical strength, and chemical resistance. Its inherent structural rigidity and film-forming capability make it an ideal base material for water treatment membranes. However, PSf alone can exhibit hydrophobic behavior and limited adsorption capacity for certain contaminants. To enhance its performance, carbon nanotubes (CNTs) are often incorporated into the polymer matrix. CNTs, especially multi-walled variants, offer remarkable surface area, high mechanical strength, and the ability to be functionalized with various chemical groups, which significantly improves their affinity for heavy metal ions. Their hollow, tubular structure also contributes to increased porosity and water permeability in composite membranes. When embedded into PSf, CNTs not only enhance the membrane's structural integrity but also introduce active sites for metal ion adsorption through mechanisms such as electrostatic interactions,  $\pi$ - $\pi$  stacking, and surface complexation. The synergistic combination of PSf and CNTs thus results in nanocomposite membranes with improved hydrophilicity, adsorption efficiency, and mechanical robustness, making them highly effective for the retention of heavy metals like zinc and iron from contaminated water <sup>1,2</sup>.

This work focuses on the synthesis and characterization of polymeric nanocomposite membranes based on polysulfone (PSf) and carbon nanotubes (CNTs) for the removal of heavy metals from wastewater. The membranes were fabricated via the phase inversion method, incorporating varying amounts of CNTs to enhance physicochemical and mechanical properties. Comprehensive characterization was performed using Fourier-transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and Raman spectroscopy—both before and after the metal retention process—to evaluate structural changes and surface interactions. Scanning electron microscopy (SEM) was used to analyze membrane morphology, while mechanical tests assessed the impact of CNT integration on membrane strength. The membranes were evaluated for their efficiency in retaining zinc ( $Zn^{2+}$ ) and iron ( $Fe^{2+}$ ) ions from simulated wastewater.

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## Development of novel double-reinforced supports for bone tissue engineering

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### INTRODUCTION

The research work aims to build new biomimetic supports based on calcium alginate (A)/ cellulose nanofibrils (CNFs) double-reinforced with chitosan/polyethylene oxide nanofibers (NFs) and graphene oxide (GO), using the 3D printing technique 1. The applicability of these composite scaffolds lies in bone tissue engineering and regeneration.

### EXPERIMENTAL METHODS

The precursor composite inks were synthesized starting from the polymer matrix consisting of A/CNF (AC) in a (v/v) ratio of 3/1, in which both the nanofibrous component (NFs) with a constant content of 2% (w/w) and graphene component (GO) in variable concentrations between 0.1 and 0.5% (w/v) were added. The next step after charging the composition into the cartridge was testing the filament for each ink and optimizing the printing parameters.

### RESULTS AND DISCUSSION

The printability evaluation was carried out to determine the shape fidelity of the 3D printed supports; the AC and the composite ink with 0.5% GO (ACNG 0.5) presented the highest (1.03) and the lowest (0.71) printability index, respectively, which correlates with the rheological results, that demonstrated the gel-like behavior of all precursor inks 2. Surface morphology and 3D internal microstructure were analyzed using scanning electron microscopy (SEM) and micro computed tomography ( $\mu$ -CT), which evidenced a synergistic effect of the reinforcing and functional fibers addition, as well as of the GO sheets that seem to govern materials structuration. The nanoindentation results emphasized the improvement of nanomechanical properties with the increase of GO content within the composite scaffolds from 96 kPa in the case of sample consisting of only calcium alginate (A), to 172 kPa for ACNG 0.5 sample. In vitro assays performed on MG-63 osteoblast cells confirmed the biocompatibility of the calcium alginate-based scaffolds and highlighted the osteostimulatory and mineralization enhancement effect of GO.

### CONCLUSIONS

In virtue of their biocompatibility, structural complexity similar with the one of native bone extracellular matrix, and biomimetic mechanical characteristics, these novel materials were considered appropriate as guided supports for bone tissue formation.

### ACKNOWLEDGEMENT

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## Bicomponent Scaffold for Osteochondral Tissue Engineering

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Osteochondral reconstruction remains a significant challenge in regenerative medicine due to the complex hierarchical structure of articular cartilage. Articular cartilage consists of multiple layers with distinct properties, ranging from the superficial zone, which is aligned parallel to the surface and resists shear forces, to the deep zone, which is perpendicular to the surface and provides resistance to compressive stresses<sup>1</sup>. This multilayered architecture, coupled with a high-water content and specialized extracellular matrix (ECM), enables cartilage to perform its critical function in load-bearing and joint mobility<sup>2</sup>.

In this study, we propose a novel bicomponent scaffold that mimics the native cartilage architecture. The scaffold integrates electrospun fibrous meshes derived from fish gelatin<sup>3</sup>, providing an ECM-like environment conducive to cell attachment and proliferation, with a poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel matrix to improve mechanical resilience and water retention.

The scaffolds were characterized in terms of chemical composition, surface properties, mechanical performance, and stability under physiological conditions.

Our findings suggest that this bicomponent material holds promise for osteochondral tissue engineering, offering a structurally and mechanically suitable environment for cartilage regeneration.

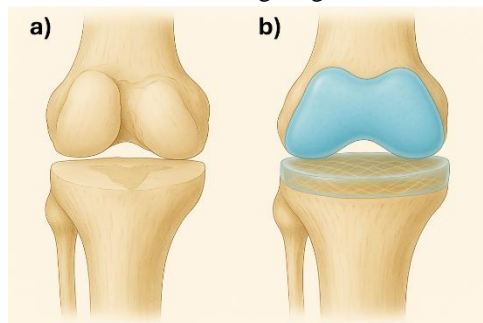


Fig.1 Schematic representation of: a) degenerated cartilage and subchondral bone damage and b) articular cartilage reconstruction with the proposed bicomponent scaffold integrating electrospun fibers and a hydrated matrix. Image generated with AI tools.

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## Polymeric host based on modified Chitosan for encapsulation of 5-aminosalicylic acid

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Encapsulation of active substances in polymeric hosts can be considered an attractive method used to solve certain limitations such as low stability of the drug in a certain environment and possible degradation initiated by the presence of certain enzymes [1]. The encapsulation technique also ensures advantageous modification of the release profile [2].

In this work, we proposed and tested a polymeric host for encapsulation of 5-aminosalicylic acid (5ASA). The polymeric component involved in the preparation of this host is represented by modified chitosan with a polyamidoamine dendrimer (PAMAM). The results of UV-Vis analysis indicated that polymeric host exhibits a high drug encapsulation efficiency and can be classified as pH-responsive host in terms of drug release profile.

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## The Use of Biopolymers as Encapsulating Agents for Probiotic Strains

