Novel 3D Extrusion Bioink Containing Processed Human Articular Cartilage for Application in Cartilage Tissue Engineering

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Introduction

Cartilage damage is challenging to treat due to its limited self-healing capacity caused by its avascular and aneural nature. However, 3D tissue engineering approaches that utilize biomaterials, additives, and cells offer a promising solution to regenerate functional cartilage. These innovative techniques have the potential to restore native cartilage properties, prevent the development of osteoarthritis, and restore joint function. In this study, we investigate a novel alginate-based 3D bioink composed of human articular cartilage for cartilage tissue engineering.

Materials & Methods

Cartilage Bioink Formation

- **Cartilage**: C20A4 human articular chondrocytes encapsulated in 20% medium viscosity sodium alginate (Alginate), 5% polyvinyl alcohol (PVA), 5% Gum Arabic (GA), and 5% pulverized human articular cartilage **(Figure 1)**

Construct Fabrication, Culture and Analysis

- Printed using a BIO-X 3D Bioprinter
- Concentric disk of cartilage bioink with 100% infill
- Constructs cross-linked with calcium chloride, rinsed, and cultured
- Cellular viability of overall construct analyzed after 1, 7 and 14 days in culture
- Gene expression analysis of the cartilage construct performed after 1, 7, and 14 days in culture
- Acellular constructs visualized by scanning electron microscopy (SEM) to examine microstructure

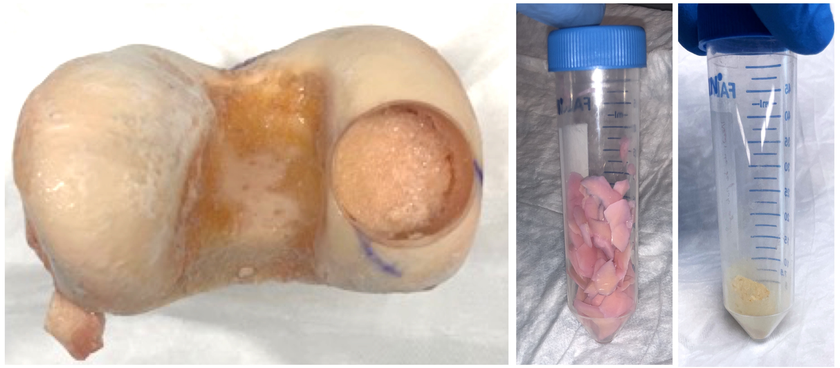
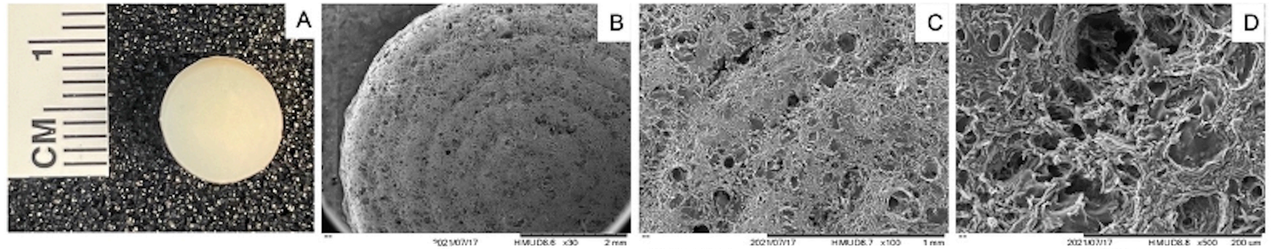


Figure 1. Human Articular Cartilage Harvest. (Left) Distal femur showing area of cartilage harvest. (Middle) Cartilage shavings attained from harvest. (Right) Final pulverized cartilage for use in bioink.

