Effect of mechanical properties in mimetic hydrogels for cartilage formation

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INTRODUCTION

Articular cartilage regeneration remains a major clinical and scientific challenge due to its avascular nature, low cellularity, and limited intrinsic repair capacity. These factors contribute to degenerative joint diseases such as osteoarthritis, a significant global health issue (1). Current treatments often produce mechanically inferior fibrocartilage and face limitations in tissue integration and donor site morbidity. Therefore, advanced strategies are needed to restore both the structural and functional properties of native cartilage (2,3).

This project aims to design and evaluate a biomimetic hydrogel system that supports cell viability, promotes chondrogenesis, and enables the formation of functional cartilage-like tissue. Methacrylate-based polymers are used to tune the hydrogel's mechanical properties, optimizing the cellular environment for proliferation and differentiation, alongside cell models that enhance extracellular matrix production and tissue maturation

METHODS

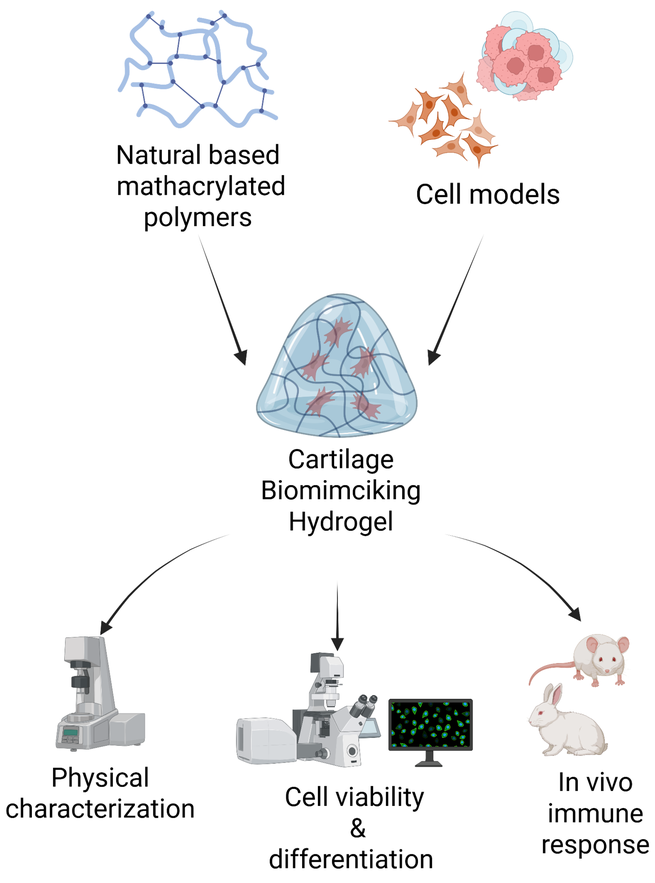


Fig. 1.

Project workflow and techniques. The first stage focused on selecting Methacrylate Hyaluronic Acid (HAMA) and Gelatine Methacrylate (GelMA) for their biocompatibility and similarity to native extracellular matrix. Various formulations were developed to obtain a range of suitable mechanical properties. Human mesenchymal stem cells (hMSCs) and ATDC5 cells were used as biological models.

Mechanical properties were analysed through rheology. In vitro studies assessed biocompatibility, cell morphology, and differentiation using confocal microscopy, qPCR, and histology. Finally, constructs were implanted in subcutaneous mouse models to evaluate the in vivo immune response, and rabbit for tissue formation.