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Lately, research results based on the use of biopolymers as encapsulation material for biologically active products paves the way for new horizons in the field of health. Due to their properties, but mainly due to the possibility of controlled and targeted release of constituents, the use of biopolymers for drug development has gained increasing popularity, both from the industry and from the consumer. Thus, research has focused on the development of biopolymer-based capsules with the addition of powders, essential oils<sup>1</sup>, pharmaceutical ingredients such as vitamins<sup>2</sup> or drugs<sup>3</sup>, polyphenols<sup>4</sup> or probiotics<sup>5</sup>. The results indicated the ability to improve sensory features, antioxidant and antibacterial properties, and to enhance the bioavailability, as well. According to literature results, the viability of encapsulated probiotics was maintained for a longer period of time than non-encapsulated samples<sup>5</sup>. Among the most used and tested biopolymers, alginate is the most used, especially due to its increased encapsulation capacity and easy processing. The present study aims to encapsulate *Bifidobacterium longum* strain in a biopolymer matrix based on sodium alginate, starch, and a combination of these two hydrocolloids. Developed through extrusion method, physical, microbiological and encapsulation efficiency of probiotic capsules were evaluated. The physical evaluation included microstructure, diameter, and color through CieLAB method. Transmittance and opacity were spectrophotometrically analyzed. Antioxidant capacity was evaluated using DPPH radical scavenging assay and total polyphenols were determined using the modified Folin–Ciocalteu method. The survival rate of *Bifidobacterium longum* was tested in simulated gastric and intestinal fluids. Swelling index and mass of the capsules were evaluated, as well. Encapsulation of the probiotic strain in biopolymer matrices improved the viability and stability of cells, regardless of the type and composition of sodium alginate or starch used. The capsules had a diameter ranging from 2.89 to 4.15  $\mu\text{m}$ . Although with an irregular surface, no pores or cracks were observed in the biopolymer matrix. The color presented slight variations, being influenced by the intensity of the biopolymers used. Thus, the luminosity increased in the order sodium alginate capsules < starch - sodium alginate mix capsules < starch capsules. Immersed in simulated gastrointestinal fluids, the capsules changed their size; thus, it decreased slightly in gastric juice and increased in intestinal juice. The highest swelling index was observed in the capsules with the highest starch content, while the lowest was found in the composition with the highest sodium alginate amount. According to the results obtained, both sodium alginate and starch represent a viable solution for encapsulation of active compounds. Tested *in vitro*, the capsules maintained their stability in simulated gastric juices and disintegrated in simulated intestinal juices. The addition of starch proved to be a cost-effective solution that facilitated the disintegration of the capsules in intestinal fluids. Future studies should involve testing of the characteristics of biopolymer-based encapsulated probiotics stored under refrigerated conditions during various periods.

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## Polycations-mediated gene delivery: a comparative study

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Gene therapy is the modulation of gene expression in particular cells by introducing exogenous genetic material to treat pathological conditions. The mainstay factor in utilizing the full potential of gene therapy technology represents the design of suitable gene carriers, capable of delivering efficiently the therapeutic gene to the targeted cell/tissue, facilitating intracellular trafficking<sup>1</sup>. Currently, the “library” of gene therapy drugs mainly comprises plasmid DNA (pDNA), small interfering RNA (siRNA), microRNA (miRNA) and short hairpin RNA (shRNA) and antisense oligonucleotides (ASO)<sup>2</sup>. Even so, exploiting the full potential of gene-based therapy in academic or clinical practices entails some strategies that are able to tackle the drawbacks faced in gene delivery. Thus, packaging of therapeutic genes in a nanocarrier, using viral or non-viral vectors is highly desirable both to protect the therapeutic gene from degradation and enhance its delivery to target cell.

In this study, we aim to formulate and investigate the hydrodynamic characteristics and biological performance of various gene delivery carriers based on different types of polycations. Two types of synthetic polyethylene imine: linear (PEI-L) and branched (PEI-B) along with the natural chitosan with two different Molecular weight (CS-LMW) and (CS-MMW) were used for the precipitation of DNA. A comprehensive review of hydrodynamic (DLS) and morphological (SEM/TEM) features along with stability *in vitro* biocompatibility (MTT, LDH) investigations were performed. Small aggregates were observed regardless of the polycation type used in the complexation reaction. However, the uniformity of the formulated colloidal systems was significantly influenced by the nature of the polycation (natural or synthetic) (Fig. 1), while the biocompatibility was affected by their structure.

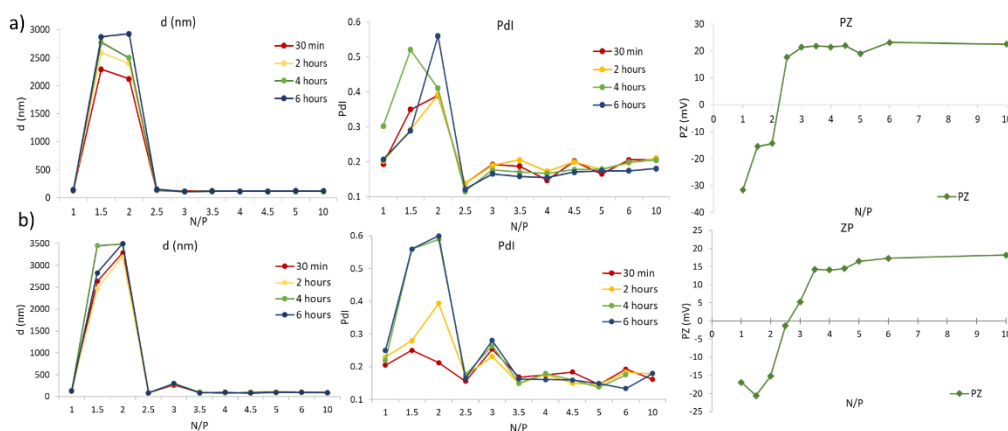


Fig.1. Hydrodynamic features and stability of complexes formulated with a) - liner PEI, b) - branched PEI

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## Polymeric Membranes with Increased Biomineralization Ability

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### INTRODUCTION

The mineralization ability is a crucial feature of biomaterials designed for applications in osseointegration since numerous studies showed that mineralized biomaterials have improved bone regeneration enhancement ability compared to their non-mineralized counterparts. Preliminary studies performed to assess the mineralization ability in vitro are mainly based on chemical methods (e.g., simulated body fluid incubation, alternate soaking in calcium and phosphate ionic solutions) or biological methods such as mineralization by osteogenic cells [1]. We present here two different studies in which the ability of polymeric membranes (with potential uses in osseointegration) for biomineralization at their surface have been synthesized.

### EXPERIMENTAL METHODS

In the first study, magnetic nanoparticles synthesized by the co-precipitation method were used for the preparation of cellulose acetate composite coatings through the phase-inversion method [1]. In the second study, the surface of commercial cellulose acetate membranes was functionalized with 4'-aminobenzo-15-crown-5 ether, using a covalent bonding approach. The main goal was the improvement of the membranes biomineralization ability, thus making them prospective materials for bone regeneration applications [2].

### RESULTS

The biomineralization ability of the membranes was tested through the Taguchi method, and it was found that nanostructured hydroxyapatite was formed at the surface of the composite membrane (with a higher organization degree and purity, and a Ca/P percentage closer to the one seen with stoichiometric hydroxyapatite, compared to the one deposited on neat cellulose acetate) [1]. For the second study, the proposed reaction mechanism was confirmed by XPS and NMR analysis while the presence of the functionalization agents in the membranes structure was showed by ATR FT-IR and Raman spectra. The effects of the functionalization process on the morphology, thermal and mechanical properties of the membranes were studied by SEM, TGA and tensile tests.

### CONCLUSIONS

The results obtained indicate a potential new application for magnetic nanoparticles in the field of orthopedics [1]. For the second study, the obtained results revealed that the cellulose acetate membranes were successfully functionalized with crown ether and provided a good understanding of the interactions that took place between the polymer and the functionalization agents. Moreover, promising results were obtained during the Taguchi biomineralization studies. SEM images, EDX mapping and XRD spectra indicating that the CA-AB15C5 membranes have a superior  $\text{Ca}^{2+}$  ions retention ability, this causing an accentuated calcium phosphate deposition on the modified polymeric fibers, compared to the neat CA membrane [2].

