AR.8.S1-K1 Architected Biomaterials for skeletal regeneration

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Abstract

Every year worldwide ten million orthopedic procedures involve fixing fractured or broken bones and injured or arthritic articular cartilage. The gold standard for bone and cartilage repair today is still represented by autologous or allogenic grafts. While these methods have shown evident signs of clinical success, they are also associated with intrinsic drawbacks and risks such as donor variability, morbidity, pain, infections, fracture due to compromised mechanical properties, and limited availability. When articular damage is too severe, prosthetic implants are chosen instead of grafts. These implants are characterized by anatomically shaped biomaterials that replace the functions of the impaired joint for 15–25 years after surgery. Most biomaterials used are metals, with a mismatch to the mechanical properties of the surrounding bone that leads to tissue remodeling and consequently to revision surgery. One prominent alternative to prosthetic implants is to use regenerative medicine strategies where scaffolds are designed as bioactive supports for cells to regenerate new tissues and to integrate with their surroundings. Similar to the native ECM, these scaffolds should ideally exhibit biocompatibility, biodegradability, and suitable mechanical, physico-chemical, and biological features at different scales.

To achieve these targets, the spatiotemporal control over biological signals at the interface between cells and materials is often aimed for. Alternatively, biological activity can be triggered through the control of mechanical cues, harnessing more fundamental know-how in mechanobiology that could be combined with biofabrication strategies. Here, some of the most recent advancements in merging mechanobiology with biofabrication that enabled the control of cell activity are presented, moving towards enhanced tissue regeneration through engineered mechanical stimulation that can be controlled on demand.

AR.8.S1-K2 3D printing of synthetic and natural polymer based inks for 3D in vitro models of muscoloskeletal tissues

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Abstract

Introduction. Investigation of bone and skeletal muscle pathologies in vitro is now a challenging field that requires the design of scaffolds with suitable morphological, structural and biocompatible properties [1, 2]. In this work, 3D printed models are designed with different patterns and proposed as scaffolds for *in vitro* models of bone and skeletal muscle tissues.
Materials and Methods. *In vitro bone models*. TPU and PLLA scaffolds were fabricated by FDM. Different patterns in terms of filament orientations in the structures' layers and pore sizes were selected. GelMA/nHA scaffolds were (bio)printed by extrusion-based printer with a 0/90° fiber orientation. Morphological, physico-mechanical characterization was performed. *In vitro* direct cytocompatibility test was performed by seeding bone marrow-derived human mesenchymal stem cells (hMSCs). Data were statistically analyzed by ANOVA.
In vitro skeletal muscle model. Chitosan-gelatin blend hydrogels were prepared as (bio)inks. Rheological properties were studied. An optimization of the process parameters was performed. Different crosslinking conditions were considered. Shape fidelity investigation, SEM observation, FTIR analysis, and mechanical characterization were performed to find the optimal crosslinking conditions.
Results and Discussion. *In vitro bone models*. The morphology of the printed scaffolds matched the one of the CAD-designed models. All scaffolds are stable in culture medium up to 21 days. The compressive modulus varied depending on the printed pattern and material. An increase in cell metabolic activity was detected from 1 to 14 days of culture on the printed scaffolds. Osteo-differentiated hMSCs colonize the pores of the scaffolds with inorganic ECM deposition, proving cell adhesion, proliferation and differentiation.
In vitro skeletal muscle model. Rheological characterization allowed to detect the sol-gel transition of the blends. Evaluation of the maximum shear stress to which cells could be subjected during the 3D bioprinting process detected that an increase in gelatin content caused an increased value of shear stress. The investigation of the physical-mechanical properties of the printed scaffolds evidenced that shape fidelity and mechanical properties were strictly dependent on the crosslinking conditions.
Conclusions. Based on the obtained results, scaffolds can be appropriately designed to match the properties of different tissues. Morphological and structural properties of the scaffolds here presented are suitable for mimicking the bone and skeletal muscle tissue, in order to produce a 3D *in vitro* model useful for tumor and pathology research.

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AR.8.S1-O1 3D printing of green and antibacterial ceramics for bone regeneration

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Abstract

Antibacterial metal-doped calcium phosphates ceramics show promising efficacy for addressing infection and promoting bone regeneration at one same time, also thanks to the possibility to exploit phosphates having different solubility.

To this regard, the use of marine-derive materials, to obtain the phosphates, appears promising, for two main reasons: because it entails the use of green materials, thus reducing the environmental burden of the devices, and because minerals (calcium carbonates and phosphates) from marine origin contain several ions in the matrix, such as magnesium, and sodium, which have a positive effect on bone cells.

Here we show combination of antimicrobial calcium phosphates from synthetic and natural origin and additive manufacturing, by pursuing two strategies, i.e. (i) the use of antibacterial ceramic-based inks for ceramic 3D printing and (ii) the development of nanostructured coatings, to be applied to 3D printed polymeric or metallic substrates.

The coatings and scaffolds are obtained both by using synthetic calcium phosphates (beta-TCP, with/without silver doping) and by natural marine sources (calcium carbonate from seashells for 3D printing and lingula seashell, a natural multi-doped fluorapatite, for the coatings). Prior to 3D printing, calcium carbonate from mussels and oysters shells is converted into phosphates, exploiting a mild-wet synthesis at room temperature and purposely obtaining different degrees of conversion, to tune the stability profile and ion-release of the powders.

Our results show that both approaches are promising to obtain antibacterial and bioactive coatings, having tunable architecture, mechanical properties, and dissolution profile. Indeed, powders can be effectively converted obtaining carbonated hydroxyapatite in the desired percentage and 3D printed. Coatings, obtained by Ionized Jet Deposition, permit to maintain the same composition of the target, either synthetic or natural and, hence, its antibacterial activity. They show high reproducibility, adhesion to substrate, biocompatibility and a suitable dissolution profile. When combined with 3D printed scaffolds, deposition is conformal and does not alter the scaffolds fibers or porosity. No damages are observed due to interactions with plasma with the substrate.

AR.8.S1-O2 Cuttlefish bone as a sustainable osteoinductive biomaterial in 3D printed biocomposite scaffolds promoted osteogenesis

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Abstract

Sustainability focuses on reducing waste products by reintegrating them in other useful production cycles, thereby providing financial advantages and minimizing environmental pollution levels. Marine derived food waste from shrimps, crabs, squids, lobsters, and cuttlefish are usually discarded away. In particular, cuttlefish bones consist of aragonite, a calcium carbonate polymorph with osteoinductive properties. Aragonite is a common skeletal mineral in marine organisms, while calcium carbonates have been used as bio-fillers in polymers 3D printing. Considering these points, the aim of this work was to develop 3D biocomposite scaffolds consisting of the thermoplastic polylactic acid and the cuttlefish bone with tailored properties for bone tissue engineering.

Aragonite powder was obtained from the bones, purified from organic compounds and impurities by alkaline treatment. PLA and composite PLA/aragonite filaments were prepared, containing 2.5, 5 and 10% w/w of aragonite. 3D printed scaffolds were manufactured using the fused filament fabrication (FFF) technology. All raw and composite materials were characterized by Differential Scanning Calorimetry, Thermogravimetric analysis, X-ray Diffraction, Scanning Electron Microscopy and Fourier Transform Infrared spectroscopy. The hydrophilicity and compression modulus were also evaluated. The cell adhesion and proliferation were investigated by culturing pre-osteoblasts on the scaffolds. The osteogenic potential was examined by quantification of the alkaline phosphatase activity, collagen and calcium production in vitro.

Alkaline treatment led to the removal of organic compounds and did not alter the aragonite polymorph. The incorporation of aragonite increased the hydrophilicity of PLA and the Young modulus. Pre-osteoblastic cells cultured on 3D printed PLA/aragonite scaffolds exhibited high adhesion. Alkaline phosphatase activity, calcium and collagen production were significantly elevated with increasing aragonite content, indicating enhanced osteogenic differentiation. Calcium production, detected by alizarin red staining, showed an almost two-fold increase from day 7 to day 14, indicating that PLA/aragonite scaffolds stimulated matrix mineralization. Moreover, biogenic aragonite significantly increased collagen production, particularly at the highest aragonite concentrations of 5 and 10% w/w, supporting extracellular matrix formation.

In this study, different composite scaffold formulations were produced containing a thermoplastic PLA matrix and three different concentrations of aragonite (2.5, 5 and 10% w/w) from cuttlebones as a sustainable filler with osteoinductive properties. The results demonstrate that 3D biocomposite PLA/aragonite scaffolds promote cell adhesion, proliferation and osteogenesis in vitro, with the highest aragonite content of 5 and 10% w/w indicating significantly higher levels of all osteogenic markers.

AR.8.S1-O3 Volumetric Biofabrication Strategies for Regenerative Medicine Applications and Tissue Vascularization

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Abstract

Vascularization of engineered constructs remains a critical challenge in tissue engineering, as a functional vascular network is essential for oxygen and nutrient exchange, bioprinted cell survival and tissue functionality. Several biofabrication strategies have been explored to incorporate microcapillary networks into 3D-engineered constructs. However, achieving high-fidelity bioprinting of cell-laden biomaterials with low mechanical properties (~500-1500 Pa), which better support cellular functions and microvasculature self-assembly, remains a key bottleneck. Here, we aim to showcase novel approaches developed in our group to enhance capillary self-assembly and endothelial cells recruitment within volumetrically printed constructs for regenerative medicine applications.

As volumetric bioprinting is still in its infancy, only a limited array of printable materials is available. Thus, we focused on developing bioinks that better support cellular functions. To enable microcapillary network formation, we engineered a photo-crosslinkable micro-resin (μResin) composed of jammed gelatin methacryloyl (GelMA) microgels by optimizing the milling time of thermally-gelated gelMA. μResin-based constructs were volumetrically bioprinted in under 22 seconds, with a positive feature resolution of 244 ± 16 μm. The void spaces present between the jammed microgels allowed the embedded endothelial cells to stretch, migrate and form pre-angiogenic capillary networks throughout the 3D structure. To further improve the printing resolution in a permissive microenvironment, we then developed a hybrid bioink, where GelMA backbone was functionalized with adamantane. In the presence of acrylated β-cyclodextrin, hydrogels with both supramolecular host-guest interactions and covalent bonds were formed upon visible light exposure through methacryloyl chain-growth polymerization. Hybrid hydrogels exhibited a higher compressive modulus than GelMA control hydrogels with only covalent bonds, improving printing fidelity. Nevertheless, the presence of reversible supramolecular bonds also allowed plastic remodeling of the hydrogel network by the embedded cells, creating a more favorable environment for cell migration and microvasculature formation. Finally, to promote endothelial cells recruitment within specific areas of the engineered constructs, we exploited thiol-ene click chemistry and sequential volumetric bioprinting to locally photograft pro-angiogenic growth factors with a resolution of ≈50mm.

These results demonstrate that by tailoring the bioinks properties, it is possible to engineer cm^3-scale constructs with high shape fidelity while supporting microcapillary network development. By integrating multiple biofabrication strategies, we can further enhance the biological complexity of bioprinted constructs, potentially replicating the hierarchical structure of native vasculature.

AR.8.S2-K3 Scaffolded Spheroids – A New Strategy for Osteochondral Tissue Engineering

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Abstract

Multicellular aggregates, also referred to as spheroids or pellets, have become increasingly popular as building blocks for tissue engineering (TE). They show improved biological properties with regard to regenerative capacity and provide a route to generating tissue constructs with high initial cell density. Nevertheless, the use of such building blocks also comes with substantial challenges - prolonged culture of spheroids often results in rather inhomogeneous size and shape distribution, while their compaction and fusion can lead to substantial volume loss. We have recently introduced a novel synergetic TE strategy enabled by the use of 3D printed microscaffolds, which allows to solve these issues. The specialized microscaffolds help to reduce the compaction of the individual spheroids that they are hosting, as well as maintain their shape and roundness over extended cell culture periods. Scaffolded spheroids show high viability and preserve chondrogenic and osteogenic potential of human adipose-derived stem cells (ASCs). They were successfully used for bottom-up assembly of larger tissue constructs. Furthermore, it was demonstrated that the presence of microscaffolds improves the fusiogenic capacity of spheroids differentiated towards chondrogenic lineage and characterized by substantial deposition of according extra-cellular matrix (ECM). Our findings indicate that the microscaffolds carrying high density of cells are promising building blocks for cartilage and bone TE. This synergetic approach provides a route towards recreating more complex tissues by combining scaffolded spheroids differentiated towards different phenotypes, as demonstrated on the example of osteo-chondral construct. In this contribution our recent progress, as well as perspectives for further TE applications of scaffolded spheroids, will be discussed.

