Devitalized Cell Derived Polymer/Extracellular Matrix Composite Scaffolds for Cartilage Regeneration

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Statement of Purpose: It has been shown that the extracellular matrix (ECM) components, collagen and glycosaminoglycans (GAGs), which are prevalent in native cartilage are useful in inducing and directing chondrogenesis of mesenchymal stem cells (MSCs). Most commonly chondrocytes are used to produce cell derived cartilage-like ECM within a polymer scaffold in tissue engineering efforts[1]. However, chondrocytes are difficult to obtain, expand, and maintain in culture while retaining the chondrogenic phenotype making the fabrication of cell derived polymer/ECM composite scaffolds less clinically feasible. This study investigates the use of co-cultures of chondrocytes and MSCs on fibrous polymer scaffolds to produce polymer/ECM composites with the objective of reducing the number of chondrocytes needed while still producing ample cartilage-like ECM such that acellular polymer/ECM composite scaffolds might be chondroinductive upon implantation. To accomplish this, the affect cell type ratio had on the deposition of cartilage-like ECM *in vitro* was examined. Also, the efficacy of a freeze thaw method of devitalization was investigated and the composition of the acellular scaffolds was evaluated.

Methods: Co-cultures of bovine chondrocytes and rabbit mesenchymal stem cells (MSCs) were seeded on electrospun poly(ε-caprolactone) (PCL) fibrous scaffolds (Ø= 3mm, 1 mm thick) at a density of 35,000 total cells/scaffold in three different ratios of chondrocytes to MSCs: 1:1, 1:3, and 1:5 with 1:0 and 0:1 seeded scaffolds as controls. These cellular constructs were cultured in serum containing medium without growth factors for durations of 7, 14, and 21 days. At the end of the three culture periods, constructs were either rinsed with PBS or devitalized in sterile water using three 10 minute freeze thaw cycles followed by 10 minutes of sonication generating cellular and acellular scaffolds respectively. Collagen and GAG content of the scaffolds was measured to determine if cell-cell interactions between MSCs and chondrocytes had an impact on cartilage-like ECM development as well as determine the impact of devitalization on the scaffold composition. To assess the efficacy of the devitalization process, cellular and acellular polymer/ECM hybrid scaffolds were analyzed using the picogreen assay for DNA content.

Results: Cellular and acellular polymer/ECM hybrid scaffolds were successfully produced by co-culturing various formulations of chondrocytes and MSCs on electrospun PCL scaffolds. Only cellular scaffolds cultured with 1:0 or 1:1 chondrocytes to MSCs led to significantly more collagen or GAG deposition than scaffolds produced by co-culture ratios with lower concentrations of chondrocytes (Figure 1, "$"). However, all four cellular day 21 PCL/ECM scaffolds formed with chondrocyte containing cultures resulted in significantly greater amounts of collagen and GAGs than MSC generated controls (Figure 1, "♦"). Furthermore, there was

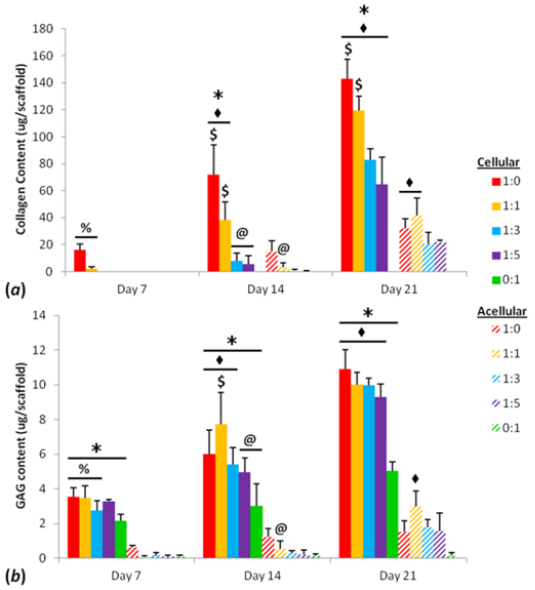

Fig. 1: Collagen and GAG contents of cellular and acellular PCL/ECM scaffolds.

no significant difference in collagen content among decellularized scaffolds formed by chondrocyte containing cultures and MSCs alone generated minimal amounts of collagen. With respect to culture duration, by increasing chondrocyte concentration significant increases in ECM deposition occur sooner (Figure 1, "%" indicates a significant increase in ECM content over all three culture durations, "@" indicates a significant increase in ECM content after day 14). The only decellularized PCL/ECM scaffolds to exhibit a significant temporal change in collagen and GAG content were the 1:1 co-culture generated scaffolds. Devitalization processing successfully removed most cell material as determined by DNA content. The freeze thaw process also resulted in a significant decrease in GAG content for all co-culture ratios at all three ECM maturities when compared to the corresponding cellular co-culture ratio (Figure 1, "✱"). The devitalization process also had an adverse effect on collagen content leading to significant losses for all four day 21 PCL/ECM scaffolds generated with chondrocyte containing cultures (Figure 1, "✱").

Conclusions: Co-culturing MSCs with chondrocytes is a viable option for producing cartilage-like ECM within a fibrous polymer scaffold. The inclusion of MSCs in co-cultures enables the reduction of chondrocytes needed for ECM production. Devitalization by freeze thaw is successful at removing cellular matter from the scaffolds yet does reduce the amount of matrix present in the scaffold. Further work should be performed to improve the devitalization process and to assess the ability of the devitalized polymer/ECM hybrid scaffolds to direct chondrogenesis of MSCs.

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References: Liao J.et al. *Biomat.* 2010; 31: 8911–8920.