BIOL

Sphingosine 1-Phosphate Functionalized Nanopatterned Scaffolds for Engineering Vascularized Skeletal Muscle Tissue

Eve Byington
Biological and Environmental Engineering, Cornell University

NNIN REU Site: Washington Nanofabrication Facility & Molecular Analysis Facility, University of Washington, Seattle, WA
NNIN REU Principal Investigator: Dr. Deok-Ho Kim, Bioengineering, University of Washington
NNIN REU Mentor: Jonathan Tsui, Bioengineering, University of Washington
Contact: emb356@cornell.edu, deokho@uw.edu, jhtsui@gmail.com

Abstract:

Duchenne muscle dystrophy (DMD) is a genetic disorder that affects one in 3,600 males, leading to early death due to a lack of dystrophin in muscle tissue [1]. Implanted primary muscle cell patches have previously been shown to increase myogenesis and dystrophin expression in DMD mouse models [2]. This project involves cultivating muscle cells on biomimetic nanopatterned poly(lactic-co-glycolic acid) (PLGA) scaffolds that are fabricated using capillary force lithography. These scaffolds mimic topographical cues presented by the aligned collagen fibers of the extracellular microenvironment in skeletal muscle. Sphingosine 1-phosphate (S1P) is a circulating lipid metabolite known to promote angiogenesis, myoblast differentiation and satellite cell proliferation. By functionalizing the nanopatterned scaffolds with S1P, we hypothesize that the muscle tissue will be more mature and vascularized prior to implantation, therefore integrating better with the host tissue to ultimately improve function in dystrophic muscles. The optimum concentration of S1P will be determined using immunostaining and qRT-PCR data regarding myogenic, endothelial and neurogenic genes.

Introduction:

Duchenne muscle dystrophy (DMD) is the most common type of muscle dystrophy, affecting one in 3,600 males. The genetic disorder results from a mutation in dystrophin, which is integral to the structural stability of muscle tissue. Dystrophin forms a protein complex that connects muscle fibers to the extracellular matrix (ECM) via the cell membrane. DMD patients therefore suffer from muscle degeneration, fibrosis and early death—living an average of only 25 years.

Current treatments for DMD are mostly limited to palliative care. Attempts to directly inject stem cells or myoblasts into DMD patients' muscles have been largely unsuccessful, resulting in poor cell survival rates and low dispersion capabilities. Our proposed solution is to use implantable tissue patches to restore muscular function. These patches can provide long-lasting dystrophin expression, due to the presence of both mature muscle cells and satellite cells that provide a pluripotent cell reservoir. They also promote neovascularization due to the presence of endothelial cells, allowing the patches to integrate easily into the host tissue.

Engineering tissue requires a cell culture environment that is as close to the tissue's native microenvironment as possible. We used nanopatterning on the biodegradable polymer poly(lactic-co-glycolic acid) (PLGA) to mimic the collagen fibers present in the skeletal muscle ECM. The patterning—aligned ridges that are 800 nm wide and 600 nm in height—is similar to the dimensions and anisotropic topography of collagen fibrils (Figure 1). We also functionalized the scaffolds with the metabolite sphingosine 1-phosphate (S1P), which is known to promote angiogenesis, myoblast differentiation and satellite cell proliferation.

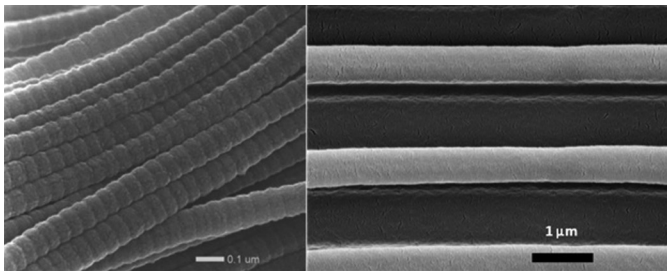

Figure 1: SEMs of collagen fibers on left (Dr. Claus Burkhardt, NMI, Reutlingen, Germany) compared to our scaffold on right, functionalized with 50 µM S1P.

Experimental Procedure:

The PLGA scaffolds were fabricated using solvent-assisted capillary force lithography (CFL). PLGA was dissolved in choloroform at 15% w/v and deposited on glass coverslips mounted on polydimethylsiloxane (PDMS) gel. PDMS is pressed onto the solution for five minutes to absorb the solvent. The film is then left open to air for five minutes on a hot plate at 120°C. A nanopatterned polyurethane-acrylate (PUA) mold is placed on top of the film and pressure is applied for 15 minutes. The CFL process is shown in Figure 2.

The coverslips are glued onto bottomless wells using NOA83H, which is cured in UV overnight. A solution of 10 μM Tris buffer and 3,4-dihydroxy-L-phenylalanine (DOPA) at 2 mg/mL, along with the appropriate concentration of S1P, was used to functionalize the scaffolds. Concentrations of S1P were 0 μM, 50 μM, 100 μM, 175 μM, and 250 μM respectively.

Primary mononuclear muscle cells were isolated from mice, and were seeded at 100,000 cells per scaffold. Each [S1P] group included flat and patterned scaffolds, and there was an additional control group seeded on tissue culture plates with no S1P. The cells were cultured for ten days.