AR.8.S2-K4 Engineering Hard-Soft Tissue Interfaces via 3D Printing and Melt Electrowriting

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Abstract

Hard-soft tissue interfaces, such as tendon-to-bone or cartilage-to-bone connections, are critical for musculoskeletal function. These interfaces exhibit gradual transitions in architecture, mechanics, composition, and biochemical signaling over micro- to nano-scale dimensions, making them challenging to regenerate after injury and difficult to replicate in laboratory conditions. In our studies, we aim to closely mimic the gradient structure of native hard-soft tissue interfaces using advanced biofabrication techniques. By employing 3D printing and melt electrowriting, we strive to create biomimetic gradient structures with precise control over material deposition at the micrometer scale.

We designed graded and smooth transitional scaffolds with controlled architecture and material composition, followed by cell seeding with different cell types. The constructs were cultured under both static and dynamic conditions, including cyclic mechanical stretching, to assess their impact on cell behavior and tissue formation. Our findings demonstrate that the substrate designs and mechanical stimulation significantly influence cellular responses (Figure 1). Tenocytes responded distinctly to cyclic mechanical loading, showing enhanced proliferation and alignment, while osteoblasts exhibited a less pronounced response to mechanical cues. These observations highlight the importance of mechanical microenvironments in directing cell fate at tissue interfaces.

Our study paves the way for gradient-functional scaffold design, facilitating the engineering of complex, hierarchical, and heterogeneous tissue interfaces. The combination of melt electrowriting, 3D printing and mechanical stimulation offers a promising approach to recapitulating native tissue gradients, ultimately contributing to improved strategies for orthopedic regeneration.

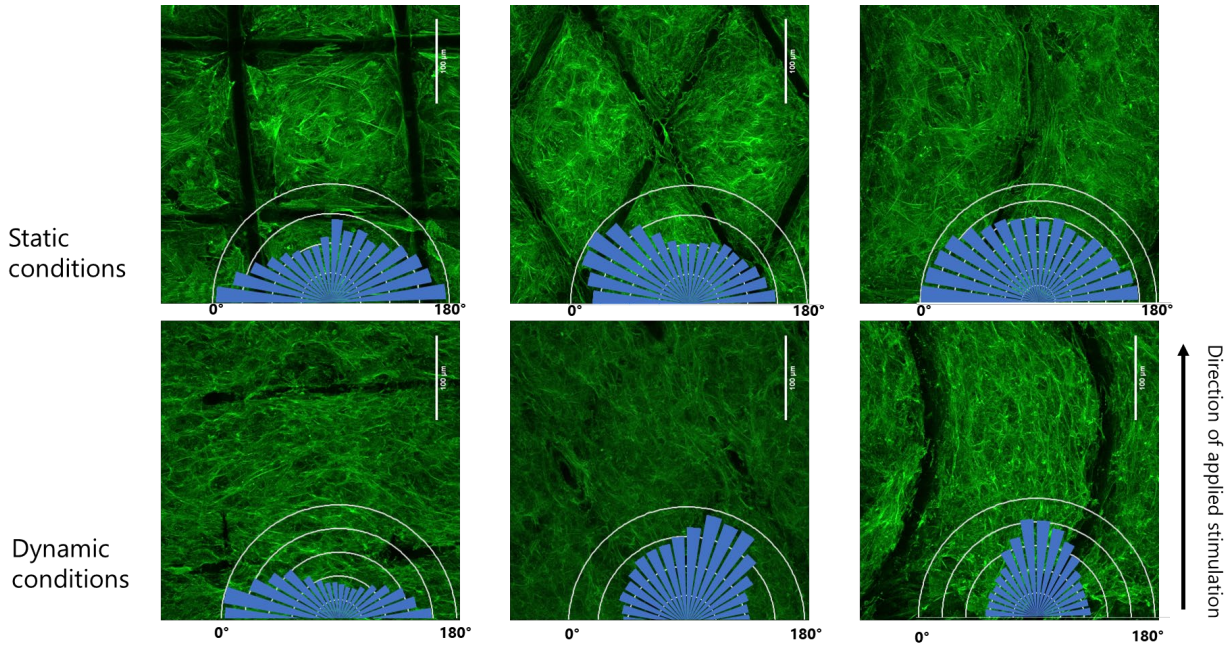

Figure 1. Alignment of cells reaching confluence when cultured on melt electrowritten scaffolds is restored after mechanical stimulation. An exemplary response of the cells to stimulation is shown here with primary human fibroblasts cultured for 28 days (phalloidin staining). The half-circular graphs indicate cell growth directionality.

AR.8.S2-O1 Personalized Treatment of Cleft/Lip Palate Deformities of Canine Patients with Organo-Mineral 4D scaffolds

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Abstract

Introduction: Advancements in additive manufacturing have revolutionized scaffold design for treating bone defects, incorporating physicochemical and architectural cues to stimulate bone regeneration. However, challenges persist in achieving precise fit between scaffolds and patients' bone defects due to workflow inaccuracies, which can detrimentally impact regenerative outcomes. Additionally, current scaffolds often lack appropriate mechanical properties, biodegradation rates, and potential for promoting vascularized bone tissue formation. To address these issues, we 3D-printed organo-mineral scaffolds with elastic behavior before setting to treat canine patients with spontaneous cleft lip and palate deformities (CLP).

Methods: Ethical approval was obtained for canine patient treatment (CERVO-2022-14-V). To date, 18 puppies with spontaneous CLP have been recruited and treated using either patient-specific 4D scaffolds (experimental group) or autologous bone graft (control group). Patient specific scaffolds were designed based on pup CT-scan 2 weeks prior intervention, and after validation of the surgical strategy to adopt. Patented organo-mineral formulation was developed combining α-tricalcium phosphate, silanized hyaluronic acid and hydroxypropyl methylcellulose. This paste was printed by robocasting (27G cones, BioX CellInk), using if needed, 40% $_{w/v}$ Pluronic F127 as a support material. Surgical intervention involved soft tissue reconstruction and placement of scaffolds soaked in autologous bone marrow. Bone formation was monitored at 3- and 6-months post-reconstruction, with detailed analyses conducted at 6 months (µCT, histology, SEM).

Compulsory, influence of several sterilization methods of the scaffolds were also assessed to ensure the transfer of the developed technology to human clinical settings. This implied extensive physico-chemical, morphological and mechanical characterization before and after sterilization.

Results: Scaffolds exhibited excellent handling properties (elastic deformation) and were effectively inserted into complex bone defects, achieving significant contact between bone edges and scaffolds. Osteointegration was observed at 3 months, with evidence of bone formation within the scaffold's macroporous network. Results at 6 months are comparable if not better with the personalized scaffolds (compared to autograft) when soft tissue dehiscence did not occur at early timepoint. Ethylene oxide seems a suitable sterilization modality, with no degradation of the flexible behavior before cement setting (*in situ* once implanted).

Discussion & Conclusions: Preliminary results demonstrate the promising potential of our strategy in treating spontaneous CLP deformities in a veterinary setting. Sterilization of scaffolds after printing is achievable, even with the presence of fragile macromolecules) which represents a major step toward human clinical translation. Animal patients represent an ethical way for the development of innovative treatments within the OneHealth concept.

AR.8.S2-O2 Development of an integrated melt-electrowriting and cell jetting biofabrication platform to generate 3D cellular gradients and regional mechanical properties for cartilaginous tissue regeneration

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Abstract

Introduction/Objectives. The meniscus plays a vital role in shock absorption and stabilisation in the knee joint. Injuries to the meniscus can lead to osteoarthritis if left untreated. Current surgical therapies provide minimal tissue regeneration and so have poor long-term success rates, thus there is a distinct clinical need for novel tissue engineered approaches. Restoring the structure-function relationship of the native meniscus is critical, but current tissue engineering approaches often fail to consider the spatial organisation of cells, or the different biomechanical requirements of the tissue. Thus, the objective of this study was to apply a sophisticated biofabrication pipeline, seamlessly integrating melt-electrowriting (MEW) and microvalve bioprinting techniques to craft meniscal tissue analogues containing cellular and mechanical gradients mirroring native tissue.

Methods. MEW process parameters were optimised using a design-of-experiments approach to facilitate the deposition of layers of PCL microfibres with a range of defined geometries, creating microchambers that could reinforce a soft hydrogel matrix. The effect of process parameters on fibre geometry was assessed through SEM measurements. Microvalve jetting of alginate into MEW microchambers was the optimised to facilitate precise droplet patterning, enabling the encapsulation of human mesenchymal stem cells (MSCs) with high viability. Patterning of cellular gradients was confirmed through fluorescent cell labelling and mechanical properties of the hybrid scaffold were confirmed through mechanical testing. MSC-seeded hybrid scaffolds with varying fibre spacings were cultured in chondrogenic media with or without compressive dynamic mechanical stimulation and the resulting differentiation and matrix formation assessed using qPCR, histology, immunohistochemistry and quantitative biochemical assays.

Results. Aligned polymeric fibre walls were generated using MEW, which emulated geometries of collagen bundles in native tissues, offering tunable fibre diameters and microchamber sizes. Fine-tuning fibre diameter and spacing resulted in scaffolds approaching physiologically relevant stiffness, potentially allowing us to tailor the mechanosensory environment for encapsulated cells. Multi-nozzle microvalve jetting attained intricate patterns of bioink droplets within polymeric microchambers, showcasing high cell viability and enabling precise patterning of cellular gradients. After 28 days in culture, the reinforced scaffolds closely emulated the compressive stiffness of native cartilaginous tissue, featuring aligned collagen deposition along the polymeric fibres and glycosaminoglycan (GAG) deposition within the hydrogel matrix.

Conclusions. These hybrid constructs exhibit significant potential for engineering mechanically robust, cartilaginous tissue analogues with defined regional properties, promising advancements in meniscus tissue regeneration and preclinical disease modelling.

AR.8.S2-O3 Physicochemical characterization of high β-TCP content polymeric composite for 3D printed bone scaffolds: Investigating the effect of calcium phosphate coating on composite properties under simulated physiological conditions

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Abstract

The use of commercial medical-grade biomaterials is an essential requirement for moving a lab-based technology to a higher Technology Readiness Levels. Over the past decade, a greater range of Good Manufacturing Practice-manufactured biomaterials of different formulations has become available, including material in filament form specifically for Fused Deposition Modelling 3D printing. Composites with high bioceramic content are beneficial for scaffold-guided bone regeneration due to their potential to enhance biological performance. However, 3D printing of composites with a high bioceramic phase (> 20%) remains challenging, as it negatively impacts both the accuracy and speed of the printing process. A common approach to further improve the bioactivity of bone scaffolds is surface modification through the deposition of calcium phosphate (CaP) layer. However, there is limited research on how such coatings affect the mechanical properties of the material under simulated physiological conditions.

This study aims to characterize the physicochemical properties of 3D printed scaffolds fabricated from a medical-grade 60/40 blend of Lactoprene® 7415 and β-tricalcium phosphate, both before and after CaP deposition.

This study optimized the 3D printing process to manufacture solid samples that accurately represent the bulk material properties. FTIR analysis was performed to investigate the molecular interactions between the composite material and water molecules, while DSC was used to assess the thermal properties of the material. A subset of the samples was then coated with CaP. All samples were subjected to uniaxial compression testing according to ASTM D695-23 standards under simulated physiological conditions to evaluate the impact of the CaP coating on the mechanical properties. The coating was characterized in terms of morphology, thickness, and weight. Additionally, water absorption and recovery behaviour were evaluated for both coated and uncoated groups.

