Development of PDMS Microstructures for the Investigation of Cardiac Cell Function

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Abstract and Introduction:

Polydimethylsiloxane (PDMS) is a silicone-based polymer that may serve as a flexible substrate for cell culture. The goal of this project was to microfabricate PDMS structures to study contractile forces and intracellular organization of cardiac cells. Mutations in the nuclear envelope proteins lamin A/C cause approximately 10% of inherited cases of dilated cardiomyopathy, a disease responsible for a third of all heart failures. By comparing lamin mutant and healthy cells, we can develop a better understanding of how the mutations affect cellular function, gain new insights into the origin of the disease, and identify potential treatment approaches.

We used soft lithography techniques to create thin, flexible PDMS micropillars for contractile force assessment of cardiac cells. When cells adhere to the tips of these pillars, their spontaneous contractions cause deflections in the pillars allowing for direct calculation of the contractile forces generated by the cells [1]. The deflection, δ, can be used to calculate the applied contractile force, F, using the equation $F = 3EI\delta/L^3$ where E, I, and L represent Young's modulus, moment of inertia, and length of pillar, respectively [1].

In addition, we used PDMS surfaces with equally spaced ridges to assess the organization of cells and their cytoskeleton grown on lined substrates. Because cardiac cell function depends of the organization of the cytoskeleton and previous reports had shown that lamin mutant cells have defects in mechanosensing, we were interested in using the linear ridge substrates to determine how nuclear mutations affect cytoskeletal organization [2].

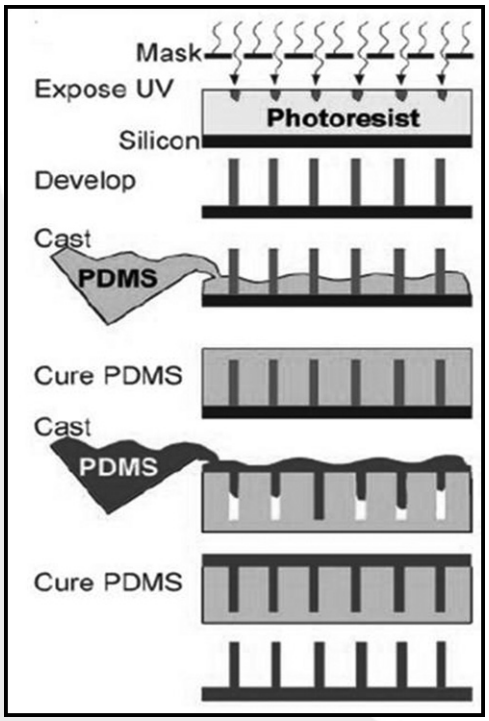

Figure 1: Overview of device fabrication process [1].

For the initial studies, we cultured human and mouse fibroblasts on these two microfabricated device types, pillars and linear ridges, for preliminary testing and imaging by fluorescent microscopy.

Experimental Procedure:

Micropillar and linear microridge devices were fabricated using photolithography and SU-8 negative photoresist spun onto a silicon wafer with a thickness of 11 μm for the micropillars and 3 μm for the linear ridges. A negative PDMS mold was cast and cured from the SU-8 devices (Figure 1) [1]. This double molding approach was employed to avoid adhesion of PDMS features inside of SU-8 features during the casting and curing process. Substrates were coated with silane between each molding steps to prevent adhesion. Finally, a drop of PDMS was placed on a glass slide, onto which the second mold was applied, and cured for 22 hours. The final devices were then carefully unmolded to obtain thin PDMS pillars and ridges.

Once fabrication was completed, the devices were prepared for fibroblast cell cultures. Fibronectin, an extracellular matrix protein, was micro-contact printed onto the micropillars to encourage cell growth on the tops of the pillars. These devices were incubated in a 0.02 g/mL Pluronic® F-127 solution to inhibit cell adhesion on the sides of the pillars and beneath the pillars. Linear ridge devices were incubated in a 50 μg/mL fibronectin solution to obtain an even coating and encourage

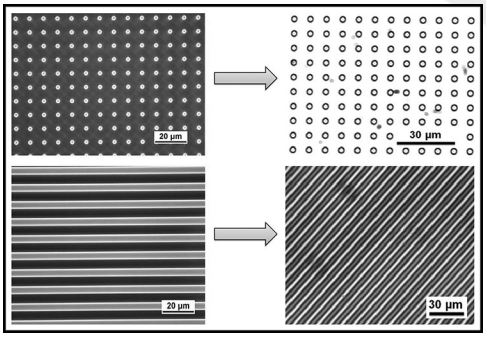

Figure 2: Optical microscope images of micropillars and linear microridges in SU-8 (left) and PDMS (right).