Results



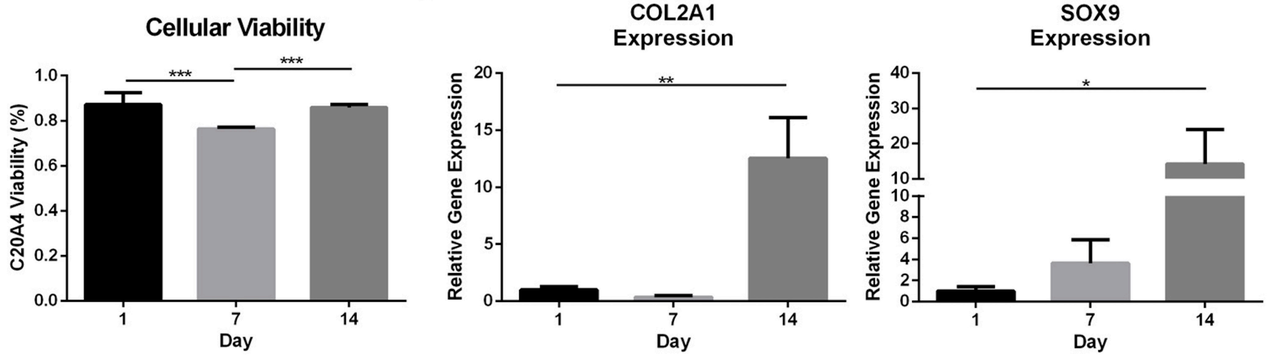


Figure 2. Visualization of Printed Constructs **A.** Unmagnified image of cartilage construct 1cm in diameter, **B.** SEM image taken at 30x, **C.** SEM image taken at 100x, **D.** SEM image taken at 500x.

Figure 3. Viability and Transcriptional Activity of C20A4 Chondrocytes (Left) Percent cell viability calculated by intensity of live dead staining. (Middle) Col2A1 transcriptional activity. (Right) Sox9 transcriptional activity. Error bars represent SD, *p<0.05, **p<.01, ***p<0.001.

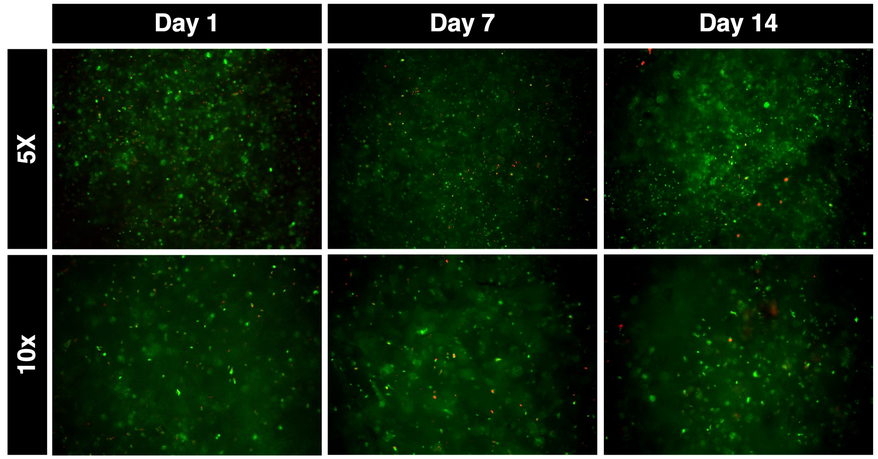


Figure 4. Cellular Viability within 3D Printed Constructs. Live (green) Dead (red) imaging of cells within 3D printed constructs taken after 1, 7 and 14 days in culture using 5x and 10x objectives.

Discussion and Conclusion

The bioink successfully formed porous 3D constructs that withstood the printing process. Although cellular viability initially decreased, it gradually recovered over a two-week culture period. Moreover, the upregulation of chondrogenic genes indicates the potential of the bioink to promote cartilage regeneration. These findings suggest that the developed bioink holds promise as an effective approach for cartilage tissue engineering, offering possibilities for future therapeutic interventions in cartilage repair and regeneration.

Acknowledgments

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