The results showed that a uniform coating was formed on the composite surface and improved water absorption, which impacted the mechanical properties of the composite and reduced the compressive modulus from 11.3 MPa to 6 MPa when exposed to simulated physiological conditions. However, the compressive performance of both groups under simulated physiological conditions demonstrated highly elastic behaviour, with a 90% recovery after compression. The study also revealed that water molecules can induce plasticization by modifying the thermal and chemical properties under physiological conditions.

The composites exhibit advantageous physicochemical properties for scaffold-guided bone regeneration, together with a high ceramic content that could benefit the biological performance in future in vivo assessments. Such scaffolds are, therefore, promising candidates for bone tissue engineering.

AR.8.S3-K5 3D Printing and Bioprinting in Orthopaedics: Opportunities and Limitations

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Abstract

Additive manufacturing technologies have developed heavily in the last few decades. Respective 3D printing methods are commonly used in the meanwhile for the fabrication of patient-specific implants (PSI), e.g. for cranial defects. However, in orthopaedic surgery utilisation of PSI is still limited and advantages for patients are continuously under discussion. In addition, 3D printing of patient-specific solutions is restricted to nondegradable materials whereas a lot of studies demonstrated opportunities of utilising also biodegradable biomaterials for applications in regenerative medicine.

Beside (bio)materials also live cells, mostly in combination with hydrogels, can be utilised in additive manufacturing and the respective technologies, summarised as 3D bioprinting, are intensively under investigation. Bioprinting allows the direct manufacturing of tissue engineering (TE) constructs – but in contrast to conventional TE methods allow the integration of several cell types with good spatial resolution. This enables the fabrication of complex tissue constructs including those for the treatment of defects at tissue interfaces, for example osteochondral lesions.

The presentation will give an overview about recent developments in the field of both PSI and bioprinting, specifically for applications in orthopaedic surgery. Important topic also will be the discussions of opportunities and limitations of these technologies in clinical applications.

AR.8.S3-O1 Towards Transforming the Current Clinical Approach to Bone Tumors with Innovative Customized 3D-printed Bioresorbable Implants

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Abstract

Osteosarcoma (OS) is the most common malignant bone tumor in children and adolescents, yet survival rates have stagnated for over four decades. Current treatment involves tumor resection combined with neoadjuvant and adjuvant chemotherapy, but this approach faces significant challenges. Up to 50% of patients with localized disease experience recurrence, often at the primary site, and disease relapse drastically reduces survival rates. Additionally, chemotherapy causes severe systemic toxicities, impacting quality of life. Limb-salvage surgery (LSS) is performed in 85–90% of OS patients to avoid amputation. However, LSS is associated with high complication rates: 30–40% of endoprosthetics fail due to growth-related loosening or infection, and ~23% of bone grafts fail due to rejection or incomplete healing. Addressing these limitations requires innovative solutions that simultaneously control tumor recurrence and restore bone integrity.

To address these challenges, we developed 3D-printed bioresorbable scaffolds with a Voronoi-tessellated design for two complementary concepts: localized chemotherapy delivery and scaffold-guided bone regeneration (SGBR). In an orthotopic OS rat model, lactoprene/β-TCP (60/40%) scaffolds combined with doxorubicin (DOX)-loaded gelatin methacryloyl (GelMA) hydrogels provided localized drug delivery after tumor resection. This approach significantly inhibited tumour regrowth for up to six weeks and reduced systemic toxicity compared to intravenous chemotherapy. Interestingly, local DOX also appeared to prevent the development of lung metastases compared to untreated and systemic chemotherapy groups.

In a separate study, the same scaffold design, combined with recombinant human bone morphogenetic protein-2 (rhBMP-2), was evaluated for its ability to regenerate bone in a critical-sized defect model. Micro-CT imaging demonstrated that the combination of scaffold and rhBMP-2 could support critical sized defect healing within 16 weeks. Histological analyses revealed robust cortical and trabecular bone formation, with extensive integration into the scaffold's porous architecture, demonstrating the feasibility of this combination in SGBR.

Future studies will investigate the convergence of localized chemotherapy and SGBR into a single-step surgical intervention. Combining DOX-loaded hydrogels with rhBMP-2-enhanced scaffolds aims to simultaneously prevent OS recurrence and regenerate bone after tumor resection. This dual-function strategy represents a significant advancement in orthopaedic oncology, with the potential to improve outcomes and quality of life for OS patients by addressing both tumor control and bone regeneration in a single surgical procedure.

AR.8.S3-O2 Enabling Hospitals to Print Patient Specific Models at the Point of Care

Jakob Föhres

Materialise N.V., Leuven, Belgium

Abstract

Abstract: The integration of 3D printing technology into orthopedic care is transforming personalized medicine by facilitating the creation of patient-specific models directly within hospital settings. This presentation explores the current state of the art in point-of-care 3D printing, highlighting technological advancements that enable rapid and accurate production of anatomical models, surgical guides, and custom implants. The process of 3D printing at the point of care involves crucial stages: data acquisition; digital model creation; the right choice of material given different requirements for biocompatibility and durability; printing; and post-processing. Each stage presents unique challenges and opportunities, requiring interdisciplinary collaboration among radiologists, orthopedic surgeons, and biomedical engineers to ensure successful clinical implementation.

Case studies demonstrating the practical application of patient-specific models in pre-surgical planning, intraoperative guidance, and postoperative assessment illustrate the benefits of customized 3D printed solutions. These examples show how tailored models can lead to more precise surgical interventions, reduced operative times, and improved patient outcomes.

Strategies for overcoming common obstacles in translating 3D printed biomaterials to clinical use, such as regulatory compliance, quality control, and cost-effectiveness, will be discussed. By adopting systematic workflow integration and leveraging best practices from early adopters, hospitals can effectively incorporate 3D printing into their orthopedic departments.

In summary, point-of-care 3D printing represents a paradigm shift in personalized orthopedic care. This technology offers the potential to revolutionize surgical precision and patient outcomes by enabling the production of patient-specific models within hospital settings. This presentation aims to provide healthcare professionals with the knowledge and tools needed to successfully implement and optimize 3D printing solutions, ultimately enhancing the standard of care in orthopedics.

AR.8.S3-O3 State-of-the-art Patient-Customized Bone Graft Harvesting Strategies

Lucas P Weimer[1], Alexandra C Bruckner[1], Tina Frankenbach-Désor[1], Susanne Mayer-Wagner[1], Dietmar W Hutmacher[2,3], Flavia M Savi[2,3], Siamak Saifzadeh[3,4], Jacqui McGovern[2,3], Nathalie Bock[2,3], Stefan Milz[5], Boris M Holzapfel[1], Markus Laubach[1,3]

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Abstract

Treatment of critical-size bone defects has been enhanced by advancements in scaffold-guided bone regeneration (SGBR), for which the usage of osteogenic bone graft material is crucial. To optimize the extraction of bone graft, the aspirator + reaming-aspiration (ARA) concept has recently been evaluated in a preclinical in-vivo study as an alternative intramedullary harvesting strategy to the well-known Reamer-Irrigator-Aspirator 2 system (RIA 2 system). As part of the in-vivo study, bone graft obtained from sheep was applied together with a 3D-printed bioresorbable scaffold construct in an ectopic rat model. Fluorochrome labelling with calcein green (day 8), alizarin complexone (day 22) and xylenol orange (day 36) was performed at different timepoints post-surgically. After 8 weeks, probes from five experimental groups (see Table 1) were explanted resulting in a total of 58 resin probes for further analysis.

Table 1: Five different experimental groups showing implant, bone grafting method and bone graft type

Group	1 - Sc	2 - ScRIA2	3 - ScA	4 - ScRA	5 - ScARA
Implant + method	Only scaffold	Scaffold + RIA 2 system	Scaffold + Aspirator	Scaffold + Reaming-Aspiration	Scaffold + Aspirator Reaming-Aspiration
Bone graft type	-	Bone chips + bone marrow	Bone marrow	Bone chips	50% Bone chips + 50% bone marrow

Fluorescence of 198 samples was assessed semi-quantitatively through applying a three-stage evaluation scale (“none”, “minimal”, “intense”) by two assessors (LPW and ACB) independently. No fluorescence was defined as the absence fluorescent clusters over the entire sample section after manual screening at 20x magnification. Minimal fluorescence was defined as the presence of a maximum of three fluorescent clusters at independent image sections at 20x magnification. Intense fluorescence was defined as a minimum of three fluorescent clusters at independent image sections at 20x magnification. Preliminary descriptive statistical results are shown in Figure 1. All group 1 “Sc-samples”

and 58% of group 3 “ScA samples” show no fluorescence. Minimal fluorescence was observed for 5% of group 2 “ScRIA2 samples”, 32% of group 3 “ScA samples”, 14% of group 4 “ScRa samples” samples, and 41% of group 5 “ScARA samples”. Intense fluorescence was observed for 95% of group 2 “ScRIA2 samples” samples, 10% of group 3 “ScA samples”, 86% of group 4 “ScRA samples” and 53% of group 5 “ScARA samples”. In conclusion, our preliminary data indicate comparable active bone regeneration and bone formation of bone graft material harvested with the new ARA concept compared to the RIA 2 system. In a next step, quantification of the fluorescent sample surface will be performed. Furthermore, advanced statistical analysis and putting the results in relation with histological and immunohistochemical analysis of paraffin samples are required.

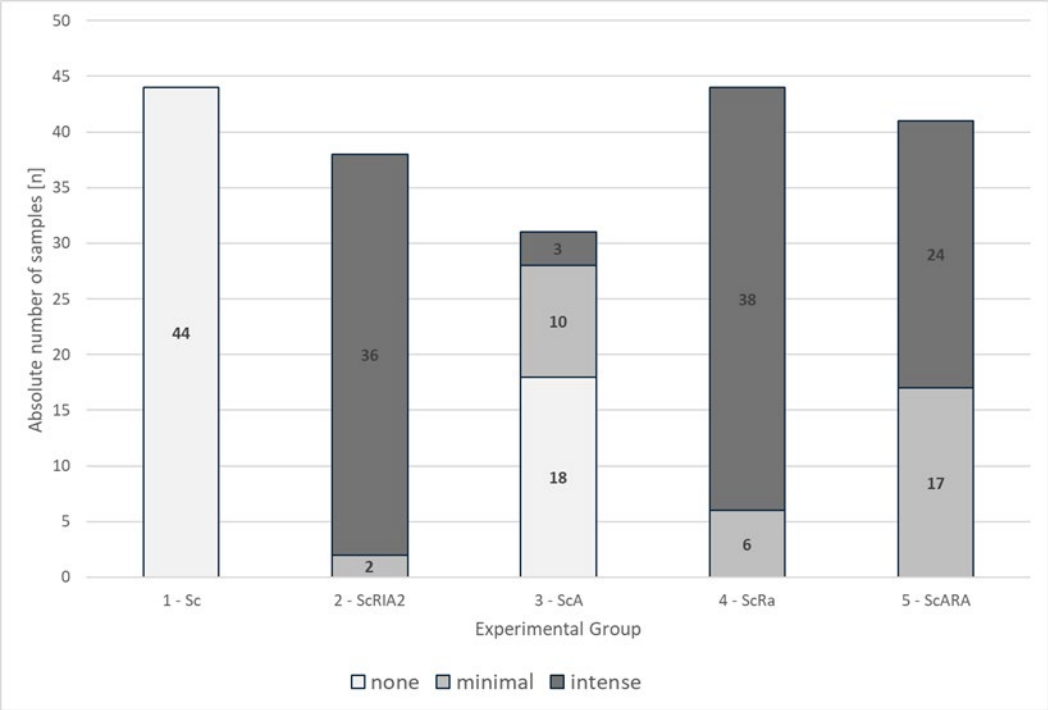

Figure 1: Absolute distribution of fluorescence per experimental group (n=198) after semi-quantitative evaluation

AR.8.S3-O4 Current innovations in 3D-printed implants and instruments for knee and hip arthroplasty

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Abstract

The introduction of 3D printing technology in knee and hip arthroplasty has transformed orthopaedic surgery, offering patient-specific solutions for addressing complex anatomical and pathological challenges.

