Micropatterns and PDMS Microdevices for the Investigation of Cardiac Muscle Cell Structure and Function

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Abstract and Introduction:

Heart disease is the leading cause of death in the United States, responsible for an estimated 787,000 deaths in 2011 [1]. Genetic mutations are the cause of up to one-third of cases of dilated cardiomyopathy; mutations in the gene encoding lamin A/C alone accounts for 10% of inherited cases. Lamin A/C is an important component of the nuclear envelope: these proteins are essential for many cellular functions, such as preservation of the nucleus' shape, DNA replication, regulation of transcription, and chromatin organization [2]. One hypothesis is that these mutations interfere with heart muscle cell organization and/or the exertion of contractile forces, which are essential for these cells to properly function. In this project we designed and built polydimethylsiloxane (PDMS) devices to (a) examine the organization of the cytoskeleton and (b) assess the contractile forces of healthy and lamin A/C mutant human cardiac cells. By developing these two devices for the examination of cardiac cell functions, we hope to understand the role lamin A/C has on dilated cardiomyopathy.

Experiment Procedure:

The first device consisted of PDMS stamps that were used to pattern rectangular shapes of extracellular matrix proteins with various aspect ratios. The stamp design consisted of 10 mm × 10 mm squares containing rectangles with an area of 50 μm^2, but different aspect ratios, with rectangles spaced 200 μm apart. From the design, we fabricated a mask and prepared the molds for the stamps using SU-8 photolithography techniques. The resulting features on the wafer were rectangular pillars 20 μm tall. Figure 1 shows a schematic of the procedures used to produce the micropattern device.

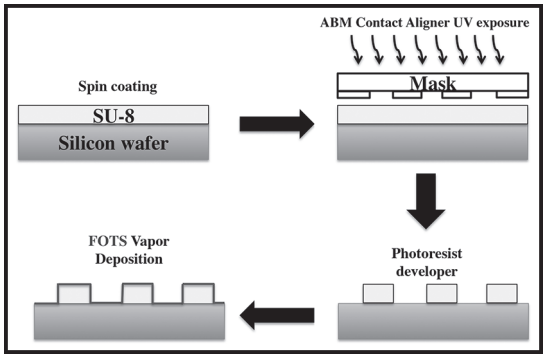

Figure 1: Overview of SU-8 photolithography used for the development of the micropattern device.

After microfabrication, molds were fabricated using PDMS. A 50 μg/mL solution of fibronectin, an extracellular matrix protein, was incubated on the PDMS stamps for an hour. The fibronectin-coated PDMS was placed into contact with a plasma cleaned glass coverslip to print rectangular fibronectin areas. The glass coverslips were then immersed in poly(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG), which deposited on the glass that was not covered in fibronectin and prevents cell adhesion, so that cells could only adhere to the rectangular fibronectin patterns.

The glass coverslips were prepared for tissue culture by incubating in antibiotic solution, and human fibroblasts were cultured and then observed on the microscope.

The second device consisted of flexible PDMS micropillars, ranging in sizes and center-to-center distances. As the cells exerted forces onto the pillars, the quantification of cardiac cell contractile forces could be determined from their deflections. A mask, consisting of circles with a 1 μm diameter and 2 μm pitch, was used to microfabricate the pillars using standard lithography techniques.

Hexamethyldisilizane (HMDS) and nLOF-2020 (negative photoresist) was spin-coated on a silicon wafer, which was then exposed on the Autostep, post-exposure baked and developed. The wafer was placed in the Oxford 80 and processed for oxygen descum (straightening the pillar edges) and then oxide-etched to remove the silicon wafer's native oxide. The wafer was placed in the deep reaction ion etcher to perform a Bosch process, followed by oxide stripping to remove the photoresist.

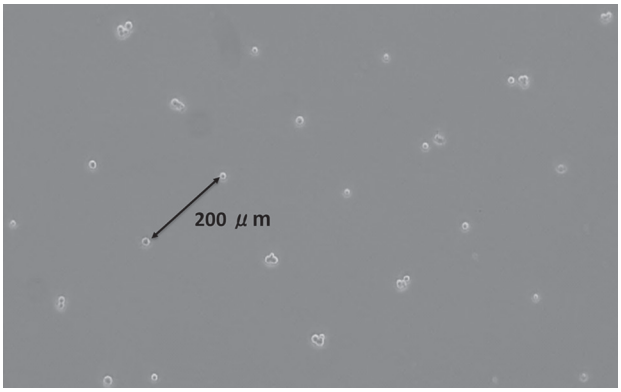

Figure 2: Bright-field contrast of the 70 × 35 μm² stamp, spaced at 200 μm, imaged at 5× objective.

