INTRODUCTION: Ruptures of the anterior cruciate ligament (ACL) are common to athletics, resulting in more than 100,000 reconstructive surgeries per year. The ACL is composed primarily of fibroblast cells and type I collagen organized in a parallel structural alignment consistent with their biomechanical function in resisting tensile loading. The ACL possesses a 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, polypropylene, polytetrafluoroethylene, carbon fibers) were evaluated in ACL reconstruction. However, a widely accepted prostheses has not been achieved due to mechanical mismatch relative to the native tissue and the inability to obtain long-term, stable fixation.

Nanofiber channelized constructs fabricated by melt extrusion have aligned micrometer scale surface channels that may serve as biomimetic templates for tissue growth and regeneration. This inherent surface structure offers a unique and potentially industrially viable approach for cellular topographic guidance on three-dimensional constructs.

The goal of this research was to determine whether capillary channel polymer fibers, subjected to uniaxial cyclic strain, could be used to guide cell behavior and induce neo-ligament synthesis. Hypotheses: 1) Cellular alignment will not be affected by the application of cyclic strain. 2) T cell synthesized type I collagen will increase the tensile strength of the cell seeded CC-P fiber scaffolds when subjected to uniaxial cyclic strain.

METHODS: The CC-P fibers were prepared via melt extrusion of polyethylene terephthalate (PDT) (Celanex®) and collected on Sonoco bobbins using a Leesona winder. CC-P fiber dimensions were measured from prepared cross-sections using light microscopy.

For cell seeding, eight parallel lengths of eight-filament yarn, 2 cm in length, were anchored to both sides of a rectangular mylar film frame and attached at the ends using a medical grade UV curable adhesive. The fibers were sterilized in 70% ethanol and then coated with 20 µg/ml fibronectin protein for 1 hour to promote cell adhesion. Two frames were placed in the chamber of a bioreactor, submerged in 50 ml of Dulbecco’s Modified Eagle Medium (DMEM) with 5 ml of 1 mM ascorbic acid 2-phosphate (AA2P). AA2P, an essential precursor for collagen synthesis, was with held from the “cells without collagen” groups. Each length of yarn was then seeded with 10⁶ normal human dermal fibroblast cells. The samples were incubated and cyclically strained (4% strain at 1Hz for 15 minutes, 3 times per day) for 24 hours and 2 weeks at 37°C and 5% CO₂.

At the conclusion of each time point one set (n=16) of the cell seeded CC-P fiber frames (9 and 19 dpf) were fixed in 4% paraformaldehyde for 20 minutes at room temperature for fluorescence microscopy. The second set (n=16) of the fiber containing frames were fixed in 2.5% glutaraldehyde, dehydrated in graded ethanol, and dried using a critical drying technique for scanning electron microscopy (SEM). The third set (n=16) was snap frozen in liquid nitrogen for cellular quantification and measuring total collagen content. The fourth set (n=16) was taken directly from the incubator to the Instron for tensile testing, following ASTM standard 2256 for a single end yarn break.

RESULTS: The linear densities of the CC-P yarns produced on the Hills Research and Development melt extruder were measured to be 9 dpf and 19 dpf. These fibers displayed ellipsoidal cross-sections with two major and six minor grooves (Figure 1). The approximated diameters varied with the varying linear density (Table I).

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FIGURE 1: SEM image of a resin imbedded CC-P fiber cross-section (900x).

TABLE 1: Channel dimensions of the two CC-P fiber liner densities.

<table>
<thead>
<tr>
<th>Capillary Channel Polymer Fiber Dimensions</th>
<th>Major Channel</th>
<th>Minor Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width</td>
<td>Height</td>
<td>Width</td>
</tr>
<tr>
<td>9 dpf</td>
<td>25±3.3</td>
<td>17±3.0</td>
</tr>
<tr>
<td>19 dpf</td>
<td>30±5.9</td>
<td>25±3.2</td>
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</tbody>
</table>

DISCUSSION: 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.

The application of uniaxial cyclic strain was found to increase cell numbers, type I collagen synthesis, and the tensile strength of these tissue scaffolds. The 9 dpf CC-P fiber demonstrated characteristics that could possibly lead to the development of a tissue engineering approach to ligament regeneration. This geometry imposed greater cellular and type I collagen alignment, as compared to the 19 dpf CC-P fiber, and responded favorably to cyclic loading. The fiber’s channel dimensions were large enough to promote cell growth, but small enough to provide the necessary restriction to maintain cellular and type I collagen alignment throughout the loading period.

Future studies will focus on the utilization of a more physiologically relevant cell type for greater type I collagen synthesis.

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REFERENCES: