Novel Fibers as Structural Templates for Ligament Regeneration.

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Introduction

The anterior cruciate ligament (ACL) is composed primarily of fibroblasts and extracellular matrix organized in a parallel structural alignment consistent with their biomechanical function in resisting tensile loading. The ACL possesses limited capacity for intrinsic healing and regeneration, and therefore injuries require surgical reconstruction to restore joint stability and prevent the premature onset of degenerative joint disease. Over the last thirty years, a variety of non-degradable, synthetic fibers (polyethylene terephthalate (PET), polypropylene, polytetrafluoroethylene, carbon fibers) were evaluated in ACL reconstruction; however, a widely accepted prosthesis has not been achieved due to differences in mechanical properties relative to the native tissue and obtaining long-term, stable fixation.[1] Numerous degradable fibers are currently under investigation as scaffolds for ACL regeneration and tissue engineering. Capillary channel polymer (CC-P) fibers possess a novel cross-sectional architecture that provides increased surface area relative to smooth fibers, as well as supporting the capillary-like transport of fluids. The objective of this study was to investigate the ability of the CC-P fiber architecture to support adhesion, growth, and organized alignment of fibroblasts and extracellular matrix.

Materials and Methods

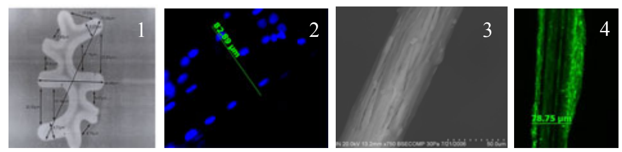
19 dpf CC-P, 19 and 98 dpf round fibers were prepared by melt extrusion of PET (Wellman 0.84 IV) and collected on Sonoco bobbins using a Leesonia winder. Fiber dimensions were measured from cross-sectional SEM images.

For cell seeding, fibers were coiled around supporting frames and attached at the ends using UV curable adhesive. The fibers were sterilized with 70% ethanol and then coated with 20 µg/ml fibronectin for 1 hour. Four hundred microliters of a dense solution of fibroblasts was allowed to precipitate down on to the fibers for to provide a thorough coating of the fiber surfaces. The samples were incubated for 24 hours, 7, and 14 days at 37°C, and then fixed with 4% paraformaldehyde.

Two of the frames from each time point were stained using DAPI, rhodamine-phalloidin, and conjugated antibody stains for visualization and imaging of nuclei, actin filaments, and collagen fibers by fluorescence microscopy. The angle of orientation of the cell nuclei was measured from the DAPI images with respect to the fiber axis. The other frames underwent a secondary fixation in 3.5% glutaraldehyde for SEM imaging.

Results

CC-P fibers displayed elliptical cross-sections with 2 major and 6 minor grooves with an approximate diameter of 20 µm and 8 µm, respectively (Figure 1). At 24 hours, fibroblasts were densely adhered to all fiber types. DAPI staining demonstrated alignment of cellular nuclei parallel to the underlying fiber topography on the CC-P fibers(Figures 2 and 3). Large amounts of collagen was produced on all fiber types, however, axial alignment was only found on the CC-P fibers (Figure 4). SEM analysis further confirmed that the fibroblasts adopted a bipolar, aligned morphology parallel to the channels of the CC-P fiber, growing both on top of and within the channels.



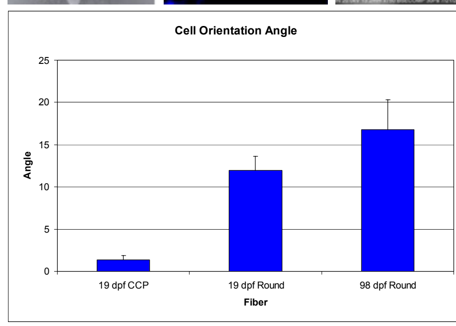


Figure 1*. Cross section of CC-P fiber (SEM 1.0k), Figure 2. Longitudinal view of CC-P fiber (SEM 750x), Figure 3*. DAPI stained nuclei (20x), Figure 4. Antibody labled collagen on CC-P fiber (400x).
Figure 5. CC-P shape provides greater cell orientation than round fiber geometries.

Conclusions

These studies demonstrate the superior ability of the capillary channel polymer fibers to support adhesion, alignment, and organization of fibroblasts; as compared to round fibers. Additionally, these fibers demonstrated grater uniformity of seeding and accelerated formation of multi-layered three-dimensional biomass relative to round fibers. CC-P fibers offer a novel approach to translating principles of topographic guidance into 3-dimensional constructs that may serve as templates for the regeneration and tissue engineering of organized cellular structures such as the ACL.

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References: [1] E.Liljensten, K. Gisselfalt, et.al., Journal of Materials Science: Materials in Medicine 13 (2002) 351-359.