Applications and Advantages. 3D printing enables the creation of customized implants and surgical instruments specifically tailored to the unique anatomy of patients. This technology significantly enhances preoperative planning by producing precise anatomical models, allowing surgeons to effectively address severe bone defects and complex anatomical challenges. The current data indicate that the use of 3D-printed patient-specific instruments in arthroplasty can lead to consistently accurate positioning of implants. (Henckel et al., 2018) Furthermore, the variability in surface structures, such as porous surfaces, of 3D-printed implants has been shown to promote osseointegration, thereby enhancing long-term stability. (Wang et al., 2022) In addition, 3D-printed femoral stems for primary hip arthroplasty show a reduced level of stress shielding. (Johnston et al. 2016)

Clinical Outcomes. Recent investigations into the clinical performance of 3D-printed implants and instrument have demonstrated promising results. Recent data suggest that 3D-printed cutting guides for individualized implants in primary knee arthroplasty yield favorable clinical outcomes, at least during short-term follow-up. (Moret et al., 2021) Porous 3D-printed femoral or tibial cones also offer excellent metaphyseal fixation, demonstrating strong survivorship and minimal complications in patients with severe bone loss undergoing complex revision TKA. (Tetreault et al. 2020) In cases of complex patient anatomy, such as severe valgus knee osteoarthritis, the use of 3D-printed cutting guides and individually manufactured knee implants has shown excellent early survivorship and restoration of the leg axis. Furthermore, the use of these implants has been associated with superior short-term clinical outcomes compared to conventional techniques. (Tücking, submitted 2025)

Challenges and Future Directions. While 3D-printed implants hold immense promise, challenges such as high manufacturing costs, regulatory barriers, and limited long-term data hinder their widespread adoption. Addressing these issues will require interdisciplinary collaboration to establish robust clinical evidence. Additionally, advancements in biomaterials and artificial intelligence may further refine implant design and patient outcomes.

Conclusion. 3D printing is transforming knee and hip arthroplasty by enabling personalized and precise surgical solutions. Early clinical outcomes suggest that 3D-printed implants enhance function and durability in primary and revision arthroplasty.

AR.8.S3-O5 3D Bioprinting of Osteochondral Units with Human Nasal Chondrocytes Using a Granular Composite of Hyaluronic Acid, Collagen, and Hydroxyapatite

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Abstract

Introduction. Replicating the zonal structure of osteochondral tissue in engineered constructs remains challenging. This study explores 3D bioprinting of osteochondral units using human nasal chondrocytes (hNCs). While hNCs are effective for fabricating hyaline cartilage layers, their interaction with hydroxyapatite (HAp) remains poorly understood. HAp, known to promote hypertrophic and osteogenic differentiation of mesenchymal stem cells, may similarly induce hypertrophic differentiation in hNCs. To test this, we developed a bioprintable granular hydrogel composite incorporating HAp, enabling simultaneous deposition of bioink and hNCs.

Methods. High molecular weight hyaluronic-acid functionalized with tyramine (THA) was combined with collagen fibrils and HAp particles then crosslinked enzymatically with a combination of horseradish peroxidase and hydrogen peroxide. The bulk hydrogel was fragmented via extrusion through a cell strainer. The resulting granular gel was supplemented by a solution of low molecular weight THA, hNCs and a photoinitiator system based on ruthenium/sodium persulfate. Constructs were extruded into cylindrical molds using a pneumatic bioprinter and crosslinked using blue light. The printed constructs were cultured in chondrogenic medium for 28 days.

Results. We have successfully 3D bioprinted composites with homogeneously distributed HAp particles and hNCs. The cell viability was preserved throughout encapsulation and printing, as shown by an initial assessment with trypan blue before printing (Fig.1A) and Live/Dead staining on days 1, 3, and 7 (Fig.2). The constructs maintained their shape throughout the 28-day culture period and exhibited various cell morphologies within and around the constructs. By day 28, a proliferative layer of hNCs was observed surrounding the constructs (Fig.1B,1C).

Conclusion. We developed a composite bioink for joint encapsulation of HAp particles and hNCs in biofabricated constructs, demonstrating promising cell viability, shape retention, and printability. Combining this granular system with bioactive factors and multi-material 3D bioprinting could enable the fabrication of multizonal tissues, providing a potential solution for osteochondral defect repair.

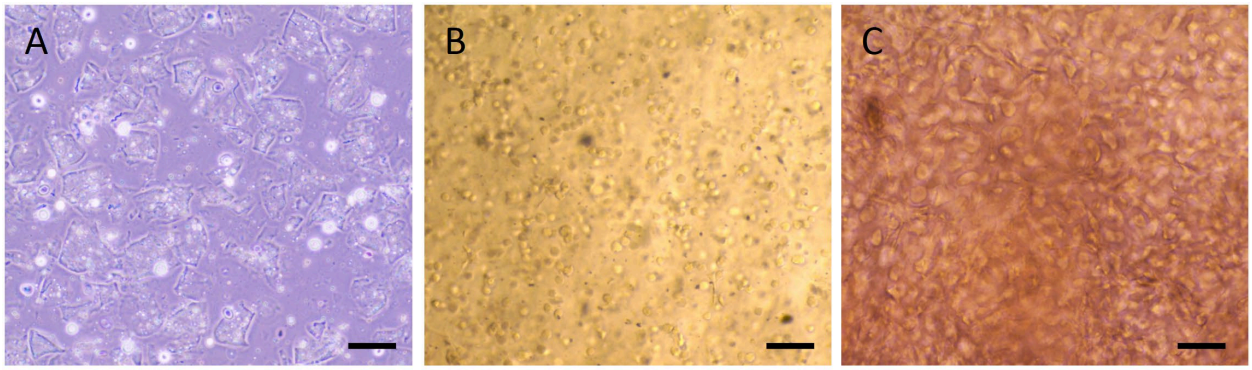

Fig. 1. *Phase-contrast images of hNCs in a granular hydrogel with HAp. (A)Bioink with trypan-blue showing hNC viability and varied granule sizes post-printing. (B)Day 1 post-printing, with black dots indicating HAp particles. (C)Day 28 in chondrogenic medium, showing diverse cell morphologies and a proliferative hNC layer. Scale bar=100 µm.*

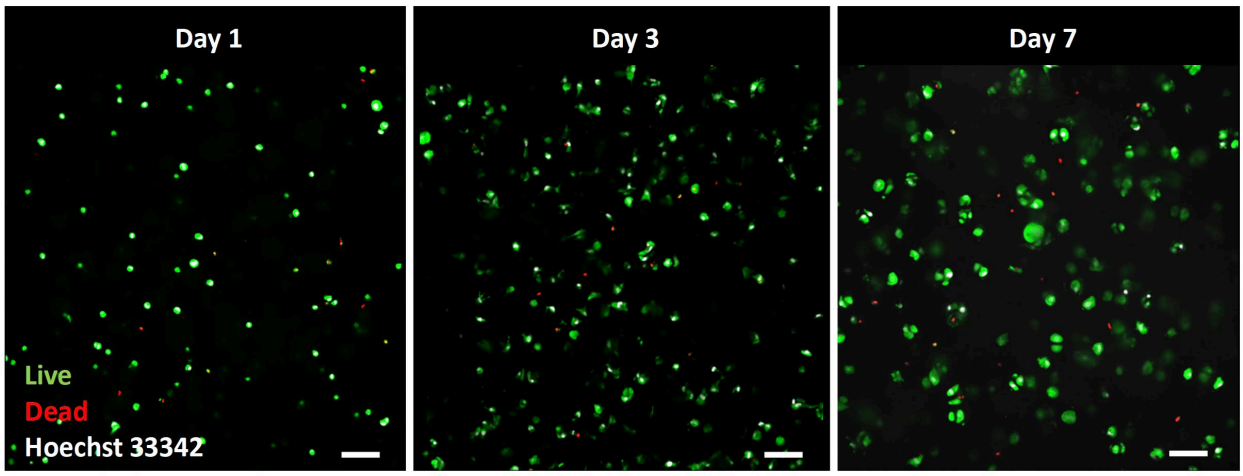

Fig. 2. *Confocal Live/Dead images of hNCs in a granular hydrogel with HAp after bioprinting. Scale bar=100µm.*

AR.8.S3-O6 3D-Printed Patient-Specific Instruments for Corrective Osteotomy in Pediatric Cubitus Varus Deformity

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Abstract

Introduction: Cubitus varus deformity is characterized by a complex three-dimensional deformity with varus angulation, internal rotation, and hyperextension, resulting in both cosmetic and functional impairments. Traditional techniques like lateral closing wedge osteotomy often fail to address its multidimensional nature. Recent advancements in medical imaging and 3D printing enable computer-assisted surgical planning and patient-specific instruments (PSI) for precise corrections with reduced surgical complexity. This study evaluates the efficacy of 3D-printed PSI in corrective osteotomy for cubitus varus deformity.

Methods: A retrospective analysis was conducted on 16 pediatric patients with cubitus varus deformity treated at Chang Gung Memorial Hospital, Linkou form 2020 to 2024. Three-dimensional computed tomography was utilized to facilitate deformity analysis, surgical planning, and simulation. Customized 3D-printed surgical guides were used to enable precise osteotomies and fixations. Outcomes were evaluated using radiographic measurements, elbow range of motion (ROM), carrying angle, and Flynn's criteria.

Results: Postoperative evaluations revealed significant improvements. The mean Baumann’s angle corrected from 84.4° to 67.3°, while the mean lateral humerocapitellar angle improved from 19.5° to 30.8°. The mean carrying angle improved from -15.8° to 5.9°, with the mean elbow ROM increased from 120° to 145°. Flynn's criteria rated outcomes as satisfactory in 14 cases. One case of postoperative infection resolved with early intervention.

Conclusions: 3D-printed PSI offers a promising solution in achieving precise, multidimensional corrections for cubitus varus deformity with reduced surgical complexity. Challenges such as radiation exposure, cost, and limitations including small sample size and a retrospective study design necessitate further investigation.

AR.8.S4-K6 Biofabrication Approaches for the Generation of Small Diameter Vascular Grafts

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Abstract

Vascularization plays a crucial role in bone tissue engineering, as it ensures the supply of nutrients and oxygen necessary for tissue regeneration and integration with host tissue and is essential for maintaining tissue health. Biofabrication aims to mimic the hierarchical structure of natural tissues to enhance the performance of fabricated constructs. Biofabricated tissue models should replicate not only the macroscopic structure and shape but also the microarchitecture and biomechanics of the tissues they replace. These design criteria are vital for generating models with improved function and tissue specificity.

The objective of this work is to demonstrate the compatibility and options for convergence of fabrication techniques to generate biomimetic small diameter vascular grafts. Potential applications in bone tissue engineering will be highlighted. The demonstrated bi-layered tubular constructs are fabricated using a combination of solution electrospinning (ES) and melt electrowriting (MEW). To fine-tune the mechanical properties of these scaffolds, various combinations of materials, such as polycaprolactone (PCL) and poly(ester urethane) (PEU), are employed in ES to influence the overall properties of the grafts. The mechanical properties of the cell-seeded constructs are compared and adjusted to match those of native human tissues.

Additionally, the synergy between MEW and Volumetric Bioprinting (VBP) is explored. VBP enables printing around MEW tubes, enhancing the mechanical properties of hydrogel-based constructs and allowing the creation of cell-containing three-layered constructs that mimic the layered structure of human blood vessels. This approach is particularly beneficial for bone applications, where vascularization is critical for successful tissue integration and regeneration.

In conclusion, this presentation demonstrates how the combination of different fabrication methods can be utilized to create hierarchical, multi-layered tubular constructs with biomimetic architectures and adjustable mechanical properties.

AR.8.S4-K7 Biofabrication of complex tissues for bone regeneration via bioprinting

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Abstract

We report on the biofabrication of complex tissues for bone regeneration using elastic bioactive bioinks with encapsulated cells and extrusion bioprinting to create osteochondral (OC) and vascularized bone (VB) constructs as an integrated approach for sustained osteogenesis.

