Regulation of Human Tendon Fibroblast Response by Fiber Diameter of Electrospun Polymer Scaffolds

N.M. Lee, C.E. Erisken, W.N. Levine, H.H. Lu

Biomaterials and Interface Tissue Engineering Laboratory, Columbia University, New York, NY.

Statement of Purpose: Connective tissues such as tendons and ligaments are characterized by a collagenous extracellular matrix which displays a hierarchical architecture. As these tissues play critical roles in musculoskeletal mechanics, injuries to and degeneration of these tissues often occur. Soft tissue healing typically results in scar tissue formation which is compositionally and mechanically inferior to the native tissue, notably failure rates as high as 94%[1] have been reported after primary repair procedures. It is known that the collagen fiber diameter of these connective tissues vary with health and age[2,3]. Structural characteristics, such as fiber diameter and organization may serve as critical components to promote host cell-mediated healing. Electrospinning can be used to fabricate scaffolds in which substrate architecture and morphology can be modulated to resemble native tissue and healing scar tissue. The **objective of this study** is to assess the impact of fiber diameter of unaligned nanofiber scaffolds on fibroblast cell response. It is **hypothesized** that the diameter of unaligned polylactide-co-glycolide (PLGA) scaffolds can be used to guide cell alignment, proliferation, matrix deposition, and gene expression. The elucidation of cell-biomaterial interactions is anticipated to provide critical insight into biomaterial scaffold design parameters to augment connective tissue repair.

Methods: *Scaffold Fabrication:* Unaligned scaffolds: were produced by electrospinning solutions of PLGA dissolved in N,N-dimethylformamide (DMF) and Acetone. Nanofibers were collected on a stationary plate. By varying the solvent ratio, weight% polymer, flow rate, needle gauge and distance from the needle to the collector three fiber diameter groups were produced (39, 740, 1420nm). For aligned scaffolds nanofibers were collected on a rotating mandrel. Scaffolds were characterized using scanning electron microscopy and fiber diameter was measured (ImageJ). *Cells & Cell Culture:* Human rotator cuff fibroblasts (hRC-FB) were derived from explant culture of tissue obtained from patients (51-76yo) undergoing surgery. Cells (p3-5, 30,000 cells/cm^2) were cultured on PLGA scaffolds (1x1x0.12-0.18cm) in DMEM+10% FBS, with monolayer culture as controls. *Cell Response:* Cell viability (n=3) was assessed using Live/Dead. Live-stained images were analyzed for cell alignment (n=3) using circular statistics software.[4] Total DNA (n=5, PicoGreen) and collagen (n=5, hydroxyproline) was quantified. RT-PCT (n=3) was performed at Day 14, with expression normalized to GAPDH. Samples were sectioned to 10µm and analyzed histologically. *Statistical Analyses:* A two-way ANOVA was performed and the Tukey-Kramer test was used for all pair-wise comparison at $p<0.05$.

Results: Cell alignment analysis (MA approaching 0, MVL approaching 1, CSD approaching 0) indicates that initially, micron sized fibers promote the greatest degree of cell alignment, while by Day 28 there were no significant differences noted between any of the unaligned groups. While a similar degree of cell proliferation was seen between groups over time, a statistically greater value was observed on both nanometer diameter scaffolds. H&E staining (Day 28) indicates matrix deposition by all groups, with a more intense degree of staining noted on both the nanometer diameter scaffolds. Notably, cell penetration through the depth of the scaffold appears to be greater on the unaligned scaffolds (H&E). Gene expression indicates an upregulation in Col III and Col V expression in the 740nm group compared to both the unaligned micron fiber and aligned group.

Discussion & Conclusions: Fibroblast cell growth, alignment and differentiation can be modulated by the underlying culture substrate. Cell growth is similar for unaligned nanofiber scaffolds and is greater than that observed on the microfiber group. Furthermore unaligned (740nm) cell growth is greater than that observed on the aligned (640nm) group. Collagen production also appears to be enhanced on nanofiber substrates. These observations suggest that nanofiber unaligned scaffolds may resemble matrix in a state of injury thus stimulating cells for matrix deposition and cell proliferation as part of the tissue repair process.

References: (Galtz L. J Bone Joint Surg Am 2004;86:219, Alaseirlis DA, Connet Tissue Res 2005;46:12, Svensson M. Am J Sports Med 2007;35:301, Costa KD. Tissue Eng 2003;9:567, Moffat KL.Tissue Eng. 2008;14:1, Erisken C. Tissue Eng. 2012)

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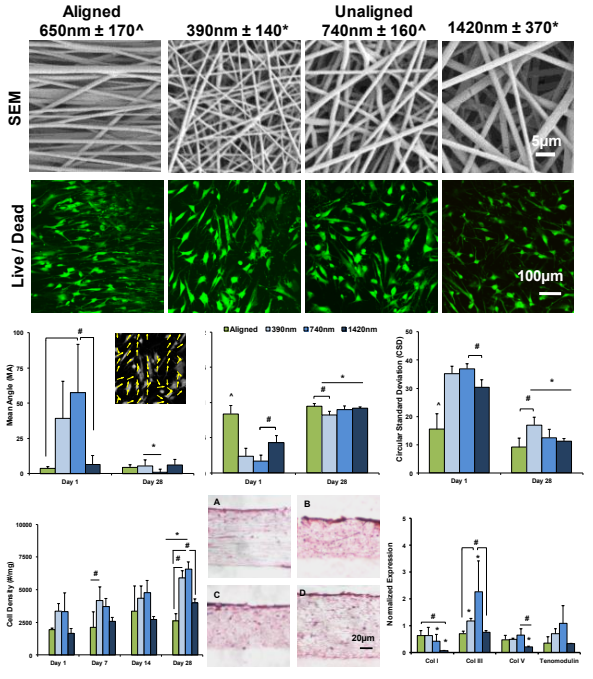

Figure. *Top:* SEM, live/dead confocal Mid*dle:* Cell Alignment analysis, vector map of cells used for analysis (inset) *Bottom:* Cell density, (A-D) Aligned, 390nm, 740nm, 1420nm Day 28 H&E, Day 14 Gene expression (significant difference between *time points, #groups)