RESULTS

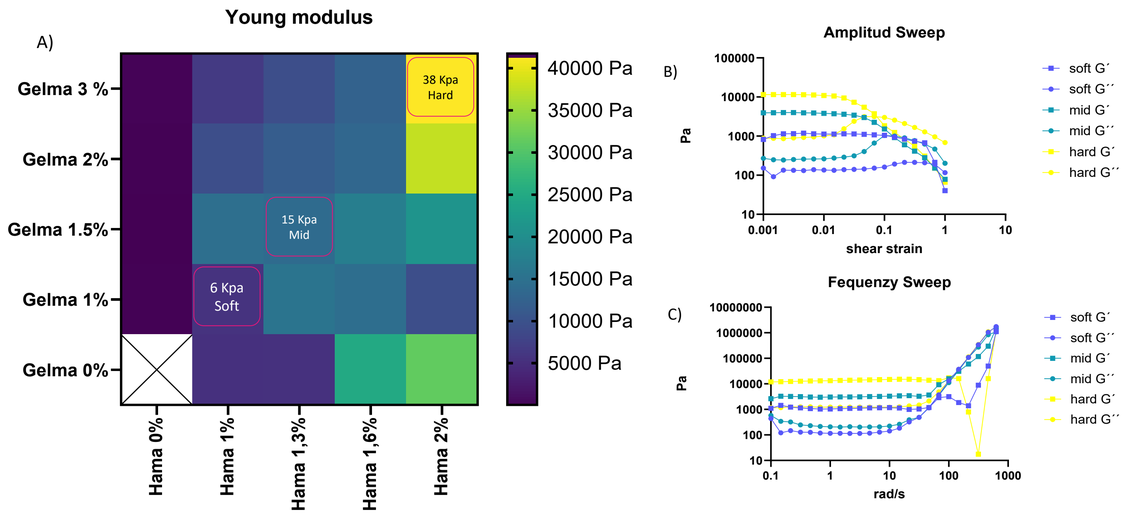


Fig 2. A) Heatmap representation of the hydrogel library generated from different combinations of HAMA and GelMA concentrations. Based on this screening, three representative conditions were selected for further studies: a soft formulation (6 kPa), a medium formulation (15 kPa), and a hard formulation (38 kPa). B) amplitude sweep of selected formulations. C) frequency sweep of selected formulations.

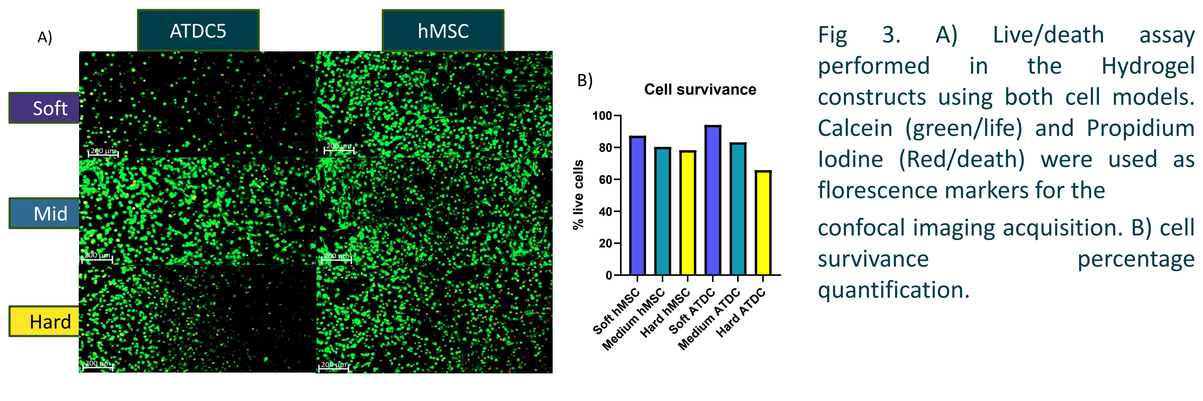


Fig 3. A) Live/death assay performed in the Hydrogel constructs using both cell models. Calcein (green/life) and Propidium Iodine (Red/death) were used as florescence markers for the confocal imaging acquisition. B) cell survivance percentage quantification.

