Development of 3D In Vitro Human Osteosarcoma Constructs for Studying Cell–Biomaterial Interactions

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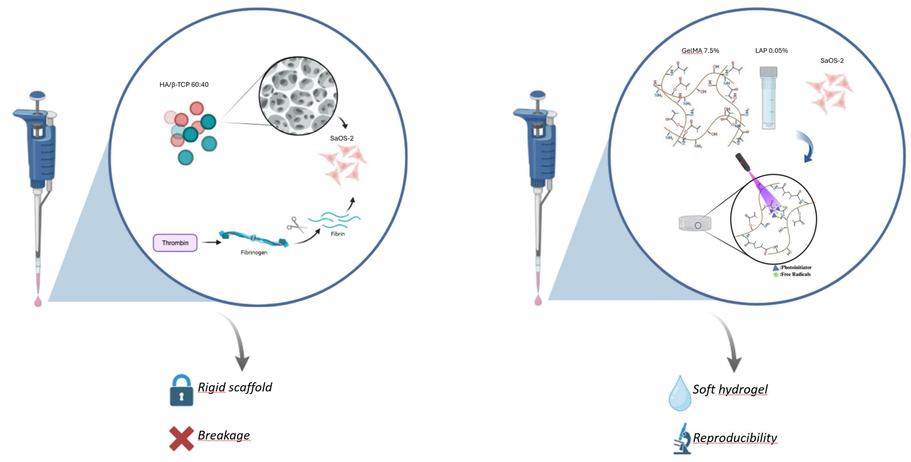
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INTRODUCTION & AIM

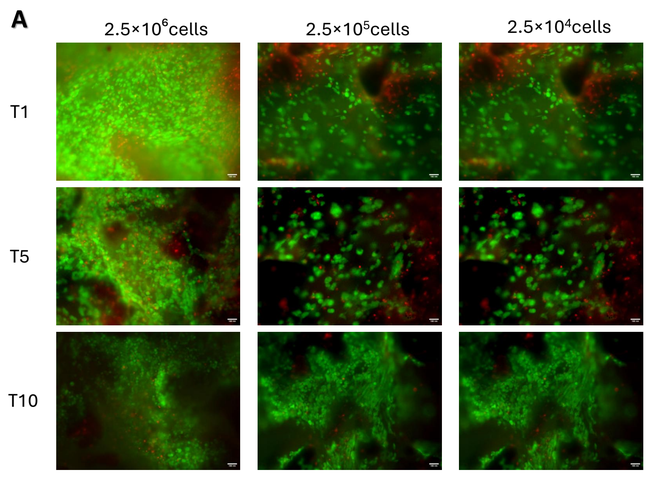
Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents, with limited improvement in survival rates. Conventional 2D models fail to reproduce the tumor microenvironment, making **3D in vitro systems** essential for more predictive studies. Biomaterials used in bone regeneration, such as biphasic calcium phosphate (BCP), may not be suitable for tumor modeling.

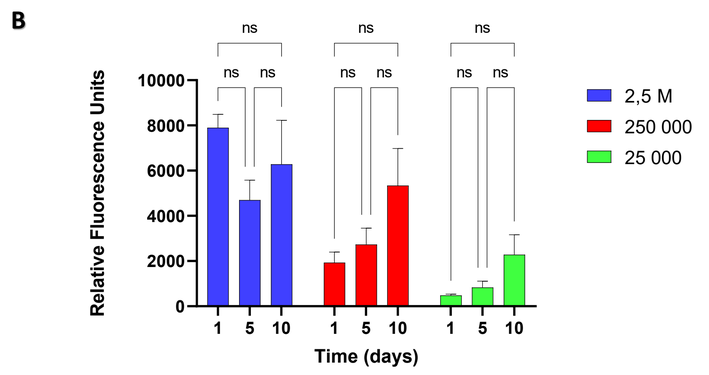
This study aims to evaluate biomaterial compatibility and optimize a reproducible **3D osteosarcoma model** by tuning scaffold composition and cell density.

