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

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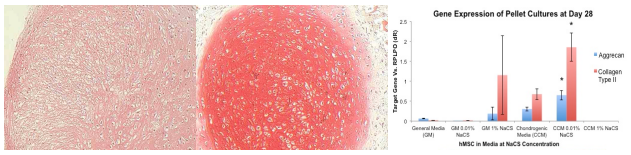
Introduction: Articular cartilage has a limited ability to heal and thus, remains a problem for the orthopedist and patient. Current surgical procedures to repair cartilage result in poor integration with surrounding tissue and formation of fibrocartilage instead of hyaline cartilage. The presence of fibrocartilage may suggest 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-βeta (TGF-β) is a commonly used growth factor for mesenchymal stem cell (MSC) 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), a semi-synthetic derivative of cellulose, is a sulfated polysaccharide with structural similarity to the GAGs found in the cartilage extracellular matrix and has yet to be explored for cartilage repair. This study evaluated the effect of NaCS on MSC chondrogenesis and its effect on maintaining the bioactivity of TGF-β3 in comparison to other sulfated GAGs.

Methods: *NaCS on Chondrogenesis:* Human MSCs derived from adult bone marrow were grown in pellet cultures at 200,000 cells/pellet over a 28-day period in 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. All samples were evaluated histologically and by gene expression for aggrecan and collagen type II. *TGF-β3 Activity:* Solutions of TGF-β3 were 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). 10 ng/ml of TGF-β3 was used for all solutions, the same concentration used in CCM. The amount of TGF-β3 still in its active form was measured after 2, 4, and 7 days 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 NaCS/gelatin mats of varying NaCS concentration (0%, 0.1%, 1%, and 5%) was evaluated by gene expression, cell morphology, and collagen type II production.

Results: *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). Cells in GM on NaCS scaffolds had early expression of chondrogenic genes and produced more collagen type II over time than gelatin alone (Figure 4). In CCM, cells on 0.1% NaCS scaffolds produced the most collagen type II over all other groups at all time points (Figure 4).



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.

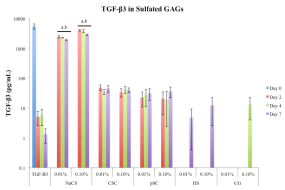


Figure 2. TGF-B3 in solution over 7 days, where Day 0 is TGF-B3 activity immediately after adding PBS. a: significantly different from all groups at all time points, p<0.05. b: Day 7 significantly less than other time points, p<0.05.

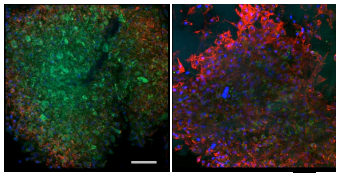
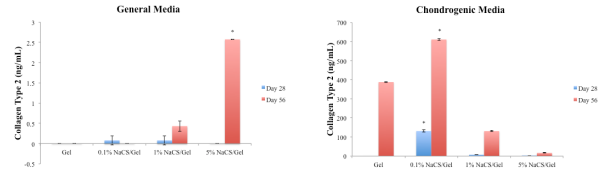


Figure 3. Confocal images of hMSCs on 5% NaCS/gelatin in [left] GM and [right] CCM after 28 days, F-actin red, nucleus blue, and collagen type II green. 20x objective, scale bar 100 um.



A B

Figure 4. Type II collagen production by hMSCs on scaffolds in [A] GM and [B] CCM after 56 days.

Conclusions: NaCS in pellet culture or scaffold environment promoted chondrogenic differentiation. 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. The combination of TGF-β3 and NaCS could contribute to the enhanced chondrogenesis seen in the pellet culture and scaffold environment. This study shows there may exist an optimal NaCS concentration that enhances production of ECM molecules. 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. [2] Barry, F. et al, *Exp Cell Res*, 2001.