For the 3D bioprinted OC constructs, we designed bioinks containing an elastic polymeric matrix comprising gellan gum (GG) and polyvinyl alcohol (PVA) and zinc-doped bioactive glass (BG) with antimicrobial properties. The bioink was combined with human adipose tissue derived stromal cells (ADSCs). The prepared inks were examined for their printability, rheological properties, thermal behavior, crystallinity, water uptake and degradation rate in the presence of cells. The constructs were assessed biologically in vitro for their osteogenic and chondrogenic capacity, as well as for their antibacterial activity. The bioprinted constructs showed a printing accuracy of 65-75%, with the composite bioinks being higher. The bioinks demonstrated excellent capability regarding extrusion printability at extrusion pressures of 100 kPa, which is significant to ensuring high cell survival rates. Rheological analysis of the bioinks revealed 90% viscosity recovery. The gene expression of relevant osteogenic and chondrogenic markers, as well as the biochemical markers and the extracellular matrix molecules were significantly elevated in the composite bioink.

For the VB constructs, a complex 3D bioprinted model was biofabricated comprising (i) an outer zone from a photocrosslinkable hydrogel with electrically conductive properties aiming to enhance osteogenesis under dynamic culture conditions, and (ii) an inner zone including a vascular-like tubular structure from a biofunctional nanocomposite hydrogel to induce angiogenesis. The angiogenic matrix was prepared by dispersing gellan gum in a laponite suspension. Wharton's jelly mesenchymal stem cells (WJ-MSCs) were incorporated in the nanocomposite forming a bioink functionalized with platelet-rich plasma (PRP). The osteogenic matrix was prepared from a blend of poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), polyvinyl alcohol, gelatin and polyethylene glycol diacrylate combined with bone marrow mesenchymal stem cells (BM-MSCs). Angiogenic and osteogenic differentiation were examined through immunofluorescent staining, determination of VEGF/BMP-2 secretion by ELISA, gene expression via quantitative polymerase chain reaction (qPCR), alkaline phosphatase activity, and quantification of produced calcium and collagen. All markers were significantly increased in the complex VB constructs under dynamic culture conditions.

Both types of bioprinted constructs were implanted in a subcutaneous mouse model for two weeks, revealing the absence of adverse immunological reactions.

AR.8.S4-O1 Regional transcriptional and proteomic characterisation of human meniscus informs parameters for generation of 3D bioprinted meniscus analogues

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Abstract

Introduction/Objectives. Meniscal injuries affect over 1.5 million people across Europe and the USA annually. Injury greatly reduces knee joint mobility and quality of life and frequently leads to the development of osteoarthritis. Tissue engineered strategies have emerged in response to a lack of viable treatments for meniscal pathologies. However, to date, constructs mimicking the structural and functional organisation of native tissue, whilst promoting deposition of new extracellular matrix, remains a bottleneck in meniscal repair. This project aimed overcome these limitations through regional histological and proteomic analysis of native human tissue and transcriptional analysis of their resident cells. 3D bioprinting was then employed to deposit and pattern cellular bioinks with high spatial resolution to enable development of a biomimetic 3D bioprinted meniscal substitute with appropriate structural and functional properties.

Methods. Histology, gene expression and mass spectrometry were performed on native human meniscal tissue to investigate tissue architecture, matrix components, cell populations and protein expression regionally across the meniscus. 3D laser scanning and magnetic resonance imaging were employed to acquire the external geometrical information prior to fabrication of a 3D printed meniscus. Bioink suitability was investigated through regional meniscal cell encapsulation in alginate and blended alginate-collagen hydrogels, with the incorporation of growth factors (TGFβ and/or CTGF) and assessed for their suitability through rheology, scanning electron microscopy, histology and gene expression analysis. Bioprinting of 3D meniscal constructs were fabricated through zonal deposition of regionally tailored bioinks and regional differences in cell and matrix biology assessed.

Results. Meniscal tissue characterisation revealed regional variations in matrix compositions, cellular populations and protein expression. The process of imaging through to 3D printing highlighted the capability of producing a construct that accurately replicated meniscal geometries. Regional meniscal cell encapsulation into bioinks revealed a recovery in cell phenotype, with the incorporation of growth factors stimulating cellular re-differentiation and improved zonal functionality. Bioprinting of bioinks regionally enabled the fabrication of a 3D meniscal construct with regional variations in cell and matrix deposition.

Conclusions. Detailed characterisation of native tissue provides a crucial benchmark for design of novel regenerative therapies. Meniscus biofabrication highlights the potential to print patient specific, customisable meniscal implants. Achieving zonally distinct variations in cell and matrix deposition highlights the ability to fabricate a highly complex tissue engineered construct.

AR.8.S4-O2 Custom 3D-Printed Intramedullary Nails: A Novel Preclinical Model for Addressing Critical-Sized Bone Defects

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Abstract

Background. Critical-sized bone defects (CSBDs) represent a major challenge in orthopaedic and trauma surgery. Translational research requires preclinical models that reliably replicate human conditions. This study aimed to develop and evaluate a custom-made 3D-printed titanium intramedullary nail (IMN) specifically designed for CSBDs in minipigs. The main objectives were to determine the feasibility of designing an anatomically adapted IMN for minipig femurs, evaluate its capacity for consistent and reproducible surgical procedures, and assess its ability to promote bone healing.

Materials and Methods. The IMN was custom-designed using CT data from minipig femurs, optimizing parameters such as femoral curvature, length, and medullary canal diameter. Following 3D printing in titanium, the IMNs underwent *in vitro* testing before their use in *in vivo* experiments. Female Aachen minipigs underwent bilateral femoral surgeries, wherein CSBDs were created and stabilized using the custom IMNs, specific cutting guides clipped onto an ancillary. Post-operative follow-up included blood tests, radiographs and CT imaging every two weeks, and femurs were explanted after three months to assess mechanical stability and consolidation.

Results. The custom IMNs fit with minipig femoral anatomy, enabling reproducible CSBD creation and consistent surgical procedures. Symmetric double osteotomies were performed successfully, and bone allografts showed minimal discrepancies in morphology. While distal osteotomy sites demonstrated stable consolidation in most cases, proximal fixation posed challenges, leading to non-union in certain instances. These issues underscore the need for improved screw placement and additional mechanical support.

Discussion. The custom 3D-printed IMN offers significant potential for preclinical modeling of CSBDs, facilitating the study of both surgical procedures and biological responses to bone grafts. Furthermore, this model serves as a platform to explore advanced reconstructive strategies, such as the induced membrane technique pioneered by Prof. Masquelet, which promotes bioactive responses around allografts. Proximal fixation challenges identified in this study highlight the importance of iterative design improvements to enhance stability and mimic human clinical scenarios more effectively.

Conclusion. This preclinical model using custom 3D-printed IMNs represents a promising step forward in the study of CSBDs. It enables the evaluation of innovative

surgical techniques and supports translational research aimed at improving outcomes for patients with complex orthopaedic conditions.

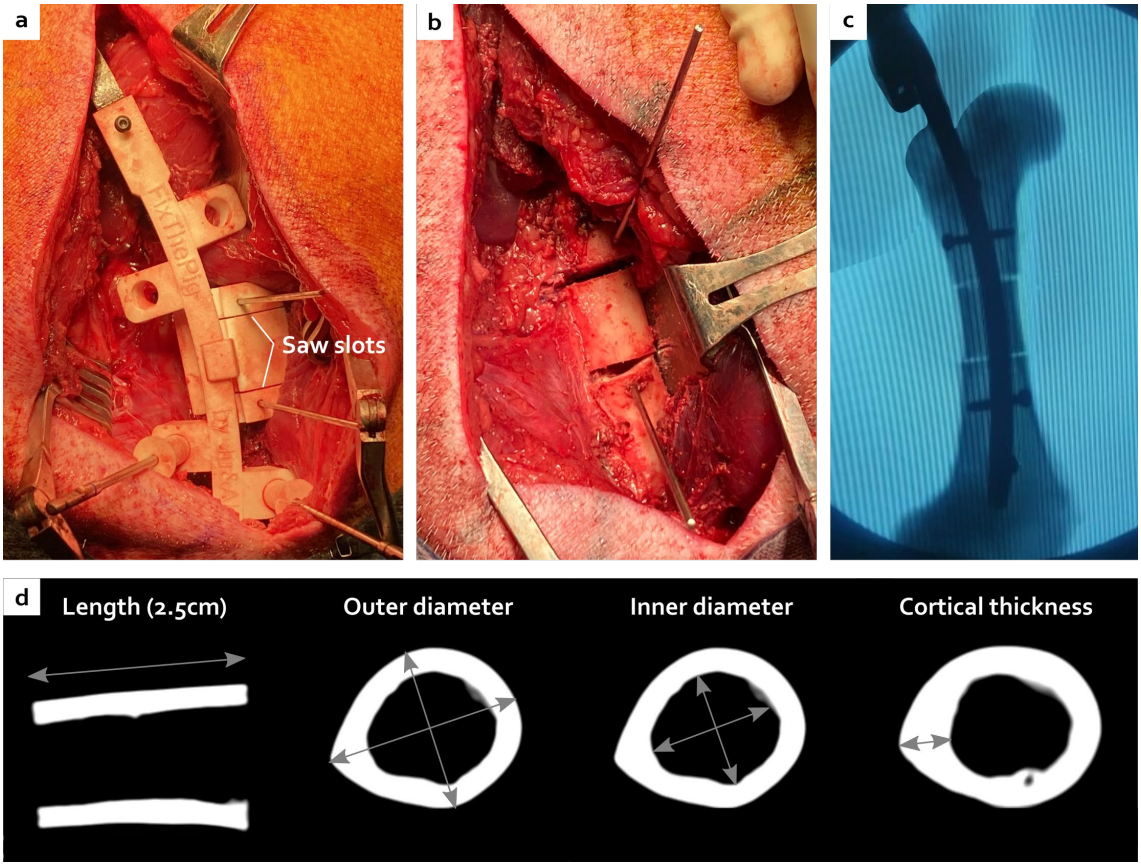

Figure : Creation of the critical-size bone defect with the ancillary and the cutting guide (a) leading to a centred ostectomy with reproducible morphology visible during the surgery (b), under fluoroscopy (c) and after CT scan analyses (d).

AR.8.S4-O3 4-D bioprinting of vascularised and innervated tissue engineered bone for repairing large segmental bone defects

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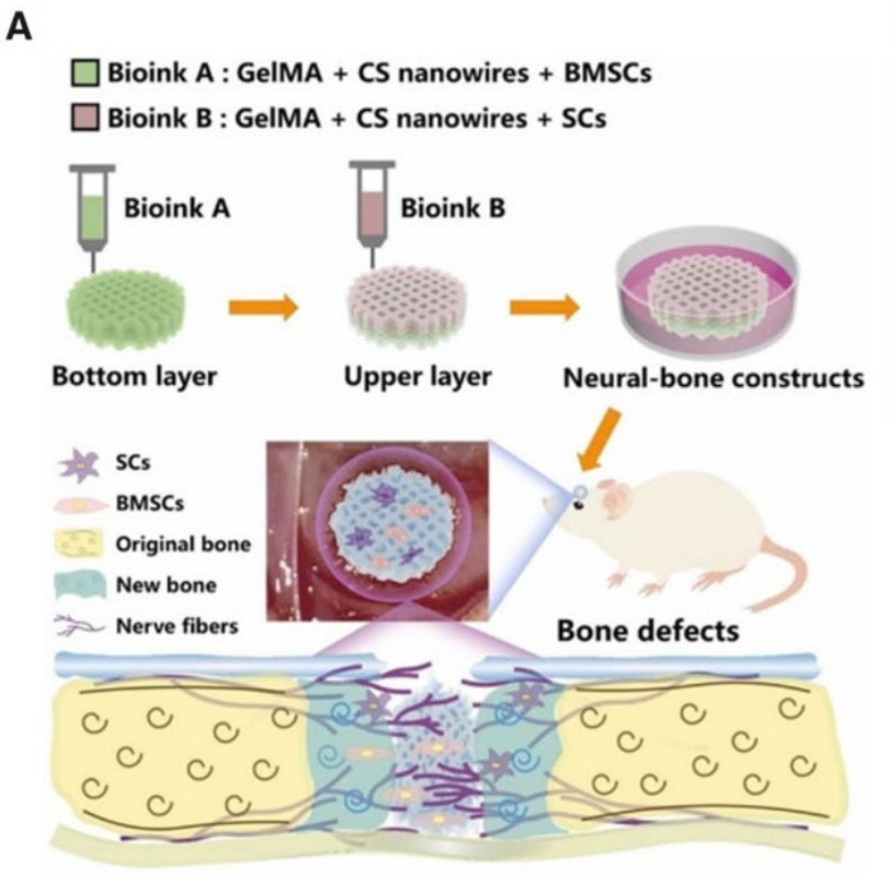
Abstract