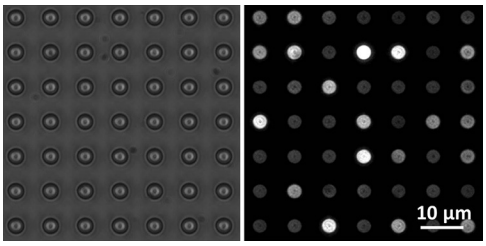

Figure 3: Micropillars stamped with fluorescent dextran to validate µCP.

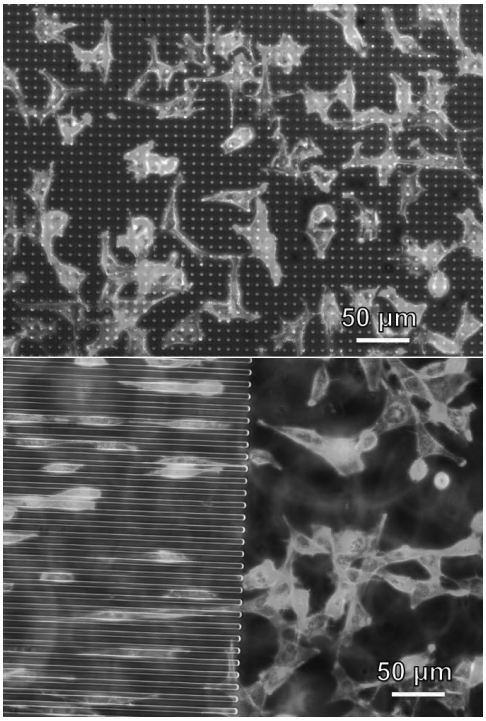

Figure 4: Fibroblasts grown on micropillars (top) and linear microridges (bottom) stained for DNA and F-actin. (See full color version on page xxxvi.)

fibroblast growth over the entire device. Fibroblasts were cultured on the devices for 24 hours, fixed, and then fluorescently stained for imaging.

Results and Conclusions:

The final PDMS micropillars were 11 μm tall, 2 μm in diameter and had a 9 μm center-to-center distance. Ridges were 3 μm tall and 5 μm wide with 5 μm spacing between ridges. Initial microfabrication problems with adherence of the SU-8 pillars to the silicon wafer were overcome by incorporating a flat SU-8 adherence layer and the mask design was altered to allow sufficient pillar-to-pillar distance to prevent adherence of the tall flexible pillars to themselves. SU-8 features were replicated onto PDMS molds (Figure 2), and silanization between molding steps effectively prevented adhesion of PDMS to substrate during curing process. Micro-contact printing (μCP) techniques were verified by stamping fluorescent dextran onto pillar tops and visualization by confocal microscopy (Figure 3).

Cells successfully grew on and adhered to both device types (Figure 4); however, we were unable to observe obvious deflections of the pillars. Based on the equation above, a contractile force of approximately 15.5 nN is required to cause a 1 μm deflection in pillars with a height of 11 μm. The pillars may have been too stiff to see a deflection from the few nanoNewtons of forces generated by the fibroblasts in comparison to the myocytes. Another possible reason for the absence of noticeable pillar deflection is cell adherence between the pillars, rather than on top. PDMS devices may be optimized by improving cell adhesion to the top of the pillars, i.e. by soaking in Pluronic for longer or finding a better cell-repellent.

Linear ridge device images confirmed that fibroblasts aligned with the linear ridges on the substrate. In contrast, the flat PDMS surface surrounding the linear device showed cells growing in random orientations with no particular pattern. Thus, the PDMS devices worked well in providing a substrate suitable for analysis of cell function specifically aligning cells in the predicted direction.

Future Work:

Once the experimental procedures and design for the micropillar substrate are optimized, devices will be seeded with cardiac myocytes so that they can be used to compare contractile forces between healthy and lamin mutant cardiac myocytes. Linear ridges will be used to assess variations in cytoskeletal and nuclear organization in the mutant and wild-type cells. Taken together, these devices will help us to develop a better understanding of the diseases caused by mutations in the nuclear envelope proteins lamin A/C in cardiac myocytes.

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References:

[1] Tan, J, et al. PNAS. 100. 1484-1489. 2002.

[2] Bray, M, et al. Biomaterials. 31. 5241-5150. 2010.