Cellular Deformations in Micro-integrated Elastomeric Electrospun Scaffolds under Biaxial Stretch

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Statement of Purpose: Vascular smooth muscle cells (SMCs) have been electrosprayed concurrently with electrospun biodegradable elastomeric poly (ester urethane) urea (PEUU).[1] The resulting cellularized scaffolds exhibit soft tissue-like elastomeric mechanical properties[2], and are thus promising candidates for repair or replacement of diseased cardiovascular tissues. Cellular deformations during in-vitro mechanical training will strongly influence the extracellular matrix formation. However, cellular deformations within intact polymer scaffolds are likely complex function of scaffold properties and cellular interactions with the surrounding polymer fibers. Our objective is therefore to quantify cell deformations in response to tissue-level scaffold stretch, using the nuclear aspect ratio as an index of overall cellular deformation. Knowledge of cell responses to mechanical stimulation can provide guidance to mechanical training protocols of the construct.

Methods: To make SMCs integrated PEUU scaffold, 1 x 10^7/ml SMCs were fed into a sterilized capillary charged at 10 kV and located 4 cm from the target mandrel. 12 wt% PEUU/HFIP solution was fed into a capillary charged at 13KV and located 24 cm from the target mandrel, which was charged at 4 kV and rotating at 250 rpm while translating 8 cm along its axis at 8 cm/s. After 30 min electrospinning, the microintegrated tube was taken away from the mandrel and then cultured in a spinning flask at rotation rate of 15 rpm. 12 mm x 12 mm square specimens were then cut and stained with DRAQ 5, a cell permeant and far red-fluorescing DNA probe for viable cells. With the capability of distinguishing different components and subsurface imaging, an inverted Laser Scanning Confocal Microscope (LSCM) was chosen to observe living cells and scaffold *in-situ*. A custom biaxial stretching device then incorporated into the LSCM. Test specimens were mounted on the biaxial stretcher with stainless steel hooks attached to flexible sutures. Graphite markers attached to the surface were used to monitor the tissue-level deformation. LSCM images were taken via a coverslip window below the specimen. Cell nuclear images were taken under CY5 channel and PEUU structure were imaged under CY3 channel via polymer autofluorescence. Cell nucleus aspect ratio and fiber structure were then analyzed using custom software.

Results/Discussion: LSCM images taken at ~15 μm depth showed that cells were embedded in subsurface of scaffold and distributed evenly (Fig. 1). Cell nuclei were found to have an average aspect ratio of 2.5. Under biaxial stretch, substantial cell deformation was observed. The aspect ratio of cell nucleus was found to increase to ~ 4.0 under an areal strain of 40% (Fig. 2). Corresponding fiber alignment increased with strain, while the fiber angular distribution remained relatively constant since the specimens were subjected to equi-biaxial strain (Fig. 3).

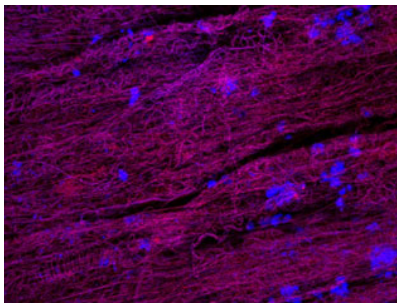

Figure 1. LSCM image of cell integrated scaffold.

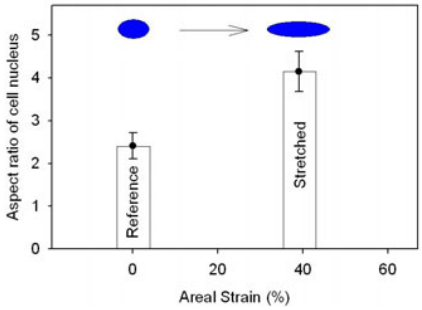

Figure 2. Aspect ratio of cell nucleus.

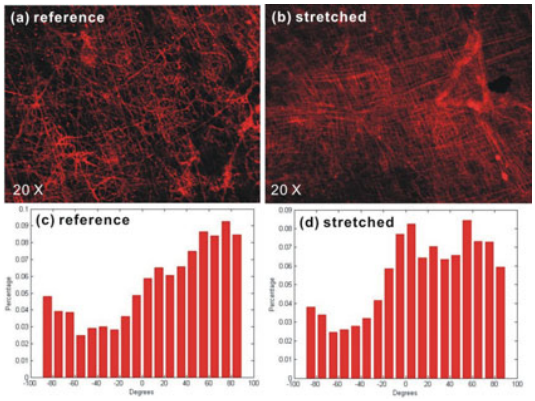

Figure 3: Fiber angular distribution analysis.

Conclusions: Utilizing biaxial stretch regimes and LSCM, we were able to simultaneously visualize the cell nuclear deformations and changes in fiber architecture under biaxial stretch, which enabled us to begin to establish a relationship between global material deformations, fiber structure, and cellular deformation.

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References: 1) Stankus et al., Biomaterials. 2006; 27: 735-44, 2) Courtney et al., Biomaterials, 27, 3631-38