Fiber Reinforced Collagen-GAG Scaffolds for Tendon Bone Junction Repair

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Statement of Purpose: The tendon bone junction (TBJ) is a common injury site that also displays poor healing properties. We are developing a collagen-glycosaminoglycan (CG) scaffold which mimics elements of the biophysical and biochemical heterogeneities of the native TBJ [1]. However, this highly porous, bioactive scaffold is orders of magnitude weaker than healthy native tissue at the TBJ. Our goal is to develop a platform to increase the mechanical properties of collagen-GAG scaffolds while simultaneously developing a method to deliver growth factors (GF) in a spatially selective manner. This biomaterial will provide adipose derived stem cells (ASC) a mechanically robust platform during the early healing stages on which differentiation can take place as a precursor to improved biological fixation between tendon and bone. We can characterize and tune the fiber reinforcement mechanics through inclusion of sinusoidal patterns. Additionally, we can covalently or non-covalently tether GFs to the surface of the fibers to elicit a cellular response. We propose to generate prototype CG scaffolds with improved mechanical and bioactive properties to aid regenerative repair of the TBJ.

Methods: ABS fiber variants (straight, 1 mm sinusoidal amplitude, 2 mm sinusoidal amplitude) were printed using a MakerBot Replicator 2X (MakerBot Industries, Brooklyn, NY). ABS fiber-reinforced scaffolds were created by embedding fiber variants into a liquid suspension of type I collagen and chondroitin sulfate followed by lyophilization. ABS fiber-reinforced scaffolds were characterized via mechanical tensile testing. For cellular experiments, ABS fibers were functionalized via an oxygen plasma treatment followed by carbodiimide crosslinking in the presence of a biomolecule. ABS fiber-reinforced scaffold variants were seeded with ASCs and cellular viability was established via Alama Blue and Hoechst assays.

Results: The elastic modulus of the ABS fiber-reinforced scaffolds increased significantly (~70-fold) between groups, with the straight fibers having the highest elastic modulus (15.05 ± 1.73 MPa), followed by the 1 mm sinusoid fibers (8.01 ± 0.51 MPa), 2 mm sinusoid fibers (2.47 ± 0.32 MPa), and finally the scaffold alone (0.22 ± 0.01 MPa). Similarly, the peak stress of the ABS fiber-reinforced groups increased significantly (~30-fold) with the straight fibers having the highest peak stress (0.91 ± 0.063 MPa), followed by the 1 mm sinusoid fibers (0.65 ± 0.075 MPa), 2 mm sinusoid fibers (0.30 ± 0.015 MPa) and finally the scaffold alone (0.03 ± 0.001 MPa). Finally, the toughness of the samples increased significantly (~55-fold) with the straight fibers having the highest toughness (151.9 ± 15.43 MPa), followed by the 1 mm sinusoid fibers (75.44 ± 0.12.59 MPa) and 2 mm sinusoid fibers (77.30 ± 6.3 MPa) having similar toughness, and finally the scaffold alone (2.77 ± 0.11 MPa) (Fig. 1). No significant differences were found between ABS fiber and ABS fiber-reinforced samples. Cellular viability (Alamar Blue, Hoechst) of ABS fiber-reinforced scaffolds was tested. After 7 days, there were no significant differences in cellular proliferation and viability between the fiber-reinforced scaffolds and the scaffolds alone (control). ABS fibers were then functionalized with bovine serum albumin (BSA), a model protein. ABS fibers were visualized using a fluorescent microscope to confirm the attachment of BSA (Fig. 2). Fibers were then functionalized with PDGF to increase cellular proliferation. After 1 day, we saw an increase in cellular proliferation and bioactivity on functionalized ABS when compared to non-functionalized controls.

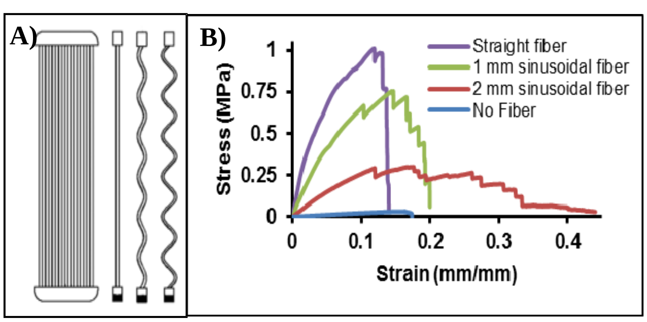

Figure 1A) Left to right: top view, side view of straight fiber, 1 mm amplitude sinusoidal fiber, and 2 mm amplitude sinusoidal fiber constructs. **1B)** Representative stress-strain curves of each fiber reinforced scaffold

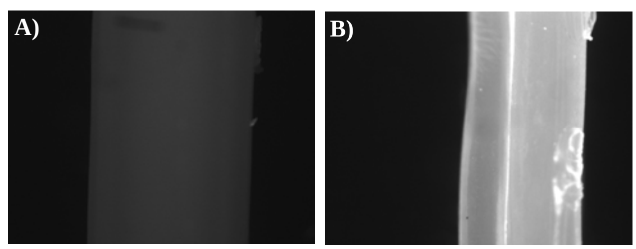

Figure 2A) ABS fiber without BSA functionalization (control) **2B)** ABS fiber functionalized with BSA via an oxygen plasma treatment

Conclusions: We have demonstrated the ability to enhance the mechanical properties of CG biomaterials through ABS fiber-reinforcement. By varying the geometry of the fiber-reinforcements, we can tailor the mechanical properties of our overall construct. We have also demonstrated the ability to immobilize PDGF to enhance cellular proliferation. In the future, fiber reinforcements can be created from poly(lactic acid) (PLA), which has enhanced bioactivity but similar mechanical properties compared to ABS. Fiber reinforcements can be functionalized with a variety of growth factors in order to elicit a range of responses.

References: [1] Genin, et al. Biophys J. 2009;97:976-985.