

Fabrication Patterned Substrates for Cell Alignment

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Abstract:

Microcontact printing (μ CP) is a soft lithography technique that allows for the controlled patterning of cells in an organized fashion. Here, we were interested in better understanding how surface geometry and tissue alignment affect myoblast differentiation and cellular contraction. To study this, we used Cornell's NanoScale Science & Technology Facility (CNF) to design and develop μ CP substrates containing microgrooved patterns with single-micron features that could promote tissue morphogenesis and cell alignment. These were fabricated using SU-8 photolithography to form patterned silicon wafer masters with the DWL2000 Mask Maker and ABM Contact Aligner. The wafer masters were then used to create PDMS stamps, which were applied to gelatin-coated acrylic sheets to generate grooved cell culture surfaces. Throughout the project, we explored several fabrication parameters, including SU-8 thickness, baking conditions, and UV exposure time, while troubleshooting issues such as incomplete photoresist coverage and mask adhesion during exposure. After establishing a reliable fabrication process, the patterned substrates were used to culture C_2C_{12} skeletal muscle cells, allowing the formation of aligned cells that grew parallel to the tissue patterning direction, suggesting that local topological cues play an important role in guiding cellular organization.

Summary of Research:

The goal of this project was to investigate how microscale surface geometry promotes muscle cell alignment. To create patterned cell culture substrates, we used photolithography techniques as available at the Cornell NanoScale Science & Technology Facility (CNF), fabricating silicon wafer masters containing parallel line patterns. These designs consisted of two patterned regions with 4 μ m line widths, 20 μ m spacing, and a 14 mm gap between the regions. The wafers were then fabricated using SU-8 photoresist, the DWL2000 Mask Maker, and the ABM Contact Aligner.

Briefly, to enable device fabrication, we investigated optimizing the photolithography process to allow for precise definition of micron scale features. Initial challenges included incomplete SU-8 coverage, manual edge bead removal, mask adhesion during exposure, and determining appropriate baking and UV exposure conditions. After iterating upon these parameters, we established a reliable process. This allowed for the consistent production of high-quality wafer masters, which could be used in the development of micropatterned tissue chips, enabling the controlled formation of aligned muscle tissues.

Gelatin tissue chips were then fabricated using PDMS micromolding. This allowed us to evaluate how microscale topologies could be used to guide cellular organization. Here, master wafers were used as molds to form PDMS stamps, which in turn could serve as repeatable, soft-lithographic molds for use in gelatin chip manufacture. Next, the PDMS stamps were pressed into gelatin-coated acrylic sheets to generate a micro patterned surface (Figure 1). These were then seeded with C_2C_{12} skeletal muscle cells, an immortalized mouse myoblast cell line. After culturing for fourteen days, C_2C_{12} formed aligned, multinucleated myotubes. As evidenced by brightfield microscopy, these showed that the cells elongated and aligned in the direction of the patterned substrates (Figure 2), demonstrating that surface patterning can provide effective cues for promoting skeletal muscle differentiation and alignment. These results suggest that engineered geometries can influence cell behavior and may serve as useful tools for studying tissue regeneration and cellular organization.

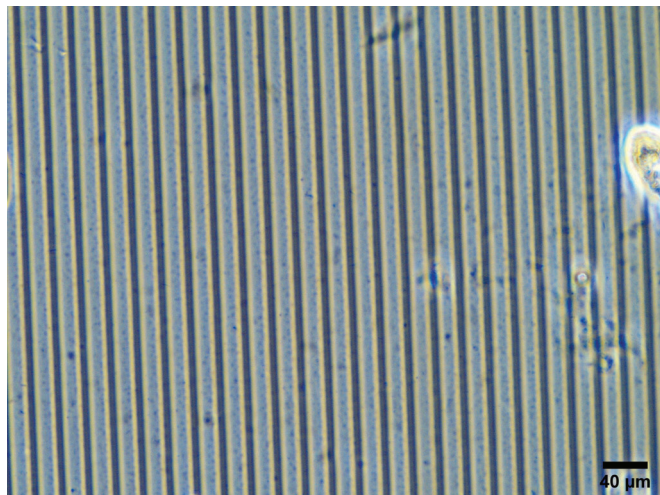


Figure 1: Gelatin micromold produced using a PDMS stamp derived from the SU-8 patterned silicon wafer. Parallel grooves with a line width of 4 μm and spacing of 20 μm were successfully transferred onto the gelatin surface.

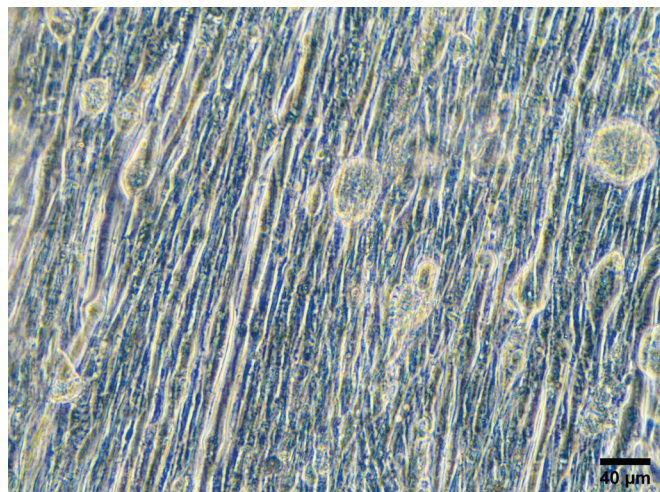


Figure 2: C_2C_{12} skeletal muscle cells cultured on the patterned gelatin substrate. Cells exhibit elongation and alignment parallel to the direction of the grooves, demonstrating that the engineered surface geometry provides effective guidance that influences cell organization.

Conclusions and Future Steps:

Collectively, this project successfully established a workflow for fabricating micropatterned substrates using CNF's photolithography equipment. Through multiple rounds of iteration, high-quality SU-8 wafer masters and PDMS stamps were produced. C_2C_{12} cells cultured on the patterned gelatin surfaces showed clear alignment along the pattern, demonstrating that surface geometry can effectively guide cellular organization.

Going forward, we hope to use these processes as a basis for developing geometrically controlled tissue chips to study how infarct geometry and tissue alignment impact action potential propagation in myoblasts following ischemic injury. By establishing a baseline photolithography process, we plan to use this as a platform for varying further construct parameters, including pattern spacing, relative alignment, and overall geometric patterning. By doing this, we hope to better understand how these features impact emergent cell behaviors, such as tissue conductance, syncytium formation and myoblast differentiation. In using this approach, our goal is to better understand how these basic structure-function relationships can give rise to aberrant physiological behaviors following ischemic injury.