Spontaneous Alignment of Myotubes through Satellite Cell Migration

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Statement of Purpose: In large volume muscle injuries, muscle fibers as well as the surrounding connective tissue are damaged, preventing therapeutic muscle stem cells, satellite cells (SCs), from reaching the injury site and initiating repair [1]. There is a clinical need to rapidly fabricate in vitro muscle tissue constructs that mimic the native tissue organization, with aligned myotubes, for insertion and integration at the patient's injury site. Most strategies for myotube alignment require microfabricated structures or prolonged orientation times. We utilize the SC's natural propensity to close gaps across an injury site to guide alignment on an extracellular matrix (ECM) protein substrate. Through the binding affinity of biotin and streptavidin molecules, we pre-position SCs in straight-line patterns separated with small gaps, mimicking small-scale muscle injuries. This temporary positioning initiates the migratory nature of the SCs to align and form myotubes across the gaps. The rapid and highly specific binding of biotin-streptavidin allows for quick formation of temporary patterns, with SC alignment based on natural regenerative behavior rather than complex fabrication techniques.

Methods: All studies were completed on BioCoat collagen 1 coated slides (Corning, Corning, NY). The slides were first coated with BSA to add a protein layer with amine groups for biotin adhesion. EZ Link TFPA-PEG3-Biotin (Thermo Scientific, Waltham, MA), a UV reactive form of biotin, was added to the surface and covered with a chrome photomask that was etched with a pattern of 200 µm line widths and 800 µm spacing. The slide+photomask was placed under 1 mW/cm^2 UV light, creating a reactive biotin that bound to the surface amines only in optically activated regions. Cy3-Streptavidin was added to the surface to label the biotin surface. Human primary SCs were cultured in Hams-F12 media in 10 cm^2 Primaria culture flasks (Corning). The SCs were removed from culture and conjugated with EZ Link Sulfo-NHS-LC-Biotin (Thermo Scientific, Waltham, MA) via amino acids in the cell membrane. The biotinylated SCs were seeded on the patterned slides at 1.17x10^5 cells/cm^2. We developed a centrifugation washing technique to maximize cell removal outside of the patterned regions (weak adhesion) while minimizing cell removal within the patterned regions (strong adhesion). The slides were placed in a 4-well rectangular dish and centrifuged at 43.3 rcf in 1 minute increments using the centrifuge plate attachment. Data analysis of cell adhesion was completed using a Hoechst nuclear stain and ImageJ cell counting. Cell viability was verified using calcein and ethidium assays. Once clear cell patterns were achieved, the slides were placed in SC differentiation media (DMEM + 2% Horse Serum) for 8 days. Samples were fixed with formaldehyde buffer and myosin heavy chain immunohistochemistry (IHC) confirmed myotube formation. ImageJ directionality was used to study the alignment of myotubes relative to the original patterns.

Results: Fluorescent microscopy verified successful biotin-streptavidin patterns conjugated on collagen 1. After biotinylated cells were seeded on the surface and weakly adhered cells were removed through centrifugation, there was a significant difference in the cells/cm^2 in the patterned regions of the sample compared to the unpatterned regions ($p<0.05$). Centrifugation provided an easily reproducible method for removing these weakly bound cells. After 8 days in differentiation media, SCs in the patterned structures migrated from their original position into the gaps and formed myotubes.

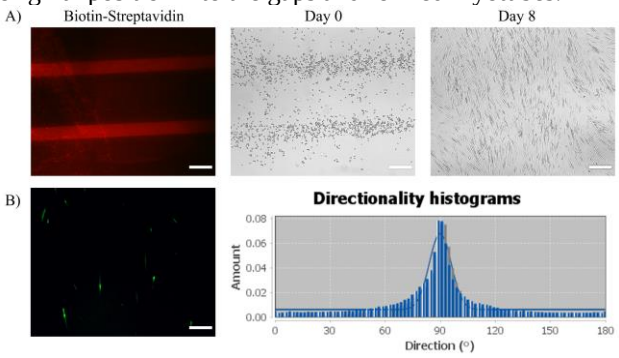

Figure 1. A) Biotin-Streptavidin and SC patterns on collagen 1 after initial seeding on Day 0 and after differentiation on Day 8. B) Myotubes stained for myosin heavy chain and their directionality after differentiation on Day 8. Scale bars represent 200 µm.

During differentiation, there was a directional component to the SC migration perpendicular (90°) to the original biotin-streptavidin surface patterns. Cells migrated into the spaces between patterns towards other cells but did not migrate in directions where no cells were present. Myosin heavy chain IHC demonstrated that cell biotinylation, seeding, and centrifugation did not impact viability or long-term functionality. Myotubes formed from biotinylated SCs seeded on a patterned surface were not significantly different ($p>0.05$) from unmodified SCs seeded on unmodified collagen (control) based on myotube length or number of nuclei per myotube.

Conclusion: Myotube alignment can be achieved by utilizing the SC's innate regenerative response. Biotin and streptavidin provide a rapid means of positioning SCs in temporary structures with intentional spacings. These spacings create a small-scale injury model that initiate the SCs to migrate over the gaps and form myotubes. Subsequent studies will focus on live tracking of SCs as they migrate from the initial patterns and form myotubes to analyze the cause of this directional behavior.

References: [1] Grasman JM. Acta Biomater. 2015;25:2-15.