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Development of injectable composite hydrogels containing hydroxyapatite nanoparticles and hyaluronic acid

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Osteoporosis is a degenerative bone disease characterised by progressive loss of bone mass and deterioration of bone's mechanical properties. The aim of this work is to develop injectable bioactive composite hydrogels for osteoporotic bone regeneration based on hydroxyapatite nanoparticles (nHAp) and hyaluronic acid (HA). *In situ* forming HA/nHAp composite hydrogels with nHAp mass ratios of 0, 30, 40, 50, 60 and 70 wt% were fabricated by covalently cross-linked HA (molar ratio of EDC to NHS was 1:1) and chemically precipitated nHAp. The effect of nHAp phase on the hydrogels' swelling degree, gel fraction, enzymatic and bio-degradation, injectability, viscoelastic properties, morphology, and molecular structure was investigated.

It was observed that the incorporated nHAp into the hydrogel synthesis medium increased the gel fraction by 1.5 times, increased the enzymatic degradation time by five times, reduced the mass loss after biodegradation by two times, reduced the swelling degree by four times and increased the injection force by two times compared to the pure covalently cross-linked HA hydrogel. All samples showed homogenous morphology and an excellent ability to recover viscosity after injection. FTIR analysis showed the formation of ester bonds, confirming that the fabricated HA/nHAp composite hydrogels were covalently cross-linked.

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Investigating HA/PLL based hydrogel influence on cell metabolism

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Research into hydrogels is rapidly advancing due to their potential applications in the biomedical field. Physically cross-linked HA/PLL hydrogels, specifically the 70/30 and 60/40 (HA/PLL) compositions, have demonstrated multifunctional antibacterial capabilities but varying degrees of biocompatibility in both direct and indirect cultures. In this study, we investigate the impact of physically cross-linked HA/PLL hydrogels on intracellular metabolomic changes in NIH/3T3 fibroblast cells. Our approach involved toxicity assessments over three days, a time-course quantitative metabolomics analysis was also conducted using liquid chromatography coupled with massspectrometry, followed by pathway analysis. Additionally, we measured cystine uptake and conducted a Glutathione (GSH) assay using specific kits. Our results highlighted disruptions in several metabolic pathways upon hydrogel exposure, with the temporal fluctuations aligning with the viability outcomes—greater biocompatibility for the hydrogel 70/30. However, both hydrogel types induced substantial damage to cell metabolism after three days. Utilizing metabolomics, we identified the SLC7A11 transporter as a significant player, with cystine uptake and GSH assays validating our findings. These results underscore the efficacy of metabolomics in revealing the underlying molecular pathways of biomaterial-induced cytotoxicity, potentially guiding material development and facilitating the creation of novel in vitro screening tools.

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Gallium containing amorphous calcium phosphate crystallization kinetics in different media

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Amorphous calcium phosphate (ACP) is a metastable phase of any calcium phosphates with characteristic short-range order. ACP tends to spontaneously crystallize in liquid solutions, therefore it is essential to anticipate its stability in different environments. Additionally, Ca^{2+} ions could be substituted with Ga^{3+} ions, which inheres antibacterial properties. Our research aimed to develop a method for obtaining gallium-containing ACP (GaACP) and investigate the influence of gallium on the stability of ACP in different media.

GaACP was obtained via conventional precipitation method. NaOH solution was rapidly added to Ca^{2+}, PO_4^{3-} and Ga^{3+} ions containing solution resulting in precipitation of GaACP. For implementation of crystallization kinetics experiments, lyophilized ACP and GaACP powders were suspended in different media (Dulbecco's Modified Eagle Medium (DMEM), Phosphate Buffered Saline (PBS) and deionized water (DI H_2O)) and placed in incubator-shaker at 37°C and 80 rpm. After specific time samples were taken from the incubator-shaker, centrifuged, washed, and lyophilized.

The X-ray diffraction results revealed that the GaACP powders are most stable in DI H_2O, however, its crystallization in PBS occurred most rapidly. Pure ACP showed lower stability in all media compared to the GaACP. A tendency of enhanced stability was observed with increasing Ga concentration in the ACP.

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High hydrostatic pressure application for the inactivation of E. coli and ESBL bacteria

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High hydrostatic pressure (HHP) sterilisation method is widely applied in the cosmetics and food industry and recently it has been applied in the sterilisation of biomaterials (hydrogels) for medical applications.

The sterilization of drug-loaded hydrogels is complex because traditionally used methods may affect the physical and mechanical properties of the hydrogel, the loaded drug activity and its release profile. Therefore, a new reproducible, safe and resource-reducing sterilization method is needed after the drug-loaded hydrogel's production process without compromising the material's properties. HHP benefits from a low-temperature load, applicability to liquid/gel-like materials, and preservation of unstable structures. High pressure treatment of microbial cells induces many changes in the bacterial cellular functions responsible for survival and reproduction.

