**The Influence of Organized Nanofiber Alignment on the Myotube Formation**

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**Statement of Purpose:** Current treatment options for restoring large skeletal muscle tissue defects due to trauma or tumor ablation are limited by the host muscle tissue availability and donor site morbidity of muscle flap implantation. Creation of implantable muscle tissue that could restore muscle function may be a possible solution [1,2]. To engineer functional muscle tissue for reconstruction, scaffolds that mimic native fibers need to be developed [3]. In this study we examined the feasibility of using poly(ε-caprolactone) (PCL)/collagen based nanofibers as a scaffold system. We investigated whether electrospun nanofibers could guide morphogenesis of skeletal muscle cells and enhance cellular organization.

**Methods:** Electrospun meshes were fabricated using a blend of PCL and collagen with the ratio of 1:1 in weight. The PCL/collagen solution (5% wt/vol, in HFP) was electrospun using a high voltage at 20 kV potential between the solution and the grounded surface. The solution was delivered with a syringe through a blunt needle at a flow rate of 3.0 mL/hr. Fibers were collected onto a grounded mandrel at a distance of 10 cm from the needle. The mandrel was rotated at different rotation rate to achieve different fiber orientations. Fiber morphologies of the meshes were examined by scanning electron microscope (SEM) and analyzed by ImageTool 3.0 software. The tensile properties were performed on the meshes with different fiber orientations in the parallel and perpendicular directions. Human skeletal muscle cells were isolated, grown, and seeded onto the aligned PCL/collagen meshes. Cell adhesion, proliferation, alignment, and differentiation were analyzed.

**Results:** The electrospun PCL/collagen nanofiber meshes showed homogenous fibers with an average diameter of approximately 300 nm. It was evident that the fiber orientation can be controlled by the rotation rate of mandrel, in which the aligned nanofibers were obtained at 2350 rpm (Figure 1). Fiber alignment had a profound effect on the mechanical properties of the meshes. The tensile strength of the aligned nanofiber meshes showed significant differences between parallel (4.9±0.2 MPa) and perpendicular (3.1±0.1 MPa) directions ($P < 0.05$).

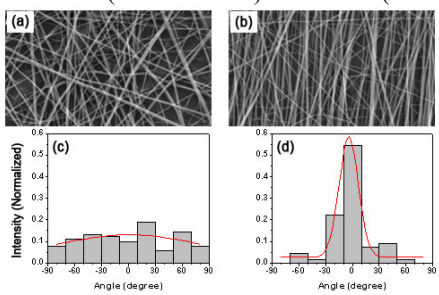


Figure 1. SEM images of (a) randomly oriented and (b) aligned nanofibers and fiber angles of (c) randomly oriented and (d) aligned nanofibers.

Human skeletal muscle cells were successfully isolated and cultured under high serum conditions where the cells continued to proliferate. When human skeletal muscle cells were seeded on the nanofiber meshes, aligned nanofibers significantly enhanced muscle cell alignment and myotube formation as compared to the randomly oriented nanofibers (Figure 2). Phenotypic expression of desmin, myosin heavy chain, and sarcomeric actin was confirmed on the nanofiber meshes after cell growth and myotube formation. The length of myotubes on the aligned nanofiebr meshes was more than twice length of myotubes on the randomly oriented meshes ($P < 0.05$), which support the hypothesis that aligned nanofibers promote myotube formation. However, the diameter of myotubes was not significantly different between the aligned and randomly oriented nanofiber meshes.

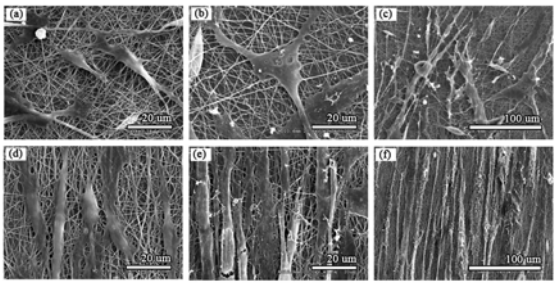


Figure 2. SEM images of human skeletal muscle cells on the electrospun PCL/collagen nanofiber meshes: (a-c) randomly oriented and (d-f) aligned nanofiber meshes, (a, d) 1 day and (b, e) 3 days after cell seeding and (c, f) 7 days after cell differentiation.

**Conclusions:** Electrospun PCL/collagen nanofiber meshes fabricated by electrospinning show a unidirectional fiber orientation**.** These results demonstrate that orientation of electrospun meshes can guide muscle cell alignment and enhance myotube formation. The aligned nanofiber scaffolds seeded with skeletal muscle cells may provide implantable functional muscle tissues for patients with large muscle defects.

**References:**

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