Biomimetic Polymeric Scaffolds for Cell-based Skeletal Muscle Regeneration

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Introduction

- Satellite cells: a cell population that has remarkable regenerative capabilities and is essential for repair of minor muscle injuries [1].
- Natural regenerative mechanism (Figure 1) fails to be employed in conditions such as muscle degenerative diseases and volumetric muscle loss due to lack of satellite cell niche, the supportive microenvironment regulating cell behaviors [2].

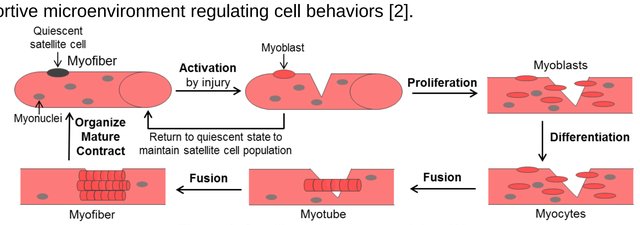

Figure 1: Satellite cell response to injury [2]

- Current cell-based strategies such as direct injection of myogenic cells are limited by the low survival and poor distribution of transplanted cells [3].
- **Objective:** To engineer an implantable biomaterial scaffold (Figure 2) that will provide a suitable microenvironment to support muscle cell function and promote muscle regeneration [4].

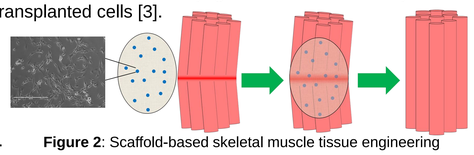

Figure 2: Scaffold-based skeletal muscle tissue engineering

Methods

- Scaffold Synthesis: Scaffolds were fabricated via electrospinning onto a plate (random fibers) or rotating drum (aligned) (Figure 3) [4].

Parameter	Condition
Polymer	Poly(lactic-*co*-glycolic acid) (PLGA 85:15), Mw ~94 kDa
Solvent	Tetrahydrofuran:dimethylformamide (3:1)
Concentration	20, 30, 40 w/v %
Rotation speed	0 rpm (plate), 1000 rpm (drum)
Field strength	1 kV/cm
Flow rate	2 mL/h

Table 1: Electrospinning parameters

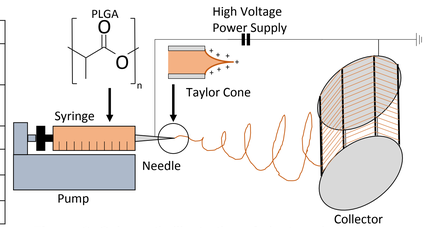

Figure 3: Schematic illustration of electrospinning

- Scaffold fiber morphology and structure were characterized by scanning electron microscopy (SEM) coupled with Image J.
- Cell Culture: Cells were cultured in media consisting of high glucose DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin Streptomycin (PS). Media was changed every other day.
- Cell Seeding: Scaffolds were placed in a cell crown insert and sterilized under UV light prior to seeding 10,000 cells/scaffold.
- Cell adhesion was analyzed after 2 hours and 24 hours of cell seeding by fluorescence microscopy with immunostaining.

Results

Electrospun Fibers

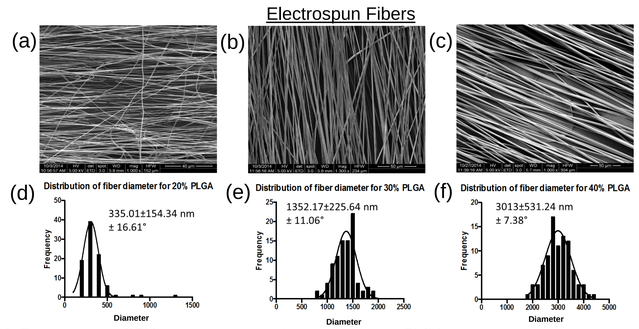

Figure 4: Representative SEM images and diameter distributions of PLGA fibers with different concentrations. (a and d) 20%, (b and e) 30%, and (c and f) 40% PLGA fibers. Fiber diameter increases with solution concentration, with a 10-fold increase (nanoscale to microscale) from lowest to highest concentration, and the rotating drum collector induces alignment.

Elongation and Organization on Random and Aligned Fibers

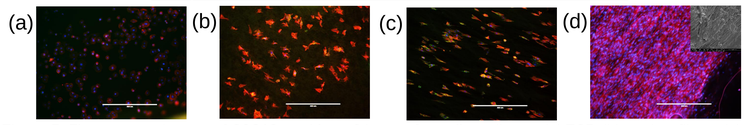

Figure 5: Fluorescence images of C2C12 myoblasts 2 hours after cell seeding on (a) TCP, (b) random fibers, (c) aligned fibers. (d) Representative cell alignment 24 hours after seeding (inset: SEM image). Red: cytoskeletal actin, Green: vinculin, and Blue: DAPI. While there is no preferred direction of growth on TCP and random fibers, cells spread along the direction of fiber orientation on aligned scaffolds indicating the effect of contact guidance.