The aim of this study was to evaluate time, pressure and cycle oscillation interaction on sterilisation efficiency against *E.Coli* and *ESBL* bacteria for developing a resource-reducing sterilization method of drug-loaded hydrogels by HHP and novel artificial intelligence/machine learning (AI/ML) strategy.

Our research offers a simple, cost-saving green chemistry approach for fast-growing regenerative medicine. Furthermore, the applied strategy could solve a wide range of design tasks moving forward to the Quality by Design concept to predefine objectives and emphasize product and process understanding.

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Comparison of commercially available stereophotogrammetry system outputs

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Facial surface models created via stereophotogrammetry are widely used for diagnostics and surgical planning. The devices and algorithms, which generate the models, are done by commercialised solutions. Therefore, the underlying method for model construction is a commercial secret. While said solutions are deemed to be clinically precise (an error margin of ± 2mm), there is no basis to indicate that these results can be comparable between systems. Hence, a need arises to understand if this is an intrinsic limitation for all systems or if there is an underlying issue regarding the reconstruction of a model. To investigate, the same set of physical objects are planned to be scanned on three commercially available systems with the results being compared qualitatively (indicating problematic regions and features) and quantitively (RMS distance in select regions). It is expected that the output models are similar for all used systems, implying that the same limitation in precision is present for all cases. These results can be further used for developing guidelines for current system selection and identifying areas for future system improvements.

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Detecting differences in serum metabolites to diagnose fracture related infection (FRI)

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Fracture related infection (FRI) represents one of the major complications in orthopaedic and trauma surgery, with Staphylococcus aureus reported up to 40% of times. New biomarkers would be of great value because the classical markers (white blood cell count or C-reactive protein) is ineffective for low-grad infection. We analysed 46 metabolites in serum from Swiss alpine sheep over the time course of bone infection an resolved infection. They received a 2 mm defect in the tibia fixed with a steel plate and inoculated with *Staphylococcus aureus* MSSA. After 3 weeks they had revision surgery and were put on antibiotic treatment. Last samples were taken 2 weeks after antibiotic stop. We found 5 metabolites significantly changed in infected sheep. One was significantly upregulated: hydroxy proline (collagen breakdown). The rest was downregulated: cysteine, cystathionine (redox), citrulline (immune regulation), and myristoyl carnitine (fatty acid oxidation). This is the first paper to show that RFI impact serum metabolites in a measurable and significant way.

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Navigating combinations of Platelet-rich fibrin with Biomaterials in Dental Surgery: A Comprehensive Literature Review

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Platelet-rich fibrin (PRF) is a protein matrix with growth factors and immune cells extracted from venous blood via centrifugation [1]. Previous studies have proved it is a beneficial biomaterial for bone and soft tissue regeneration in dental surgeries [2]. Researchers have been combining PRF with other biomaterials because it is biocompatible and easily modifiable for composite preparation [3–5]. Although *in vitro* and *in vivo* studies have tested PRF and biomaterial composites and proved superior to any of the components separately, it is difficult to compare the results due to varied research methods [6, 7]. Here, we review literature from open-source databases to help readers navigate the field of PRF and biomaterial composites and aid them in selecting a composite that suits their planned research or medical case.

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Platelet-rich fibrin immunological testing methodology using elisa assay

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Keywords: Platelet-rich fibrin (PRF), growth factors, TGFβ1, VEGF, EGF

Introduction: Immunological properties of PRF rely on its ability to release autologous growth factors and cytokines in supraphysiologic concentrations. Various studies are describing ELISA immunological assay as the method to evaluate the concentration of various proteins in PRF. The lack of information in the literature regarding methodology and used PRF dilutions make protein evaluation a challenging task.

Materials and methods: As the experimental part antibacterial activity testing was done using the ELISA method. Peripheral blood samples from 18 healthy volunteers were drawn with the butterfly blood collection method in 10 mL S-PRF tubes. Tubes were placed in a centrifuge and centrifuged at 700 rpm for 3 min according to Choukroun's protocol to obtain i-PRF and frozen immediately at -80^{0}C. Aliquots of i-PRF samples were prepared by thawing frozen i-PRF samples on ice and frozen again. Before the performing ELISA assay, i-PRF samples aliquots were thawed on ice and placed at room temperature to clot for 30 minutes. The supernatant around clots was used to perform an ELISA assay according to instructions from the kit manufacturer and i-PRF samples were diluted to make 4 different concentrations to be in the range of protein detection.

Results: ELISA assay demonstrated high concentrations of TGFβ1, EGF and VEGF in i-PRF. TGFβ1 was found in the highest concentration compared to EGF and VEGF, which were more similar to each other. The differences in each patient's protein content pattern were found. Tested proteins were in a range of detections using i-PRF supernatant dilutions: 1:4, 1:15, 1:100 and 1:200 for EGF; 1:1, 1:4, 1:20 for VEGF; and 1:100 and 1:400 for TGFβ1.

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Conclusion: i-PRF contains various growth factors in supraphysiological concentration. Between tested proteins TGFβ1 is found to be in much higher concentrations.

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