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## Innovative 3D Printed biomaterials

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Incorporating inorganic nanofillers into hydrogels can significantly improve their low mechanical characteristics. Hydrogels are networks of three-dimensional polymers that have the remarkable capacity of absorbing water or biological fluids without breaking down. Hydrogels can be categorized as either natural or synthetic depending on their origin. Flexibility, biocompatibility, and raw material availability are the main attributes of natural hydrogels. Moreover, hydrogels' structural resemblance to the extracellular matrix allows for a wide range of applications. The main natural biopolymers used in the production of hydrogels include chitosan, alginate, collagen, dextran, cellulose, hyaluronic acid, DNA, and fibrin<sup>1-3</sup>. A practical solution to overcome the limitations of mechanical properties is to incorporate nanoparticles into a hydrogel material, as previously mentioned<sup>1-3</sup>. Nanofillers and polymeric networks can interact chemically and/or physically with nanoparticles to create nanocomposites with unique topologies that are crosslinked either physically or covalently. Because nanoparticles can differ in shape and structure, carbon nanotubes, ceramic, polymeric, and metallic nanoparticles can be used to provide enhanced properties and customized functionality<sup>1-3</sup>. With a focus on the impact of Cloisites clay type on the printability and morpho-structural features, this paper offers research investigations pertaining to the characterization and printability of 3D nanocomposite hydrogel structures based on natural polysaccharide. The use of natural clay was anticipated to result in increased structural reinforcement. The hydrogels' structure was ascertained by FTIR and SEM investigations. It was also assessed how the concentration of clay affected the mechanical characteristics, water absorption, antibacterial activity, and rheological characteristics of the polysaccharide-based hydrogel. Due to the appropriate qualities the developed 3D printed nanocomposites structures are suitable for use in future bone tissue regeneration applications.

### ACKNOWLEDGEMENTS

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## Nanostructured systems containing temozolomide for Glioblastoma treatment

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**INTRODUCTION:** Glioblastoma (GBM) is the most common and aggressive malignant tumor of the central nervous system, with high mortality and low survival rates. Temozolomide (TMZ), an alkylating agent that causes DNA damage, is the first-line chemotherapy for the treatment of this tumor. However, TMZ has some limitations, such as its short plasma half-life, difficulty in resisting the cellular mechanisms of drug efflux, and cellular non-specificity, presenting cytotoxicity even to healthy cells, leading to several systemic side effects<sup>1</sup>. In this scenario, the use of intranasally administered nanostructured systems emerges as a potential alternative to circumvent the limitations of conventional treatment. The intranasal route of administration will allow TMZ to be delivered directly to the brain via the olfactory and trigeminal nerves, without entering the systemic circulation, thereby reducing side effects<sup>2</sup>. To improve nasal absorption and transport to the brain, the drug can be incorporated into polymeric nanoparticles to protect TMZ from enzymatic degradation and increase its retention time in the nasal mucosa, bypassing mucociliary clearance, particularly using mucoadhesive polymers such as chitosan<sup>3</sup>. In the present work, chitosan (CS) polymeric nanoparticles (NP) were developed to encapsulate TMZ for intranasal administration.

**EXPERIMENTAL METHODS:** NP was produced through the ionotropic gelation technique, in which sodium tripolyphosphate (TPP), a crosslinking agent, was dropped onto the chitosan dispersion (pH 5.5) under constant magnetic stirring at 600 rpm. For this purpose, three types of CS were tested, namely: A) oligomeric CS (OC); B) Low molecular weight CS (LMWC); and C) Medium molecular weight CS (MMWC). For each type of polymer, four different NP were developed, with the following CS concentrations and CS:TPP ratios: 1) 1.125mg/mL and 3:1; 2) 1.120mg/mL and 4:1; 3) 1.50mg/mL and 3:1; and, 4) 2.0mg/mL and 4:1. The particle size diameter (PSD) and polydispersity index (PDI) were evaluated by the dynamic light scattering technique, as well as determination of the zeta potential (ZP) by electrophoretic mobility of the NP.

**RESULTS AND DISCUSSION:** Formulations based on OC in proportions of 3:1, in both CS concentrations, underwent precipitation immediately after preparation. For the other NP, the PSD diameter ranged from 133.6 nm to 697.6 nm, according to the increase in the molecular weight of the CS used. The PDI values ranged from 0.271 to 0.639, and the PZ ranged from +16.2 mV to +29.1 mV. Similar to reported in other studies in the literature, the size of the NPs increased as the molecular weight of the polymer used increased, with the lowest values obtained for OC<sup>4,5</sup>. For an efficient drug delivery to the brain via the intranasal route, it is desirable that the NP have a reduced PSD, as well as low PDI values. Based on this, only the NP prepared from OC at concentrations of 1.5 mg/mL and 2.0 mg/mL were followed for TMZ incorporation. The drug (300 and 500 µg/mL) was added to the CS dispersion and the NP preparation was carried out as previously mentioned. The PSD and PDI of the NPs containing TMZ in the CS composition of 2.0 mg/mL were higher than those obtained with 1.5 mg/mL of CS. The latter presented 186.27 and 177.0 nm at TMZ concentrations of 300 and 500 µg/mL, respectively, and were selected for determination of the TMZ encapsulation efficiency using the filtration ultracentrifugation method to separate the free drug and high-performance liquid chromatography (HPLC) to quantify TMZ. The drug encapsulation efficiency for NPs containing 300 and 500 µg/mL of TMZ was 88.4 and 92.0%, respectively, with drug loading of 16.7% for the first concentration and 25.0% for the latter.

**CONCLUSION:** Considering that both presented similar PSD, these results indicate that the NP containing 500 µg/mL has a higher drug loading ability. Further studies are needed to confirm the stability and applicability of the system obtained. However, these results point to a direction to be followed in obtaining nanosystems capable of improving drug delivery to the brain and optimizing chemotherapy in GBM.

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## Formulation of chitosan nanoparticles loaded with therapeutic agent with optimal physicochemical characteristics

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### INTRODUCTION

There are numerous studies for chitosan nanoparticles as a carrier in drug delivery systems, due to its biodegradability and biocompatibility properties<sup>1</sup>. Although progress has been made, additional research is necessary to improve the manufacturing process of drugs loaded nanoparticles. The concentration, pH value, average molecular weight and deacetylation degree, are some of the factors which influence the structural and morphological characteristics of the chitosan nanoparticles<sup>2</sup>.

### EXPERIMENTAL METHODS

The aim of our research is to develop and compare two types of chitosan nanoparticles (ChNps), with low and medium molecular weight, loaded with two therapeutic agents: 5-Fluorouracil (5-FU, with 1.25 and 2.5 mg/ml) or Resveratrol (RSV, with 1.25 and 2.5 mg/ml). The chitosan nanoparticles were prepared based on ionic gelation in the presence of tripolyphosphate (TPP).

Hydrodynamic and structural characterization were carried out for blank and drugs loaded ChNps, utilizing dynamic light scattering (DLS) and FTIR. Also, encapsulation efficiency (EE%) and *in vitro* release profile of drugs from ChNps were investigated.

### RESULTS AND DISCUSSION

The formulated ChNps showed a mean hydrodynamic diameter in the range of 166 to 216 nm with the polydispersity of PDI < 0.194 (LMW), PDI < 0.231 (MMW). The encapsulation of therapeutics influenced the hydrodynamic characteristics of colloids in different ways. The encapsulation of RSV determined an increase in the average diameter of ChNps, compared to 5-FU which presented an opposite effect, the drug loading slightly decreased the hydrodynamic characteristics of colloids. FTIR spectral analysis of 5-Fu loaded ChNps showed C=O vibration at 1662 cm<sup>-1</sup> and amide vibration at 1247 cm<sup>-1</sup>. The FTIR spectrum of RSV loaded ChNps displayed C=C stretching of aromatic ring and benzene ring vibration at 1579-1500 cm<sup>-1</sup>. All the formulations presented an EE% above 64%, with the exception of 5-Fu-2.5 mg/ml which had an EE% of about 17-18% for both types of chitosan. *In vitro* release studies highlighted a classical biphasic release of 5-FU, the first phase characterized by a burst release (26-36%) followed by a more controlled release phase (40-45%), regardless of the type of the ChNps used.

### CONCLUSION

In this work, formation of nanoparticles for biomedical applications was successful for LMW and MMW at 0.125% chitosan concentration. The results of DLS and FTIR studies confirmed the incorporation of drugs within the prepared ChNps. EE% of the drugs showed a slightly increase for LMW chitosan.

