Promoting Chondrogenesis and Maintaining the Bioactivity of TGF-β3 using a Biomimetic Material

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Introduction: Articular cartilage has a limited intrinsic ability to heal and thus, remains a persistent problem for the orthopedist and patient. Current surgical procedures to repair cartilage result in poor integration with surrounding hyaline cartilage and the formation of fibrocartilage instead of normal hyaline cartilage. The presence of fibrocartilage suggests that there may be deficient bioactivity to promote the chondrocyte phenotype. This tissue engineering approach uses cells capable of chondrogenesis and promotes their differentiation with a glycosaminoglycan mimetic. Glycosaminoglycans (GAGs) have been shown to interact and maintain the bioactivity of growth factors due to their level and spatial distribution of sulfate groups [1]. Transforming growth factor-β (TGF-β) is a commonly used growth factor used for mesenchymal stem cell chondrogenesis. Of the two isoforms most investigated, TGF-β3 has been shown to have a higher chondrogenic potential than TGF-β1 [2]. Sodium cellulose sulfate (NaCS), which is a semi-synthetic derivative of cellulose, is a sulfated polysaccharide with structural similarity to the GAGs found in the cartilage ECM and has yet to be explored for cartilage repair. This study evaluated the effect of NaCS on mesenchymal stem cell chondrogenesis and its effect on maintaining the bioactivity of TGF-β in comparison to other sulfated GAGs.

Methods: Effect of NaCS on Chondrogenesis: Human mesenchymal stem cells (hMSCs) derived from adult bone marrow were grown in pellet cultures at 200,000 cells per pellet over a 28-day period in standard growth media (GM: DMEM, 10% fetal bovine serum, 1% antibiotic) and chondrogenic induction media containing 0.01 µg/mL TGF-β3 (CCM). 1% and 0.01% of NaCS was added to both GM and CCM medium. All samples were evaluated histologically and by gene expression for aggrecan and collagen type II. TGF-β Activity: The growth factor TGF-β3 was prepared with 0.1% and 0.01% of the sulfated GAGs: NaCS, chondroitin sulfate (CSC), partially sulfated cellulose (pSC), heparan sulfate (HS), and carrageenan (CG). The amount of TGF-β3 used for this study was 10 ng/mL, which is the same amount used in chondrogenic induction media. TGF-β3 DuoSet ELISA (R&D Systems) was used to measure TGF-β3 that is still in its active form after 2, 4, and 7 days in solution at 37°C. Fabrication of NaCS/gelatin scaffolds and Cell Study: Bovine gelatin was mixed with NaCS followed by the addition of the crosslinker, diisosorbide bisepoxide. This solution was then electrospun to create a fibrous mat. Chondrogenic differentiation of hMSCs cultured on 5% NaCS/gelatin mats was evaluated by gene expression and cell morphology using F-actin and immunostaining for type II collagen. Protein Interaction on Scaffolds: The scaffolds made of gelatin and 5% NaCS/Gelatin were immersed in solutions of lysozyme (200 U/mL), a model protein for growth factors, for a period of seven days at 37°C. The lysozyme assay was used to determine the amount of active lysozyme in solution and on the scaffold. Scaffolds were immersed in a solution of lysozyme that was rhodamine-labeled (NHS-Rhodamine Antibody Labeling Kit, Fisher Scientific) allowing the lysozyme to fluoresce in red. After two days at 37°C, the scaffolds were rinsed with PBS and lysozyme distribution was observed with confocal microscopy. Statistical Analysis: All statistical analyses were performed using multi-factorial analysis of variance (ANOVA) and post-hoc Tukey test for statistical differences at p<0.05 (SPSS Statistics Version 21, Student Version).

Results: Effect of NaCS on Chondrogenesis: After 28 days, cultures containing 0.01% NaCS displayed a more uniform chondrocyte morphology and production of cartilage matrix (Figure 1) as compared to standard pellet cultures. Cells in NaCS also expressed significantly higher collagen type 2 and aggrecan genes as compared to control cultures without NaCS (Figure 1). TGF-β3 Activity: After 7 days, both concentrations of NaCS had significantly higher amounts of TGF-β3 than all the other sulfated polysaccharides or PBS alone (Figure 2). NaCS/gelatin scaffolds and Cell Study: On NaCS scaffolds, cells produced collagen type II and had round chondrocyte morphology in both general media and chondrogenic media (Figure 3). Early expression of chondrogenic genes was seen on NaCS scaffolds in general media (Figure 3). Protein Interaction on Scaffolds: There was a higher percentage of active lysozyme found on the scaffold of 5% NaCS/Gelatin than in solution. While on the gelatin scaffold, there was more active lysozyme in solution, not the scaffold (Figure 4). The imaging of rhodamine-labeled lysozyme confirmed the greater presence of lysozyme on 5% NaCS/gelatin than gelatin alone scaffolds.

Discussion: NaCS added to pellet culture or used as a scaffold promoted chondrogenic differentiation. NaCS maintained the bioactivity of the growth factor, TGF-β3, that can promote chondrogenesis. The use of TGF-β3 for human MSC chondrogenesis has been reported to have a greater effect over other TGF-β isoforms [2]. Our studies demonstrated that TGF-β3 maintains its bioactivity with NaCS significantly better than chondroitin sulfate, the naturally occurring GAG and a material widely investigated for cartilage repair applications. NaCS also performed better than heparan sulfate, a GAG known for its growth factor binding capabilities. Studies have reported that heparin and heparan sulfate will bind to TGF-β1 and TGF-β2, but not TGF-β3.
β3 [3]. The combination of TGF-β3 and NaCS could contribute to the enhanced chondrogenesis seen in the pellet culture and scaffold environment. Furthermore, a scaffold fabricated with NaCS is able to complex with the model protein, which suggests it may have a similar effect on the growth factor. This study demonstrated the feasibility of NaCS as a potential scaffolding material for cartilage tissue engineering.

Significance: There is an unmet need for a treatment that can offer long-term functional repair to cartilage injury, especially in an emerging younger population. This study demonstrates the feasibility of NaCS as a scaffolding material for cartilage tissue engineering by providing aspects of the cartilage environment in order to promote human mesenchymal stem cell chondrogenesis and maintain growth factor bioactivity.

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Figure 3. Confocal images of hMSCs on crosslinked 5% NaCS/gelatin in [A] GM and [B] CCM after 28 days, F-actin red, nucleus blue, and collagen type II green. 20x objective, scale bar 100 um. [C] Gene expression for hMSCs on 5% NaCS/gelatin scaffolds in general media. * p< 0.05 between gelatin and NaCS/gelatin.

Figure 4. Percent of active lysozyme at day 2, 4, and 7 for gelatin [A] and 5% NaCS/gelatin [B]. *p<0.05 significant difference between scaffold and solution.