Influence of Sparse Electrospun Fibers on the Differentiation of Mesenchymal Stem Cells in Collagen Gels

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Statement of Purpose: Engineered connective tissues have the potential to overcome intrinsic limitations with current autologous and allogeneic treatments, but they require the development of scaffolds that can support cell infiltration, proliferation, and organization into tissue-like structures. Electrospinning can produce aligned micro-fiber meshes that can guide cells to adopt a spindle shaped morphology. However, thick micro-fiber meshes can impede cell migration, proliferation, and tissue formation. Inclusion of hydrogels such as collagen within electrospun meshes may both increase inter-fiber spacing a provide substrate that cells can attach to, migrate through, and degrade. In this study, model composites were formed by embedding a low density of aligned electrospun micro-fibers in collagen gels. Using this model system we sought to determine if mesenchymal stem cells (MSCs) incorporated into composites 1) sensed the presence of the fibers, 2) differentiated in a manner that depended on fiber stiffness, and 3) could be mechanically stimulated by cyclic tensile loading of composites.

Methods: Micro-fibers were prepared by electrospinning and deposited between silicone rubber strips on a rapidly rotating mandrel to create thin (10-20 µm thick) aligned meshes. Blends of polyurethane (PU) and polycaprolactone (PCL) were used to achieve micro-fiber meshes with different moduli characterized, and mechanical properties and architectures of resultant meshes were determined by tensile testing and and scanning electron microscopy (SEM), respectively. Meshes were then mounted under uniaxial tension onto glass slides using silicone adhesive. A 0.5 wt% collagen gel was cast beneath the suspended meshes, and after gelation, rat bone marrow-derived MSCs within another 0.5 wt% collagen gel was cast on the surfaces of meshes. The resultant composites were analyzed for cell morphology after 2 days by F-actin staining, and for expression of ligament markers by PCR after 7 days of culture. Separately, composites containing fluorescently labeled fibers were stained for F-actin and nuclei and imaged by confocal microscopy to characterize spatial distribution of cells within composites. Finally, composites were cultured within Flexcell© wells and cyclically stimulated 30 min/day at 4% strain.

Results: Micro-tensile testing confirmed the fabrication of meshes with distinct elastic moduli ranging from 5.6 MPa (for a 75/25 wt% PU/PCL blend) to 31 MPa (for 100% PCL). Analysis of SEM images indicated that fiber diameters were 0.6-0.8 µm and angular standard deviations were 13-21° (corresponding to a high degree of alignment). MSCs seeded within the composites oriented parallel to the axis of fiber alignment within 2 days of culture (**Figure 1**). Quantification of morphological characteristics revealed that cells oriented and spread less on the stiffest fibers compared to the softer alternatives, while the collagen controls had no preferential orientation. Confocal images revealed close association of the cells with the fibers (**Figure 2a**). Cells not in direct contact (100 µm and 200 µm above the fibers) with the fibers were also oriented in the direction of fiber alignment. Finally, cells on the softest fibers expression higher levels of collagen 1, tenomodulin, and scleraxis compared to stiffer fibers (**Figure 2b**).

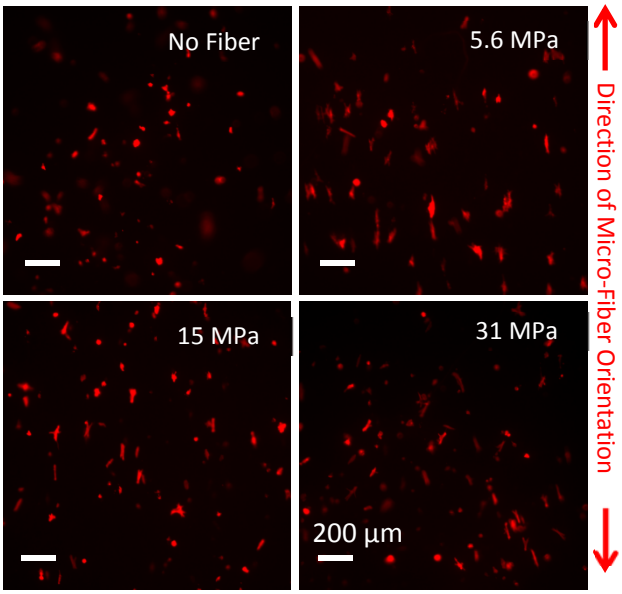

Figure 1. Effect of fiber stiffness on cell morphology. Cells were more oriented on the softer materials compared to the stiffer materials while those in collagen gels (no fiber) remained unoriented.

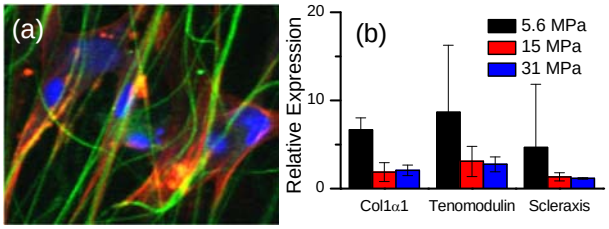

Figure 2. (a) Confocal images of MSCs (red F-actin and blue nuclei) attached to micro-fibers (green), and (b) mRNA expression by MSCs after 7 days of culture in micro-fiber/collagen composites.

Conclusions: In this study model micro-fiber/hydrogel composites were constructed to assess the impact of the microenvironment on MSC behavior. Cells migrated through the gels to attach and align to micro-fibers. In addition, variation of the elastic moduli of the micro-fibers influenced the morphology and phenotype of MSCs. Ongoing research is focusing on determining the response of MSCs to cyclic mechanical stimulation. Through careful control of fiber architecture and topography, this platform may have application in engineering a variety of soft tissues, including tendon, muscle, cardiac and vascular.