# 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 to aid in inducing cellular differentiation towards chondrogenesis. Electrospun gelatin scaffolds are 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:** 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) or 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 at 10 minutes, 1 hour, 1 day, and 7 days. 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:** SEM imaging confirmed that the addition of GAG mimetics to fabricated gelatin scaffolds maintained the fibrous morphology (Figure 1). Fiber diameters ranged from 1-3 microns for both native GAG and GAG mimetic-containing gelatin scaffolds. NaCS-containing scaffolds demonstrated the greatest sequestration of TGF-β3 (in both conditions with and without serum) compared to all other groups (Figure 2).

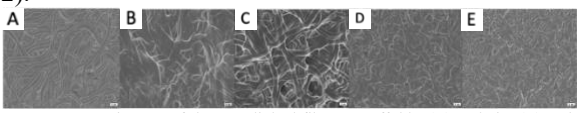


**Figure 1.** SEM images of the crosslinked fibrous scaffolds. (A) Gelatin, (B) Gel-CSC, (C) Gel-pSC, (D) Gel-HS, (E) Gel-NaCS. (A-E) Imaged at 2000x magnification.

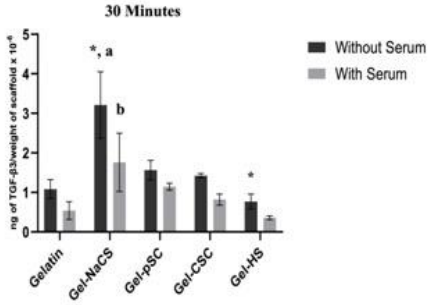


**Figure 2.** Sequestration of TGF-β3 at 30 minutes. *p<0.05 for each scaffold group without serum compared to with serum. [a]p<0.05 for Gel-NaCS without serum compared to all other without serum groups. [b]p<0.05 for Gel-NaCS with serum compared to all other with serum groups except for Gel-pSC.

**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 *in vivo*. In all, these studies determined the potential of gelatin scaffolds containing GAG mimetics for cartilage regeneration.

**References:**

[1] Leifer, V. P., Katz, J. N., & Losina, E. (2022). The burden of oa-health services and economics. *Osteoarthritis and Cartilage*, *30*(1), 10–16. https://doi.org/10.1016/j.joca.2021.05.007

[2] Huang, G. P., Molina, A., Tran, N., Collins, G., & Arinzeh, T. L. (2014). Promoting Chondrogenesis and Maintaining the Bioactivity of TGF-beta using a Biomimetic Material.

[3] Griffith, L. G., & Naughton, G. (2002). Tissue engineering--current challenges and expanding opportunities. *Science*, *295,* 1009–1014. https://doi.org/10.1126/science.1069210

[4] Huang, G. P., Shanmugasundaram, S., Masih, P., Pandya, D., Amara, S., Collins, G., & Arinzeh, T. L. (2014). An investigation of common crosslinking agents on the stability of electrospun collagen scaffolds. Journal of Biomedical Materials Research Part A, 103(2), 762–771. https://doi.org/10.1002/jbm.a.35222

[5] Garrity, C., Arzi, B., Haus, B., Lee, C. A., & Vapniarsky, N. (2022). A fresh glimpse into cartilage immune privilege. *CARTILAGE*,*13*(4),119–132. https://doi.org/10.1177/19476035221126349

[6] Menezes, R., & Arinzeh, T. L. (2020). Comparative study of electrospun scaffolds containing native gags and a gag mimetic for human mesenchymal stem cell chondrogenesis. *Annals of BiomedicalEngineering*,*48*(7),2040–2052. https://doi.org/10.1007/s10439-020-02499-9

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