Leveraging native human femoral head bone as a physiological platform for biomaterial testing and in situ tissue engineering

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Background

Biomaterial development for clinical use is complex, costly, and usually requires *in vivo* testing during the preclinical phase.

Ex vivo models interpose between *in vitro* and *in vivo* methods and help to advance 3R efforts, as they enable testing of biomaterials in a native human tissue environment. Human femoral heads are widely available for research due to high numbers of hip arthroplasties in Germany and are therefore a promising resource for an *ex vivo* bone model.

Hydrogels are widely used biomaterials. They are three-dimensional crosslinked polymeric networks with high water content and can mimic extracellular matrix (ECM) to facilitate cellular migration, proliferation, and differentiation. Collagen is the primary protein in mammalian ECM and plays a pivotal role in bone regeneration, making collagen-derived hydrogels of particular interest to bone tissue engineering approaches.

Aim

Validation of test platform with various collagen hydrogels

Methods

An *ex vivo* model from human femoral head tissue was developed including a 3D-printed toolkit for standardized fabrication. Eight types of hydrogels were implanted into drilled defects after 14 days of pre-cultivation. Collagen based hydrogels were preferably used due to their proximity to the native ECM. Methacrylated gelatin was used as reference hydrogel, accompanying every implanted hydrogel, to compensate tissue variability. Polyethylene glycol diacrylate (PEGDA) was implanted as negative control lacking cell adhesion motifs. After culture, hydrogels were extracted from the explants and analyzed using fluorescence microscopy for staining of cells and mineralization and resazurin metabolic assay for indirect cell counting.

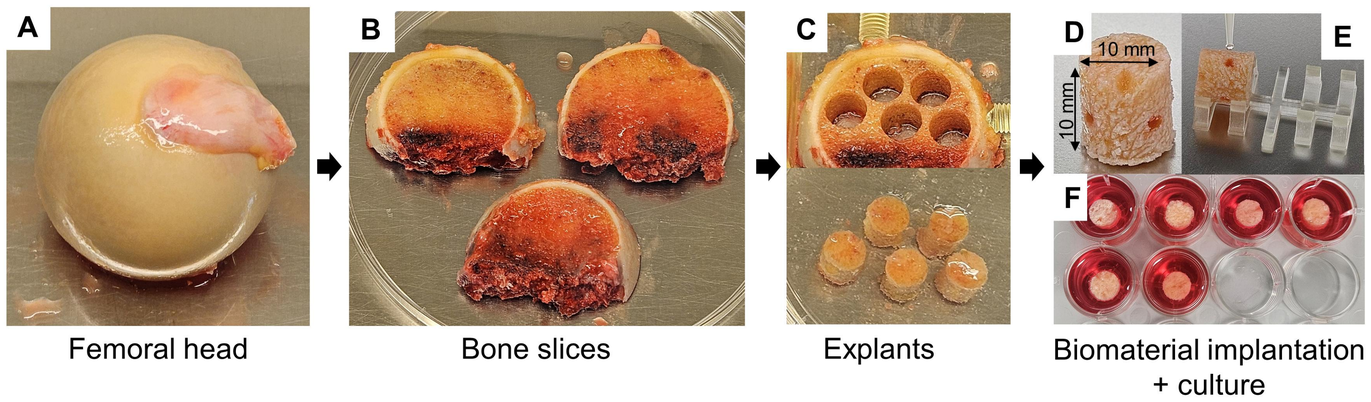


Figure 1: Fabrication workflow for *ex vivo* cultures. Human femoral heads **(A)** are segmented into slices **(B)** using a 3D-printed template. Explants are drilled from bone slices **(C, D),** defects are set and filled with hydrogels **(E)**. Final explant cylinder **(D)** and culture in 12 well plate **(F).**

Results

Explant metabolic activity is maintained over 42 days

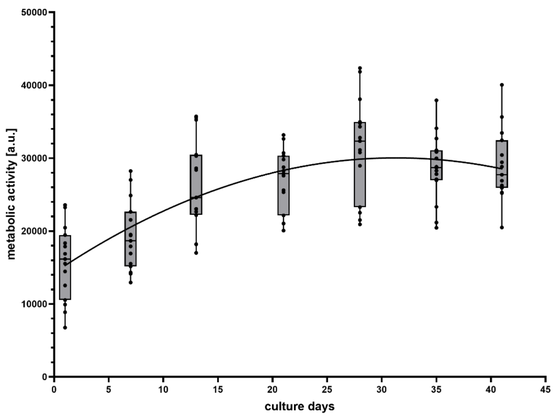


Figure 2: Explant metabolic activity over six weeks culture period

Whole explant cylinders maintain high metabolic activity during six weeks culture period in repeated resazurin assay.
The metabolic activity peaks on day 30. A 14-day pre-cultivation period allows for tissue regeneration following the fabrication trauma before implantation and covers the phase with the highest metabolic activity.

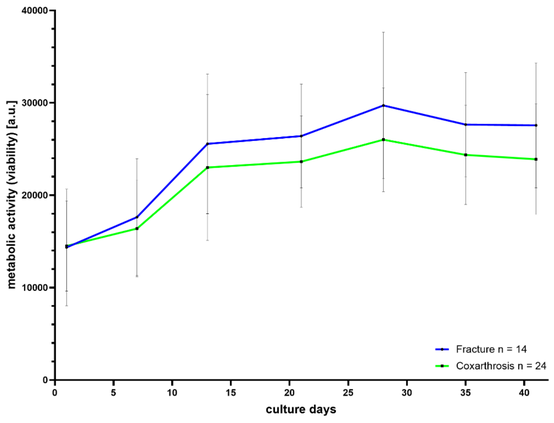


Figure 3: Metabolic activity of explants compared to tissue source

Differences in metabolic activity between tissue sources can be observed.
Samples obtained from femoral neck fractures tend to show higher activity levels than those from patients with coxarthrosis. Despite high variability between donors, metabolic kinetics are comparable over the culture period.