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## Advancements in influenza vaccines: from egg-based to cell-based technologies

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### INTRODUCTION

The Influenza virus, responsible for some of the worlds most semnificant pandemics, including the 1918 „Spanish Flu”, or „The mother of all pandemics”, still poses a great risk, being responsible for a great number of deaths every year. Today, vaccination is one of the most important prevention strategies, in order to control the spread of the virus and to prevent seasonal outbreaks.

### RESULTS AND DISCUSSION

Influenza vaccine manufacturing is a complex process and it depends on the serotypes and the subtypes of the virus that can cause an outbreak every year, each one with its own particularities. There are two serotypes of flu virus that affect humans, Influenza virus type A, with 3 antigenic variants of hemagglutinin (H1, H2 and H3) and 2 antigenic variants of neuraminidase (N1 and N2), and Influenza virus type B. Type A is more virulent and presents a higher pandemic risk because of its high genetic variation that can be either point mutations (antigenic drift) or recombinations (antigenic shift).

Most Influenza vaccines are egg-based, using embryonated chicken eggs for viral multiplication. Despite being used for decades, this process has some disadvantages: a long production time, poor viral multiplication, allergic responses to egg proteins, egg availability and it also poses ethical concerns.

A promising alternative is the use of mammalian cell cultures, usually MDCK cells for viral multiplication. This relatively new method makes the manufacturing process more reproducible and has some advantages compared to the egg-based process: a shorter production time, a higher initial purity, the absence of egg proteins, and the possibility of a rapid response in the case of a pandemic.

We conducted a number of tests in order to optimize the critical stages of the conventional process, to obtain a higher immunogenicity, purity and stability of the vaccine, using different methods of viral purification and splitting.

### CONCLUSION

The transition from the conventional egg-based process to a cell-based one is desired and development on research and technological steps were possible within Cantacuzino Institute.

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## Multi-Functional Hybrid Terpolymer Thermosets with Tunable Properties Based on Thiols and Bio-Based Epoxy and Benzoxazine Monomers

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Polybenzoxazines, a versatile emerging class of thermosetting resins, are well recognized for their exceptional properties, including dimensional stability, chemical resistance, low flammability, hydrophobicity, minimal shrinkage, and outstanding mechanical performance. Despite their promising properties, benzoxazine-based materials still present certain limitations. Most benzoxazine monomers are inherently solid and require melting prior to processing. Their polymerization typically occurs at elevated temperatures ranging from 220 to 260 °C, and the resulting materials often exhibit brittleness after curing<sup>1,2</sup>.

A possible strategy to overcome benzoxazine disadvantages would be blending with another thermoset. For instance, copolymerization of benzoxazine with different compounds such as epoxy monomers and thiols will lead to significant modifications in both thermal and mechanical properties of the resultant hybrid networks in comparison with benzoxazine homopolymer.

The present study aims to develop complex bio-based thermoset networks by combining the advantages of benzoxazine with epoxy monomers. The free amino groups present on the structure of eugenol-based benzoxazine monomers have the potential to chemically interact with the oxirane ring of epoxidized linseed oil leading to homogeneous network with high crosslinking density. At the same time, the potential of thiols as common crosslinking agents was assessed by studying their influence over polymerization temperature and network properties. It was already demonstrated that these functional groups have a high reactivity towards both oxazine and epoxy moiety. Thiol groups facilitating ring opening reactions under mild conditions and have the potential to improve toughness and flexibility of thermosets while acting as chain extenders at the same time. Thus, by combining benzoxazine with epoxidized oil chains and thiols, brittleness can be diminished and the reactivity of the system can be improved leading to novel hybrid copolymeric networks with unique features.

The resulting terpolymers were characterized in terms of thermomechanical properties by employing TGA, DMA and nanomechanical performance. The curing dynamic and kinetics were monitored by differential scanning calorimetry (DSC) and Fourier transform-infrared spectrometry (FTIR). FTIR results showed that the curing process takes place in multiple steps and depends on the concentration of thiol used as crosslinker. At the same time, the complexity of the reactions that take place within each system was highlighted by the curing profiles obtained by DSC.

### ACKNOWLEDGEMENT

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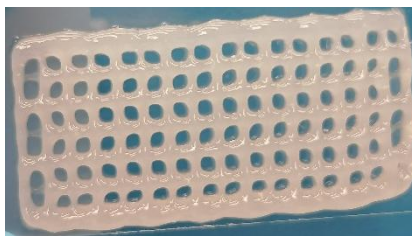
## Potential applications of methacrylated xanthan in 3D bioprinting

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The skin, the largest organ of the body, has limited regeneration capacity in the case of certain pathologies. Therefore, in these cases, it is necessary to use some treatments to help the wound healing. Polysaccharide-based biomaterials have huge potential for wound healing because they ensure proper wound moisture, allow gas exchange, and absorb exudate, which are fundamental features for the healing process [1].

Xanthan, an anionic polysaccharide, can be considered for development of biomaterials with applications in the treatment of dermatological diseases (wound healing) being recommended for its anti-inflammatory and antibacterial effects. Additionally, its double helix structure provides a pseudoplastic behavior, which makes xanthan a good candidate for 3D bioprinting applications. The uniform dispersion of halloysite (a natural layered silicate) within polymeric matrix promotes cell adhesion and re-epithelialization [2, 3].

In this work, different bioink formulations based on modified (methacrylated) xanthan and halloysite nanotubes have been investigated



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## Tri-Polymer Chitosan/Gelatin/ $\kappa$ -Carrageenan Hydrogels: Toward Stable and 3D-Printable Bioinks for Tissue Engineering

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Recent advancements in 3D printing technologies have broadened their applications in biomedical engineering, particularly in the fabrication of complex, patient-specific structures. In tissue engineering, state-of-the-art 3D bioprinting enables the precise deposition of cell-laden bioinks to create architectures that closely mimic native tissue structures<sup>1</sup>. Polymer-based hydrogels remain central to this progress due to their high water content, biocompatibility, and tunable physicochemical properties. Natural polymers such as gelatin, chitosan, and  $\kappa$ -carrageenan are of particular interest for their bioactivity and ability to support cellular functions<sup>2</sup>. To address the limitations of single-component systems, multimaterial hydrogel formulations have gained increasing attention, combining the advantages of individual polymers to better control rheology, degradation, and mechanical properties<sup>3,4</sup>.

The progression of 3D bioprinting technologies requires the development of bioinks that balance printability, structural fidelity, and long-term stability under physiological conditions. Thus, in this study, we present a tri-polymer ink composed of chitosan, gelatin methacryloyl (GelMA) and  $\kappa$ -carrageenan, designed for extrusion-based bioprinting and subsequent tissue engineering applications.

To comprehensively evaluate and optimize the ink formulation, a range of characterization techniques was employed. Fourier transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance (<sup>1</sup>H NMR) were used to confirm the successful chemical modification of gelatin to GelMA by identifying characteristic functional groups and quantifying the degree of methacrylation. Rheological assessments were performed to characterize shear-thinning behaviour and viscosity recovery—critical parameters influencing extrusion consistency. A mathematical model was used to define a theoretical printability window based on rheological data and printing conditions. Experimental 3D printing trials were then conducted to optimize these parameters, using the theoretical window as a benchmark. Printability was further evaluated through various parameters, whilst mechanical testing was used to assess the structural integrity of printed constructs. Swelling behaviour and stability in simulated physiological conditions were also examined to predict *in vivo* performance.

Different crosslinking strategies were employed and evaluated, such as post-printing photocrosslinking for GelMA, chemical crosslinking with genipin for gelatin and chitosan, and ionic thermo-gelation with potassium chloride for carrageenan. The crosslinking strategy was optimized to balance mechanical stability, cytocompatibility, and degradation, ensuring suitability for tissue engineering applications.

Overall, this tri-polymer hydrogel system presents a versatile platform with tunable properties and multiple crosslinking capabilities that enhance long-term scaffold performance. Thus, this work establishes a robust foundation for future development of cell-laden constructs and tissue-specific bioprinting strategies.