Adhesion and Morphology on Varying Diameters

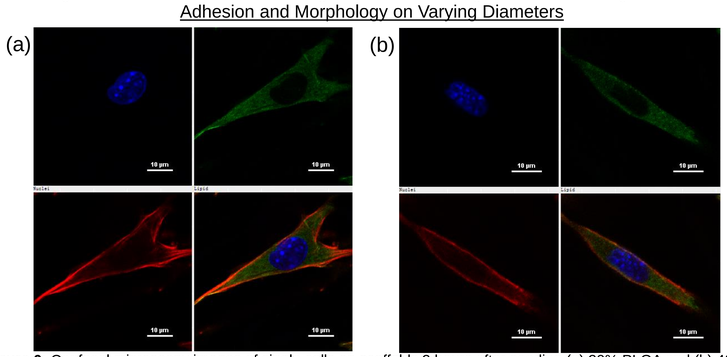

Figure 6: Confocal microscopy images of single cells on scaffolds 2 hours after seeding (a) 20% PLGA and (b) 40% PLGA. Red: cytoskeletal actin, Green: vinculin, and Blue: DAPI. Cells respond to the differences in fiber diameters by reorganizing their cytoskeleton indicating the cell sensing ability to substrate topographical cues.

Conclusions

- Aligned fiber scaffolds were achieved by electrospinning to mimic oriented muscle fibers constituting the natural cell microenvironment using a novel rotating collector.
- Fiber diameters ranging from the microscale to nanoscale were obtained by changing polymer solution concentration.
- Aligned fiber matrices supported cell alignment demonstrating the effect of contact guidance.
- These experiments provided critical insight on the role of scaffold topography governing cellular responses as well as design considerations for the development of an engineered cell niche that supports the regenerative response.

Future Directions

- To elucidate the cell-signaling pathway governing cell topography sensing.
- To optimize scaffold biophysical properties (e.g. substrate stiffness) to facilitate cell transplantation for muscle regeneration.

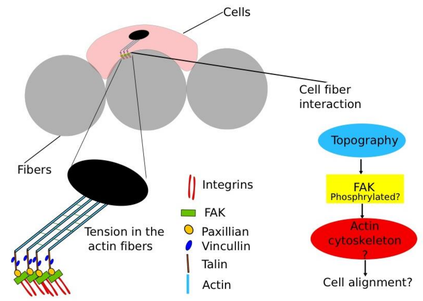

Figure 7: Cell-material interactions

References

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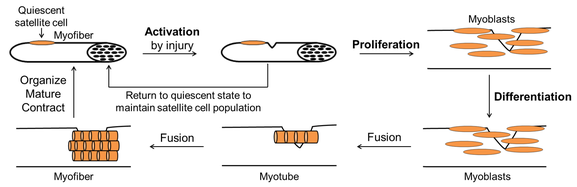
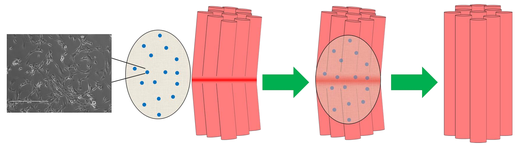
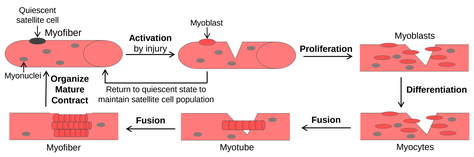

Quiescent satellite cell
Myofiber
Activation by injury
Proliferation
Myoblasts
Organize
Mature
Contract
Return to quiescent state to maintain satellite cell population
Differentiation
Fusion
Fusion
Myofiber
Myotube
Myoblasts

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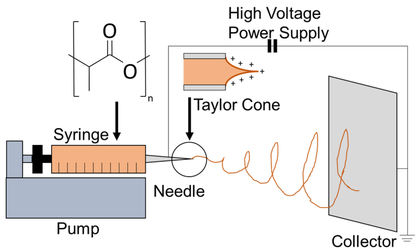
Myocytes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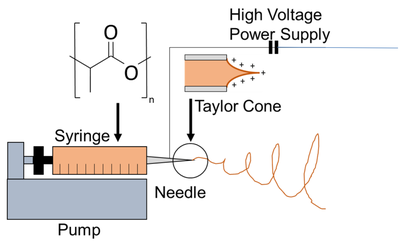
Taylor Cone
Syringe
Needle
Pump
Collector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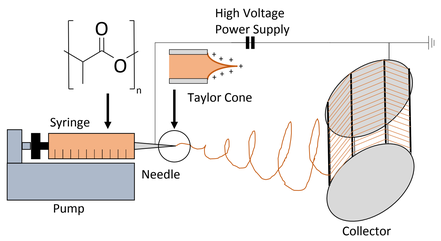
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