Novel Ridge Geometries for Use in Microfluidic Devices for Cell Sorting

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Abstract

As cell-based therapies continue to grow in popularity, advanced techniques are required to maintain consistent quality between patient samples. Accurate measurement and filtering based on cell viability will improve the efficacy of medicines and the replicability of the manufacturing process. The microfluidic devices used in this project sort cells by viability through exploitation of the flexibility and compressibility properties of cells. Cells of a singular type are suspended in a buffer solution and processed through the device's primary channel, where overhanging angled ridges sort the cells. The ridges sort the cells by deflecting stiff dead cells and allowing compliant cells to flow unaltered through the channel. Outlets collect the subpopulations for further study. The goal of this project is to determine the effect of altering the cross-sectional geometry of the ridges, rectangular up to this point, on the sorting effectiveness of the devices. Specifically, the desired geometries were triangular, trapezoidal, rounded, and concave. To make these devices, polydimethylsiloxane (PDMS) is cast into a mold made using the Photonic Professional *GT* from Nanoscribe. After the devices are prepped, their effectiveness at deflecting nonviable cells is tested.

Introduction

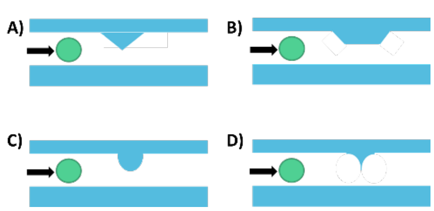

Figure 1

A) Triangular
B) Trapezoidal
C) Rounded
D) Concave

The Sulchek Research Group has been exploring the use of microfluidic devices to sort cells based on their viability. The devices are made by casting polydimethylsiloxane (PDMS) into a mold fabricated from SU-8, a permanent crosslinking polymer. Due to limitations of this method, the ridges of devices can only have a rectangular cross-sectional ridge geometry, which increases the flow resistance of the device. By changing the ridge geometry, such as to the ones shown in Figure 1, one can reduce the overall flow resistance of the device and possibly improve the device's capabilities. Since standard photolithography with SU-8 cannot make molds with these geometries, the Photonic Professional *GT* from Nanoscribe located in the Marcus Organic Cleanroom at Georgia Tech was used instead, as its two-photon lithography technology allows for the fabrication of virtually any ridge geometry on the micron scale. However, the IP-S resin used by the Nanoscribe is susceptible to degradation from solvents, of which trace amounts are found in PDMS. To mitigate this risk, the use of double casting to create a reusable mold from PDMS was explored.

Experimental Procedure

When using the Nanoscribe to fabricate the molds, the following conditions were used. The molds were printed on a 25 mm by 25 mm ITO glass slide from Nanoscribe using the 25X objective. A single drop of

IP-S resin is used for the fabrication with laser intensity set between 0.8 – 0.9 intensity. After the print is finished, the slide is submerged in SU-8 developer for 20 minutes and rinsed by submersion in isopropanol (IPA) for 2 minutes (consult cleanroom staff for general operating procedure).

Molds were also made using SU-8 photolithography for performance comparison. Molds were made on 4-inch silicon wafers in two layers using the Karl Suss Mask Aligner and custom exposure masks (consult cleanroom staff for mask fabrication procedure). Layer one constituted the gap height and was 5 microns thick (SU-8 2005, spun in two steps; Ramp: 3, RPM: 500, Dwell: 5, followed by Ramp: 5, RPM: 3000, Dwell: 40 followed by a 2 minute bake at 95 °C, a 105 mJ/cm^2 exposure and a 3 minute bake at 95 °C) and the second layer was 15 microns thick (SU-8 2010, spun in two steps; Ramp: 3, RPM: 500, Dwell: 5, followed by Ramp: 4, RPM: 1600, Dwell: 40 followed by a 3 minute bake at 95 °C, a 140 mJ/cm^2 exposure, a 4 minute bake at 95 °C, and 3.5 minutes of submersion in SU-8 developer). This process resulted in molds capable of making devices with a 5 micron gap height and a 20 micron channel depth, consult the SU-8 2000 series datasheet to alter the gap height and channel depth as desired [1].