Collagen fibril-forming hydrogels display higher levels of cell migration

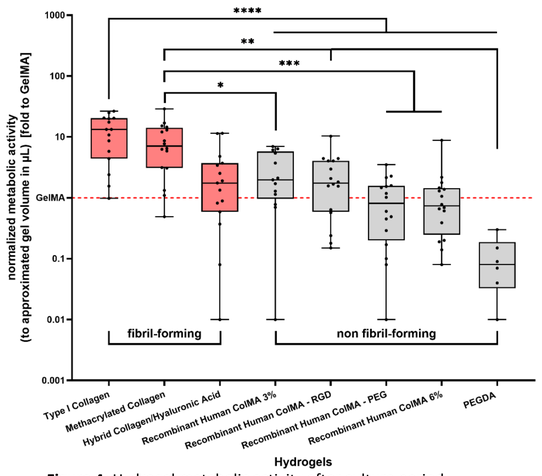


Figure 4: Hydrogel metabolic activity after culture period

The implantation of hydrogels forming collagen fibrils leads to significantly higher cell infiltration in tested hydrogels. Native collagen type I shows 10-fold higher infiltration than the reference gelatin methacrylate. PEGDA, providing no cell adhesion motifs, displayed negligible cell migration levels.

Migrated cells show hydroxyapatite deposition within implanted hydrogels

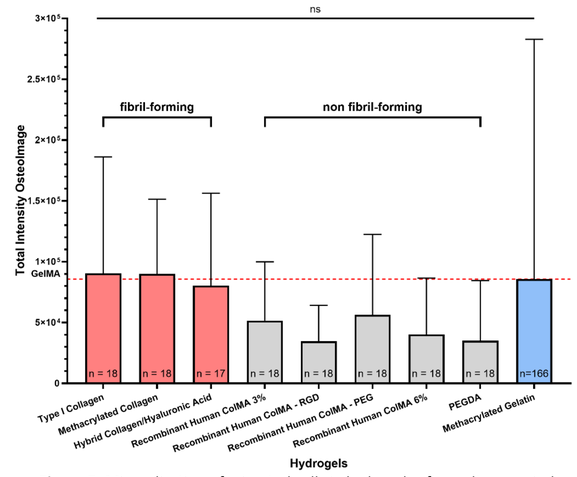


Figure 5: Mineralization of migrated cells in hydrogels after culture period

Migrated cells spontaneously remodel hydrogels and mineralize. Fibril-forming hydrogels tend to show more mineralization than non-fibril-forming ones. However, no significant differences could be observed. High sample number of reference hydrogel results from the accompany of every tested hydrogel.

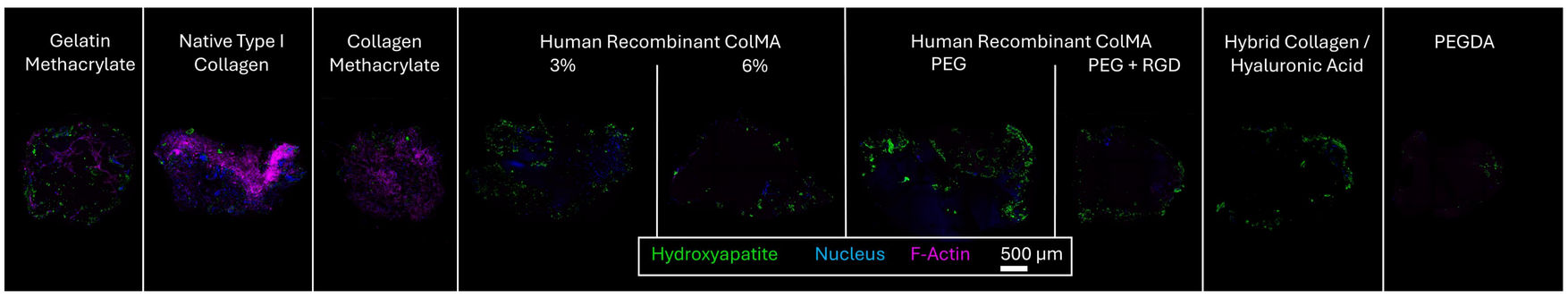


Figure 6: Sample images from fluorescence microscopy of tested hydrogels

Native type I collagen and collagen methacrylate show the highest tendency for matrix formation. Human recombinant ColMA subtypes differ slightly in degree of mineralization.

Limitations and Outlook

A characterization of the migrated cells is lacking and highly needed. In addition, insights into the development of the cellular composition of the explant cylinder during the culture period should be gained. As mechanical stimulation is an important factor in the process of bone healing, an addition of this to the culture system could be beneficial. The impact of media flow on the explant culture and the role of immune cells are also important questions to investigate in future studies.

Conclusion

The expected differences between the tested hydrogels in comparison to the reference hydrogel could be confirmed. Collagen fibrils enhance cell infiltration, which corresponds with the prevailing opinion in the literature. The *ex vivo* model is capable of displaying expected differences and poses a viable alternative to early *in vivo* experiments.

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