Fabrication of two and three-dimensional aligned nanofiber/skeletal muscle -like constructs

Vince Beachley,[1, 2] Xuejun Wen [1,2]
[1]Department of Bioengineering, Clemson University, Clemson, South Carolina, United States
[2]Department of Cell Biology, Medical University of South Carolina, Charleston, South Carolina, United States

Statement of Purpose: Three-dimensional tissue engineered constructs can be fabricated by seeding cells into a 3-dimensional matrix or by assembling cell containing components using techniques such as rapid prototyping with 1-dimensional cellular components or layer-by-layer assembly of 2-dimensional cellular components. One novel approach to fabricate 2-dimensional cellular components developed by Ishii et al.[1] utilized ultrathin (10um) randomly oriented electrospun nanofiber sheets collected on a 15mm diameter ring as a scaffold. Limitations to this technique include a relatively small surface area and a lack of control of fiber orientation. Nanofiber orientation becomes especially important in the case of aligned tissues such as nerve, muscle, tendon, and ligament because cells cultured on aligned topography adopt an aligned morphology. Electrospinning techniques developed in our laboratory [2] allow for the fabrication of ultrathin nanofiber sheets with controlled morphology and greatly increased surface areas (ex. 7x7cm). Ultrathin aligned polycaprolactone (PCL) nanofiber sheets were fabricated to allow for the formation of 2-dimensional cellular components with unidirectional alignment. 3-dimensional structures were fabricated from these components using layer-by-layer technologies.

Methods: Polycaprolactone (PCL, M_n=80,000, Sigma) was dissolved in 3:1 dichloromethane/ dimethylformamide (Sigma) at 20% w/v. The solutions were feed through a 21 gauge needle at 0.015-0.020 ml/min and a voltage of 8kV was applied to the needle tip to initiate electrospinning. DiI was added to the polymer solutions at a concentration of 0.075 mg/ml. Aligned fiber arrays were collected across a rack using a parallel mobile track device designed in our lab. Individual nanofiber sheet layers were fabricated on frames (Fig. 1A), sterilized, seeded with mouse myoblast cells, and cultured to confluence. Multilayered structures were easily fabricated by stacking the individual cell-nanofiber layers. Multilayered constructs were cultured in both standard (DMEM) and differentiation media (DMEM + 10% horse serum). The tensile strength of multilayer integrated cell-nanofiber constructs was evaluated using a Shimadzu EZ Graph tensile tester (Nakagyo-ku, Kyoto, Japan) with Trapezium 2.32 software. All constructs that were not mechanically tested were fixed in 4% paraformaldahyde. Cells were stained with AlexaFluor Phalloidin for actin filament, DAPI for cell nuclei, Live/Dead Cell Double Staining Kit for cell viability, myosin heavy chain and α-actinin antibodies to identify phenotype. Cell alignment was quantified from the shape of the cell using Image Pro.

Results: Highly aligned ultrathin loose PCL nanofiber sheets with dimensions up to 7x7cm were easily fabricated using our previously developed electrospinning techniques. Thickness could be estimated as the diameter of few nanofibers (diameter≈600nm). Fiber alignment was quantified with a resulting standard deviation of +/-6.3º. Myoblast cells seeded on these ultrathin nanofiber scaffolds attached, proliferated, and aligned in the direction of the fibers. Cell alignment was quantified with a resulting standard deviation of +/-8.7 º and the average direction of cell alignment was found to be within one degree of the average direction of fiber alignment. 3-dimensional multilayered structures of 1-20 layers were fabricated using layer-by-layer technologies. Myoblasts were able to differentiate into multinucleated myotubes within multilayered nanofiber/myoblast constructs and the mechanical strength of tissue-like constructs increased with increasing maturation time and increasing number of layers.

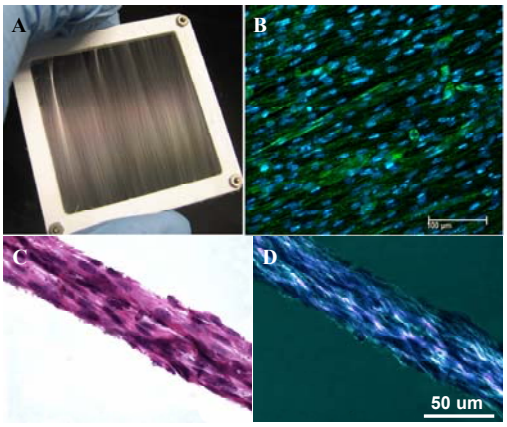

Fig. 1: (A) Ultra thin aligned PCL nanofiber scaffolds; (B) Aligned myoblasts on nanofiber scaffold; (C, D) Cross-section view of engineered skeletal muscle-like tissue based on several layers of highly aligned loose nanofibrous structure. (C) Bright field (HE staining). (D) DIC image showing the loose nanofiber layers.

Conclusions: Ultrathin highly aligned nanofiber sheets were able to support the attachment, proliferation, and differentiation of mouse myoblasts. Aligned topography of the nanofibers induced the alignment of myoblast cells. Individual 2-dimensional nanofiber/myoblast sheets could be easily combined into 3-dimensional constructs using layer-by-layer technologies because they were assembled while fixed at their edges. Increases in mechanical strength during cell maturation indicated the functionality of these constructs and highlights their potential as skeletal muscle grafts. Ultrathin nanofibers may possess several advantages as scaffolds for 2-dimensional cellular constructs, including promotion of cell alignment and ease of handling and assembly into multilayered structures.

References:

1.) Ishii et al. *J Thorac Cardiovasc Surg* 2005;130:1358-1363
2.) Beachley V WX. Society for Biomaterials Meeting 2007; Chicago, IL, USA