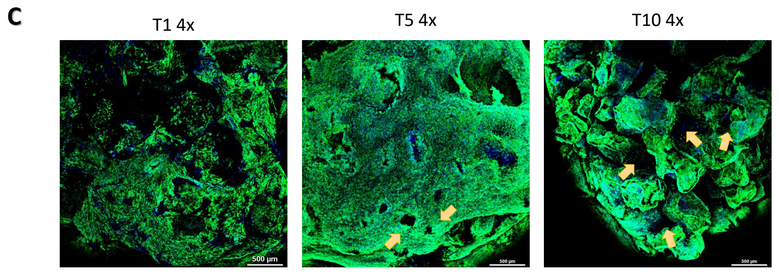
MATERIAL & METHOD

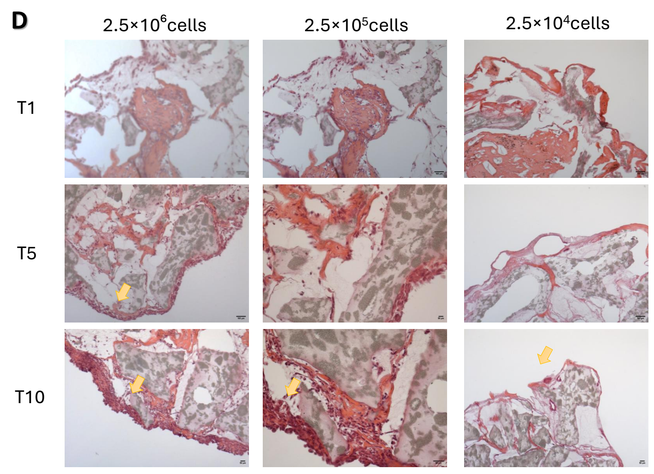


BCP SCAFFOLDS ARE NOT SUITABLE FOR OSTEOSARCOMA 3D CULTURE





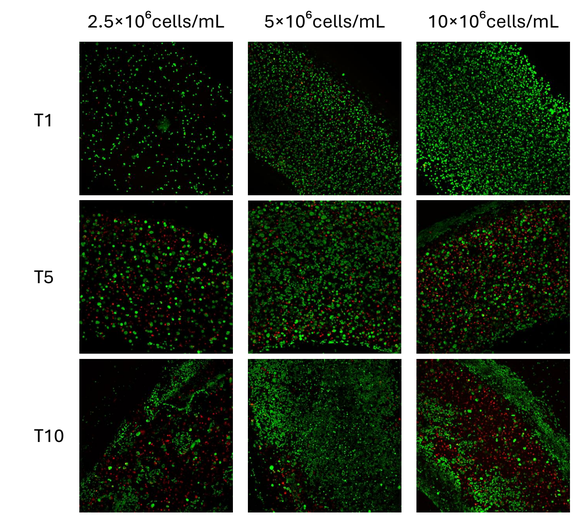


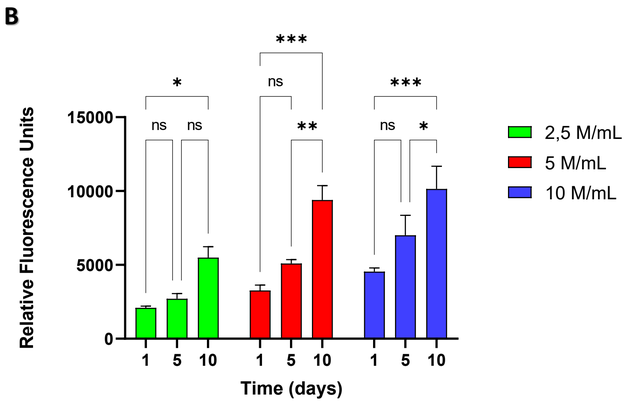


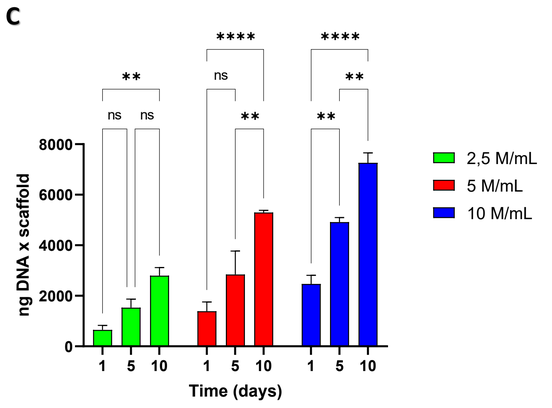
Each test was performed in triplicate at time points t1, t5, and t10 using three cell densities: 2.5 × 10^6 cells/construct, 2.5 × 10^5 cells/construct, and 2.5 × 10^4 cells/construct.

