Comparison of Anterior Cruciate Ligament Fibroblast Response on PLGA and PCL Nanofiber Scaffolds

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Introduction: The anterior cruciate ligament (ACL) is the most frequently injured ligament of the knee, with over 100,000 reconstructions performed annually[1]. Our long term goal is to develop a functional and integrative scaffold system for ACL tissue engineering, which possesses physiologically relevant mechanical properties, while also exhibiting biomimetic structural organization in order to promote *guided* tissue in-growth and ligament regeneration. Specifically, scaffold mechanical properties must adequately match that of the native bulk tissue while also providing a mechanical environment that promotes the formation of such tissue by native cell populations.
Nanofiber scaffolds fabricated from polymers such as polylactide-*co*-glycolide (PLGA) and poly-ε-caprolactone (PCL) have been evaluated for a variety of orthopaedic applications[2]. However, scaffold mechanical properties are composition-dependent (Table 1) and the effect of these properties on ligament fibroblast biosynthesis and gene expression has not been well described. Therefore, the **objective of this study** is to compare ACL fibroblast proliferation, biosynthesis, and gene expression on PLGA and PCL nanofiber scaffolds. **It is hypothesized** that these cell responses will be regulated by substrate composition and mechanical properties.

Methods: *Scaffold fabrication & Cell Culture:* Aligned PLGA (85:15, Lakeshore) and PCL (Lakeshore) nanofiber scaffolds were fabricated via electrospinning[3]. Bovine ACL fibroblasts were seeded on nanofiber scaffolds at a density of 30,000 cells/cm^2, and cell response analyzed after 1, 7, 14, and 28 days of culture. *End-Point Analyses:* Cellular attachment and morphology were assessed using the Live/Dead cell viability/toxicity assay. Total DNA (n=5) was determined by the Picogreen DNA assay, while collagen synthesis (n=5) was measured via the Sircol assay. Collagen distribution was also visualized by Picrosirius Red staining (n=2). The expression (n=3) of ligament fibroblast markers (collagen I, III, fibronectin , tenascin C) was determined by RT-PCR. Gene expression was normalized by the housekeeping gene GAPDH. *Statistical Analysis:* ANOVA and the Tukey-Kramer *post-hoc* test were used for all pair-wise comparisons (*p<.05).

Results: *Cell Attachment and Growth:* Cells proliferated uniformly on both groups of scaffolds (Fig. 1A). After four weeks of culture, cell number was significantly greater on the PCL scaffolds (Fig. 1B), as compared to that of the PLGA scaffolds. No significant difference in collagen production was found between the samples in either group (Fig. 2A). A similar level of matrix was produced on both polymer types with a more uniform surface morphology evident on the PLGA scaffolds, as compared to the undulating surface noticeable on the less stiff PCL scaffolds (Fig. 2B). *Fibroblast Phenotype:* The expression of relevant fibroblast-specific markers remained relatively constant over 4-weeks of culture (Fig. 3). Interestingly, a significant decrease in collagen I expression was measured on the PCL scaffolds after 28 days, while elevated collagen III expression was observed on the PCL scaffolds after 14 days (p<0.05).

Discussion: It is observed in this study that by day 28, cell proliferation on PCL scaffold was greater than that on the PLGA scaffold. This difference may be attributed to the difference in degradation rate between PLGA, which degrades more rapidly via bulk erosion, and PCL, which degrades slower and via surface erosion. After 28 days of culture, it is likely that more of the PCL scaffold remains resulting in increased available surface area for cell proliferation. In addition to composition, our results suggest that differences in scaffold mechanical properties regulate the expression of key extracellular matrix components. Specifically, collagen type I expression was down-regulated while collagen type III expression was up-regulated by fibroblasts after 28 days of culture on PCL scaffolds. Studies examining the expression ratio of type I to type III have seen similar trends in injured tendons and ligaments, indicating that the PCL substrate may direct fibroblasts towards the injury repair process[6]. The mechanical properties of the PCL substrate are lower than those of the native ACL and the PLGA scaffolds, which may promote the formation of scar tissue as opposed to native ligament tissue. These findings indicate that substrate mechanics and composition influence fibroblast response. Future studies will aim to decouple the effects of polymer composition and mechanical properties on ligament fibroblast response, in order to optimize these degradable nanofibers scaffolds for ACL tissue engineering.

References: *1)Glickson et al., AAOS Bulletin, 2004. 2)Moffat et al., Tissue Eng., 2008. 3)Reneker et al. ,Nanotechnology, 1996. 4)Lee et al., Biomaterials, 2005. 5)Zong et al., Biomacromolecules, 2003. 6) Lin et al., Clin. Orthop. Rel. Res., 1995 7)Ge et al., J. Biomed. Mat. Res. A., 2006.* **Acknowledgements:** Funding from NIH/NIAMS (AR056459-02, AR055280-02), Wallace H. Coulter Foundation.

	Fiber Diameter (nm)	Elastic Modulus (MPa)	Yield Strength (MPa)	Ultimate Stress (MPa)
hACL	45	180	46.7	75.8
PLGA	615±152	341±30	9.8±1.1	12.0±1.5
PCL	700±140	131±13	9.1±0.6	21.5±1.1

Table 1: Comparison of mechican properties between PCL/PLGA scaffolds and the native human anterior cruciate ligament [7].

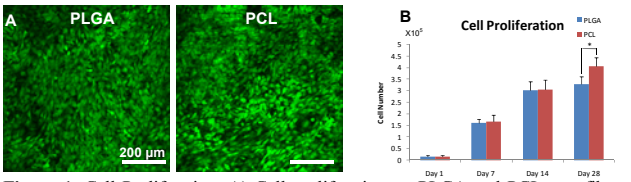

Figure 1: Cell Proliferation. A) Cell proliferation on PLGA and PCL nanofibers (Day 28, 20X). B) Quantitative analysis of cell proliferation over the duration of culture.

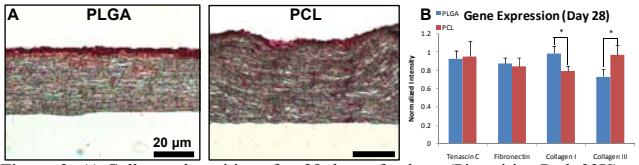

Figure 2: A) Collagen deposition after 28 days of culture (Picrosirius Red, 32X). B) Gene expression of key fibroblast markers (*p<.05).