### ACKNOWLEDGEMENT

This research study was supported by a grant of the Romanian Alliance of Technical Universities. The authors acknowledge the support of the project "Novel smart hydrogels based on biopolymers and graphene oxide for photothermal therapy", contract 23/09.10.2023.

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## Peptoid molecules with antibiotic complexation properties: rational design, purification and structural characterization

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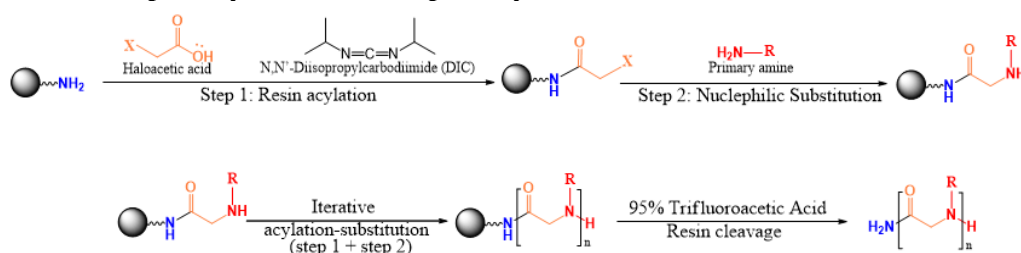
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One of the most important research directions in healthcare focuses on the development of novel antibiotic bioconjugates that would bring innovation in areas like drug delivery systems or advanced sensing devices. Inappropriate antibiotic therapeutic strategies have been noticed to have an increasing effect on undesired microorganism antibiotic resistance. Thus, the development of materials capable of conjugating antibiotic molecules offer a promising path towards limitation of antibiotic resistance. In this context, our work focuses on the rational design of N-substituted polyglycine oligomers (peptoids) capable of antibiotic complexation, benefiting from various biotechnological applications. Due to their structural particularities, peptoids benefit from a peptide-similar backbone, while being less susceptible to enzymatic degradation<sup>1</sup>. Typically, they are synthesized through sub-monomeric solid phase synthesis protocols, involving iterative acylation and nucleophilic substitution on a -NH<sub>2</sub> terminal growing chain. In this work we report the successful synthesis of rationally designed peptoid molecules with tetracycline complexation properties, as one of the most frequently used antibiotics<sup>2</sup>. The peptoid sequences contain N-(s)-(1-phenylethyl)glycine (Nspe) and N-(1-naphtylethyl)glycine (N1npm) units, which promote tetracycline conjugation both via  $\pi$ - $\pi$  stacking interactions, and hydrogen bonds. 7-mers containing these structural units were synthesized, purified via solid phase extraction and structurally characterized. Their complexation capacity was investigated and a correlation between side-chain positioning and conjugation capacity was revealed, highlighting a very promising platform for different biotechnological applications.

### General solid phase synthesis reaction pathway:



### ACKNOWLEDGEMENT

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## Self-healing Guar gum/pectin hydrogels for wound healing applications

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Guar gum is a natural polymer, a polysaccharide obtained from the seeds of the guar plant, consisting of  $\beta$ -D-manopyranose and  $\alpha$ -D-galactopyranose units linked in the 1,4-position. It is biocompatible, biodegradable, abundant in nature, affordable and low cost. Its most important property is that it is soluble in water (even if cold), forming solutions with high viscosity. Pectin is a natural polysaccharide with an anionic character consisting of D-galacturonic acid units esterified with methanol, linked in the 1,4-  $\alpha$ -positions. It is usually extracted from the cell wall of citrus peels, apples and sugar beets. In the last decade, pectin has attracted great interest in many applications due to its biocompatibility, biodegradability, non-toxicity [1].

Fast self-healing, highly flexible hydrogels based on guar gum and pectin have been developed. They have shown the ability to repair themselves and return to their original structure, appearance and functions after damage. Responsible for this characteristic were the hydrogen bonds that are established between the two polysaccharides and the reversible ester-borate bonds that were formed upon cross-linking with borax solutions of different concentrations [2]. The structure of the obtained materials was revealed by FT-IR spectrometry. The demonstration of self-healing ability was evidenced by optical microscopy, determination of rheological properties and macroscopic verification. Other characterization methods that were used to identify the properties of the hydrogels were: thermogravimetric analysis, determination of the swelling degree at different pH values, degradation study, determination of the sol-gel transition temperature and adhesion evaluation to different substrates.

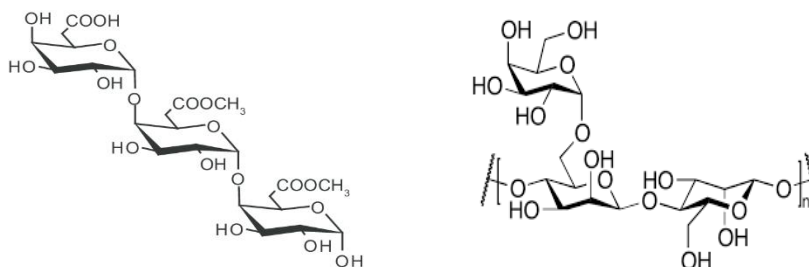


Fig. 1. The chemical structure of the raw materials used for the synthesis of the self healing hydrogels

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## Synthesis and evaluation of poly(propylene fumarate)-grafted graphene oxide as nanofiller for porous scaffolds

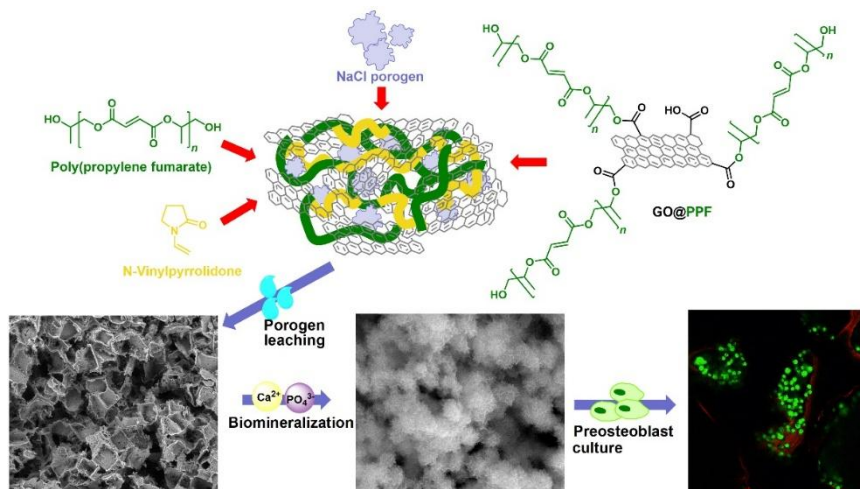
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Recent advances in nanotechnology and polymer chemistry enabled the development of many advanced scaffolds for tissue engineering and regenerative medicine. Therefore, a variety of materials including hydrogels, porous nanocomposites and nanofibers have been investigated as potential candidates for bone repair [1]. Several aspects should be considered when designing scaffolds for tissue engineering applications, including mechanical properties, biodegradation rate, and biocompatibility in order to allow cell adhesion, differentiation and proliferation [2]. In an effort to obtain porous scaffolds with improved mechanical properties and biocompatibility, the current study discusses nanocomposite materials based on poly(propylene fumarate)/N-vinyl pyrrolidone (PPF/NVP) networks reinforced with polymer-modified graphene oxide (GO@PPF). The GO@PPF nanofiller was synthesized through a facile and convenient surface esterification reaction, and the successful functionalization was demonstrated by complementary techniques such as FT-IR, XPS, TGA and TEM. The PPF/NVP/GO@PPF porous scaffolds obtained using NaCl as porogen were further characterized in terms of morphology, mechanical properties, sol fraction, and in vitro degradability. SEM and nanoCT examinations of NaCl-leached samples revealed networks of interconnected pores, fairly uniform in size and shape. We show that the incorporation of GO@PPF in the polymer matrix leads to a significant enhancement in mechanical properties, which we attribute to the formation of denser and more homogenous networks, as suggested by a decreased sol fraction for the scaffolds containing a higher amount of GO@PPF. Moreover, the surface of mineralized PPF/NVP/GO@PPG scaffolds is uniformly covered in hydroxyapatite-like crystals having a morphology and Ca/P ratio similar to bone tissue. Furthermore, the preliminary biocompatibility assessment revealed a good interaction between PPF/PVP/GO@PPF scaffolds and murine pre-osteoblasts in terms of cell viability and proliferation.



