Novel (Poly)caprolactone (PCL) Scaffold Architecture for Tendon Tissue Engineering Applications

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Statement of Purpose: With approximately $2x10^5$ tendon and ligament repairs performed per year in the United States, the poor healing capability of tendons, and the significant pain associated with tendon injuries, tendon tissue engineering is a significant research area.[1] Tendons are connective tissues that join muscles to bones and function to transmit forces, permit locomotion, and enhance joint stability.[1] These musculoskeletal tissues have complex structures with multi-unit hierarchical structures, minimal ground substance, and low cell content.[2] Tendon tissue engineering requires development of a load-bearing tissue that is capable of withstanding tensile, compressive, and shear stresses, multi-directional mechanical forces, and complex motions. Requirements for appropriate scaffold design include an extensive list of factors for consideration including supporting matrix for cell attachment and proliferation, biodegradability, biocompatibility, biological cues such as growth factors, cost-effectiveness, porous for cell incorporation, and sufficient mechanical properties.[1] Pitfalls of current solutions to tendon injuries include limited availability of allografts and autografts and failure to meet stability and mechanical requirements of synthetic grafts. A need exists in tendon tissue engineering to create a successful tendon replacement. It is hypothesized that a PCL scaffold design with bi-axially aligned nanofibers and optimal growth factor concentration may create a microenvironment to direct cell growth while maintaining mechanically effectiveness for tendon tissue engineering applications.

Methods: To synthesize the scaffold, electrospinning was utilized. PCL was dissolved at 18% (wt/vol) in an 85:15 glacial acetic acid:acetone mixture. The electrospinning parameters were a voltage of 14 – 16 kV and flow-rate of 5 mL/h with the collector target about 25 cm from the syringe tip. To create a tube-shaped scaffold with criss-crossing fibers, the polymer was spun onto an oscillating rod target. The nanofiber alignment was controlled with magnets flanking the target area. The polymer collected as aligned fibers perpendicular to the magnets. Scanning electron microscopy (SEM) was used to image the scaffolds and confirm nanofiber alignment and arrangement. To investigate the optimal conditions for tenogenic differentiation, a gradient of fiber diameter versus growth factor concentration of GDF-5 will be investigated. Wet isotropic etching will be used to create various diameters used for experimentation.[3] At time points of 3, 7, and 14 days, quantitative in cell western blotting will be used to analyze Scleraxix (Scx) protein levels normalized to DNA through the use of primary and secondary antibodies for Scx detection and DRAQ5 for DNA. Scx, a tendon specific cell growth and differentiation marker, will be targeted to observe tenogenic differentiation.

Results: The electrospinning technique in combination with the oscillating rod and magnet set-up demonstrated parallel, aligned fibers as shown in **Figure 1A**.

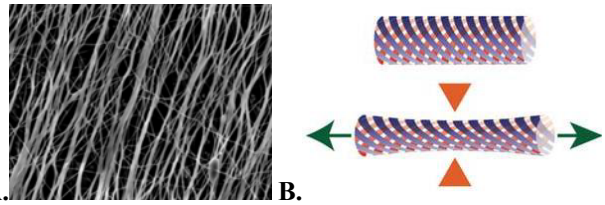

Figure 1: **A.** SEM image at 2000x of aligned nanofibers, **B.** Criss-crossed fiber design with application of tension

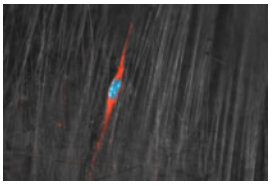

Figure 2: Cells were seeded on the electrospun scaffolds and immunostained to investigate how the cells grew in response to the fiber alignment and structure.

The project innovation lies in the Chinese finger trap concept, shown in **Figure 1B**, with an overlapping criss-cross design to improve graft retention and mimic natural mechanical properties. The tube-shaped scaffold was synthesized as one piece rather than as a sheet to reduce the potential for points of weakness. **Figure 2** illustrates cell alignment to the parallel fiber arrangment. This is important in the potential to use bioinstructive geometry in nanofiber structure to replace growth factors or augment their response.

Conclusions: Controlling fiber diameter and growth factor concentration suggest a way to create the optimal conditions for tenogenic differentiation. The results suggest potential for bioinstructive geometry as a way to use structure and shape to encourage cell growth down specific lineages. Future studies include mechanical tests such as tensile tests to determine toe regions and Young's moduli which the ultimate goal to reach human tendon values (toe region typically falls in the 0% to 3% range and E=~1.2GPa).[4, 5] The cellular interactions will also be investigated by seeding cells on the constructs with optimized diameter, pattern, and GDF-5 concentration and culturing with time in a bioreactor which will simulate an environment for the cells with incorporation of physiological cyclic stretch. This proposal suggests a solution for tendon regeneration that could span to further ligament and other bone insertion research.

References:

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