Design and Application of Microfluidic Devices to Study Cell Migration in Confined Environments

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Primary CNF Tools used: Heidelberg DWL 2000 mask writer, ABM contact aligner, SÜSS MA6 contact aligner, Plasma-Therm 770, Anatech SCE-110-RF resist stripper, Trion Minilock III ICP etcher, Tencor P-10 profilometer, MVD

Abstract:

For multicellular organisms, cell migration can act as a double-edged sword. While being vital for embryonic development, wound healing, and immune responses, wayward cells may also disrupt essential biological processes that can detrimentally affect the long-term survival of a life form. This is particularly true for the ability of metastatic cancer cells to translocate from the primary tumor, invade into surrounding tissues, and colonize distant organs. Metastatic cancer cells are able to penetrate through tight interstitial spaces of only 1-30 µm in diameter. The squeezing through such confined spaces places substantial physical stress on the cell nucleus, leading to nuclear envelope ruptures, chromatin herniation, and significant DNA damage. To study these processes in more detail, we created a microfluidic device that models the tight 3D constrictions that metastatic cancer cells may encounter during the metastatic process. The device gives us a high-throughput method for observing the short- and longer-term effects mechanically induced nuclear deformation has on the tumor cells. Originally, we constructed our intricate PDMS microfluidic devices from SU-8 molds, which lacked reliability and inconsistently reproduced the most critical features of our designs. Thus, we shifted the nanofabrication process to deep-reactive-ion etching (DRIE) and reactive-ion etching (RIE) of silicon. This revised approach has enabled us to improve the fidelity of our critical features, while also reducing the fabrication time and costs. The precision of silicon etching has opened doors for creating more complex microfluidic designs and other novel ideas. For example, we recently created a set of five devices that mimic different densities of the extracellular collagen fiber networks that form in many tissues. These devices are now finding use in the study of cancer cell migration and immune cell motility in confined spaces. We also have explored the use of fluorinated ethylene propylene (FEP Teflon®) as a substitute for PDMS molded devices. Nanoimprinted FEP has a reflective index near that of water and could give our devices the capability of super-resolution microscopy. Taken together, these examples illustrate new uses of the available nanofabrication technologies to create improved *in vitro* models to study cancer cell migration.

Summary of Research:

For decades, cell biologists have relied on two-dimensional (2D) migration assays for their convenience and seamless integration with many common imaging tools [1]. While being impactful in the field of cell biology, 2D studies are limiting for studying cancer cell migration [2], as they do not accurately depict how a cancer cell moves *in vivo* [3]. In order for cells to move throughout the body, they must navigate three-dimensional (3D) matrixes of different compositions, pore sizes and stiffnesses. Our goal was to design polydimethylsiloxane (PDMS) based microfluidic devices that recreate the confining 3D environment cells encounter *in vivo*, using approaches that provide precisely controlled and consistent geometries, and that enable high resolution imaging of the cells as they migrate through the devices [4]. These devices support a wide range of cell lines, and enable high-quality fluorescence imaging of nuclear lamina bucking, chromatin strain, DNA damage and nuclear rupture/blebbing and repair [4,5].

Over the past two years, we have explored alternate nanofabrication methods for the microfluidic migration devices. SU-8 was effective in creating these migration devices, but suffered from a few common fabrication issues. First, the reproducibility of the fine (~1 µm) features was difficult to achieve consistently, which led to an overuse of the few successful device wafers. The repeated molding and removal of PDMS would overtime weaken the SU-8/silicon substrate bond, eventually resulting in the delaminating of features and device failure. In order to reliably reproduce and preserve our most critical features, we decided to forgo SU-8 and instead etch the fine constrictions of the devices directly into the silicon substrate. We accomplished this "bottom-down" approach by using a negative photoresist mask and deep-reactive ion etching (DRIE). Not only were the critical dimension sizes reproduced with fidelity (Figure 1), but the fabrication time was cut in half.

Another advantage to this process was the ease of creating 1 µm features through contact lithography with photoresist compared to SU-8. With this in mind, we revisited some of our alternative migration device designs. One design that was now achievable with DRIE was creating large (~25 mm × 25 mm) arrays of randomly spaced 5 µm diameter circular pillars, mimicking collagen matrixes with different densities (Figure 2). While the

DRIE process successfully created the desired geometries, we noticed that the holes etched into the silicon wafer to mold the 5 µm PDMS pillars frequently contained remnants of the PDMS pillars after molding. We determined that the scalloped-sidewalls from the DRIE etch likely contributed to this problem, and that smoother sidewalls were required to facilitate removal of the PDMS replicas. To achieve this, we developed a reactive-ion etching (RIE) protocol, which was subsequently used for all device fabrication. The photonics etch on the Plasma-Therm Unaxis SLR 770 was chosen for its unique capability to produce smooth anisotropic sidewalls in silicon and compatibility with our original DRIE lithography. As another demonstration of the versatility of the new RIE approach, we created another migration design for the observation of single cells passing a precisely defined constriction (Figure 3). We highly recommend this process to other researchers aiming to produce microfluidic devices with smooth walls, feature heights of several micrometers, and width in the sub-micrometer range.

