Nanofiber Matrices for Tendon Augmentation: Effect of Cell Seeding and Extracellular Matrix Components on Proliferation and Tensile properties

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Statement of Purpose: These studies optimize a viable augmentation device for surgical repair of massive rotator cuff tears based on the combination of electrospun Poly (e-caprolactone) (PCL) and extracellular matrix (ECM) component nanofiber matrices. Current autografts and allografts have issues with availability, disease transmission (allografts), and mechanical properties (allograft). ECM based materials have shown favorable *in vivo* histology but do not posses the required tensile properties for immediate ambulation; a key factor in preventing adhesions and maximizing tendon regeneration. An alternative is to incorporate synthetic fibers into an ECM component matrix, creating a biomimetic connective tissue surface both in the form of electrospun microfibers and collagen self assembly nanofibers. The resulting bioactive scaffold would possess tunable, reproducible mechanical properties, be widely available and be inexpensive in contrast to current technology. The resulting PCL matrix displayed robust biocompatibility in regards to cell proliferation, which subsequently enhanced mechanical properties over four weeks of *in vitro* culture. Further, proliferation varied dependent on the tendon ECM components that were used for scaffold modification.

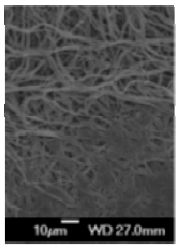

Figure 1: 400x SEM image of PCL scaffold seeded with rat tenocytes. Confluent cell front continuing under scaffold at 28 days of culture.

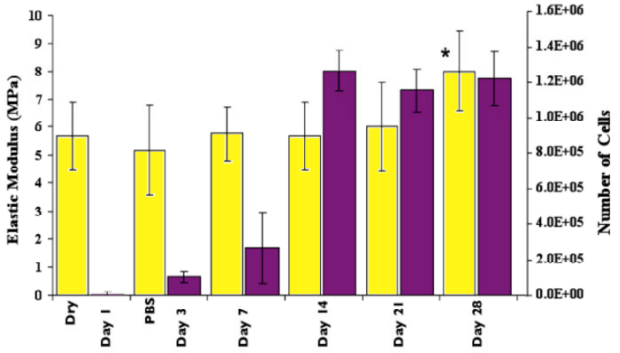

Figure 2: Cell proliferation (**purple**) and mechanical response (**yellow**) of primary rat tenocytes to PCL scaffold after 4 weeks of culture. Cell proliferation ceased at 14 days while remodeling of PCL matrix by cells resulted in improved tensile modulus at 28 days of culture.

Methods: PCL was electrospun to produce fiber matrices approximately 1 mm thick using optimized parameters to obtain bead free structures. ECM component surface modification was performed by incubating 0.1% (wt/v) collagen I, collagen I with fibronectin and collagen I with hyaluronic acid at 37^{O}C for 24 hours. Rat primary tenocytes were acquired from Achilles tendon explants dissected from freshly sacrificed male Sprague-Dawley rats. Sterile fiber matrices were incubated with 50,000 cells/scaffold for proliferation studies. Fiber matrices of 2 x 4 cm samples were seeded with 50,000 cells to measure tensile properties. Proliferation was assessed with the MTS assay. Tensile modulus was acquired with an Instron, exposing a 2 x 1 cm section between the Instron clamps according to ASTM standards.

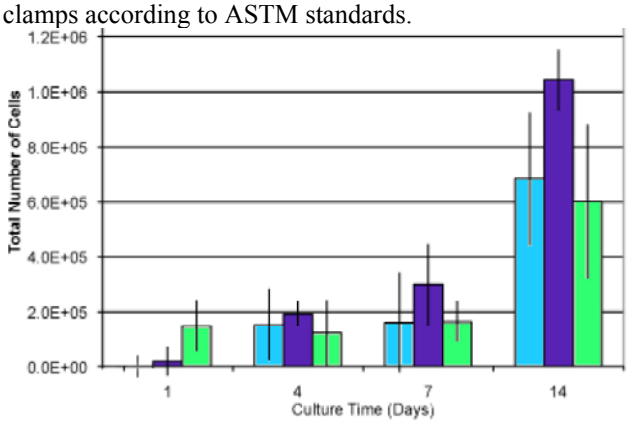

Figure 3: Proliferation of primary rat tenocytes on PCL matrices modified with collagen I (**blue**), collagen I/fibronectin (**purple**) and collagen I/hyaluronic acid (**green**). Addition of fibronectin improved cell proliferation on the PCL fiber matrices.

Results: PCL matrices supported progressive tenocyte growth, attaining confluency by day 14 (**Fig.2**). Tenocytes wrapped around the underside of the scaffold, demonstrating the aptitude of electrospun PCL fibers to support cell attachment and proliferation (**Fig.1**). Despite the plateau at day 14 there was evidence of cells depositing and modifying the extracellular matrix as tensile modulus measured from wet seeded samples significantly increased by day 28 (**Fig.2**). To improve cell adhesion, proliferation and differentiation PCL fiber matrices were modified with collagen I self-assembled networks alone or with the addition of fibronectin or hyaluronic acid, all ECM constituents that play active roles in tendon development and repair. The addition of fibronectin to self-assembled collagen fibers resulted in drastically faster proliferation through 14 days of culture (**Fig.3**), the time period in which tenocytes reach confluency under these culture conditions.

Conclusions: The PCL electrospun scaffold supported cell attachment and proliferation of tenocytes. Cell seeding improved scaffold mechanical properties, illustrating that cells were interacting with the scaffold in a tendon like manner. The improved mechanical properties highlights the importance of cell seeding for future *in vivo* studies. The combination of fibronectin and collagen self-assembled fibers on the scaffold resulted in enhance cell proliferation compared to other ECM components, suggestive of cells acquiring better cell attachment with this treatment. It is preferable for a clinically viable tendon augmentation device to be seeded during operation rather than being pre-cultured. Therefore enhancing cell attachment with the above components will improve the practical utilization of such a scaffold for massive rotator cuff tear augmentation with surgically extracted mesenchymal stem cells.