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## Sustainable 3D printed catalyst for PLA depolymerization

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### INTRODUCTION

The design and fabrication of polymeric-metallic composites mostly obtained by incorporating metal powders or particles into the thermoplastic matrix is a special class of composite filaments. The primary objective of developing this class of filaments by combining the characteristics of both polymers and metals is to obtain novel functional features, such as supported 3D printed catalysts. Furthermore, in line with the concept of 3D-printed catalysts, which enhance catalytic processes through innovative structural designs, this class of filaments could be applied in this direction. In developing a polymer-metal filament for 3D printing, key parameters that should be considered include the selection of materials for obtaining homogeneous blends. Additionally, cost-effectiveness and recyclability are essential for economic and environmental sustainability. In regards with the selection of polymeric matrix, using recycled plastics as feedstock for 3D printing is a sustainable approach by employing recycled thermoplastics.

### EXPERIMENTAL AND RESULTS

This study explores incorporating organo-metallic catalysts based-on tin derivatives into polymeric matrix from mechanically recycled wastes to produce polymer-metal 3D printed catalysts for domain of polymer chemical recycling. The novelty of this study lies in utilizing PP extracted from face masks using the natural solvent limonene, with chitosan serving as a ligand for metal salts to ensure uniform metal distribution throughout the filaments. The 3D printed catalysts were used in chemical recycling of polylactic acid towards obtaining new lactic acid ester-derivates via heterogeneous catalysis.

### CONCLUSIONS

In parallel, the polylactic acid chemical recyclability was assessed within homogeneous catalysis to compare the two main recycling pathways. These findings revealed that 3D printed polymeric materials incorporating commercial catalyst demonstrated a superior degree of distribution within the 3D supported matrix.

### ACKNOWLEDGEMENTS

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# Surface and Thermal Properties of Synthesised Chemically Crosslinked Poly(1-Ethenylpyrrolidin-2-One) Homopolymers

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## INTRODUCTION

Homopolymers of 1-ethenylpyrrolidin-2-one have been approved for many applications, e.g. as an additive in the food industry, in biomedical applications or as adhesives. These heterocyclic polymers containing nitrogen are typically produced as light yellow or white, amorphous and hygroscopic powder and they are generally recognized as safe (GRAS) <sup>1, 2, 3</sup>. The aim of this investigation was to prepare and characterize chemically crosslinked, three-dimensional poly(1-ethenylpyrrolidin-2-one), p(EP), homopolymers.

## EXPERIMENTAL METHODS

Poly(1-ethenylpyrrolidin-2-one), p(EP), homopolymers were synthesized by the free radical polymerization method with thermal initiation. Different amounts of crosslinker ethane-1,2-diyl bis(2-methylacrylate) were mixed with the monomer. Morphology characterization of the synthesized p(EP) homopolymers was conducted using the scanning electron microscopy (SEM) method. The differential scanning calorimetry (DSC) method was applied to detect their thermal characteristic.

## RESULTS AND DISCUSSION

The SEM micrograph showed a crosslinked, macroporous surface of p(EP) homopolymer networks. Values of glass transition ( $T_g$ ) and melting ( $T_m$ ) temperatures for p(EP) homopolymer hydrogels were found to be in the range of 130.27-130.37°C and 159.95-163.13°C, respectively, indicating their thermal stability during sterilization. The obtained results of melting temperatures were higher than p(EP) homopolymer products in powder state (139°C). The change in melting enthalpy ( $\Delta H_m$ ) was measured to be in the range of 1.416-12.88 J/g.

## CONCLUSION

Synthesized chemically crosslinked p(EP) homopolymers could serve as promising candidates in potential new biomedical applications and also as drug carriers.

## ACKNOWLEDGMENTS

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## Poly(N-vinyl formamide) nanocomposite hydrogels

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### INTRODUCTION

Hydrogels are versatile, water-absorbing polymer networks with a wide range of applications in biomedical and pharmaceutical fields (e.g., tissue engineering, wound care, drug delivery, biosensors, bioprinting), as well as in agriculture, catalysis, microfluidics, actuators, cosmetics, membranes, and water treatment. (Nano)composite hydrogels combine a hydrogel network with nanoparticles, such as carbonaceous materials, metals or metal oxides powders, or layered clays, to enhance performance and enable new applications. A special place among these nanoparticles is occupied by Laponite, a synthetic clay with the empirical formula  $\text{Na}^{+}_{0.7}[(\text{Si}_8\text{Mg}_{5.5}\text{Li}_{0.3})\text{O}_{20}(\text{OH})_4]^{-0.7}$ , acting also as crosslinker in the hydrogel network. The first such hydrogel, synthesized from N-isopropylacrylamide using Laponite XLG as the crosslinker [1], displayed remarkable mechanical and optical properties. Since then, nanocomposite hydrogels have been developed from various monomers, including acrylamides and, occasionally, non-acrylamide monomers like N-vinylpyrrolidone [2]. Poly(N-vinylformamide) (PNVF) hydrogels can be synthesized via free-radical polymerization of N-vinylformamide (NVF), typically in the presence of a divinyl comonomer. These hydrogels exhibit mechanical and swelling properties comparable to those of polyacrylamide (PAAm) hydrogels, with potential biomedical and industrial applications. NVF has lower toxicity than acrylamide (AAm) and exhibits high reactivity.

The present work investigates the synthesis and comparative properties of PNVF hydrogels prepared by free-radical polymerization of NVF in aqueous solution, using Laponite XLG, N,N'-methylenebisacrylamide (MBA), or a mixed XLG/MBA system as crosslinkers.

### EXPERIMENTAL METHODS

To obtain the hydrogels, the appropriate amounts of DW and XLG were magnetically stirred at room temperature for 2 hours to exfoliate the clay in the first step. For MBA-crosslinked hydrogels, MBA (4 mol% relative to NVF) was then added to the clear dispersion. After complete dissolution of MBA, a solution of AIBN (0.25 mol% relative to NVF) in NVF (20 wt. % relative to the total polymerization mass) was added. Depending on the hydrogel formulation, either MBA or XLG was omitted. After 10 minutes of stirring, the homogeneous solution was degassed under vacuum for a few minutes, then transferred via needle and syringe into nitrogen-purged, rubber septum-sealed glass tubes of appropriate shape and size to form cylindrical hydrogel samples for mechanical compression or tensile, respectively, tests. The tubes were then placed in an oil bath or an oven at 50 °C for 24 hours, to obtain the hydrogels, which were purified and analyzed.

### RESULTS AND DISCUSSION

For the synthesized hydrogels, the monomer conversion (C), gel fraction (GF) and the swelling degree (SD) of the samples mechanically tested were determined. The samples were also characterized by ATR-FTIR, TGA, XRD, TEM, uniaxial mechanical compressive and elongation tests.

### CONCLUSION

This study shows the successful synthesis of PNVF hydrogels via free-radical polymerization of NVF in aqueous solution, using Laponite XLG, MBA, or a mixed XLG/MBA system as crosslinkers. The comparative analysis highlights the influence of crosslinking agents on hydrogel structure, mechanical performance, and swelling DEGREE, providing valuable insights into tailoring PNVF hydrogels for potential biomedical and industrial applications.