With the mold prepared, a 10:1 silicone to curing agent solution of PDMS (aspirated for 20 minutes and all air bubbles removed) is poured over the mold and cured at 80 degree Celsius for a minimum of 1 hour, after which the device was extracted from the mold. If the extracted device was intended for use in double casting, the following procedure for alcohol passivation was used.

To passivate the device, it was placed in the plasma cleaner with the mold facing upwards. With the mold inside the cleaner, the vacuum pump on the cleaner would be turned on for a total of 10 minutes before turning on the plasma for 30 seconds. After removing the mold from the cleaner, it was submerged in 100% ethanol for 30 minutes, after which it was dried in the oven at 80 degrees Celsius for an additional 30 minutes [2]. Following the passivation, the mold is suitable to have PDMS cast into it.

Once the device was extracted from the mold, it was prepped for use by punching holes to access the inlet and outlet sites. The device and a glass slide were plasma cleaned for 40 seconds, after which the device was attached to the glass slide and placed in the oven at 80 degrees Celsius for 1 hour to complete the bonding process. With a bonded device, the experiment can begin as cells are introduced into the system via the central inlet while buffer fluid is added from the side inlets with cells being extracted from the outlet sites while a high-speed camera captures the experiment for later review.

Results and Conclusions

A mold with triangular ridges was fabricated using the Nanoscribe, and a device made from it showed deflection of non-viable SU-DHL-4 lymphoma cells during initial testing, behavior the cells did not show with rectangular ridges (Figure 2). Execution of the experiment revealed several issues to address going forward. First, the 9+ hour time of fabrication for the mold was reduced to 4 hours by removing the inlet and outlet pads and can be further reduced by increasing hatching distance and slicing size. Doing so decreases the resolution of the ridges, so

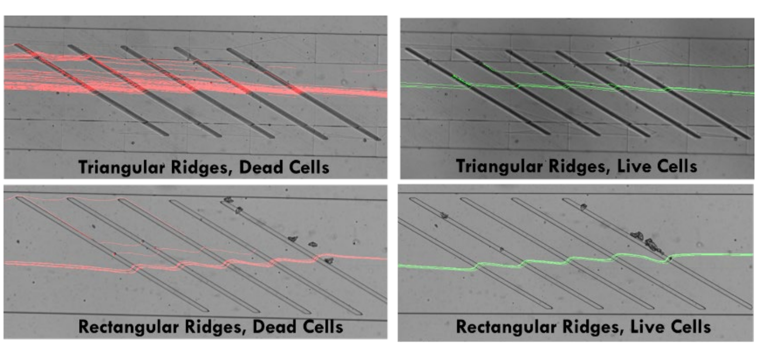

Figure 2: SU-DHL-4 Sorting Results

optimization will be required to determine the effect of this. Another issue was that the seams between the blocks of material laid down by the Nanoscribe blocked cells from deflecting further along the ridges. Further tuning of the printing parameters will be tested to decrease the seam size or redesign the ridges to fit within a single block of material. The passivation procedure requires more work to be viable, because while the first one or two devices cast from the passivated mold would show clean ridges, subsequent uses would result in severely deformed ridges. This suggests the mold needs to be passivated periodically to remain usable.

Future Work

In order to continue this avenue of research, the devices must undergo quantitative testing to determine their exact effectiveness at sorting cells, as the tests run this summer were only used to determine if deflection occurred at all. Molds with other ridge geometries such as trapezoidal, rounded, and concave will also need to be fabricated and undergo testing. Finally, the passivation process used during double casting requires refinement to be a viable technique.

[1] *SU-8 2000 Permanent Epoxy Negative Photoresist*. Microchem, microchem.com/pdf/SU-82000DataSheet2000_5thru2015Ver4.pdf.

[2] Kim, Sung-Hwan, et al. "PDMS Double Casting Method Enabled by Plasma Treatment and Alcohol Passivation." Sensors and Actuators B: Chemical, vol. 293, 15 Aug. 2019, pp. 115–121., doi:10.1016/j.snb.2019.04.145.