Aggrecan-Functionalized Methacrylated Hyaluronic Acid Bioinks for Cartilage Regeneration

Robert H. Choe[a,b], John Osborne[a,b], Blake Kuzemchak[a,b], Noah Eby[a,b], John P. Fisher[a,b]
[a]Fischell Department of Bioengineering, University of Maryland, College Park, MD
[b]Center for Engineering Complex Tissues, University of Maryland, College Park, MD

Statement of Purpose: Approximately 70% of the senior population experience cartilage degeneration with varying degrees of severity.[1] There is currently no known cure for severely damaged cartilage sites since native cartilage is structurally complex and difficult to mimic. Additionally, hyaline cartilage is composed exclusively of chondrocytes embedded within a dense extracellular matrix (ECM) consisting of water, type II collagen, and proteoglycans (Figure 1A).[2] Although proteoglycans have demonstrated the ability to promote chondrogenesis in monolayer culture studies, they still remain underutilized bioactive signals for cartilage tissue engineering.[3] Our lab has recently demonstrated that a 3D-printed scaffold covalently modified with aggrecan, the main proteoglycan within the cartilage ECM, can effectively increase cell adherence and promote hyaline cartilage formation *in vivo*.[4] In this work, we enhanced our engineered cartilage approach by incorporating aggrecan into methacrylated hyaluronic acid (MeHA) bioink solutions. The objective of this study was to investigate the printability of bioprinted, aggrecan-enhanced MeHA scaffolds.

Methods: MeHA was synthesized according to a previously established protocol. A 3.0% (w/v) MeHA bioink solution was prepared by dissolving MeHA in PBS with 0.1% Litihum phenyl-2,4,5,-trimethylbenzoylphosphinate (LAP) photoinitiator. Chondroitin sulfate or aggrecan of varying concentrations (25, 50, or 100µg/mL) were incorporated into the bioink formuation. First, the bioink formulations were casted within cylindrical molds (5 mm diameter x 10 mm height) to assess equilibrium swelling of the various hydrogel scaffolds. We then casted cylindrical discs (28 mm diameter x 1 mm height) to assess the viscoelastic properties of each bioink formulation. Lastly, a 3D square prism CAD model with a dimension of 10.0 mm (length) x 10.0 mm (width) x 0.8 mm (height) was designed in SolidWorks (Waltham, MA). A 27G needle was used to print the scaffolds. According to the manufacturer's (EnvisionTEC, Gladbeck, Germany) instruction, the scaffold was sliced into layers with a slice thickness equal to 80% of the ID of the needle before printing. All the printed scaffolds were examined for 1D, 2D, and 3D printability.

Results and Discussion: Aggrecan is a significantly larger bioactive agent compared to the traditional additives previously utilized for cartilage bioprinting. Preliminary experiments in our lab have established the feasibly of bioprinting MeHA bioinks supplemented with aggrecans (Figure 1B). Additionally, one- and seven-day equilibrium swelling measurements demonstrated that the addition of 100 ug/mL aggrecan helped the 3% (w/v) MeHA bioink retain its strut dimensions upon printing

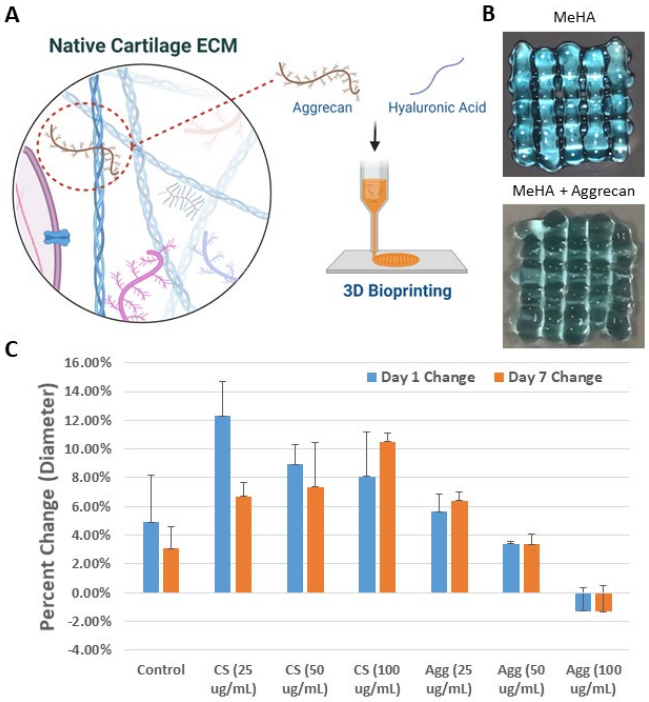

Figure 1. (**A**) Diagram of cartilage extracellular matrix, aggrecan, and 3D bioprinting. (**B**) 2-layer 3D-printed MeHA 3% (w/v) scaffolds with and without aggrecan (100 ug/mL). (**C**) Equilibrium swelling measurements of casted MeHA bioinks at Day 1 and Day 7

and storage in PBS (Figure 1C). In our future work, we will assess proliferation and chondrogenesis of hBM-MSCs within the bioprinted MeHA/aggrecan scaffolds.

Conclusions: This project seeks to develop a biofunctionalized MeHA scaffold via 3D bioprinting, which ultimately enhances neocartilage formation *in vivo*. Additionally, our future efforts will be directed towards hybrid printing these formulations with thermoplastic resin to fabricate a mechanically strong osteochondral scaffold that can withstand load-bearing forces.

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