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## Polysaccharides based materials for 3D printing

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Given its interdisciplinary character, three-dimensional (3D) printing technology has been gradually increasing in various sectors, including polymer chemistry, medicine, pharmaceutical research for creating advanced drug delivery systems, and biology. Polysaccharide-based hydrogels are highly appealing in this field because of their biodegradability, natural abundance and low cost, as well as their biocompatibility and pharmacological properties[1-3].

The goal of our research is to develop polysaccharide-based interpenetrating network materials with improved printable ink properties for biomedical application using extrusion-based 3D printing. The shear-thinning properties of the inks was assessed before 3D printing and stability of the crosslinked scaffolds was evaluated by swelling degree and degradation tests that were performed in PBS (pH=7,4) at 37 °C. In addition, morphology analysis was employed to assess the pore size and uniformity of the freeze-dried scaffold.

Based on the overall results of these experiments, 3D-printed constructs made from polysaccharide ink have the potential to be used in soft tissue engineering applications.

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## Balancing Printability and Degradation: 3D Printed Polymer-Bioglass Scaffolds with Osteogenic Potential

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### INTRODUCTION

Bone tissue engineering has increasingly relied on advanced manufacturing techniques to create scaffolds that can simultaneously provide mechanical support and promote tissue regeneration. Despite significant progress in this field, challenges remain in developing materials that offer both structural integrity and bioactivity for critical-sized bone defects<sup>1</sup>. Selective Laser Melting (SLM) has emerged as a transformative technology in fabricating porous titanium alloy scaffolds that provide excellent mechanical properties. However, titanium alone lacks the bioactive properties necessary for optimal bone integration. This research addresses this limitation by developing composite hydrogel-bioactive glass scaffolds integrated with 3D printed titanium lattice substrates, combining the mechanical advantages of metallic frameworks with the biological properties of bioactive materials.

### EXPERIMENTAL METHODS

A dual-material approach was employed in this study to create integrated bone regeneration scaffolds. First, porous titanium lattices were fabricated via selective laser melting (SLM) to provide mechanically robust, osteoconductive frameworks. Second, a bioactive hydrogel ink was formulated, composed of a biocompatible polymer matrix and doped bioactive glass (SiO<sub>2</sub>-P<sub>2</sub>O<sub>5</sub>-CaO-Na<sub>2</sub>O co-doped with europium and silver). The bioglass was synthesized using a particulate sol-gel method to improve biological properties, including antibacterial activity via Ag<sup>+</sup> ions and osteogenic stimulation via Eu<sup>3+</sup> ions<sup>2</sup>. The hydrogel composition was tailored for rheological compatibility with extrusion-based printing, similar to methods demonstrated in previous bioactive glass-hydrogel composites. Critical printing parameters—such as speed, pressure, layer resolution, and interfacial bonding strategies between hydrogel and titanium—were systematically studied to optimize structural fidelity and mechanical cohesion.

### RESULTS AND DISCUSSION

Rheological analysis confirmed that the chosen hydrogel compositions (notably 7% alginate, 8% gelatin and 3% alginate, 6% gelatin) exhibited suitable viscosity and shear-thinning behavior for high-fidelity printing. Material characterization using FTIR verified the successful incorporation of bioglass within the hydrogel matrix, while SEM and EDS analyses revealed information on the dispersion of bioglass particles throughout the scaffold. The scaffolds supported cell viability, as demonstrated by LIVE/DEAD immunocytochemistry. Swelling and degradation studies showed full degradation of the scaffolds under 28 days with 120-380% increase in fluid absorption.

### CONCLUSION

This work presents a hybrid scaffold combining mechanically robust titanium lattices with bioactive, ion-doped hydrogel composites. These findings, together with evidence from *in vitro* studies on bioactive glass-coated titanium scaffolds, highlight the potential of such composites for effective, personalized bone defect repair.

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## Aging of polypropylene tree-shelters made from mixtures of virgin and recycled plastic

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Tree-shelters (or tree guards) are simple and economical devices widely used in afforestation and agricultural activities, for example in vineyards and pistachio plantations (Fig. 1). These devices protect seedlings from predators and aggressive agronomic practices and also generate a suitable microclimate for plants by controlling relative humidity, irradiation and temperature, thus increasing survival and growth rates<sup>1,2</sup>. Polypropylene (PP) is the most used material in this application, because of its lightness, outstanding and optical properties and low-price.

The use of recycled polypropylene (rPP) in this application significant boost for the PP recycling process, since thousand tons of this plastic are used each year in the manufacture of tree-shelters in Europe. However, most of today is virgin plastic. Many users are reluctant to use rPP this material can present varying levels of degradation and contamination, which can affect its mechanical and optical properties and, especially, the lifespan of tree shelters in the field.

Within a research project dedicated to the study of the use of polyolefins in this application, the main objective of this work was to get insight on the degradation of PP tree-shelters manufactured with different contents of rPP, and the changes in the mechanical and optical properties. A postindustrial clear rPP and a commercial stabilizing additive, containing an antioxidant and a HALS, were used. Light transmission was controlled by using a commercial dye. The shelters were subjected to either accelerated aging in a Q-UV chamber during 3000 h or natural aging forestry during 1 year in a location near Madrid. Polymer degradation was characterized by using IR spectroscopy and thermal analysis. Changes in the properties were studied by using UV-Vis spectroscopy and tensile tests.

IR spectroscopy shows that both accelerated aging and outdoor exposure cause a photooxidative degradation of PP tree-shelters mainly located in the external face. There is also an increase in the crystallinity degree, revealed by the DSC analysis and related to chain scission caused by the UV radiation, which leads to increased shelter rigidity. This structural transformation results in a decrease in elongation at break. The level of degradation depends on the rPP content. While only a slight decrease is observed in the elongation at break of tree-shelters produced from virgin PP or even 50 % recycled PP, shelters manufactured from 100 % rPP lose 70% of their elongation at break. Light transmission of the shelters decreases slightly during the aging tests but is not reduced by the presence of rPP. Thus, our results show that rPP can be successfully used in the manufacture of tree-shelters, preferably in blends with virgin PP.

### ACKNOWLEDGEMENTS

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Fig. 1. PP tree-shelters

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# Visible Light-Responsive Gelatin-Based Hydrogels with Tunable Mechanics for Advanced Tissue Engineering Applications

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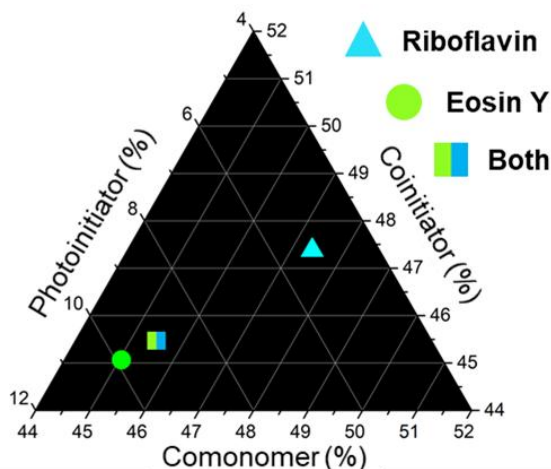
Natural biopolymers like gelatin are widely recognized for their excellent biocompatibility, biodegradability, and safety profile, making them highly attractive for tissue engineering. However, traditional chemical crosslinking approaches often involve toxic reagents that can compromise cellular viability. In this study, we present an innovative photocrosslinking strategy that leverages visible light and biocompatible photoinitiators—riboflavin and eosin Y—to fabricate gelatin-based hydrogels with controllable properties.