Another major breakthrough this past year was discovering a method to remove SU-8 features from silicon wafers, which enables reusing wafers when the SU-8 structures have become damaged after repeated use. The weakest point to any SU-8 device is its substrate adhesion, especially after repeated PDMS molding. For our migration devices, the silicon wafers contain the fine featured etched into the wafer using the RIE approach described above, and additional larger SU-8 features for the fluid handling. Typically, the wafers have to be discarded after any SU-8 component begins to delaminate from the wafer. To overcome this issue, we developed two techniques that can remove all the remaining SU-8 from our wafers while not affecting the fine photonics etch features. This approach enabled us to spin on a new layer of SU-8 and reuse the fine silicon etched wafer. Intriguingly, this process can also be used to produce and liftoff designs entirely made from SU-8 (Figure 4), which could find future use in the fabrication of small micrometer-sized parts.

Lastly, we investigated PDMS substitutes for molding our microfluidic devices for super-resolution applications that require imaging through media that matches the refractory index of water, i.e., n = 1.33 [6]. The cured PDMS typically used for microfluidic devices has a refractive index of 1.41 [7], which is not well suited for the lattice light sheet imaging. Instead, we chose to use fluorinated ethylene propylene (FEP) as an alternative material for the device fabrication. FEP is a highly transparent copolymer of hexafluoropropylene and tetrafluoroethylene with a refractive index of 1.334 (https://holscot.com/glossary/fep/), closely matching that of water. We were able to emboss thin FEP sheets with micrometer resolution and minimum damage to our existing molds with the Nanonex 2500 nanoimprint tool. The most accurate representations of our both DRIE and RIE master wafers were achieve slightly below the melting point of FEP (260°C - 265°C) (Figure 5). Ongoing work is focused on optimizing thermal or chemical bonding of FEP sheets to each other or to glass substrates to create complete microfluidic devices.

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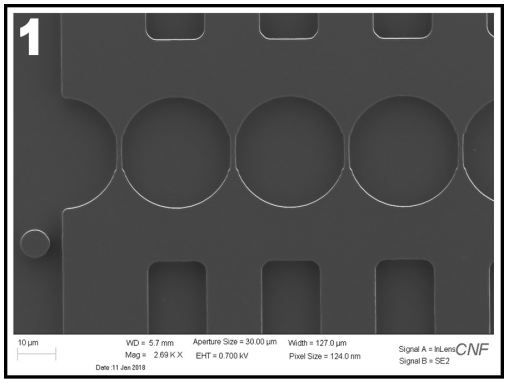

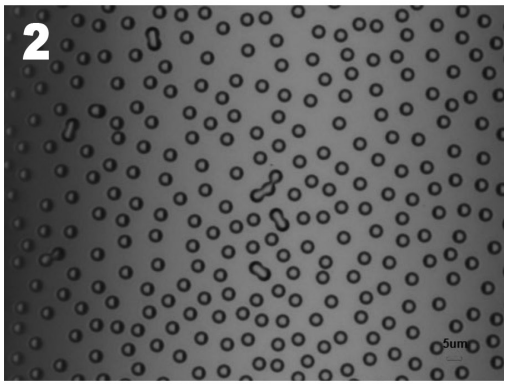

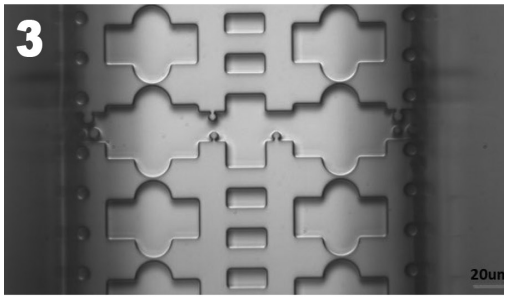

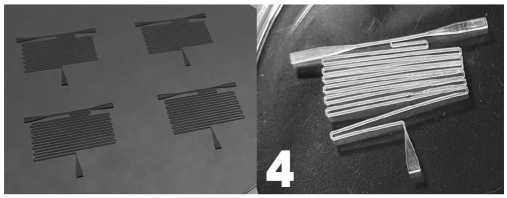

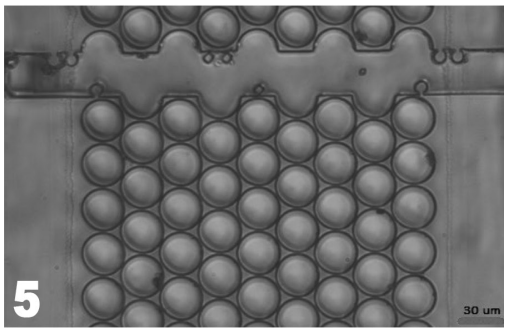

Figure 1: *SEM image of a row of 1 µm constrictions created by etching 5 µm into Si by DRIE.* ***Figure 2:*** *An example of DRIE 5 µm diameter holes intended for PDMS molding pillars to mimic collagen (50x).* ***Figure 3:*** *Single cell migration device with 1 µm constriction gaps.* ***Figure 4:*** *Left: Four SU-8 microfluidic devices ≈ 170 µm tall on a four-inch silicon wafer. Right: A microfluidic device detached from the silicon substrate.* ***Figure 5:*** *Nanoimprinted fluorinated ethylene propylene (FEP) with 5 µm DRIE master @ 265°C (20x).*