Promoting Chondrogenesis and Maintaining the Bioactivity of Proteins using a Biomimetic Material

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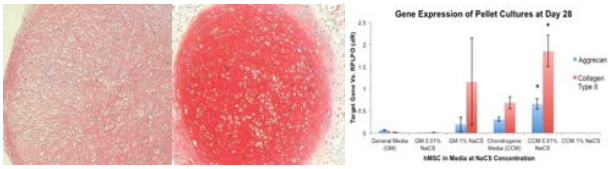
Introduction: Articular cartilage has a limited intrinsic ability to heal and thus, remains a persistent problem for the orthopedist and patient. Current surgical procedures to repair cartilage result in poor integration with surrounding hyaline cartilage and the formation of fibrocartilage instead of 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]. 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 proteins 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. *Model Protein Activity in Solution:* Lysozyme was used as a model protein to represent growth factors such as TGF-β3. Lysozyme was placed in solution with 0.1% and 0.01% of a sulfated polysaccharide: sodium cellulose sulfate (NaCS), sulfated dextran (SD), partially sulfated dextran, and chondroitin sulfate (CSC). A lysozyme activity assay (Invitrogen) was used to determine the amount of lysozyme activity present after 2 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. *Protein Interaction on Scaffolds:* The scaffolds made of gelatin and 5% NaCS/Gelatin were immersed in solutions of lysozyme 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.

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). *Model Protein Activity in Solution:* After two days, all of the solutions containing a sulfated polysaccharide maintained lysozyme activity over the solution without a sulfated polysaccharide or GAG. *NaCS/gelatin scaffolds and Cell Study:* On NaCS scaffolds, cells produced collagen type II and had round chondrocyte morphology in general media and chondrogenic 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.



A B C

Figure 1. Safranin-O stain of pellet culture at day 28 in CCM [A] and CCM 0.01% NaCS [B]. Gene expression for pellet culture at day 28 [C]. * p< 0.05 between CCM 0.01% NaCS and CCM.

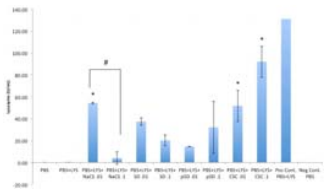


Figure 2. Lysozyme activity in solution. *p<0.05 significantly greater than lysozyme without GAG. #p<0.05 significant difference between concentrations.

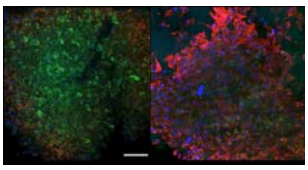


Figure 3. Confocal images of hMSCs on crosslinked 5% NaCS/gelatin in [left] GM and [right] CCM after 28 days, F-actin red, nucleus blue, and collagen type II green. 20x objective, scale bar 100 um.

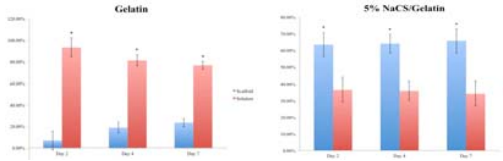


Figure 4. Percent of active lysozyme at day 2, 4, and 7. *p<0.05 significant difference between scaffold and solution

Conclusions: NaCS in pellet culture or scaffold environment promoted chondrogenic differentiation. NaCS maintained the bioactivity of the model protein, which suggests it may have a similar effect on growth factors that can promote chondrogenesis. This study demonstrated the feasibility of NaCS as a potential scaffolding material for cartilage tissue engineering.

References: 1. Gama, C.L. et al, *Nat Chem Biol*, 2006.