Development of an *in vitro* Muscle Regeneration Model using a Combination of Microfluidics and Micropatterning

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Abstract:

Mutations in nuclear envelope proteins that connect the cell nucleus to the cytoskeleton cause muscular diseases [1]. While the exact disease mechanism remains unknown, it is thought that incorrect positioning of nuclei inside the muscle cell may play a critical role. The goal of this project was to develop a microfluidic device that enables imaging nuclear position during muscle development in cultured muscle cells under physiological conditions. Our device consisted of channels to provide culture media to the cells, channels for localized perfusion of agrin, and a micropatterned substrate to induce aligned muscle fibers. We designed several variations of the device based off of a recently published design [2]. We fabricated and tested these devices, which included designing systems of delivering medium and agrin to the cells. We conducted several pilot studies in order to validate the final device. The device will now be used in the Principal Investigator's laboratory to observe the maturation of mutant and normal muscle cells.

Introduction:

Muscle biopsies of dystrophy patients show abnormal nuclear positioning, but the exact relevance and mechanism of abnormal nuclear location remains unknown. In normal muscle development, single-nucleated myoblasts fuse to form a multi-nucleated myotube. Subsequently, these nuclei migrate to the periphery of the cell. Biopsies provide only a single image of this process. The goal of our project was to use microfluidics and micropatterning to create a device for time-lapse imaging of nuclear positions during maturation of mutant and normal muscle cells.

The main design considerations for our microfluidic device were to continually provide medium to the cells and to deliver agrin to a sub-region of the cells. Agrin stimulates a subset of nuclei, known as synaptic nuclei, to migrate to the neuromuscular junction, where the muscle cells interact with the nerve cell. In the final version of the design, cells will be plated on a micropatterned substrate to induce formation of linear muscle fibers. Figure 1 shows a schematic overview of the device.

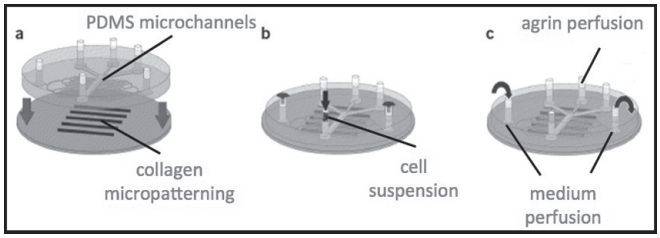

Figure 1: A schematic of the components of the device [2].

Experimental Procedure:

To create microfluidic features with different heights, we used a two-layer fabrication approach. The first layer was 25 μm thick; the second layer 250 μm thick. Figure 2 shows the design of each layer. The two-layer design allowed us to create channels of different heights for different flow rates within the same device. We created separate masks for each wafer using the Heidelberg.

We spin-coated the first layer of SU-8 2015 onto our wafer and baked it. We exposed our wafer to ultraviolet light through the first mask using the ABM contact aligner, followed by another bake. We developed only the alignment marks with SU-8 developer and then placed a piece of tape over each mark. Next, we added a layer of SU-8 2075 by spin-coating. We removed the tape, pulling the SU-8 off the alignment marks. We then baked and exposed the wafer using the second mask, this time fully developing the entire wafer. Subsequently, we deposited a layer of fluoroctatrichlorosilane (FOTS) on the wafer using molecular vapor deposition to prevent stiction of polydimethylsiloxane (PDMS). We then created our devices by casting PDMS over the wafer. After curing, the PDMS devices were removed from the wafer and bonded to glass slides by activating both the PDMS and the glass slide with oxygen plasma and placing them together.

