

Fabrication of a Novel 3D Extrusion Bioink Containing Processed Human Articular Cartilage Matrix for Cartilage Tissue Engineering

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Background: 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. In this study, we investigate a novel alginate-based 3D bioink composed of human articular cartilage for cartilage tissue engineering.

Methods: The bioink was developed by encapsulating C20A4 human articular chondrocytes in a mixture consisting of 20% medium viscosity sodium alginate (Alg), 5% polyvinyl alcohol (PVA), 5% Gum Arabic (GA), and 5% human articular cartilage. The human articular cartilage was obtained from cartilage shavings of distal femurs and ground into a powder for incorporation into the bioink (Figure 1). The bioink was printed into concentric discs using a BIO-X 3D Bioprinter, and the resulting 3D-biofabricated constructs were cross-linked with calcium chloride, rinsed, and cultured in chondrogenic media. There were 31 constructs in total. After printing, one construct was immediately visualized with SEM (n=1). The remaining 30 constructs were placed in culture for 1, 7 or 14 days and subsequently analyzed for cellular viability (n=5) and gene expression (n=5) at each time point.

Results: SEM imaging revealed that the 3D printed constructs exhibited a porous structure (Figure 2). Representative images of live/dead fluorescently stained constructs can be seen in Figure 3. Post-printing, the cellular viability was 87.2% which decreased significantly to 76.4% at day 7 (p<0.001) but recovered to 85.9% at day 14 in culture (Figure 4). Furthermore, gene expression analysis showed a significant increase in transcriptional activity of chondrogenic genes *Col2a1* (p=0.008) and *Sox9* (p=0.021) at day 14 compared to day 1 (Figure 4).

Conclusion: This study explored the use of an innovative alginate-based 3D bioink composed of human articular cartilage for cartilage tissue engineering. 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.



Figure 1. Articular Cartilage Preparation. Images shows cartilage harvest site on distal femur (left), resulting cartilage shavings (middle), and final powder used within the bioink (right).

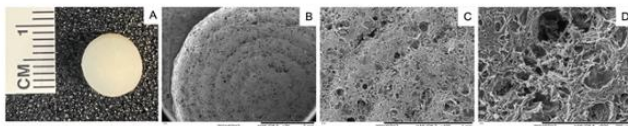


Figure 2. Visualization of 3D printed constructs. (A) un magnified sample, (B,C,D) scanning electron microscopy at 30x, 100x, and 500x respectively.

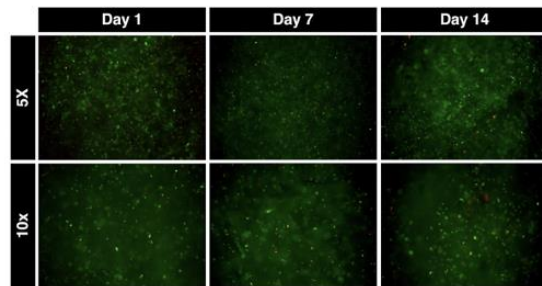


Figure 3. Fluorescence Imaging. Fluorescence Microscopy images of live (green) dead (red) staining of constructs at days 1, 7 and 14. Taken with 5x and 10x objectives.

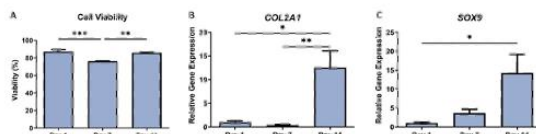


Figure 4. Cellular Viability and Gene Expression of C20A4 Cells. (A) Percent cell viability calculated by live dead staining intensity. (B) *COL2A1* transcriptional activity. (C) *SOX9* transcriptional activity. Error bars represent SD. *p<0.05, ** p<0.01, ***p<0.001.