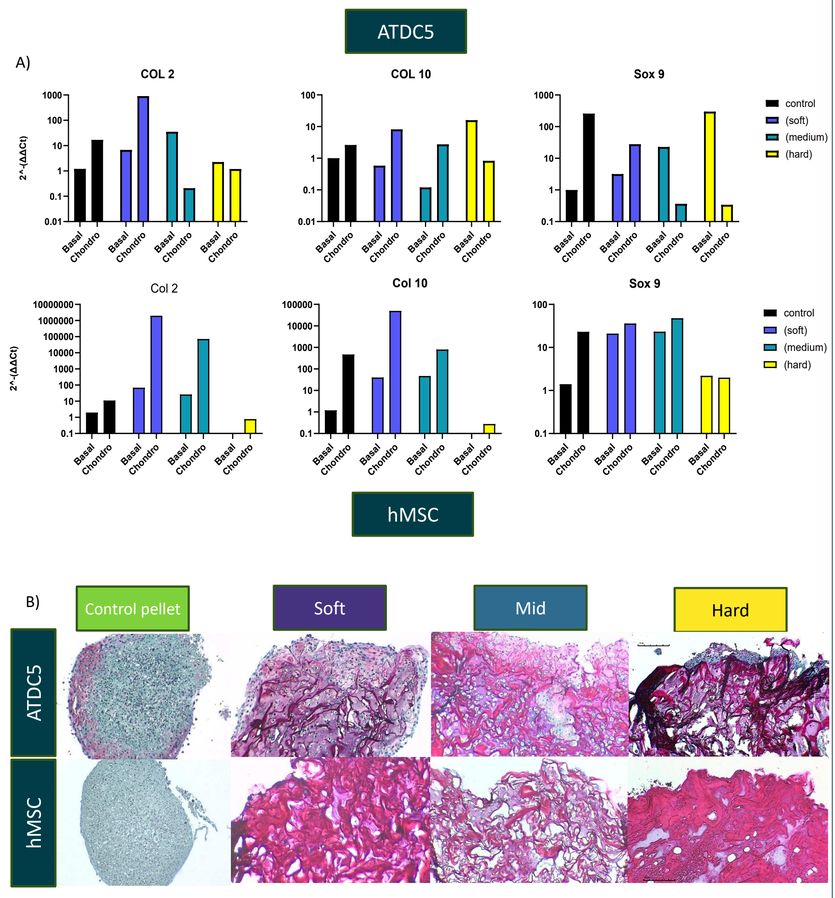


Fig 4. A) Chondrogenic markers gene expression performed via qPCR. Hydrogel construct with ATDC and hMSC cells were cultured 4 weeks in basal media and chondrogenic differentiation media. The Collagen 2, 10 and SOX 9 expression of the cell in the hydrogels was compared to cell pellets in the same medias. The presence of the hydrogel, the differentiation media or the combination of both enhanced the chondrogenic differentiation of the hMSCs cells in soft and mid condition. Meanwhile In the ATDC cells only the soft condition in combination with chondrogenic media enhanced the collagen 2 and 10 production

B) Safranin O staining of hydrogel construct with cells after 28 days in cell culture with chondrogenic media. All conditions show chondrogenic cell morphology but only ATDC started to form new extracellular matrix in the peripheric area

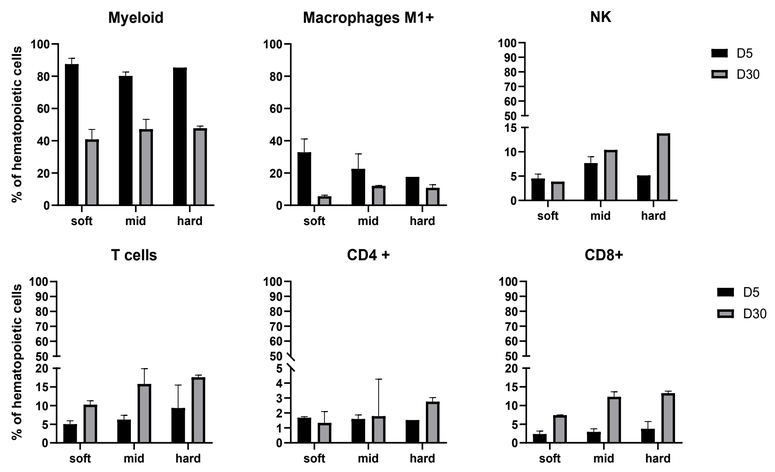


Fig 5. Immune response quantify via flow cytometry of 3 hydrogel stiffness after 5 and 30 day of subcutaneous implantation in immune competent mice's. myeloid cells decreased by day 30, including pro-inflammatory M1 macrophages indicating the absence of an acute immune response. Among the implanted materials, the Hard construct exhibited the highest number of cytotoxic $CD8^+$ T cells and NK cells, both of which are associated with sustained, long-term inflammatory responses.

CONCLUSIONS

1. All hydrogel stiffness conditions were biocompatible and allowed cell viability and chondrogenic differentiation.
2. The differences in stiffness modify the embedded cells behavior and even affected the immune response during implantation

FUTURE WORK

Test the efficacy in terms of tissue-forming capacity of cell-carrying implants, both in ectopic murine models and in orthotopic models in rabbits.

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