Investigating Sequestration of TGF-β3 on GAG Mimetic-Containing Scaffolds for Cartilage Repair

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Introduction

Osteoarthritis (OA) affects approximately 7% of the global population (528 million people) [1]. It results in the damage and breakdown of hyaline cartilage between joints. Articular cartilage has limited ability to self-repair and irreversible damage leads to OA. Current treatments for OA result in poor integration with the surrounding tissue and formation of fibrocartilage instead of hyaline cartilage, indicating an insufficient amount of bioactivity to promote chondrogenesis [2].

Transforming growth factor-beta (TGF-β) is necessary for chondrogenesis of mesenchymal stem cells (MSCs), with TGF-β3 inducing a higher chondrogenic potential than other growth factors [2].

Sulfated glycosaminoglycans (GAGs) are polysaccharide components of the cartilage extracellular matrix (ECM) that can sequester such growth factors and cytokines to aid in inducing cellular differentiation towards chondrogenesis. Electrospun gelatin scaffolds are porous, biodegradable structures fabricated into fibrous mats that mimic the structure of the cartilage ECM [3].

This study aims to compare GAGs and GAG mimetic-containing gelatin scaffolds as a strategy for cartilage tissue repair. Specifically, these studies fabricated native GAG and GAG mimetic-containing gelatin scaffolds and characterized the TGF-β3 sequestration.

Methods

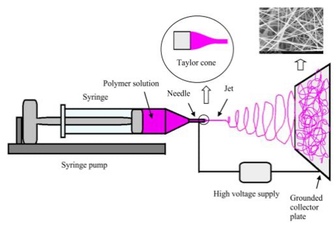


Figure 1. Diagram of the electrospinning process [4]

Scaffolds were fabricated using 24% (w/w) bovine gelatin in 63/37 acetic acid/water solutions and 5% (w/w) of chondroitin sulfate (CSC) or heparan sulfate (HS), which are naturally derived GAGs, or partially sulfated cellulose (pSC), fully sulfated cellulose (NaCS), which are GAG-mimetics. The scaffolds were crosslinked using 200 mM 1-3 ethylcarbodiimide hydrochloride (EDC)/ 40mM N-hydrosuccinimide (NHS) to increase their hydrolytic stability and maintain their fibrous structures in aqueous environments as established [4]. Scaffolds were viewed by scanning electron microscopy (SEM) to confirm fibrous morphology. Hydrolytic stability tests including changes in percent swelling, thickness, and diameter of scaffolds immersed in phosphate buffered saline (PBS) were conducted. TGF-β3 sequestration studies were conducted at 30 minutes and 1 hour in solutions with or without 10% serum. TGF-β3 was quantified using an enzyme-linked immunosorbent assay (ELISA). Statistical significance was confirmed with a one-way ANOVA and Tukey's post-hoc tests.

Results

Using SEM to Analyze Fiber Diameter

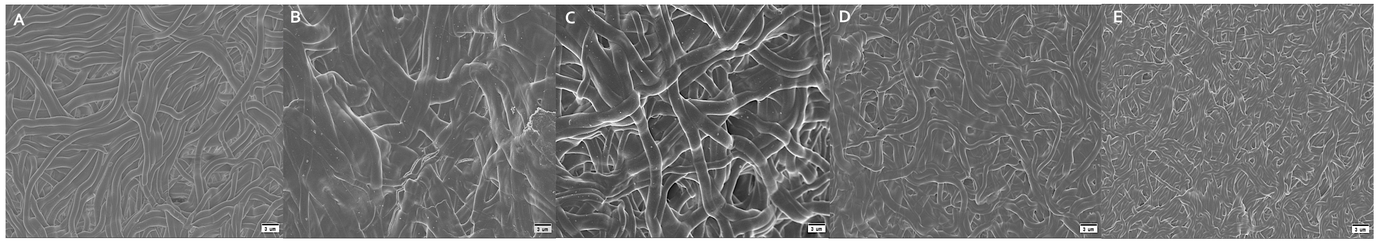
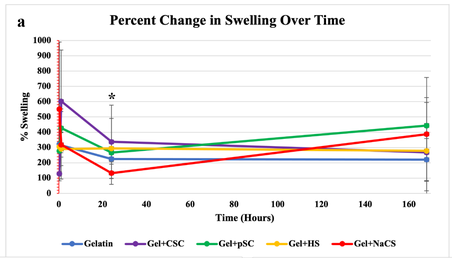


Figure 1. SEM images of the crosslinked fibrous scaffolds. (A) Gelatin, (B) Gelatin with CSC, (C) Gelatin with pSC, (D) Gelatin with HS, (E) Gelatin with NaCS. (A-E) Imaged at 2000x magnification.