Large segmental bone defects remain an unsolved clinical challenge because of the lack of effective vascularization in newly formed bone tissue. 4D bioprinting is a fabrication technology with the potential to create vascularized and innervated bone grafts with biological activity for repairing bone defects in response to patients' specific needs. Traditional bioinks have shown low mechanical strength, poor osteoinductive ability, and lacking a suitable micro-environment for laden cells. Recent advances of in situ vascularized and innervated tissue-engineered bones constructed using 4D bioprinting technology have a potential of being used for repairing large bone defects. 3D bioprinting is an additive manufacturing technique which involves the sequential, typically layer- by-layer, deposition of biocompatible materials and cells to create a 3D construct. When a printable biomaterial contains a biologic (a cell, nucleic acid or biomolecule) it is commonly referred to as a bioink. Although 3D bioprinting theoretically enables the creation of cell-laden constructs of any size and shape, maintaining the viability and function of embedded cells both in vitro (during maturation of the engineered tissue) and in vivo (following its implantation into the body) remains a central challenge in the clinical application. The use of 4D bioprinting to fabricate scaffolds with effective internal vascularization and osteoinductive bioactivity for bone defect repair has become a medical research hotspot. Combining nanocomposite bioinks and multicellular 4D bioprinting technology, biomimetic neural-bone construct can also be developed for innervated bone regeneration. A method was designed for in situ 4D bioprinting of vascularized Tissue Engineered Bone to induce osteogenesis. The in vitro evaluation of osteogenic differentiation of BMSCs has shown excellent osteogenic mineralization.

Chinese researchers have developed a biomimetic neural-bone construct delivering with BMSCs and SCs. With inorganic calcium silicate nanowires incorporated, the cells bioprinted scaffolds could simultaneously support the osteogenic and neurogenic differentiation of encapsulated cells. Furthermore, by implanting the multicellular scaffolds into the cranial defects, the authors confirmed their abilities of achieving innervated bone regeneration.

In combination with the nanocomposite bioinks and multicellular 4D bioprinting technology, vascularised neural-bone construct can be successfully developed for vascularized and innervated bone regeneration. Vascularised neural cells and bone-related cells were orderly printed to imitate the simplified spatial distribution of bone, blood vessel and nerves. Appropriate drugs can be incorporated into the bioinks to serve as "bioactive factors" to regulate multicellular behaviors, enabling the development of multicellular multiphasic tissue-engineered scaffolds, capable of delivering personalised precision medicine for patients with segmental bone defects.


A
Bioink A : GelMA + CS nanowires + BMSCs
Bioink B : GelMA + CS nanowires + SCs
Bioink A
Bioink B
Bottom layer
Upper layer
Neural-bone constructs
SCs
BMSCs
Original bone
New bone
Nerve fibers
Bone defects

AR.8.S5-K8 Personalized Bone Regeneration: Bioactive Substitutes and Synthetic Periosteum

Elisabeth Engel

Institute for Bioengineering of Catalonia, Barcelona, Spain

Abstract

The big challenges for reconstructive bone surgeons are the large volume of bone defects resulting from traumatic incidents, infections, or cancer resections. Despite advances in implant design and composition, impaired bone healing is an important complication particularly in aged patients, and in patients with diabetes or osteoporosis. Addressing the challenges of alveolar bone atrophy in dental implantology and large-volume bone defects in reconstructive surgery, this work explores personalized, resorbable, and bioactive solutions. **Part 1** focuses on guided bone regeneration (GBR) using personalized 3D-printed poly-ε-caprolactone (PCL) scaffolds incorporating calcium/calcium phosphate nanoparticles to enhance bioactivity. These synthetic grafts, designed based on patient-specific magnetic resonance imaging to ensure accurate reproduction of the missing bone, offer great adaptability to defect geometries. The porous scaffolds demonstrated stable mechanical properties and in vitro biocompatibility with improved osteogenic markers, as well as implant integration and biocompatibility in subcutaneous in vivo in mice models, showing promising bone formation in in vivo murine models. **Part 2** introduces a bioactive synthetic periosteum designed to improve bone graft healing. This electrospun polylactic acid (PLA) nanofiber matrix with embedded calcium phosphate (CaP) particles mimics the native periosteum's role in bone repair. Preliminary in vivo data from a long-bone fracture rat model indicates that matrix-revested allografts supported new bone formation, unlike allografts alone. Collectively, these personalized, biodegradable, and bioactive strategies, featuring both 3D-printed scaffolds for targeted alveolar regeneration and a biomimetic synthetic periosteum to enhance bone graft integration, offer significant promise for advancing bone regeneration therapies and addressing critical challenges in reconstructive surgery and dental implantology.

AR.8.S5-K9 Bioprinting of complex skeletal implants

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Abstract

Spheroid-based regenerative constructs offer promising solutions for restoring tissue function in compromised biological conditions, such as complex bone defects. Innovative strategies and methodologies employing bioprinting have been explored to develop complex skeletal implants that mimic the cascade of events in bone fracture healing. By leveraging progenitor cells as the driving force for tissue formation and utilizing biocompatible biomaterials to provide essential 3D cues, we aim to create spatially organized tissue constructs that facilitate bone regeneration.

Our research focused on the development of in vitro engineered fracture calluses using bioprinting techniques. These constructs were based on 3D primed human periosteum-derived cells (hPDCs) and biocompatible thiol-ene alginate hydrogels, which mimic the cells and extracellular matrix present in different zones of the callus. In vitro studies confirm cell viability and maintained osteochondrogenic differentiation upon bioprinting, while in vivo assessments demonstrate that the biomaterials provide essential 3D cues guiding the cells in their tissue-forming processes without additional stimulatory molecules.

Furthermore, the potential of spheroids or microtissues as building blocks for bioprinted skeletal implants has been explored. Our investigations reveal significant morphological changes, high viability, and osteogenic differentiation of spheroids formed from hPDCs and bone marrow-derived mesenchymal stromal cells (hBMSCs) in hyaluronic acid methacrylate, gelatin methacrylate and combination of these biomaterials. Notably, hPDC spheroids exhibit higher mineralization capabilities and superior hydrogel colonization compared to hBMSC spheroids, underscoring their potential for producing mineralized bone grafts.

Additionally, the incorporation of pre-vascularized spheroids represents a compelling approach for enhancing the osteogenic and angiogenic potential of bioprinted constructs. Our studies on co-cultures of stem cells and human umbilical vein endothelial cells demonstrate stable spheroid formation, with endothelial cells arranged in capillary-like monolayers surrounding the spheroids.

In conclusion, insights into the optimization of bioprinting parameters, co-culture ratios, and media compositions to enhance the stability, viability, and bone formation potential of bioprinted skeletal implants will be provided. The findings emphasize the importance of biomimetic approaches and advanced bioprinting methodologies in fabricating living regenerative implants, offering promising solutions for patients with critical bone defects and other orthopedic conditions.

AR.8.S5-O1 4D Bioprinted Cell-Laden Hydrogels with Gradient Crosslinking Density for Bone Tissue Engineering

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Abstract

3D bioprinting of cell-laden hydrogels enables the fabrication of complex structures and delivery of stem cells in situ for bone tissue engineering. However, certain bone defects such as those in cranial bone, possess inherent curvature, which makes it challenging for 3D bioprinted hydrogels to perfectly match the structure. Therefore, developing 4D bioprinted hydrogels with shape-morphing capacities to conform to the native structure of cranial bone defects is essential. In the current study, we prepared a photocrosslinkable bioink consisting of gelatin methacryloyl (GelMA), oxidized alginate (OAlg), magnesium-doped hydroxyapatite (MgHAp), and bone marrow-derived mesenchymal stem cells (BMSCs). Methacryloxyethyl thiocarbamoyl rhodamine (RhB), an effective UV absorber, was used to generate a gradient in crosslinking density in the hydrogel and realize its shape morphing behavior. The gradient in crosslinking density led to varying pore size and porosity across the hydrogels, causing variations in their swelling behavior and subsequently inducing shape morphing. We found that the printing inks exhibited excellent printability, and that the printed hydrogels displayed high fidelity (Fig.1). Subsequently, a comprehensive study was conducted to evaluate the effects of various parameters, including UV crosslinking time, photoinitiator concentration, RhB concentration, the thickness and length-to-width ratio of printed hydrogels, and medium types, on the shape morphing behavior of hydrogels (Fig.2). Notably, our simulation results corroborated the significant influence of these parameters and mirrored the experimental observations. Furthermore, BMSCs showed a significant survival rate (> 90%) in 4D printed hydrogels and exhibited expanded morphology due to the dynamic crosslinking network formed by GelMA and OAlg. The 4D printed hydrogels could sustainably release Ca^{2+} and Mg^{2+}, which promoted the osteogenic differentiation of BMSCs by enhancing the expression of osteogenic-related genes and proteins. *In vivo* studies using rabbit skull bone defect models indicated that the 4D printed hydrogels perfectly matched the native curvature of cranial bone defects and significantly facilitated the regeneration of the defects. As a result, the current study presents a novel and versatile approach to overcoming the hurdle of achieving shape changes in cell-laden hydrogels to match the native curvature of specific bone tissues, demonstrating significant promise in bone tissue engineering.

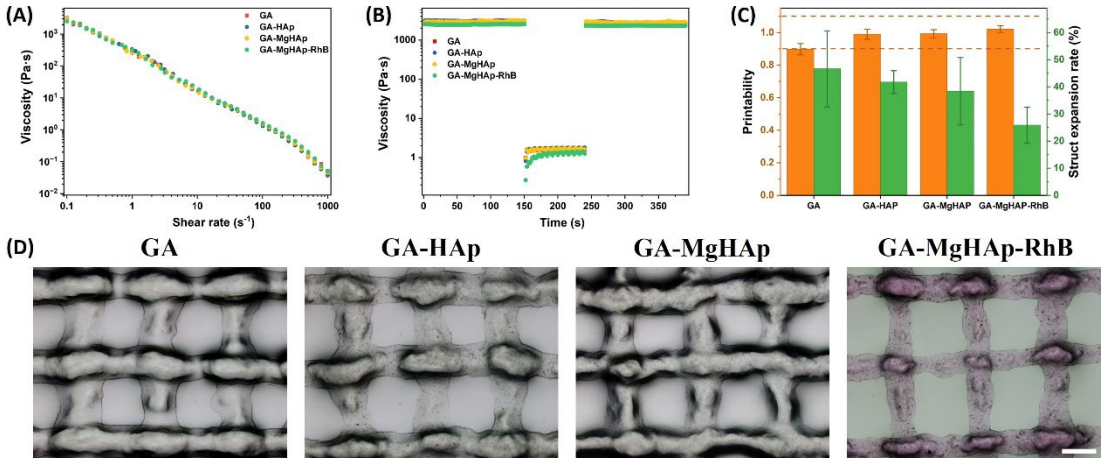

Fig.1. (A) Shear thinning and (B) thixotropic behavior of printing inks. (C) Printability of printing inks and fidelity of printed hydrogels. (D) Photos showing the structure of printed hydrogels, scale bar: 500 μm.

