3D Hybrid Printed Polycaprolactone/Methacrylated Hyaluronic Acid Scaffolds for Osteochondral Tissue Regeneration

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Statement of Purpose: Injuries to the knee affect approximately 900,000 Americans annually, resulting in more than 200,000 surgical procedures. Tissue engineering (TE) strategies have been explored to overcome the shortcomings of existing treatment modalities. Hybrid printing is a novel extrusion-based bioprinting strategy that has gained more interest in recent years. While natural polymer derivatives, such as methacrylated hyaluronic acid (MeHA), are highly printable with minimal immunogeneic response, they are still prone to undergo rapid degradation and collapse *in vivo*. Conversely, synthetic polymers may possess mechanical properties to recapitulate the biomechanics of native osteochondral tissue, but lack surface ligands for cell attachment and can generate degradation products that can negatively impact cellular metabolism. By alternately printing thermoplastic polymer and cell-laden hydrogel, a broader range of bioinks are accessible to create functional 3DP constructs that is mechanically strong and bioactive. Here we specifically investigated the relationship between MeHA hydrogel concentration (w/v %) and MeHA-to-polycaprolactone (PCL) print strut ratio within MeHA/PCL hybrid-printed scaffolds and characterize the resulting mechanical properties and protein absorption capacity.

Methods: A two factor, factorial design was devised: (i) MeHA hydrogel concentration (w/v %), and (ii) MeHA/PCL print strut ratio (Figure 1A). The first factor will be investigated at three levels and second factor will be investigated at five levels. Therefore, a 3 x 5 design, comprising of 15 formulations that was investigated. Prior to printing, MeHA was be synthesized according to a previously established lab protocol.[8] MeHA solutions will be prepared by dissolving MeHA in PBS, with 0.1% Lithium phenyl-2,4,5-trimethylbenzoylphosphinate (LAP) photoinitiator. 3D cylindrical models with various dimensions were designed in SolidWorks (Waltham, MA) to prepare scaffolds for all experiments. Scaffolds were 5 mm diameter by 10 mm height for mechanical testing and 5 mm diameter by 4 mm height for the protein adsorption study. The cylindrical models were imported into the Bioplotter RP (EnvisionTech) program for layer slicing and patterning. All model scaffolds were sliced into layers with a slicing thickness equal to 80% (0.32 mm) of the needle size (0.4 mm) before printing. Alternating print angles of 0° and 90° were used to print every two layers, and a fiber spacing of 0.7 mm from center to center of extruded fibers were used to maintain the necessary mechanical and structural properties. Compression testing was conducted on an Instron Mechanical Tester at a ramp rate of 1 mm/min. Protein adsorption behavior by the MeHA/PCL scaffold was determined by immersing the printed scaffolds (n=3) in FITC-BSA (Sigma-Aldrich) in PBS until reaching adsorption equilibrium.

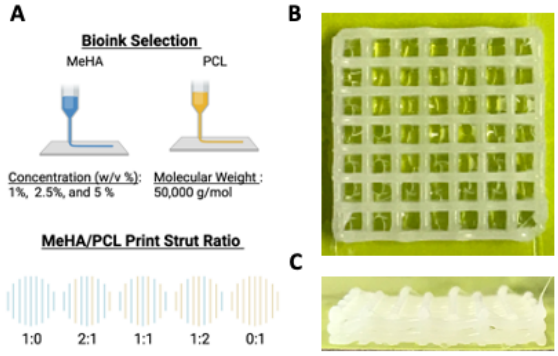

Figure 1. (**A**) Bioinks utilized for hybrid printing and print strut ratio. (**B**) 5-layer hybrid printed MeHA/PCL scaffold with 1:1 ratio.

Results and Discussion: Preliminary experiments in our lab have established the feasibly of bioprinting MeHA bioinks with a thermoplastic polymer such as PCL (Figure 1B). Moreover, we were able to demonstrate that varying the MeHA/PCL print strut ratio influenced the compressive mechanics and protein adsorption capacity of the hybrid-printed MeHA/PCL scaffolds.

Conclusion: This project is directed to engineering a scaffold that maximizes the properties of two distinct biomaterial types to create a successful biofunctionalized osteochondral scaffold. In our future work, we plan to assess the performance of these scaffolds in static and dynamic cell culture studies.

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