Results and Conclusions:

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed to measure the relative quantities of marker genes for myogenic and endothelial differentiation. Pax7 was found to have a much higher expression on the nanopatterned scaffolds, indicating a larger population of satellite cells (Figure 3). Expression of MyoG, a marker for mature muscle cells, was also slightly higher on the nanopatterned scaffolds (Figure 3).

The expression of endothelial genes had a clear correlation with the concentration of S1P—both CD31, a marker for early endothelial differentiation, and eNOS, a marker for mature endothelial cells, were more highly expressed as the concentration of S1P on the scaffolds increased (Figure 4). This indicates that cells grown in the presence of S1P may have more angiogenic potential.

Future Work:

Data is still being analyzed for the neurogenic markers of the qRT-PCR, as well as the immunostaining results for myogenic, endothelial and neurogenic proteins. *In vivo* testing of the tissue patches in DMD mouse models is the next step, which will ultimately determine how viable this treatment is for restoring muscle function in those afflicted with DMD.

Acknowledgments:

Special thanks to my principal investigator Dr. Deok-Ho Kim and my mentor Jonathan Tsui, as well as Dr. KJ Janebodin, David Yama, Hyunsoo Lim, the NNIN REU staff and the University of Washington NTUF staff for the SEM imaging. Additionally I would like to thank the NNIN REU Program and the NSF for funding this work.

References:

[1] A.D.A.M. Medical Encyclopedia. "Duchenne muscle dystrophy." Atlanta (GA): A.D.A.M., Inc. (2005).

[2] Yang, H. S., et al. "Nanopatterned muscle cell patches for enhanced myogenesis and dystrophin expression in a mouse model of muscular dystrophy."Biomaterials, 35(5), 1478-1486 (2014).

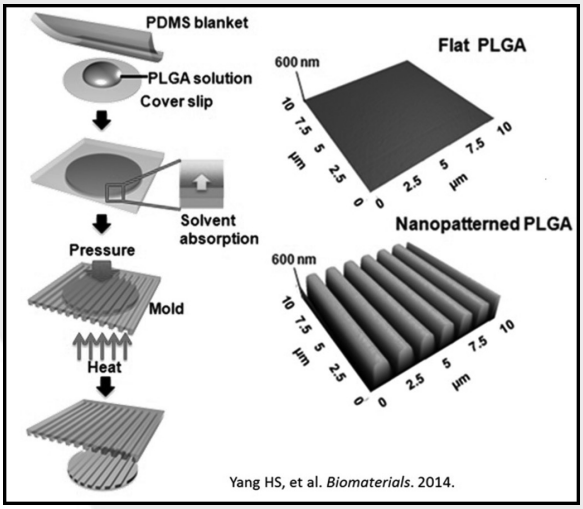

Figure 2: Fabrication technique of solvent-assisted capillary force lithography, and 3D renditions of the resulting flat and patterned films.

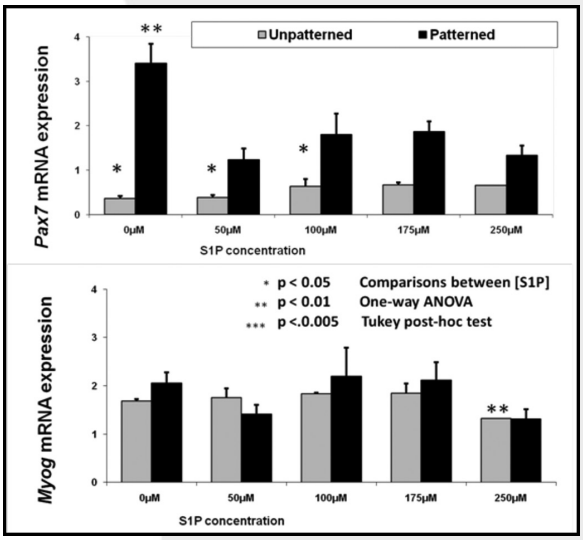

Figure 3: qRT-PCR for myogenic genes: Pax7, a marker for satellite cells, and MyoG, a marker for advanced muscle cell differentiation.

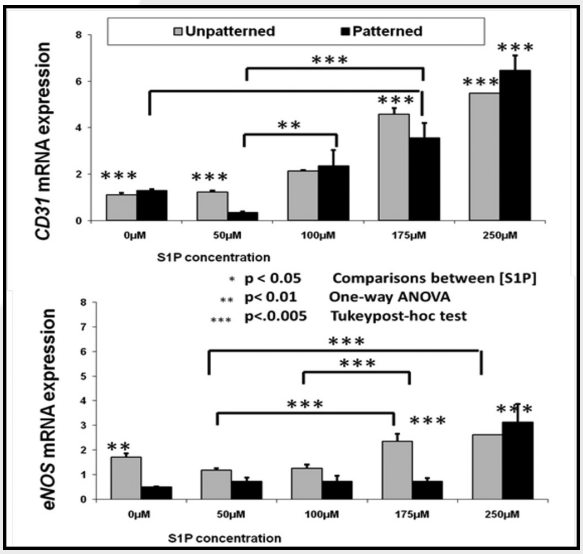

Figure 4: qRT-PCR for endothelial genes: CD31, expressed initially in differentiation, and eNOS, expressed later in differentiation.