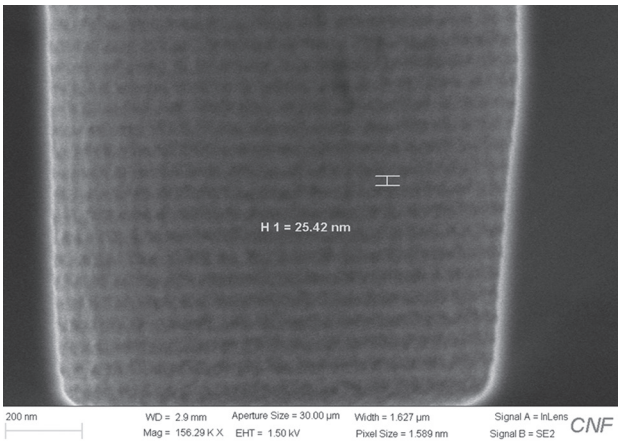

Figure 3: SEM image of the Bosch process with each scallop having a height of 25.42 nm.

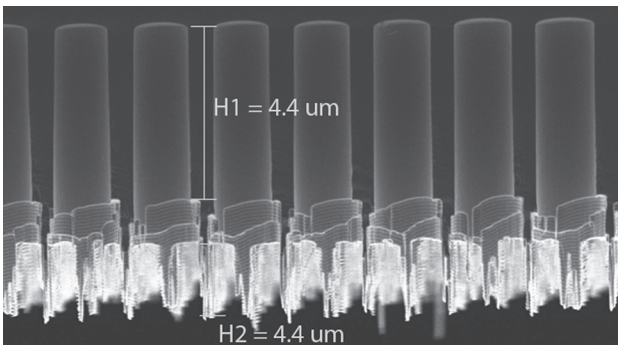

Figure 4: SEM image of the 1 μm, with a center-to-center pitch of 2 μm, device having a height of 4.44 μm. (See cover!)

Results and Conclusions:

The fabrication process for the micropattern device successfully created the desired height of 20 μm as well as obtaining PDMS stamps from the molds. This was verified by optical microscopy, where the molds had proper shape and size. Following the microfabrication process, the preliminary studies with human fibroblasts showed that the cells had adhered to the stamped patterns. The initial studies have shown that the application of a PDMS stamps with various patterns has the ability to control cell adhesion and placement. Figure 2 shows the bright-field contrast of cells adhering to a substrate patterned with the 70 × 35 μm² stamp, where (mostly) individual cells are being spaced 200 μm apart.

Initially, the micropillar device fabrication process had used similar SU-8 photolithography techniques as the micropattern device. However, this method resulted in cracked SU-8, as well as pillars floating off the device in the SU-8 developer. Thus, the method described in the experimental procedure was used. From the results, the device had successfully performed the Bosch process, where the pillars had scallops that were fairly linear, with an approximate scallop height of 25 nm as seen in Figure 3. However, issues arose in the deep reaction ion etcher process, causing the photoresist to burn, resulting in pillars not fully developing. Figure 4 shows that the pillar height was approximately 4.44 μm. Unfortunately, due to the burns, the pillar height did not meet the goal of 10 μm.

Future Work:

The micropattern device will be used to assess variations in cytoskeletal alignment between mutated and wild-type cardiac cells. The micropillar device is being finalized for the quantification of cellular force generation, where optimizing techniques being considered include a positive photoresist, changing feature sizes and the addition of an oxide layer prior to spin-coating HMDS and photoresist.

From the initial studies conducted, the fibroblasts adhered to micropattern stamps, while cardiomyoctes contracted on PDMS pillars. Using these devices, we will able to better understand the role of lamin mutations in dilated cardiomyopathy.

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References:

[1] Roger, V., et al. AHA Statistical Update. 2011.

[2] Fox, SI. Human Physiology 13th Edition 2011. McGraw-Hill Publishing. New York, New York.