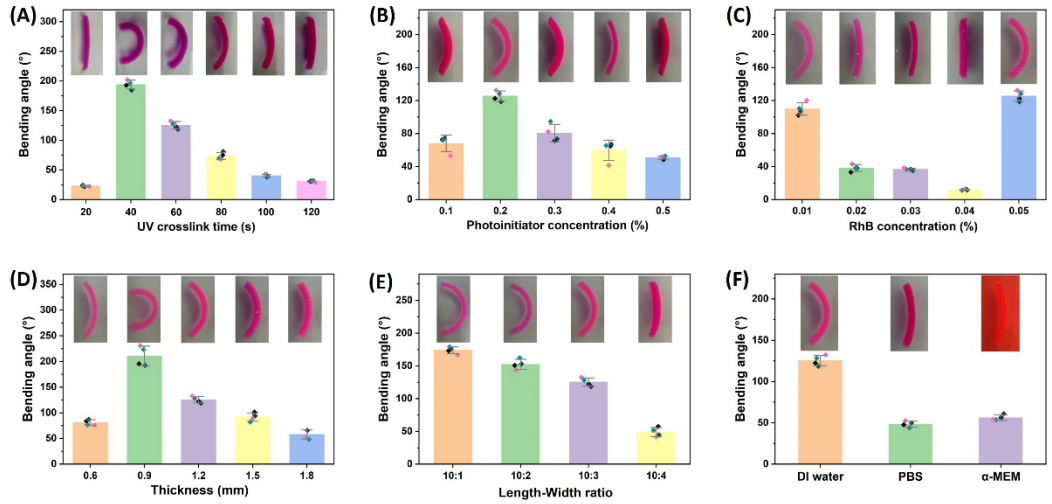

Fig.2 The effects of various parameters on shape morphing behavior of printed hydrogels.

AR.8.S5-O2 Biomedical bone biofabrication approach for modelling bone cancer pathologies *in vitro*

Francisco Javier Verdugo, Jorge Roberto Toledo, Carlos Medina, Carolina Delgado

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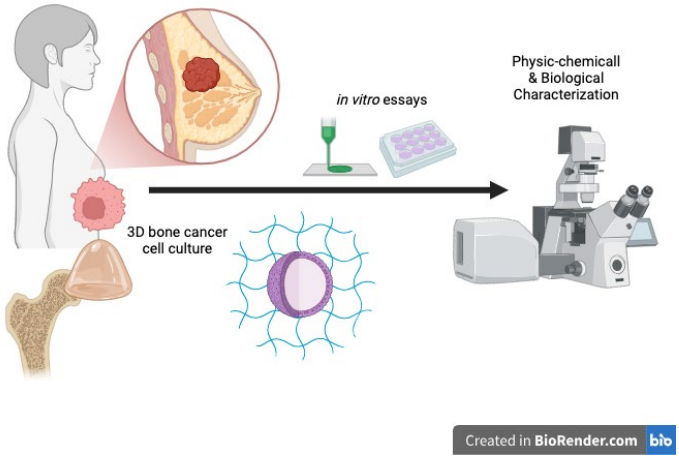
Abstract

Bone tumours and metastases are a significant healthcare concern due to their impact on health and well-being and represents a serious health risk with a decline in quality of life for these patients and their families. Although several advances in targeted therapies and drug treatments have been made and the survival results are promising with bone sarcomas, other metastatic bone tumours and late diagnosed distant malignant tumours have a decreased survival rate, being less than a year for metastatic patients. Hence, it is mandatory to develop complementary measures to improve the efficacy of current drug therapies and also to decrease the lifespan of treatment for preventing a growing economic burden in the healthcare system. *In vitro* 3D models resembling the bone microenvironment have the feasibility to introduce patient-derived cells from biopsies of bone cancer tissue in order to study personalized treatments that can lead to more effective and tailored therapies for patients

In this research we aim to develop a new 3D scaffold formulation for biofabricating bone tissue models consisting in bone cancer cells and others (MCF7, Saos2, MC3T3-E1 and adipose derived mesenchymal stem cells) based on a simple and low-cost hydrogel approach without the employment of human bone decellularized microparticles as part of a composite for several physic-chemical and biological characterization.

The hydrogel was successfully produced demonstrating shear-stress characteristics that were later demonstrated by bioprinting. Biocompatibility was tested by different assays for cytocompatibility quantification, either by imaging or by molecular measurements, and no toxic results were registered confirming the capabilities of the cells to attach and proliferate in the scaffold for several days (up to 30 days). Additionally, it was successful implemented as part of histopathology workflow with the samples, there were effectively processed as biopsy tissue for optical and inmunohistochemical characterization validating several protein and matrix responses-interactions characteristics of cancer cells. This 3D culture biomaterial has demonstrated that can be employed for simulating bone extracelullar matrix for preclinical development.



AR.8.S5-O3 A multifunctional 3D-printed scaffold with therapeutic and regenerative potential for bone cancer therapy and tissue regeneration

Ashkan Bigham[1,2], Anna Mariano[1], Xiao Yang[3], Xingdong Zhang[3], Maria Grazia Raucci[1], Luigi Ambrosio[1]

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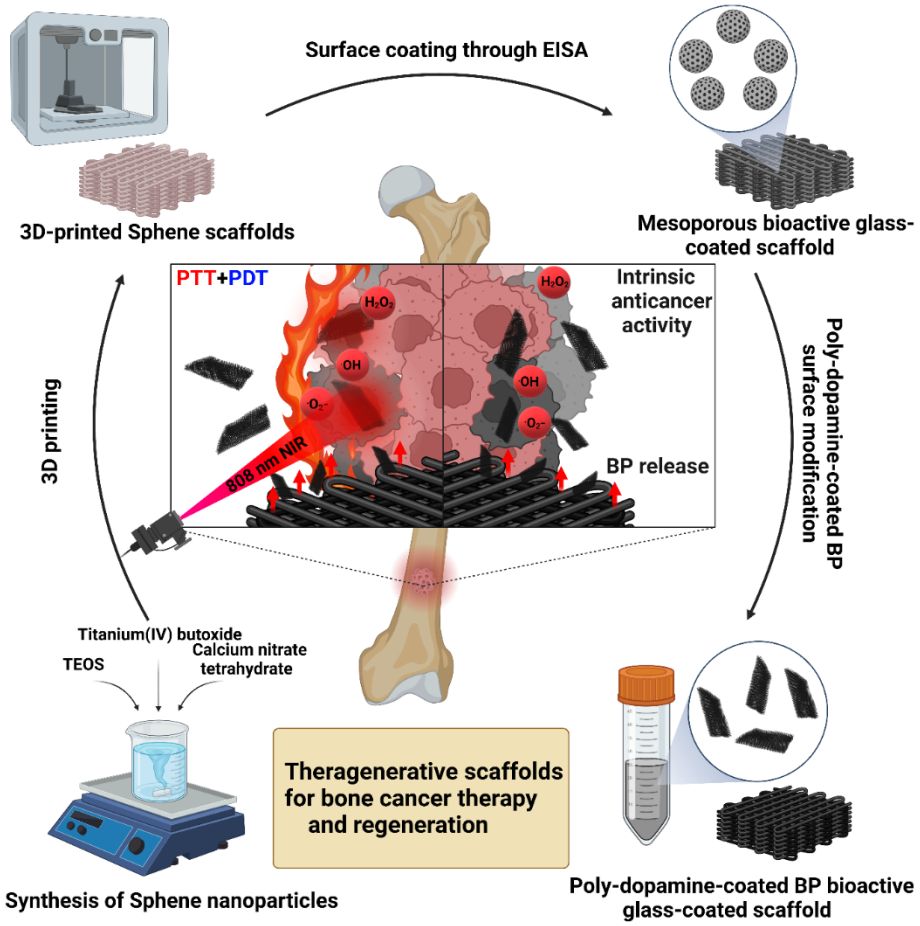
Abstract

Introduction. Malignant bone tumors pose clinical challenges, requiring both tumor removal and subsequent bone regeneration [1]. This necessitates advanced biomaterials designed through interdisciplinary strategies capable of both therapeutic and regenerative functions—a concept known as "theragenerative" biomaterials [2-4].

Methods. This study presents a novel multifunctional 3D-printed scaffold composed of Sphene ($CaTiSiO_5$) for bone cancer therapy and tissue regeneration (**Scheme 1**). Sphene nanoparticles were synthesized followed by fabrication of 3D printed scaffolds. The scaffolds were coated with mesoporous bioactive glass (MBG) to enhance bioactivity and a nanocomposite of black phosphorus (BP) and polydopamine (PD) to provide anticancer functionality and immunomodulatory effects. Study objectives included: (I) Fabrication of 3D-printed Sphene scaffolds, (II) MBG coating to improve bioactivity, (III) BP-PD deposition to confer anticancer activity, (IV) *In vitro* assessment of therapeutic and regenerative potential.



Scheme 1. *Fabrication of 3D scaffolds, and coating of the scaffolds by MBG and then BP-PD nanocomposite.*

Results. Thermal optimization during sintering revealed 1300°C as the optimal temperature, preserving scaffold integrity. *Via* an innovative sol-gel technique, MBG was formed uniformly on the scaffolds and then, the BP-PD deposition was confirmed (**Figure 1**). Scaffolds exhibited bioactivity in simulated body fluid. Mesenchymal stem cell assays demonstrated excellent cytocompatibility, and the surface-modified scaffolds stimulated the proliferation, adhesion, and osteogenic differentiation of the cells. Anticancer studies using SAOS-2 osteosarcoma cells revealed significant tumor suppression, further enhanced by near-infrared (NIR) laser irradiation. BP-PD-coated scaffolds selectively inhibited osteosarcoma cells while supporting healthy cell growth.

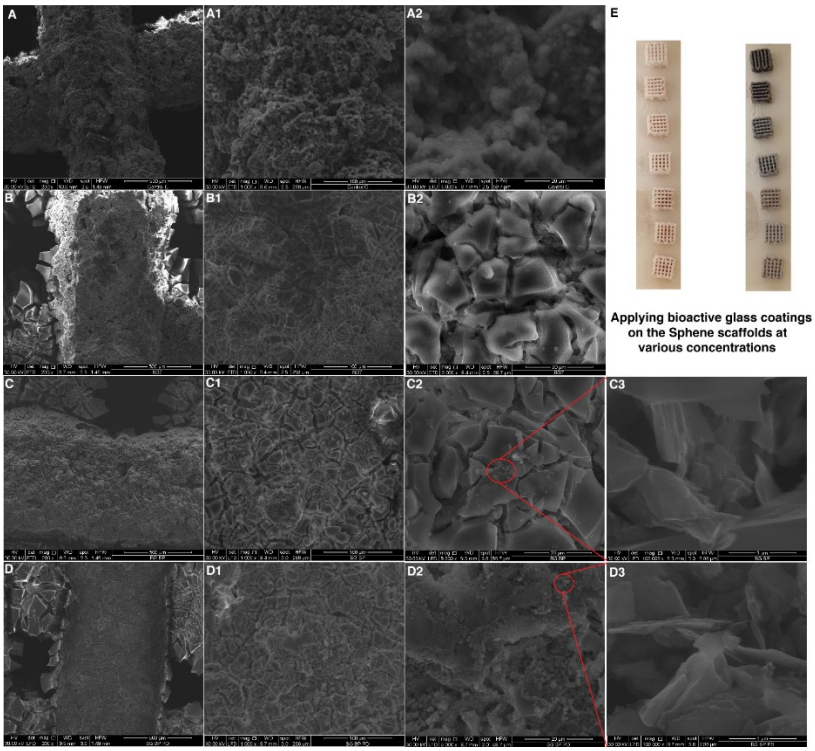


***Figure 1**. SEM of (A-A2) Sphene bare, (B-B2) MBG-coated, (C-C3) BP-deposited MBG-coated, (D-D3) BP-PD-deposited MBG-coated scaffolds. (E) Digital photographs of the Sphene scaffolds coated with MBG at various concentrations.*

Discussion. Preliminary findings suggest that BP specifically targets cancer cells while the surface coatings improve bioactivity kinetics. The scaffolds also promoted osteogenesis, as evidenced by early and late osteogenic protein expression.

Conclusion. This study introduces a novel theragenerative scaffold combining Sphene, MBG, and BP-PD, offering dual-functionality for bone cancer therapy and tissue regeneration. Ongoing investigations aim to further elucidate its physicochemical and biological properties.

