Glycosaminoglycan-mimetics Promote Mesenchymal Stem Cell Chondrogenesis and Affect Metabolic Activity

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Introduction: Osteoarthritis (OA) is a degenerative joint disease that results in the loss of articular cartilage and normal joint function. After damage, articular cartilage has a limited capacity to heal. Current therapies only alleviate symptomatic pain, and surgical interventions do not restore normal hyaline cartilage. Tissue engineering and the use of biomaterial scaffolds may be utilized to facilitate the growth of cells and production of functional, hyaline cartilage. Glycosaminoglycans (GAGs) are polysaccharides found in the native extracellular matrix and during early cartilage development. GAGs play a mechanical role in the native ECM and can also facilitate the growth of cartilage as they sequester growth factors and may affect cellular behaviors including growth, migration and differentiation [1,2]. In this study, GAG mimetics were derived from starch and dextran and their effect on mesenchymal stem cell (MSC) chondrogenesis was investigated. Starch-based materials have been investigated for several biomedical applications and are biocompatible. Dextran sulfate (DS) is generally recognized as safe (GRAS) by the FDA and dextran products have been used clinically in cosmetic applications, blood clotting factors, and pharmaceutical applications. In addition, cells may uptake starch and dextran and enzymatically degrade these materials where their byproducts may be a source of glucose. The findings of this study demonstrate that MSCs undergo chondrogenesis, producing significantly more collagen type II in culture with starch sulfate (SS) as compared to cultures without GAG mimetics and DS, demonstrating SS may promote chondrogenesis. In addition, in low glucose conditions, both GAG-mimetics promote the viability/metabolic activity of cells, as indicated by higher ATP levels as compared to controls.

Methods: Starch sulfate (SS) was synthesized by suspending starch (Sigma Aldrich) in pyridine and refluxed over pyridine-sulfurtrioxide. Products were then neutralized and purified. Dextran sulfate (DS) was purchased from Dextran Products Limited. Human mesenchymal stem cells (MSCs) were evaluated in pellet cultures in media consisting of 0.01 w/v% of GAG-mimetic added to chondrogenic induction media (CCM+), comprised of high glucose (HG – 4.5 g/L) DMEM, sodium pyruvate, proline, glutamine, antibiotic-antimycotic, ITS-Premix, and 10 ng/mL transforming growth factor beta3 (TGF-β3) and evaluated up to day 28. To evaluate if the GAG-mimetics are being utilized as glucose source, pellet cultures were also evaluated in chondrogenic induction using low glucose (LG - 1.5 g/L) DMEM. Cell number was determined via the Quant-iT dsDNA Pico Green assay kit (Invitrogen) and total GAG content was determined using the DMMB assay (N=4 per group, per time point) [1,2]. Intracellular ATP (CellTiter-Glo Luminescent Cell Viability Assay, Promega) and glucose production (EnzyChrom Glucose Assay Kit, BioAssay Systems) was evaluated in pellets for up to day 28 (N=4 per group, per time point). The production of collagen types I and II were quantified using enzyme linked immunosorbent assays (N=5 per group)(ELISA, Chondrex, USA). At day 28, pellets were processed for paraffin-embedded histology, stained for H&E and safranin-O (SafO) (N=2 per group). One-way and two-way analysis of variance (ANOVA) was used to determine significant differences between groups and time. Multiple comparisons within groups were made using Tukey's posthoc test ($p<0.05$). Statistical analysis was performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA).

Results: Significantly higher cell numbers were detected at 28 days in HG as compared to LG supplemented media for both GAG mimetic groups. Although a significant increase in cell number occurred between days 21 and 28 in cells supplemented with HG media, cell numbers in LG supplemented media were maintained over time (Figure 1A). The overall GAG content for the DS group was significantly higher than the CCM+ control in HG conditions (Figure 1B). Although there was a significant decrease in intracellular glucose in pellets in LG media in comparison to those in HG media (Figure 1C), a significantly higher intracellular ATP was detected for cells in DS and SS cultures in LG media as compared to cells without GAG-mimetics (CCM+ only) in HG media (Figure 1D). Cells produced significantly higher amounts of collagen type II for the SS group as compared to CCM+ control and DS group in HG media (Figure 2). Histology also showed that the SS group in HG media appeared to have more proteoglycan, as shown by the SafO stain (Figure 3).

