Aligned Collagen-GAG Scaffolds for Tendon Tissue Engineering

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Statement of Purpose: Within the United States there are more than 32 million injuries to tendons and ligaments per year with an associated cost of $30 billion [1]. While small tendon injuries can heal naturally, larger injuries undergo a repair-mediated process. The resultant repair tissue does not recapitulate the native hierarchical structure of tendon extracellular matrix (ECM) and displays inferior biomechanical properties. Current surgical and tissue engineering approaches have had only limited success, particularly due to high failure rates. Results in the literature suggest that successful regeneration templates for aligned tissues such as tendon must recapitulate aspects of the native tissue anisotropy [2, 3]. Collagen-glycosaminoglycan (CG) scaffolds have previously been used as successful regeneration templates for skin and peripheral nerves [4], but do not typically have requisite mechanical integrity for orthopedic applications. The objective of this study was to develop a CG scaffold variant with aligned microstructure and sufficient mechanical competence for tendon tissue engineering, while using soluble factors to improve scaffold bioactivity (cell motility, proliferation).

Methods: Composite scaffolds were created from a relatively porous CG scaffold and relatively dense CG membrane. CG scaffolds and membranes were fabricated from a suspension of type I collagen and chondroitin 6-sulfate in acetic acid [5]. CG membranes were created via an evaporative process. CG scaffolds were fabricated via lyophilization [5] in a manner that integrated the CG membrane into the scaffold structure. CG scaffolds with longitudinally aligned pore microstructures were fabricated in a Teflon mold with a copper base; the mismatch in thermal conductivity was used to promote unidirectional heat transfer through the copper bottom, resulting in aligned pores. Pore size was controlled by the final freezing temperature [5]. Scaffolds were then dehydrothermally crosslinked (105ºC, 24 hours, <25 torr).

Scaffold pore size and morphology were assessed using a stereological approach via a linear intercept macro in Scion Image. Longitudinally and transversely oriented scaffold samples were embedded in glycolmethacrylate, sectioned, stained with aniline blue, and observed using an optical microscope [5]. Tensile testing of CG scaffolds, membranes, and composites (dry, hydrated) was performed using an MTS Insight 2.

Tenocyte and tenocyte precursor cells (TTPCs) were harvested from horse tendinopathies. Soluble factor (IGF-1 and PDGF-BB) induced TTPC motility and chemotaxis was assessed using a 24 hour transwell membrane assay. TTPC motility across the transwell membrane was assessed in the presence (random motility) and absence of each soluble factor and for a gradient in soluble factor concentration across the membrane (chemotaxis).

Results: SEM analysis of CG scaffolds reveals aligned, elongated pores in the longitudinal plane compared to rounder pores in the transverse plane (Figure 1). Mean pore diameters of 41.9 ± 3.8 μm (Mean ± SD) for scaffolds flash frozen in liquid nitrogen and 92.7 ± 13.0 μm for scaffolds frozen at -60ºC were observed. Elongation of pores was assessed by measuring the aspect ratio (AR) of the best fit ellipse obtained from the linear intercept macro. Pores in the longitudinal plane (AR: 1.60 ± 0.12) were significantly more elongated than pores in the transverse plane (AR: 1.06 ± 0.04, $p < 0.0001$).

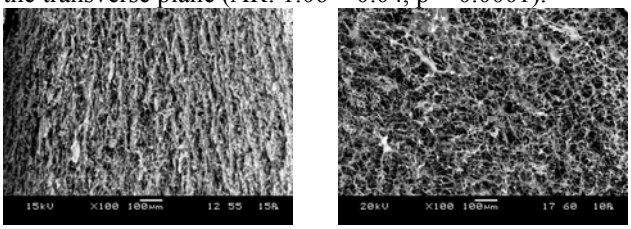

Figure 1. SEM of longitudinal (left) and transverse (right) cross-sections of CG scaffolds. Scale bars: 100 μm.

CG membranes (22.9 ± 2.6 nm thickness; 0.788 relative density) demonstrated hydrated tensile moduli of 46.6 ± 10.2 MPa; CG scaffolds (0.005 relative density) demonstrated tensile moduli of 25.3 ± 10.0 kPa; CG scaffold-membrane constructs demonstrated a greater than 15-fold increase in tensile modulus (max strain: 9%). Optimization of membrane thickness is currently being performed to further increase composite strength.

TTPCs attach and remain viable in the CG scaffolds (>48 hours). TTPC motility in response to a range of therapeutic doses for IGF-1 (50-500 ng/mL), and PDGF-BB (1-100 ng/mL) increased by 31-65% and 49-97% respectively over negative controls. No significant differences were observed .for either soluble factor between the chemotaxis and random motility groups.

Conclusions: There is an unmet need to develop clinically successful treatments for tendon injuries. Here we describe development of a CG scaffold with longitudinally aligned pores than can support TTPC viability. We have also shown that CG membrane-scaffold composites significantly increase construct tensile strength and that the presence of soluble factors PDGF-BB and IGF-1 at therapeutic doses can encourage TTPC motility. Ongoing studies are optimizing scaffold pore microstructure for maximal TTPC viability, modulating CG membrane size and scaffold-membrane geometry to further improve construct mechanical integrity while maintaining sufficient permeability, and characterizing TTPC motility within the CG scaffold in response to soluble factor supplementation. These studies are building towards a goal of spatio-temporal presentation of scaffold and soluble cues to improve TTPC bioactivity.

References:

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