Microcontact Patterning of Single Cardiomyocytes on Shape Memory Polymers

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Primary CNF Tools Used: ABM contact aligner, Heidelberg mask writer-DWL2000

Abstract:

Investigating the mechanisms of cardiomyocytes remodeling in response to the dynamic mechanical environment is critical for understanding heart disease progression. The aim of our project is to develop a dynamic platform using shape memory polymers (SMPs) and pattern single cardiomyocytes on SMPs to observe their structural and functional remodeling induced by dynamic mechanical stress. To constrain single cardiomyocytes in a predefined shape, extracellular matrix (ECM) proteins need to be deposited on SMPs at the scale of single cell. Making use of the tools at Cornell NanoScale Science and Technology Facility (CNF), we successfully generated rectangular and square patterns at single-cell resolution on SU-8 coated silicon wafers. Using these wafer molds, we fabricated PDMS stamps, and conducted microcontact printing to pattern single cardiomyocytes on the SMPs. Cardiomyocytes showed good confinement within the patterns before and after the application of dynamic mechanical stress.

Summary of Research:

Introduction. SMPs are smart materials that can undergo dynamic shape change stimulated by elevated temperature when they are programmed with internal strain [1]. Microcontact patterning is an efficient tool of controlling the shape of various cell types from tissue to single-cell level to study how the geometry confinement affects the cellular activities [2]. The combination of SMPs and microcontact patterning provides a platform for investigating remodeling of single cardiomyocytes to dynamic change of their geometry.

In this report, we focus on what we have achieved by microcontact patterning facilitated with CNF tools, which include a few steps: 1) design of a photomask with desired pattern shapes, 2) fabrication of Si wafers with opposite patterns by photolithography, 3) cure polydimethylsiloxane (PDMS) on top of wafers to make stamps and coat them with ECM proteins, 4) deposit ECM proteins though direct contact between stamps and SMPs, and 5) cell seeding on top of SMPs.

During this process, photomasks with patterns at high resolution and the efficient pattern transfer from masks to wafers ensure the perfect match of patterns between initial designs and final shapes.

Experimental Process. After estimating the areas of single cardiomyocytes, we designed rectangular and square patterns (areas: 1000 μm^2 and 2000 μm^2, aspect ratios: 1:1 and 1:3) with L-Edit software. Laser writing of photomasks was conducted by Heidelberg mask writer (DWL2000). After chrome etching and series washing steps, photomasks were ready to use. SU-8 50 photoresist was coated on top of a pre-cleaned Si wafer evenly with a photoresist spinner (spreading cycle: 500 rpm at 100 rpm per second for five seconds, spin cycle: 2000 rpm at 300 rpm per second for 30 seconds) to obtain a thickness of about 25 μm. After prebaking at 65°C for three minutes and soft baking at 95°C for seven minutes, the wafer was cooled down to room temperature on a flat surface.

Before exposure, light source with wavelength below 365 nm was filtered out to avoid overexposure of top portion of resist film. After switching on the ABM contact aligner, the photomask was vacuumed on the raised mask frame, and the wafer was vacuumed on the substrate chuck. The mask and wafer were brought into contact by adjusting the position of substrate chuck. After setting exposure timer, the light source was moved on top of the mask to crosslink SU-8.

To test the accurate exposure time for getting optimal patterns, different regions of the wafer were exposed for various time periods. Exposed wafers were post baked at 65°C for one minute and then 95°C for three minutes. Patterns on wafers should be visible after seconds of post bake. After cooled down, wafers were immersed and agitated in SU-8 developer for four minutes to wash off un-crosslinked SU-8. Developed wafers were rinsed with isopropyl alcohol (IPA) and dried with nitrogen. The quality of patterns on wafers was checked under microscope, and optimal patterns should exhibit strong adhesion to the Si substrate and same dimensions as initial designs.

PDMS was cured on top of wafers at 60°C overnight and then cut into small stamps with similar sizes to SMP samples. 20 μL of fluorescence-tagged fibronectin (100 μg per mL) was pipetted on PDMS stamps and incubated at 37°C for one hour. After transfer to an environmental control room with 50% humidity, the fibronectin residual on the stamp was wicked off with KimWipe. Then the SMP was pressed on top of the stamp with a 20 g weight for two minutes to allow fibronectin transfer from the stamp to the SMP. After blocking with 0.4 % Pluronic F-127, single cardiomyocytes were seeded on SMPs at 5×10^4 cells per milliliter. After cultured at 28°C for two days, the patterned cells were transferred to 37°C for 24 hours, and the elevated temperature triggered the shape change of patterns (Figures 1, 2).

Patterned single cardiomyocytes transformed from square to elongated shape (Figures 3, 4).

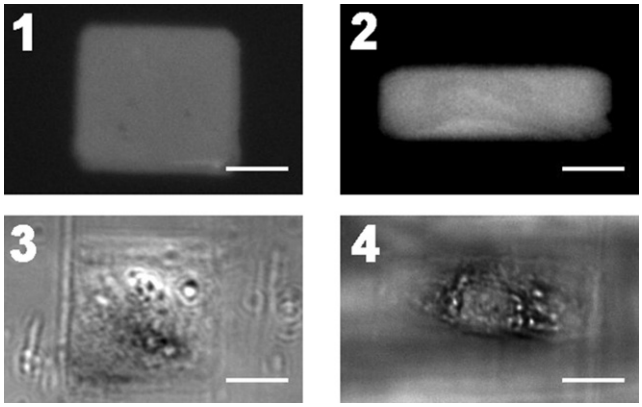

Figure 1: *Fibronectin pattern on SMPs before shape change. Scale bar: 20 μm.* ***Figure 2:*** *Fibronectin pattern on SMPs after shape change. Scale bar: 20 μm.* ***Figure 3:*** *Patterned single cardiomyocyte on SMPs before shape change. Scale bar: 20 μm.* ***Figure 4:*** *Patterned single cardiomyocyte on SMPs after shape change. Scale bar: 20 μm.*

Conclusion and Future Work:

In summary, we have fabricated Si wafers with single-cell scale patterns at CNF. We made PDMS stamps, and achieved efficient ECM protein transfer to SMPs through microcontact patterning.

We tested initial seeding of single cardiomyocytes on SMPs, and confirmed that they were elongated after triggering the shape change of SMPs. In future, we will run more cell seeding on our dynamic system to test the consistency of cell remodeling. The sarcomere organization and contractile functions will be quantified as evaluations of cellular responses.

References:

[1] Davis, K. A., Burke, K. A., Mather, P. T. and Henderson, J. H. Dynamic cell behavior on shape memory polymer substrates. Biomaterials 32, 2285-2293 (2011).

[2] Salick, M. R., et al. Micropattern width dependent sarcomere development in human ESC-derived cardiomyocytes. Biomaterials 35, 4454-4464 (2014).