Change in Scaffold Swelling and Dimensions Over Time



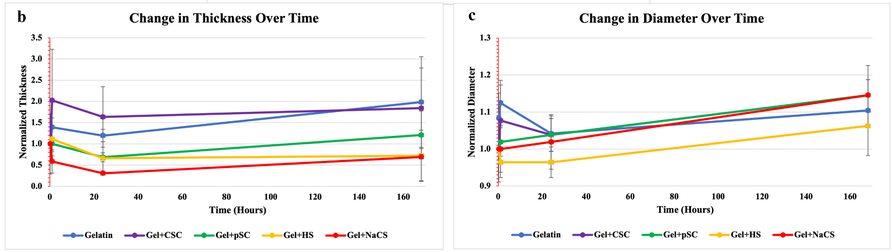
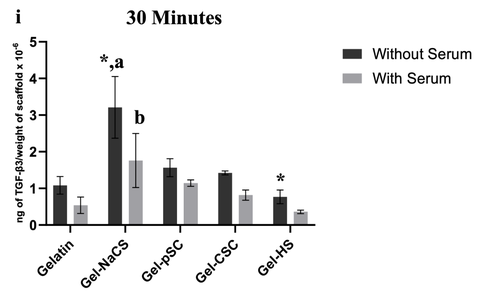


Figure 3. Change in (a) percent swelling, (b) thickness, and (c) diameter of each scaffold at 10 minutes, 1 hour, 1 day, and 7 days. Values are represented as the mean fold change with respect to 10 minutes for changes in thickness and diameter. *p<0.05 for CSC and NaCS groups at 10 minutes compared to 1 day for % swelling.

Average Fiber Diameter (μm)				
Groups	As-Spun	Standard Deviation	Crosslinked	Standard Deviation
Gelatin	0.75	1.18	1.29	0.57
Gelatin + CSC	1.96*#	0.14	2.68[a]	0.26
Gelatin + pSC	1.12#	0.37	1.81[a]	0.67
Gelatin + HS	0.95	0.20	1.22	0.29
Gelatin + NaCS	0.67	0.37	0.81	3.26

Table 1. Average Fiber Diameter. *p<0.05 is significantly different for as-spun Gelatin with CSC compared to all other as-spun groups. [a]p<0.05 is significantly different compared to other crosslinked groups. #p<0.05 is significantly different from as spun compared to crosslinked groups.

TGF-β3 Sequestration



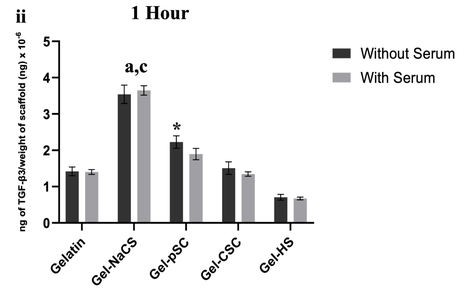


Figure 4. Sequestration of TGF-β3 at (i) 30 minutes and (ii) 1 hour. *p<0.05 for each scaffold group without serum compared to with serum. [a]p<0.05 for Gel-NaCS without serum compared to all other without serum groups. [b]p<0.05 for Gel-NaCS with serum compared to all other with serum groups except for Gel-pSC. [c]p<0.05 for Gel-NaCS with serum compared to all other with serum groups.

Discussion

SEM imaging confirmed that the addition of GAG mimetics to the gelatin scaffolds maintained the fibrous morphology. Fiber diameters ranged from 1-3 microns for both native GAG and GAG mimetic-containing gelatin scaffolds. Overall stability, as indicated by percent swelling, changes in diameter and changes in thickness, were similar across all scaffold groups. Large variability was observed with the hydrolytic stability studies, which may have been attributed to the distribution of fiber diameters. NaCS-containing scaffolds demonstrated the greatest sequestration of TGF-β3 (in both conditions with and without serum) compared to all other groups. In surgical procedures using microfracture, NaCS scaffolds may be favorable for binding TGF-β3. This may be due to TGF-β3 selectively binding to NaCS, since serum contains other growth factors and proteins that can bind to the GAGs.

Conclusions

Overall, these studies provide further understanding of how the scaffolds can contribute towards cartilage repair. GAG mimetics were previously shown to promote chondrogenesis [2]. These studies demonstrate that the GAG mimetics sequestered more TGF-β3 in serum conditions compared to the native GAGs. Specifically, NaCS-containing scaffolds performed better overall, even in the presence of serum. Next steps include performing sequestration studies with different growth factors and cytokines that may be present during repair. In all, these studies determined the potential of using fibrous scaffolds containing GAG mimetics for cartilage regeneration.

References and Acknowledgements

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