Hybrid Scaffold-reinforced Extracellular Matrix Hydrogels for Cartilage Tissue Engineering

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Statement of Purpose: Historically, cell transplantation therapies for cartilage regeneration have been limited by low retention and poor viability post-implantation. Delivery of cells in, or on, a scaffold has been demonstrated to improve both of these limitations. A number of hydrogels, including alginate, chitosan, and hyaluronic acid have been evaluated for cartilage regeneration, however, these materials do not recapitulate the native microenvironment and are not specific to the damaged tissue. Decellularized extracellular matrix (ECM) from a variety of tissue types has been shown to promote constructive remodeling and provide tissue-specific cues for progenitor cells [1]. Previously, acellular cartilage-derived materials have been used as three-dimensional scaffolds for traditional tissue engineering applications. In these proof-of-concept studies, we decellularize articular cartilage and process it into a novel hydrogel form that can be utilized in a variety of applications, including 3D-bioprinting in conjunction with synthetic polymers such as polycaprolactone (PCL). Given the tissue-specificity and bioactivity of the matrix, this scaffold may provide a superior template for chondrogenesis *in vitro* and improved cell retention and survival *in vivo*. By making hybrid materials with 3D printed plastics, these tissue engineering constructs can be better tailored for their intended applications.

Methods:

Cartilage Isolation and Decellularization: Cartilage was isolated from bovine hooves and stored in the freezer at -20 °C. Tissue was decellularized in hypotonic rinses over night. Penicillin/streptomycin was added to the decellularization solutions to retard bacterial growth. Decellularization was evaluated via DAPI and hematoxylin and eosin staining. Decellularized cartilage ECM was then lyophilized, milled into a fine powder, and partially digested with pepsin in hydrochloric acid.

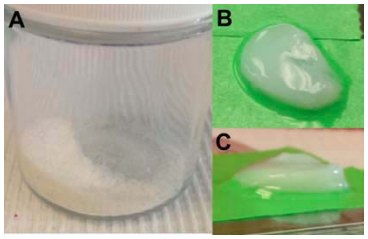

Figure 1. Lyophilized, milled ECM powder prior to pepsin digestion (A); cartilage ECM hydrogels after neutralization and incubation. (B and C)

ECM Hydrogel Preparation: ECM gels were prepared via partial pepsin digestion of the ECM in 0.1 M HCL, according to previously published protocols [2]. Briefly, the cartilage ECM was digested in the pepsin solution for approximately 48 hours, after which NaOH was added to the solution in order to bring the pH to 7.4. 10x and 1x PBS were then added to the solution to bring the solution to physiologic salt concentration. The ECM hydrogel was used at a concentration of 20.5 mg/mL.

Scaffold fabrication and seeding: In addition to the ECM-only scaffold, two different 3D bioprinted hybrid scaffolds were generated containing both PCL and ECM. Bioprinted scaffolds were fabricated according to previously published protocols [2]. The hybrid scaffolds were sterilized in 70% ethanol and then washed twice with sterile 1X PBS and then twice more with sterile alpha-MEM media. The scaffold was then seeded with human adipose derived stem cells (hASC). The scaffold was seeded with 350,000 cells and evaluated on day 3 with a live/dead cell imaging kit.

Results: Hypotonic rinses were found to be sufficient for decellularization; histologic stains of the decellularized tissue revealed no remaining nuclei. After milling, yield of decellularized ECM powder was approximately 10% of the original wet weight of isolated cartilage. When brought to physiologic conditions, the liquid ECM self-assembled into a hydrogel. The cartilage ECM hydrogel was able to be 3D-bioprinted alongside the PCL and cells attached and stayed viable in all constructs (Fig 2).

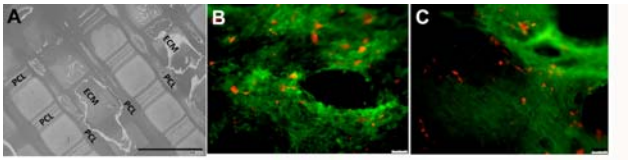

Figure 2. 3D bioprinted PCL and ECM, scale bar = 2 mm (A); Live (green) and dead (red) hASC cultured on cartilage ECM hydrogels either co-printed with PCL (B) or added after printing (C), scale bar = 100 μm.

Conclusions: With these techniques a novel hybrid natural and synthetic scaffold can be generated that harnesses the bioactivity of the cartilage ECM hydrogel and the tailorability and ease of translation that 3D printing affords. Using the gentlest decellularization technique should allow for the highest conservation of ECM content, as SDS has previously been shown to degrade ECM architecture and reduce protein content. The viability data suggest that hASC can be cultured on the hybrid materials and thus these novel scaffolds may provide promising constructs for use in tissue engineering applications, specifically for cartilage regeneration.

References:

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2. Seif-Naraghi SB. Tissue Engineering: Part A. 2010;16(6):2017-27
3. Shirwaiker R. Rapid Prototyping of Biomaterials: Principles and Applications. 2014;176-200