We investigate the distinct roles of blue (450 nm) and green (525 nm) light wavelengths in driving the photocrosslinking process. Our results demonstrate that blue light initiates a more rapid generation of reactive species, facilitating fast gelation but at the cost of structural uniformity. In contrast, green light induces a slower, more homogeneous crosslinking process, yielding hydrogels with enhanced mechanical consistency—ideal for applications requiring scaffold uniformity. By combining both photoinitiators and selectively modulating light exposure, we establish a method for generating spatial stiffness gradients—so-called "durotactic gradients"—within a single scaffold. This level of control allows for the creation of region-specific mechanical environments support diverse cellular behaviors, emulating the of native tissue architecture.

To validate this dual-wavelength strategy, we comprehensive characterization, including spectroscopy, mechanical testing, swelling degradation in physiological-like conditions, and computed tomography. These analyses confirm formation of stable, tunable networks with dynamic tissue interface engineering and drug system designs.

This study introduces a modular, light-directed engineer hydrogel scaffolds with spatiotemporal opening avenues for designing multifunctional for next-generation regenerative therapies.

**Figure 1.** Ternary representation of the result of the concentrations of photo-(co)-initiators and



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## ACKNOWLEDGEMENT

The authors would like to thank for the financial support provided by a grant from the Ministry of Research, Innovation, and Digitization, Romania's National Recovery and Resilience Plan, under the project titled "Advanced & Personalized Solutions for Bone Regeneration and Complications Associated with Multiple Myeloma", with reference CF 213/29.11.2022 and contract number 760093/23.05.2023.

## Sustainable Wound Dressings: Chitosan-Silk Fibroin Scaffolds with Green-Synthesized MgO Nanoparticles for Enhanced Healing and Antibacterial Action

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Wound healing has remained a major clinical challenge over time, especially for chronic and infected wounds where conventional bandages frequently fall short in terms of providing sufficient tissue regeneration assistance and antimicrobial protection. Although scaffolds replicate the extracellular matrix (ECM) and encourage cell growth, scaffolds made of biomimetic materials such as silk fibroin (SF) and chitosan (CS) present a possible option. By adding mechanical, antibacterial, and anti-inflammatory qualities, the incorporation of nanomaterials—such as metal oxide nanoparticles—further improves scaffold performance. Magnesium oxide (MgO) nanoparticles are notable among them due to their strong antibacterial properties, biocompatibility, and biodegradability. In this work, the group has tried to use peel extract from *Citrus sinensis* as a sustainable reducing agent to create CS/SF composite scaffolds that were integrated with green-synthesized MgO nanoparticles (0.05–0.15% w/w). In this direction, successful NP integration and scaffold porosity have been confirmed by structural analysis (XRD, SEM, FTIR), and swelling tests showed sustained water uptake (up to 837%), which is essential for preserving a moist wound environment. Related to their biological effect, the developed scaffolds have provided a significant pathogen inhibition by antibacterial tests against *Staphylococcus aureus*, MRSA, and *S. epidermidis*, especially for 1:1 CS/SF scaffolds containing 0.15% MgO. The results shown here demonstrate how MgO NPs improve scaffold performance in two ways: by increasing hydration capacity and preventing infection. Therefore, our results highlighted that the obtained environmentally friendly nanocomposites could represent suitable biomaterials as next-generation wound dressings that combine advanced therapeutic efficacy with sustainability.

## In situ synthesis of silver nanoparticles in cellulose-derived hybrid colloidal system: formation mechanism and antibacterial properties

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The synthesis of AgNPs typically involves chemical compounds that are environmentally harmful, while AgNPs have the tendency to aggregate. The *in situ* development of hybrid AgNPs onto TEMPO-oxidized cellulose nanofibers (TOCNFs) emerged as an approach for the two aforementioned drawbacks [1],[2].

Hybrid AgNPs-TOCNFs were prepared by adding silver nitrate at once or stepwise in a suspension of TOCNF (0.2% w/v). 1M NaOH was used as NP formation accelerating agent. The successful synthesis of nanoparticles is shown by the UV-VIS spectra and transmission electron microscopy (TEM) with selected area electron diffraction (SAED). The hybrid nanoparticles were characterized by Dynamic light scattering (DLS). The samples were investigated by Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrometry and circular dichroism (CD). Antibacterial assays were also performed through Live/Dead tests and minimum inhibitory concentration (MIC) evaluation for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Hybrid AgNPs-TOCNFs <15 nm were successfully obtained in the presence of TOCNF, as suggested by the UV-VIS peak between 400-420nm. As revealed by SAED analysis, the hybrid AgNPs-TOCNFs exhibit a cubic crystal arrangement, as per 04-014-0266 from the International Centre for Diffraction Data (ICDD), with intense and clear diffraction rings. All samples exhibited antimicrobial effects against both Gram-positive and Gram-negative microorganisms as shown by microbiological assays.

The amount of AgNO<sub>3</sub> added to the dispersion influences the hydrodynamic diameter of the resulting colloid with slightly different particle dimensions. Smaller concentrations lead to an increase in diameter as shown by DLS and TEM images. All samples exhibited antimicrobial effects against both Gram-positive and Gram-negative microorganisms as shown by microbiological assays.

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# Tailoring Clay-Reinforced Epoxy Networks Using Essential Oils: A Step Toward Bioactive Coatings

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In recent years, the development of bio-based epoxy nanocomposites has attracted significant interest due to their potential use in environmentally friendly coatings for corrosion protection and wood preservation. In this study, novel nanocomposites were synthesized based on epoxidized corn oil crosslinked with citric acid and reinforced with montmorillonite (MMT) clays modified with natural bioactive agents. The modification of both natural MMT and acid-activated MMT (MMT-KSF) was carried out via a solvent-free adsorption/evaporation method using palmarosa essential oil, clove essential oil, and eugenol — the major active compound in clove oil, known for its antimicrobial and antifungal potential. A 1:1 mass ratio was used in the organophilization step to ensure effective intercalation and surface modification.

The resulting nanocomposites were obtained through a multi-step process: synthesis of the bio-based epoxy precursor, functionalization of MMT and MMT-KSF, dispersion of the reinforcing agent and citric acid in the epoxidized oil, followed by thermal crosslinking. The structure and dispersion of the modified MMT in the polymer matrix were investigated by FT-IR spectroscopy and X-ray photoelectron spectroscopy, and the crosslinking reaction was monitored using DSC analysis. The activation energy of the curing reaction was calculated. Crosslinking degree and network architecture were assessed via Dynamic Mechanical Analysis (DMA). The exfoliation and interfacial interaction between the modified clays and the polymer matrix were found to be enhanced by the presence of bioactive agents, particularly in the case of eugenol-modified MMT, consistent with the known eugenol content ( $\approx 80\%$ ) in clove oil.

Thermal stability was studied by TGA and the surface hydrophobicity was evaluated through contact angle measurements. Preliminary results indicate an improvement in mechanical and thermo-mechanical properties, along with promising anticorrosive performance upon immersion in 5% NaCl solution and salt spray testing. The incorporation of palmarosa-modified MMT also suggests potential for antifungal or insect-repellent applications in wood protection systems, pending further biological testing.

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## Statistics on thyroid cancer in Parhon Institute

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Personalized medicine maximizes the chance of curing various diseases, given each patient's profile or other relevant personal traits. Cancer is the second leading cause of mortality in developed countries after cardiovascular diseases and is a generic term for a wide range of disease processes characterized by alterations of normal cells. Despite rapid technological progress, bioinformatics remains a vast and profoundly interdisciplinary field that requires precise medical information. This paper presents our approach grounded in statistics with precise details from collecting real biological data to extracting useful information for uncovering hidden patterns and relationships, supporting the move towards personalized medicine. The source of the thyroid cancer medical data is represented by Parhon's database -Hipocrate, which contains all the electronic medical records from the beginning of the institute. Based on real data, the continued advancements in data mining techniques and their integration into clinical practice are expected to yield substantial benefits in cancer treatment outcomes, thereby advancing the field of personalized medicine.

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