A. Live and Dead assay. It displays a decrease in cell viability during culture
B. Alamar blue assay. Statistical test: two-way ANOVA analysis
C. Confocal images (Oregon Green and DAPI staining) showing progressive scaffold breakage over time.
D. H/E Histology. It shows peripheral cell localization and absence of scaffold interaction.

GELMA HYDROGELS SUPPORT OSTEOSARCOMA 3D CULTURE







Each test was performed in triplicate at time points t1, t5, and t10 using three cell densities: 2.5 × 10^6 cells/mL, 5 × 10^6cells/mL, and 10 × 10^6 cells/mL.

A. Live and Dead assay. It displays an increase in cell viability during culture
B. Alamar blue assay. Statistical test: two-way ANOVA analysis
C. DNA quantification. Statistical test: two-way ANOVA analysis

DISCUSSION & CONCLUSION

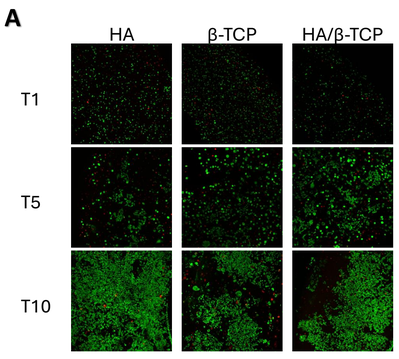
This study demonstrates that bone-regenerative biomaterials are not always suitable for osteosarcoma 3D modeling. BCP scaffolds showed poor cell compatibility, with limited infiltration and structural instability, unaffected by cell density. In contrast, GelMA hydrogels supported better cell viability and reproducibility, with an optimal density of 5 × 10^6 cells/mL.

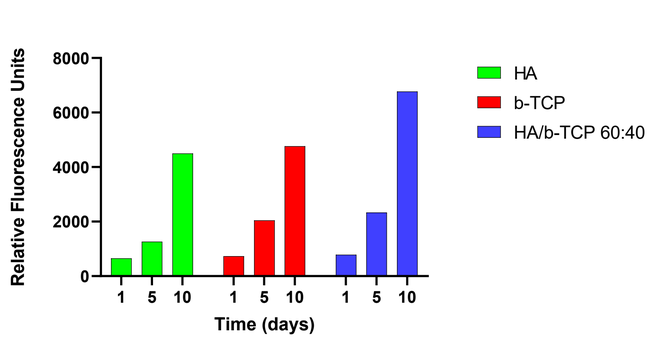
Overall, GelMA represents a more suitable platform for 3D osteosarcoma modeling, while BCP is not appropriate for this application.

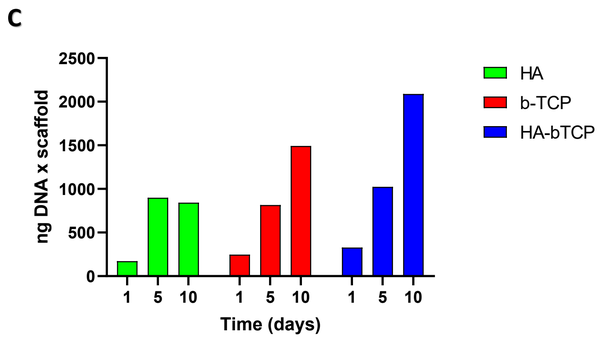
ONGOING EXPERIMENTS

Ongoing studies are focused on the development of hybrid GelMA-based systems incorporating hydroxyapatite and β-tricalcium phosphate nanoparticles. In parallel, further construct characterization is ongoing, along with improved histological processing for 3D analysis.

In addition, osteogenic differentiation will be induced, and the optimal HA/β-TCP combination will be selected based on viability, metabolic activity, and differentiation potential.







Experiments were performed at t1, t5, and t10 using 5 × 10^6 cells/mL in GelMA with HA, β-TCP, or HA/β-TCP nanoparticles (all at 1.75%, 60:40 ratio for the mixture).

A. Live and Dead Assay
B. Alamar blue assay
C. DNA Quantification

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