ABSTRACT

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Title: Collagen Type I and II Blend Hydrogels for Articular Cartilage Tissue Engineering
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Osteoarthritis (OA) is a debilitating condition that affects over 27 million Americans and is defined by degradation in articular cartilage extracellular matrix. Patients suffer from pain and stiffness in the joints associated with the onset of OA. Tissue that is damaged by OA is a major health concern since cartilage tissue has a limited ability to self-repair due to the lack of vasculature in cartilage and low cell content. Tissue engineering seeks to repair damaged cartilage by introducing an optimized combination of cells, scaffold, and bioactive factors that can be transplanted into a patient.

Collagen type II is a promising material to repair cartilage defects since it is a major component of articular cartilage and plays a key role in chondrocyte function. This work harnesses the biological activity of collagen type II and the superior mechanical properties of collagen type I by blending together the two types in different ratios of collagen type I to collagen type II (1:0, 3:1, 1:1, 1:3, and 0:1). The collagen blend hydrogels were able to incorporate both types of collagen, chondroitin sulfate (CS), and hyaluronic acid. Cryo-scanning electron microscopy images showed that the 3:1 ratio of collagen type I to type II gels had a lower void space percentage (36.4%) than the 1:1 gels (46.5%). Of the blends created, the complex modulus was larger for the 3:1 gels ($G^* = 5.0$ Pa) compared to the 1:1 gels ($G^* = 1.2$ Pa). The 3:1 blend consistently formed gels with superior mechanical properties compared to the other blends and showed the potential to be implemented as a scaffold for articular cartilage engineering.

Building on the characterization work, this study examined the chondrogenic differentiation of bone marrow-derived mesenchymal stem cells (MSCs) embedded within a 3:1 collagen type I to II blend (Col I/II) hydrogel or an all collagen type I (Col I) hydrogel. Glycosaminoglycan (GAG) production in Col I/II hydrogels was statistically higher than in Col I hydrogels or pellet culture, and these results suggested that adding collagen type II promoted GAG production. Col I/II hydrogels had statistically lower alkaline phosphatase (AP) activity than pellets cultured in

chondrogenic medium. The ability of MSCs encapsulated in Col I/II hydrogels to repair cartilage defects was investigated by creating two defects in the femurs of rabbits. After 13 weeks, histochemical staining suggested that Col I/II blend hydrogels provided favorable conditions for cartilage repair. Histological scoring revealed a statistically higher cartilage repair score for the Col I/II hydrogels compared to either the Col I hydrogels or empty defect controls. Results from this study suggest that there is clinical value in the cartilage repair capabilities of our Col I/II hydrogel with encapsulated MSCs.

There are many examples of collagen hydrogels with incorporated CS where the addition of CS has been shown to enhance the ability of MSCs to differentiation into chondrocytes. Our final study investigated the use of CS with attached collagen binding peptides (referred to as SILY) to retain, without the use of chemical crosslinking, matrix molecules in Col I/II hydrogel. It was hypothesized that the addition of CS and SILY peptides to a Col I/II hydrogel with encapsulated MSCs would better recapitulate aspects of native cartilage. The number of SILY peptides attached to a CS backbone was varied to create 3 different molecules: CS-10SILY, CS-15SILY, and CS-20SILY, with 10, 15, and 20 denoting the number of SILY peptides attached to CS. As CS retention, average fibril diameter, and mechanical properties are altered by the addition of different CS-SILY molecules, the physical properties of the desired Col I/II hydrogel can be tuned by adjusting the amount of SILY peptides attached to the CS backbone. The addition of the collagen peptide, in the form of the CS-15SILY or CS-20SILY, allowed for over 20% of the original amount of CS to be retained after 7 days where as less than 1% of the free CS remained in the same time period. When a CS-SILY molecule was added, we saw a trend of increasing G' when the number of SILY peptides on the CS backbone was increased. In addition, the scaffolds that contained CS-10SILY, CS-15SILY, and CS-20SILY had higher GAG production as compared to scaffolds with or without added CS, and this result suggests better differentiation of MSCs into chondrocytes in scaffolds that contain a CS-SILY molecule. Taken together, these results suggested that the addition of a CS-SILY molecule to a Col I/II hydrogel with encapsulated MSCs has the potential to promote cartilage repair.