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AR.8.S6-K10 Contactless Biofabrication Approaches for Tissue Modeling and Regeneration

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Abstract

This talk will provide an overview of the activities of the Field-Assisted Biofabrication (FAB) team, which is developing innovative strategies for tissue regeneration and modeling of complex multicellular systems. The team uses extrinsic fields - such as light, acoustic, magnetic and hydrodynamic waves - to spatially assemble cells, aggregates, organoids and extracellular matrices in a controlled and programmable manner. One of the team's key innovations is the enhancement of local cell density through sound-assisted assembly, which enables the creation of reproducible and morphologically relevant tissue constructs. These include spatially organized vasculature, nerve ingrowth associated with low back pain, and 3D mineralized constructs, paving the way for more predictive and human-relevant drug discovery and regenerative therapies.

AR.8.S6-K11 Microengineering of bone

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Abstract

Bone tissue exhibits exceptional structural and cellular complexity, rooted in its diverse population of specialized cells, hierarchically organized and dynamic extracellular matrix (ECM), and extensive vascular and neural networks. To faithfully model bone physiology and pathology in vitro, it is essential to integrate the critical components that constitute native bone, and to develop platforms that enable controlled investigation of their interactions.

Miniaturized in vitro bone models, or "mini-bones," have emerged as powerful tools for mimicking native bone tissue, and probing bone regeneration and disease mechanisms under physiologically relevant conditions. These models allow precise recapitulation of physiological conditions, compatibility with high-throughput experimentation, and integration with high-content imaging techniques. In this work, we focus on a subset of mini-bone models based on spheroid formation technology, which enable the formation of three-dimensional bone-like constructs within a controlled microenvironment.

Central to this approach is the incorporation of ECM-mimicking biomaterials within the spheroids. These matrices are either formed in situ through mineralization processes driven by the cells themselves, or introduced in the form of designer microparticles. The microparticles are engineered with tunable chemical and physical properties, such as composition, porosity, shape, and topography, to recapitulate the mechanical and biochemical cues of native bone ECM. Such precision-engineered elements enhance the functional relevance of the model and provide a means to systematically study ECM-mediated regulation of bone cell behavior.

Another critical aspect of bone physiology is the presence of a vascular network. To address this, we present a microengineering strategy to vascularize spheroid models. Using microwell arrays, we pattern endothelial cells either on the inner or outer surfaces of the microwells to form a vascular bed that interfaces with the spheroids during culture. This configuration supports the establishment of microvasculature, which is vital for nutrient transport, cellular signaling, and tissue maturation.

By integrating ECM biomimicry with engineered vascularization, these microengineered mini-bone models offer a miniaturized platform for studying bone biology, disease modeling, and regenerative strategies.

AR.8.S6-O1 Quantitative assessment of physical changes in facial soft tissue caused by personalised 3D-printed PEEK implants

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Abstract

PEEK (polyether ether ketone) has been used as a biocompatible material for implant development for several decades and with more recent advances in technology such implants now can also be manufactured by 3D printing. Due to this manufacturing process, such implants currently are limited to non-load bearing use, therefore being applicable for cosmetic surgeries, primarily for patients that have undergone trauma or disease which has impacted their bony tissue and led to unwelcome visual changes. As the aim of these type of procedures is to improve the aesthetic outcome, which more often than not is dictated by the visible soft tissue, it is paramount to understand the impact of the implant design itself, as the current approaches primarily focus on the correction of hard tissue defects at its core. To examine this, a total of four patients, each with at least one presonalised and 3D-printed PEEK implant used for facial correction in a different area (orbit, cheek, mandible and maxilla) had a CBCT (cone-beam computed tomography) scan taken before and approximately 6 months after surgery. These examinations were superimposed and then used to identify changes in the soft tissue volume, skin surface area and the total soft tissue thickness at the site of the implant. The change in soft tissue volume and the skin surface area varied only by up to 5% when compared to the implants volume and the surface area in contact with the soft tissue interface, respectively, in the majority of cases, while the total soft tissue thickness on average changed by a value equivalent to half of the implant thickness. These preliminary findings indicate, that while the current implant design process may lead to the desired outcome in improving the aesthetic of the patient, the soft tissue changes do not seem to be uniform, therefore implying that the implant design process could be improved by further understanding its relationship with the corresponding soft tissue response.

AR.8.S6-O2 Ensuring Reproducibility in Gel-MA-based Bioinks for Cartilage Regeneration

Didem Aksu[1], Hannah Agten[2], Aysu Arslan[1], Bjorn Vergauwen[3], Veerle Bloemen[2], Jasper Van Hoorick[1]

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Abstract

Osteoarthritis, which impacts more than 500 million individuals globally and results in annual healthcare expenditures exceeding 7.2 billion euros, poses a substantial clinical challenge due to cartilage damage which, when left untreated, can develop into osteochondral defects. The current medical procedures are often inadequate, underscoring the necessity of providing long-term approaches. Biofabrication is a promising approach to addressing e.g. cartilage defects, with Gelatin Methacryloyl (Gel-MA) becoming a material that is extensively researched in tissue engineering [1], [2]. Nevertheless, Gel-MA is frequently criticized for its perceived lack of reproducibility [3]. This issue originates from a variety of reasons including inconsistent selection of raw materials, variations in modification strategies, varying degrees of substitution, and differences in solvent and photoinitiator concentrations employed in various studies [3]. To overcome these issues, a new bioink is presented based on porcine gelatin where these issues are tackled through a combination of batch control, significant QMS protocols and a purification step to remove endotoxins from the gelatin, and significant Quality control in the following bio ink production, resulting in a true medical grade bioink. From the production of gelatin to the final formulation of ink, quality assurance protocols are implemented at each stage of material development. The printability of the ink was optimized to provide high reproducibility in 3D bioprinting. Primary human chondrocytes were mixed with the bioink and UV-crosslinked for biological validation. Biological assays for cartilage regeneration were conducted on the resulting constructs, which included metabolic activity assays, live/dead cell viability tests, and histological analysis. The bioink's compatibility with human chondrocytes was suggested by high cell viability and metabolic activity of the constructs , as indicated by preliminary results. This study introduces a GelMA-based bioink that is reproducible and ready-to-use by implementing rigorous quality assurance protocols and tackling the reproducibility issues at the earliest phases of bioink development. In the future, the primary objective will be to enhance biofabrication techniques for patient-specific constructs, with the ultimate objective of clinical translation for cartilage repair.

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AR.8.S6-O3 4-D Bioprinted Multiphasic Multicellular Scaffold for Personalised Osteochondral Tissue Engineering

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Abstract

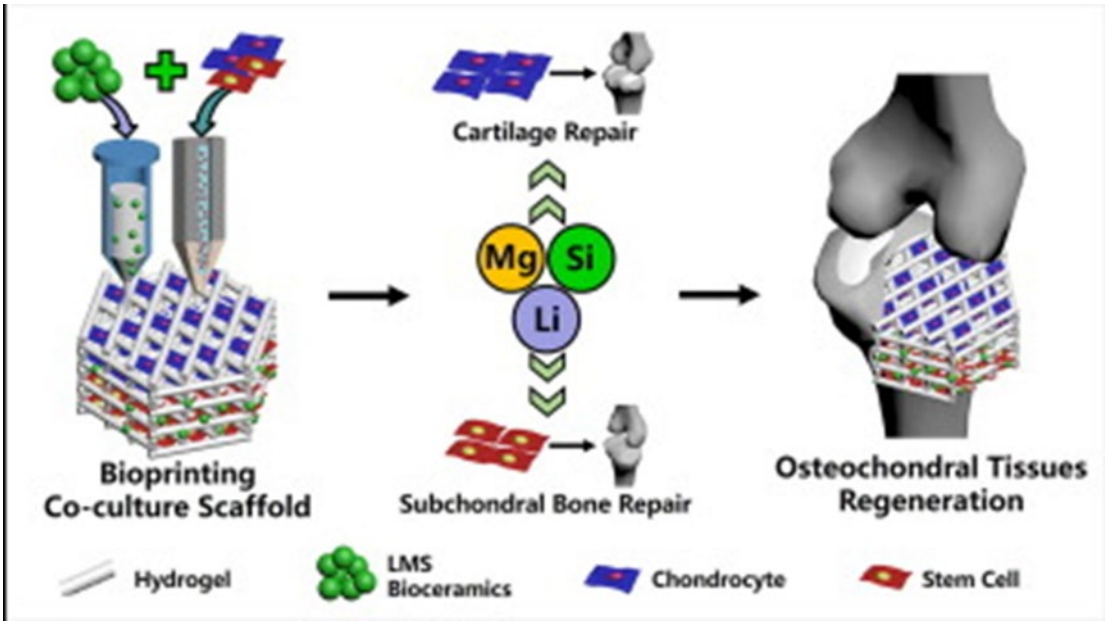
Articular cartilage defects cannot heal on their own, due to the lack of blood supply and regenerative potential. Tissue engineering technology has been showing some promise in overcoming this challenge. In recent years, advances in 3D and 4D printing technology have further enriched the structural design and composition of tissue engineering scaffolds, which also provided convenience for cell loading and cell delivery of living cells, enabling precise control of seeding cells in vivo, achieving better mimicking of tissues. When delivering the cells onto the scaffold, the cell type, cell distribution and cell retention should be fully considered. In general, cells should be delivered with its composition and arrangement similar to natural tissues, in terms of spatial-temporal relationship, in order to serve their original function. In Hong Kong, we designed a bone marrow-derived mesenchymalstem cell (BMSC)-laden 3D-bioprinted multilayer scaffold with methacrylated hyaluronic acid (MeHA)/polycaprolactone incorporating kartogenin and β-TCP for osteochondral defect repair within each region. BMSC-laden MeHA was designed to actively introduce BMSCs in situ, and diclofenac sodium (DC)-incorporated matrix metalloproteinase-sensitive peptide-modified MeHA was induced on the BMSC-laden scaffold as an anti-inflammatory strategy. BMSCs in the scaffolds survived, proliferated, and produced large amounts of cartilage-specific extracellular matrix in vitro. BMSC- laden scaffolds facilitated chondrogenesis by promoting collagen II and suppressed interleukin 1β in osteochondral defects of the femoral trochlea. Congruently, BMSC-laden scaffolds significantly improved joint function of the injured leg.

In Shanghai, through assembling cell-laden modules, the macrophage-mesenchymal stem cell (MSC), endothelial cell-MSC, and chondrocyte-MSC co-culture models are successfully established. The in vitro results indicate that the intercellular cross-talk can promote the proliferation and differentiation of each cell type in the system. Moreover, MSCs in the modular scaffolds may regulate the behavior of chondrocytes through the nuclear factor of activated T-Cells (NFAT) signaling pathway. Furthermore, the modular scaffolds loaded with co-cultured chondrocyte-MSC exhibit enhanced regeneration ability of osteochondral tissue, compared with other groups.

Overall, this work offers a promising strategy to construct a multicellular tissue engineering scaffold for the systematic investigation of intercellular cross-talk and complex tissue engineering. The scaffold modules could be easily sterilized, transported,

and stored. It would not be difficult for inexperienced staff to assemble these modules into a customized scaffold.

In addition, these modular scaffolds loaded with varying cell types could be easily stacked and disassembled, benefiting the construction of multicellular tissue engineering scaffolds, enabling desirable intercellular cross-talk.



of correlations between participant characteristics and their preferences for transitioning to a new bone regeneration technology revealed that participants with more bone defect experience are more likely to value witnessing a procedure firsthand (Spearman's rho = 0.174, p = 0.011; Kendall's Tau = 0.130, p = 0.01) and that those with more bone defect experience are somewhat more likely to value peer endorsement (Spearman's rho = -0.153, p = 0.027; Kendall's Tau = -0.116, p = 0.026). To conclude, our results substantiate the recommendation to treat complex bone defects at selected centres of excellence with large numbers of cases. The realization of fellowships at these centres could support the acceptance and implementation of new treatment options. Finally, the organization of interdisciplinary and collaborative meetings on a broad basis to discuss holistic treatment approaches is deemed beneficial.