Mechanically-Stimulated Co-cultured Tissue-Specific Scaffolds for Tendon/Bone Interface Engineering

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Statement of Purpose: The tendon/ligament to bone interface, or enthesis, is a complex transition that is essential for the functional motion because enables the transfer of loads through the musculoskeletal system[1]. The enthesis is a continuous transition from tendon to fibrocartilage to mineralized fibrocartilage to bone, with each region having specific mechanical and extracellular matrix (ECM) properties[2]. This work aims to create tissue-specific regions on a degradable fabric scaffold using co-cultured fibroblast and osteoblast deposited ECM. The ECM is further conditioned by mechanically stimulating the cells seeded on the scaffold during deposition in a custom designed mechanical bioreactor. It is our hypothesis that the mechanically conditioned ECM coating will promote increased tissue specific responses compared to scaffolds alone.

Methods: Degradable PLA fabric (X-Repair®, Synthasome, Inc, CA) was seeded with NIH 3T3 fibroblasts and MC 3T3 osteoblasts in co-culture to produce a tendon region and bone region on the scaffold. The seeded scaffolds were then clamped in a mechanical bioreactor and strained using one of two methods. One, the entire scaffold (both tendon and bone regions) was cyclically strained in tension at 5% strain for 1 hr per day at 0.5 Hz everyday for 5 weeks. Two, only the fibroblast/tendon region was strained using the same protocol as above, while the osteoblast/bone region was left unstrained. These two methods are termed All stretched and Half stretched respectively.

After 5 weeks, the ECM coated scaffolds were decellularized using combinations of freeze thaw and repeating hypo- and hypertonic solutions. The scaffolds were then cut into equal sized tendon, bone, and middle transition sections for evaluation. Rat mesenchymal stem cells (MSC) were then seeded on the stimulated ECM coated scaffold sections and a tissue culture plastic (TCP) control individually. The cells were harvested and RNA was isolated to measure tissue specific gene activation with respect to collagen III, decorin, osteocalcin, and aggrecan using real time PCR (rt-PCR). Relative gene activation was calculated using the delta delta C_t method.

Once the MSCs were removed from the scaffold sections, the ECM coatings were digesting off the scaffolds and characterized. Aliquots were taken from the digested ECM solution and DNA (Picogreen Assay), glucosaminoglycan (GAG) (alcian blue precipitation), and collagen (hydroxyproline (HYP) assay) content was analyzed for each of the scaffold sections. Histology was performed on two unsectioned ECM coated scaffolds (one each from the two stretching methods) to observe ECM deposition across the scaffold. ECM was stained using Goldner trichrome, Von Kossa, Methylene Blue-basic fuchsin, and Toluidine blue.

Results: RT-PCR showed that all tissue specific genes were increased on the ECM coated scaffolds compared to TCP. The stimulated ECM increased gene activation of the collagen, and a decrease in osteocalcin. Fibrocartilage specific aggrecan was unchanged by either strain method.

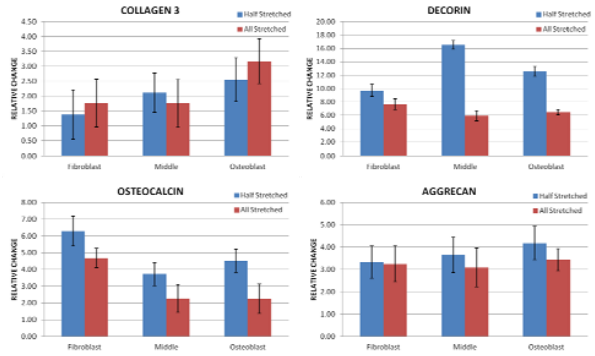
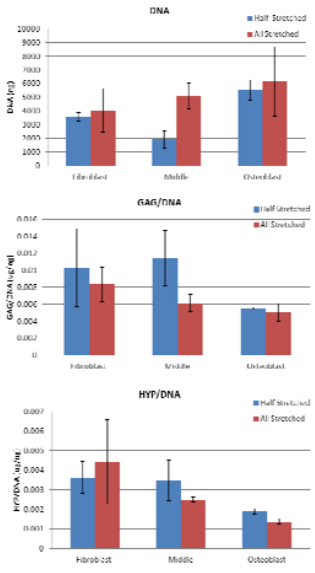


Figure 1. Relative change in gene activation of MSCs on coated scaffolds vs TCP control.



Fibroblasts deposited more ECM than the osteoblasts, and the middle region tended to mimic the trends produced by the fibroblasts. Strain did not cause an increase in ECM deposition in the osteoblast region. The ratio of collagen to GAG increased in the fibroblast and middle regions due to strain but stayed unchanged in the osteoblast region. The all-stretched scaffolds had increased cell number and HYP to GAG deposition.

Figure 2: DNA, GAG/DNA, and HYP/DNA deposited on the scaffold sections.

Conclusions: We have designed and built a mechanical bioreactor that can apply different strains to a single co-cultured scaffold to produce a more mimetic tissue specific coating. We did observe that stretching regimes in the bioreactor can affect the deposited ECM coating on the scaffold and tissue specific gene activation responds positively to the tissue specific ECM coatings compared to tissue culture plastic. Our future work is to focus on optimizing the stretching protocol for the deposited ECM coatings and evaluate the tissue specific coating in a functional tendon-to-bone animal model.

References:

[1] Zhang, X. J Shoulder Elbow Surg. 2012;21(2):266-77.
[2] Benjamin, M. Clin Radiol 2008;63(6):691-703

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