Regulatory effect of GAG-mimetics on Human Osteoarthritic Chondrocytes

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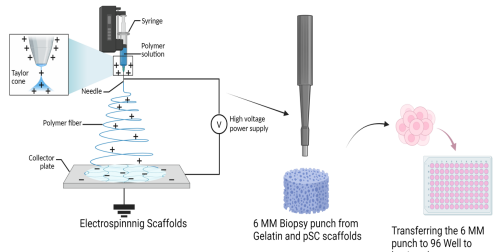
Introduction

Osteoarthritis OA is a degenerative joint disease characterized by the progressive breakdown of articular cartilage. As OA progresses, it causes pain, stiffness, and loss of joint function, affecting 528 million people, or approximately 7% of the global population [1]. Due to the limited regenerative ability of articular cartilage, damage to this tissue can result in long-term functional impairment and pain. Current treatments for OA primarily focus on mitigating pain symptoms, and there is no drug capable of effectively halting the progression of the disease [2]. Tissue engineering is a biomedical engineering discipline that creates biological substitutes to replace diseased or damaged human tissue e.g. cartilage tissue. Glycosaminoglycans (GAGs) are the main component in the cartilage tissue, essential for promoting cartilage regeneration by providing proper cues for cells to grow and differentiate [3]. Herein, we used sulfated glycosaminoglycans, a type of polysaccharide with sulfate groups, that mimics the cartilage extracellular matrix (ECM) and aids in inducing cellular differentiation towards chondrogenesis. Electrospinning technique was used to produce fibrous scaffolds that provides physical cues to enhance cell attachment and proliferation for effective tissue repair. The electrospun scaffolds were seeded with activated chondrocytes and incubated for 20 days. This work aimed to study the impact of GAG mimetics on cellular behavior under proinflammatory conditions.

Methods

Scaffolds were prepared using previously described methods [4]. Briefly, scaffolds were fabricated using 24% (w/w) bovine gelatin (Sigma) in 63/37 acetic acid/water solutions and 5% (w/w) partially sulfated cellulose (pSC, prepared as published), which is a glycosaminoglycan (GAG)-mimetic. The scaffolds were crosslinked using 200 mM 1-3 ethylcarbodiimide hydrochloride (EDC, Sigma) and 40 mM N-hydroxysuccinimide (NHS, Sigma) to enhance hydrolytic stability and retain fibrous structures in aqueous environments as previously established. Additional materials included ethanol (200 proof, Sigma), phosphate-buffered saline (PBS, Fisher Scientific), Dulbecco's Modified Eagle's Medium (DMEM, Fisher Scientific), fetal bovine serum (FBS, Hyclone), antibiotic-antimycotic (Fisher Scientific), trypsin (Fisher Scientific), poly-L-lysine (Sigma), paraformaldehyde (Sigma), Triton X-100 (Sigma), bovine serum albumin (BSA, Fisher Scientific), DAPI (Fisher Scientific), Quant-iT PicoGreen dsDNA Assay Kit (Fisher Scientific), and papain (Sigma). TGF-β1 & IL-1B were added to CCM media creating CCM+ media. Using live/dead cytotoxicity test, immunostaining, dsDNA assay procedure (PicoGreen), and MMP-13 kit, statistical significance was confirmed with a two-way ANOVA and Tukey's post-hoc tests.