Discussion: The increase in collagen type II and GAG content in pellet cultures exposed to SS containing media suggests an increase in ECM production and promotion of MSCs towards a chondrocytic phenotype. The combination of high expression of collagen type II and low expression of collagen type I may demonstrate the potential to form a more homogeneous hyaline cartilage, which is mechanically more favorable compared to fibrocartilage, which contains collagen type I. This study reinforces the necessity of glucose for cartilage production, where ECM production decreased in LG groups. SS and DS may help to maintain metabolic activity, as shown by a higher amount of ATP production in a low glucose environment. The low protein production, maintenance of cell number, and low intracellular glucose may suggest that the cells are entering a quiescent state, maintaining its survival without undergoing metabolically intensive differentiation (3). Future considerations will include further analysis of differentiation by gene expression, growth factor interactions and incorporating the GAG mimetics into fibrous scaffolds to explore a variety of cartilage tissue engineering applications.

Significance: Tissue regeneration approaches that can rebuild lost hyaline cartilage are needed. ECM mimetics could be used to enhance repair and joint function as well as provide further insight into how the ECM mechanistically contributes to cartilage development, repair and homeostasis.

References:

(1.) Menezes R, Tissue Eng, 2017, 23;1011-1021. (2.) Huang, GP, JTERM, 2018, 12; e592-e60. (3.) Valcourt JR. *Cell Cycle*. 2012;11(9):1680-1696.

Images and Tables:

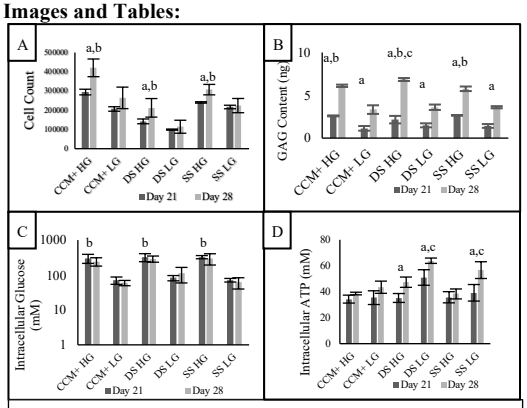

Figure 1. MSC pellets cultured in CCM+ with high glucose (HG) or low glucose (LG) media containing Gag-mimetics – DS or SS. (A) cell count, (B) overall GAG content, (C) intracellular glucose, and (D) intracellular ATP. [a.] Statistically different over time ($p<0.05$). [b.] Statistically higher than LG group ($p<0.05$). [c.] Statistically higher than CCM+HG and LG groups ($p<0.05$).

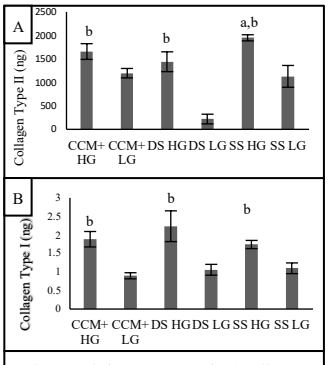

Figure 2 (left). Amounts of (A) collagen type II and (B) collagen type I for MSC pellets supplemented with CCM+ and GAG mimetics in HG and LG media conditions at 28 days. [a.] Significantly greater than all other groups ($p<0.05$). [b.] Significantly greater than LG groups ($p<0.05$)

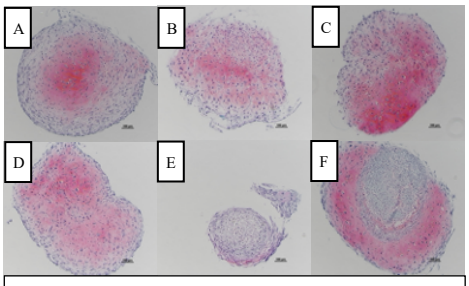

Figure 3. SafO stained histology images of MSC pellets cultured with (A) HG media only, (B) HG media + DS, (C) HG media + SS, (D) LG media only, (E) LG media + DS, (F) LG media + SS at day 28.