Introduction: Articular cartilage is composed of three zones: the superficial, the middle or transitional, and the deep zone. The chondrocytes occupying each zone demonstrate differences in mitotic and metabolic activity1 and provide unique contributions to the overall mechanical properties of the tissue.2 Photopolymerizable hydrogels are biomaterials that may be useful in designing tissue engineered constructs containing multiple layers of different cell types. This strategy may be used to create a cellular implant that mimics the native zonal organization of cartilage by encapsulating cells from the three regions in distinct layers. We hypothesize that the highly controllable photopolymerization reaction will be useful in designing multilayered hydrogels to create complex tissue structures including re-creation of the anatomic zones in articular cartilage.

Methods: Isolation of Chondrocytes from Bovine Articular Cartilage: Chondrocytes were harvested from the femoropatellar groove and medial and lateral condyles of 6-8 week old calves. The top 10%, central 20%, and lower 10% of the excised tissue was removed and digested to isolate cells from the superficial (S), middle (M), and deep (D) zones. Photopolymerization of Hydrogel-Chondrocyte Constructs: Poly(ethylene oxide) diacrylate (Shearwater Polymers) was dissolved in PBS to make a 10% w/v solution to which photoinitiator (0.05% Irgacure 2959) was added. Chondrocytes were resuspended in the polymer solution to make a final concentration of 20 million cells/mL. 100 µL of cell-polymer solution was placed in an 8mm cylindrical mold under a UVA lamp (~4 mW/cm2) for 5 minutes. The resulting polymerized gel was removed from the mold, placed in complete media and incubated for 2 weeks.

Preparation of Multi-Layered Constructs: To create multilayered constructs, 50-75 µL of the polymer solution containing one cell type was allowed to polymerize under the UVA lamp for 3 minutes (such that the solution only partially gelled), then 50-75 µL of polymer solution with a second cell type was added and exposed to UV light. In some constructs, a third layer was added. In all cases, once the final layer of polymer solution was added, the sample was placed under UV light for 5 minutes to ensure complete polymerization of all layers. Construct thickness ranged from 4-8 mm depending on how many layers were created.

Histology: Constructs were fixed in formalin at 4°C for 2 hours and prepared using standard histological technique. Sections were stained with H&E and Safranin-O.

Biochemical Analysis: Constructs were lyophilized for 48 h, homogenized and digested in 1mL of papainase for 16h at 60°C. The glycosaminoglycan (GAG) content was estimated by chondroitin sulfate determined using the dimethylmethylene blue dye method. Total collagen content was determined by quantifying the hydroxyproline content. DNA was measured using Hoechst 33258. Results are reported as means and standard deviations after values were normalized to the DNA content. Statistical analysis of these results was performed using ANOVA with post-hoc test and significance was set with a p < 0.05.

Results: Figure 1A provides a macroscopic view of a multilayered construct with the superficial layer stained blue. A demonstration of technique, PLGA microspheres were photocapsulated in the inferior layer of a bi-laminated construct. A distinct interface between the two layers was observed while adhesion of the layers was maintained (Fig. 1B). Chondrocytes from the superficial, middle, and deep layers of cartilage (constructs S,M,D) maintained differences in phenotype when photocapsulated in the hydrogel as demonstrated by the biochemical data (Fig. 2). Photocapsulated chondrocytes from the deep layer synthesized 86% more collagen and 50% more GAG than constructs containing superficial chondrocytes. Multilayered constructs were polymerized using S/D, S/M, and S/M/D chondrocytes to create bi- and tri-layered constructs. Cells survived photocapsulation and remained viable in tri-layered constructs that were approximately 8mm thick. The high magnification image in Fig. 1D demonstrates the cell survival and integration at the interface of the layers. Variable matrix staining in the different hydrogel layers (Fig.1C,D) suggests the cells maintain their preexisting discrete metabolic rates. The bi-layered constructs, S/M and S/D, produced collagen levels similar to encapsulated S cells alone. GAG levels of the S/M constructs are similar to those of S and M alone, and the S/D constructs mimic the higher production levels observed in the D constructs (Fig. 2).

Discussion: Photopolymerizable hydrogels are attractive scaffolds for tissue engineering applications since temporal and spatial control of the polymerization and gelation process is readily achieved. This control over gelation allows creation of more complex structures including multilayered hydrogels. This study presents a novel method for the photocapsulation of chondrocytes from the three zones of articular cartilage such that the regional cellular variation found in the native tissue is re-created. The polymerization conditions and hydrogel properties support cell viability and matrix synthesis, as indicated by histological and biochemical analysis. Tissue construct thickness resembling that of native cartilage was achieved without compromising cell viability or matrix production. The differences in matrix synthesis of the chondrocytes from the different zones are consistent with the current literature1,2. By reproducing the anatomical and functional characteristics of native cartilage, multi-layered composite scaffolds may improve the mechanical properties and integration of tissue engineered cartilage constructs. Furthermore, this study has important implications in the design of composite scaffolds to engineer complex tissue architectures such as an osteochondral interface.

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