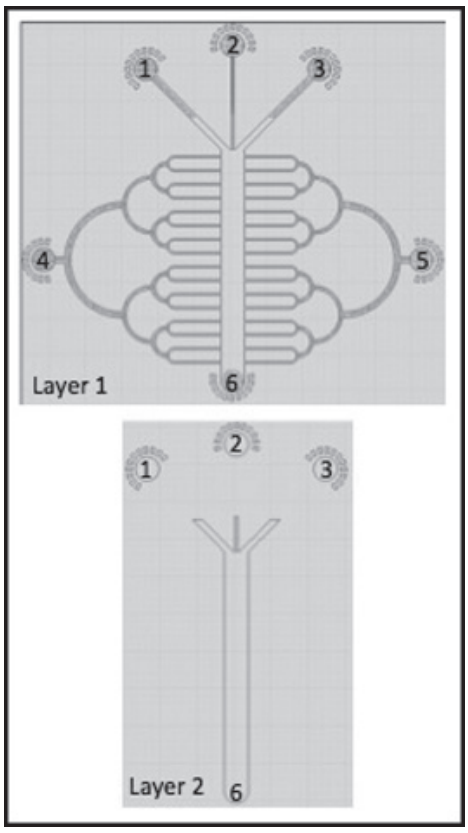

Figure 2: The design for each layer of the device.

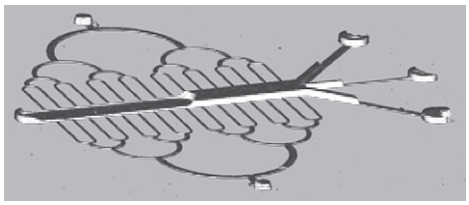

Figure 3: 3D reconstruction from confocal image stack of microfluidic device filled with fluorescently-labeled dextran to visualize the microfluidic system.

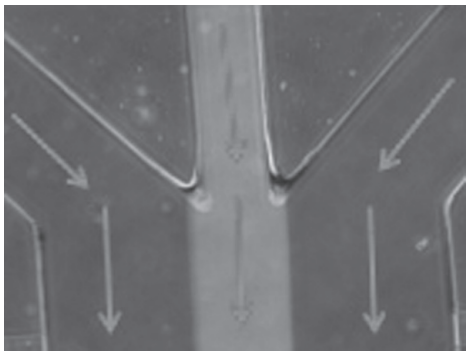

Figure 4: Dextran and sheath fluid are perfused through the device using a syringe pump.

Inlets 1 and 3 were connected to reservoirs of sheath fluid, which helped control the confined delivery of dextran. Inlet 6 was connected to a syringe pump, which aspirated the dextran and sheath fluid from the device. Both systems were validated using food coloring and dextran (Figure 4).

The technique for providing medium to the cells successfully supplied a sufficiently slow rate to prevent applying excess shear stress to the cells. We had the most success with the devices in which the second layer also contained the channel connected to the agrin outlet. This design cut down significantly on air bubbles. In conclusion, the devices performed as desired and are now ready for the seeding muscle cells.

Future Work:

After our successful pilot studies and validation, the microfluidic device will now be used in the Lammerding laboratory to study the effect of disease-causing mutation on nuclear positioning in muscle cells. The micropatterning technique will be optimized and then combined with the microfluidics device.

Results and Conclusions:

We fabricated devices using a few variations of our design with different widths and heights of the channels. We successfully created the two-layer wafers and produced PDMS replicas. We tested the complete connection of the channels on the devices by perfusing fluorescently-labeled dextran through the channels. Figure 3 shows a 3D rendering of this test.

We also evaluated different approaches of providing medium to the cells and locally delivering agrin. During our tests, a reservoir of food coloring (simulating medium) was placed approximately one foot above the device and connected to inlet 5 (refer to Figure 2). Hydrostatic pressure alone pushed medium through the device and out inlet 4. For our agrin system, inlet 2 was connected to a reservoir of fluoresecently-labeled dextran (simulating an agrin suspension).

Acknowledgments:

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References:

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[2] Tourovskaia, A., X. Figueroa-Masot, and A. Folch. Nature Protocols 1.3 (2006): 1092-104.