Myoblast Morphology and Differentiation on Topography with Model Chemistry and Chemical Patterns

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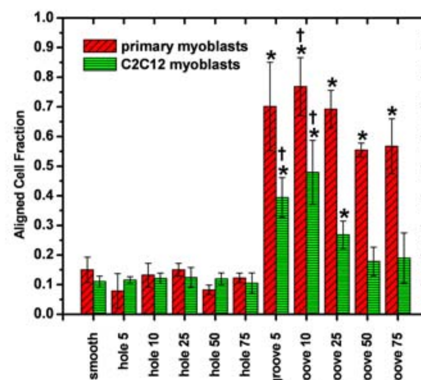
Statement of Purpose: Biomaterial surfaces influence cell differentiation due to surface modifications created through topographical [1] and chemical [2] patterning. However, topographical patterning methods may simultaneously alter surface chemistry, resulting in cell response not strictly delegated to topography. In addition, chemical patterning influences differentiation through control of cell-spreading area, but the influence of patterns controlling 2-D cell shape and cell-cell contact on differentiation has not been thoroughly evaluated. This work studies the differentiation of myoblasts on topographically patterned substrates overlaid with a previously characterized model chemical layer and chemically patterned substrates that control cell shape and cell-cell contact.

Methods: For topographic substrates, hot-embossing created fields of either holes or grooves in polycarbonate, with feature dimensions varying within each substrate from 5-75 µm. Metallization of the surface enabled chemical functionalization with a SAM presenting methyl groups for fibronectin adsorption. The SAM provides a model chemical layer previously characterized for fibronectin adsorption and activity [3]. Myoblasts were cultured on the substrates under differentiation conditions, then fixed and stained for sarcomeric myosin as an indicator of differentiation. For chemical pattern substrates, micro-contact printing created bowtie-shaped patterns [4] of SAMs presenting methyl-terminated groups on smooth, gold-coated polycarbonate substrates. Spaces between the patterns were backfilled with a SAM that suppresses cell and protein adhesion, while the patterns readily adsorbed fibronectin. The bowtie patterns provide consistent spreading area for cells, with varying levels of contact between pairs of cells. Primary myoblasts were cultured on the patterns in differentiation conditions and stained for actin to show cytoskeletal spreading on the patterns.

Results/Discussion: Topography significantly influenced cell alignment, but did not influence differentiation. Holes did not influence myoblast alignment, while grooves modulated alignment in a groove-width dependent manner with maximum alignment occurring on 10 µm wide grooves as shown in Figure 1. In contrast, the percentage of myoblasts expressing sarcomeric myocin was similar on all topographic patterns. On chemical patterns, myoblasts exhibited altered morphology with limited levels of cell-cell contact due to the bowtie patterns as shown in Figure 2.

Conclusions: Topography, when combined with a characterized chemical model layer, modulated myoblast alignment in a groove-width dependent manner but did not significantly influence differentiation. Bowtie-shaped patterns controlled cell shape and influenced cell-cell contact. Future work will evaluate the impact of controlling cell shape and cell-cell contact on differentiation. These results provide further insight of higher-order cellular response to engineered biomaterial surfaces.



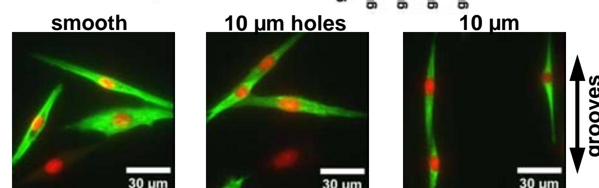


Figure 1: Grooves significantly influence myoblast alignment. Cells aligned dependent on feature shape and size, (means ± stdev, P <0.05, * vs smooth and hole, † vs groove 50 and groove 75).

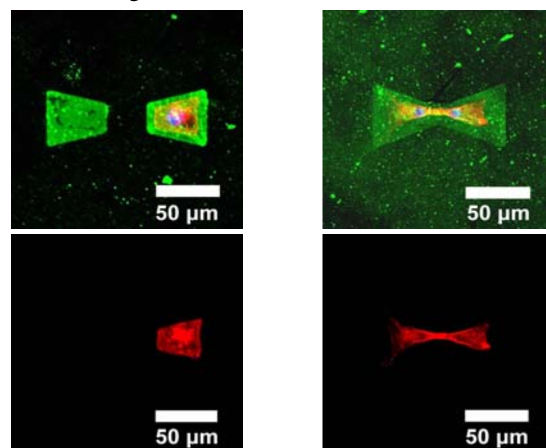


Figure 2: Bowtie patterns can control cell shape and cell-cell contact. Immunostained green fibronectin patterns, blue nuclei, and red actin show control of myoblast shape and cell-cell contact. Actin is shown separately in lower images for clarity.

References:

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[2]McBeath R. Dev Cell. 2004;6:483-95.
[3]Keselowsky B. J Biomed Mat Res. 2003;66A:247-59.
[4]Nelson CM. Mol Biol Cell. 2004;15:2943-53.