Results

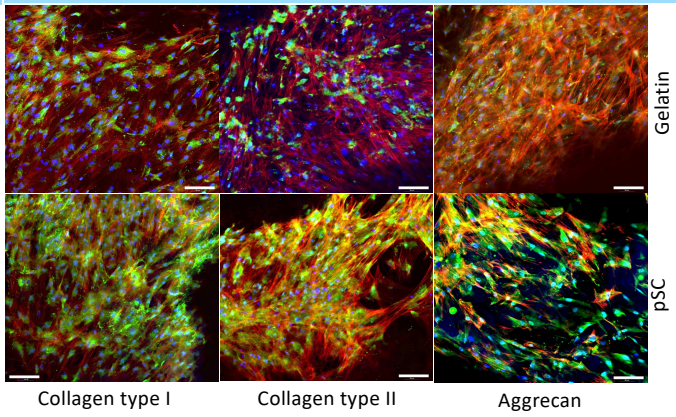


Figure 1. Immunofluorescence study of the extracellular matrix produced by activated OA chondrocytes, seeded on sulfated GAGs for 20 days. Scaffolds were treated with serum free culture media supplemented with IL-1β (10 ng/ml) and TGF-beta3 (10 ng/ml).

Immunofluorescence analysis included specific antibodies against aggrecan, type I collagen, and type II collagen (green). Phalloidein was used to stain the cellular actin filaments (red), Hoechst was used to stain nuclei in Blue. Scale Bar is 100 µm

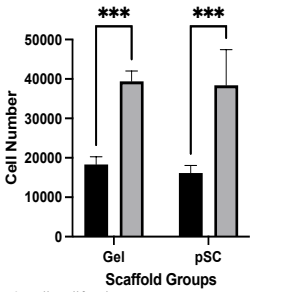


Figure 2. Cell proliferation assay. The Picogreen DNA content assay was performed on cultures of OA chondrocytes seeded on gelatin / pSC scaffolds at 6, 14 days of culture, in serum free culture and in the presence of IL-1β (10 ng/ml) and TGF-beta3 (10 ng/ml). Results were presented as mean ± SEM. A significant difference between Day 6 and Day 14 in each group was observed (***p < 0.05)

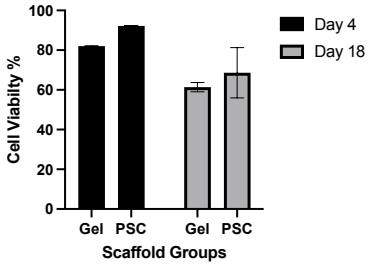


Figure 3. Live/Dead assay on cultures of OA chondrocytes seeded on gelatin / pSC scaffolds at 4, 18 days of culture, in serum free cultre and in the presence of IL-1β (10 ng/ml) and TGF-beta3 (10 ng/ml). Scaffolds exhibited high overall cell viability rate from ~ 80 % after 4 days to ~ 70% at 18 days of culture. Cell viability decreased overtime with no significance.

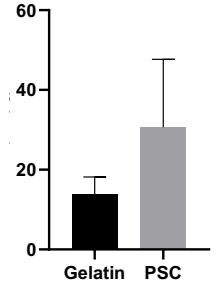


Figure 4. MMP-13 production by OA chondrocytes seeded on pSC, or gelatin, seeded scaffolds were treated with serum free culture media supplemented with IL-1β (10 ng/ml) and TGF-beta3 (10 ng/ml). Results were presented as mean ± SEM.

Discussion & Future directions

Sulfated-GAGs scaffolds demonstrated a proper microenvironment for chondrocytes to proliferate even in the presence of Pro-inflammatory cytokine (IL-1β). This was approved with Pico staining where the number of cells was increasing. This was featured with high viability rate (over 80% after 4 days, and 70 % after 8 days). OA chondrocytes were able to deposit major structural glycoproteins found in the cartilage tissue such as aggrecan as confirmed with immunostaining. Besides, cells were able to maintain their phenotype as approved with collagen type I, II staining. In addition, cells were producing MMP-13, an enzyme typically associated with OA chondrocytes. Enzymatic activity suggested that sulfated GAGs were probably able to create favorable environment to sequester exogenous IL-1β, thus, triggered pro inflammatory markers such as MMP-13. Overall, this study highlighted the importance of sulfated GAGs on maintaining cellular phenotype, and overall cellular behavior throughout the study period. In turn, this might impact cartilage repair in the presence of GAG mimetics in a further step. Next work would include sequestration studies with different growth factors and cytokines that might be present during the cartilage regeneration, more specifically IL-1β.

References

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