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

CNF Project Number: 2065-11

Principal Investigator(s): Jan Lammerding

User(s): Richa Agrawal, Maggie Elpers, Elisabeth Wang

Affiliation(s): Meinig School of Biomedical Engineering, CNF, Weill Institute, Cornell University

Primary Source(s) of Research Funding: National Institutes of Health award R01 HL082792; National Institutes of Health award R01 GM137605; National Institutes of Health award 1U54 CA210184; Department of Defense Breast Cancer Research Program Breakthrough Award BC150580; National Science Foundation CAREER award CBET-1254846; 2021 CNF REU Program via National Science Foundation under Grant No. NNCI-2025233 (Wang)

Contact(s): jan.lammerding@cornell.edu, ra664@cornell.edu, mae228@cornell.edu, emw239@cornell.edu

Website: http://lammerding.wicmb.cornell.edu/

Primary CNF Tools Used: Plasma-Therm 770 deep silicon etcher, Oxford Cobra etcher, Heidelberg mask writer - DWL2000, SÜSS MA6 contact aligner, Anatech SCE-110-RF resist stripper, P-7 profilometer, MVD 100

Abstract:

The ability of cells to migrate through tissues is an essential factor during development, tissue homeostasis, and immune cell mobility. At the same time, it enables cancer cells to invade surrounding tissues and metastasize. We have created microfluidic devices that mimic the narrow, heterogeneous interstitial spaces and that can be used to study nuclear mechanobiology during confined migration. Using these devices in combination with fluorescent imaging, we have developed a method to assess the confined migration fitness of varying cell types.

Research Summary:

During *in vivo* migration, cells such as immune cells, fibroblasts, or metastatic tumor cells traverse interstitial spaces as small as 1-2 μm in diameter. This 'confined migration' requires the deformation not only of the soft cell body but also the rate-limiting step of deforming the large (5-10 μm diameter) and relatively rigid nucleus [1]. To study these processes in more detail, we have previously designed and built polydimethylsiloxane (PDMS) microfluidic devices to model the tight three-dimensional constrictions that metastatic cancer cells may encounter during the metastatic process [2]. 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 [2-4]. However, these devices require time-consuming single-cell analysis, do not fully mimic the heterogeneously confining nature of interstitial spaces, and do not allow use of sufficient cell numbers for biological and genomic analyses of cells that have migrated through the confined spaces due to their relatively small constriction areas (Figure 1).

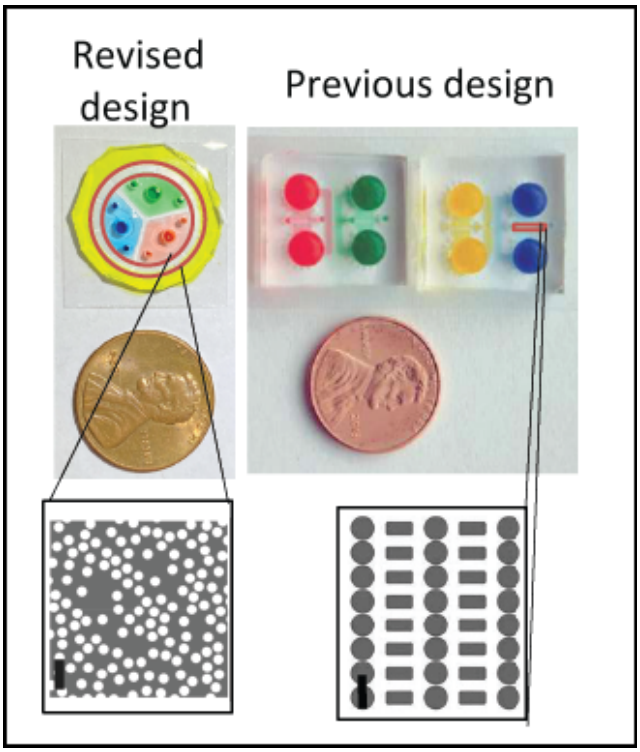

Figure 1: Overview of cancer cell migration device. Top: Partial figure reproduced from Davidson, et al. [2]. Previous PDMS microfluidic devices bonded on glass coverslips and filled with food coloring dye. Bottom: New design of "random pillar" microfluidic devices also bonded to glass coverslip and filled with food coloring. CAD for constriction areas of each design shown (outlined in red on left). Scale bars: 30 µm. All devices have migration areas with 5 µm height. Figure adapted from manuscript submitted to Methods in Molecular Biology.

To overcome these limitations, we have designed novel migration devices that mimic the intermittent confinement of interstitial environments using a precisely controlled but heterogeneous "field of pillars" with variable spacing

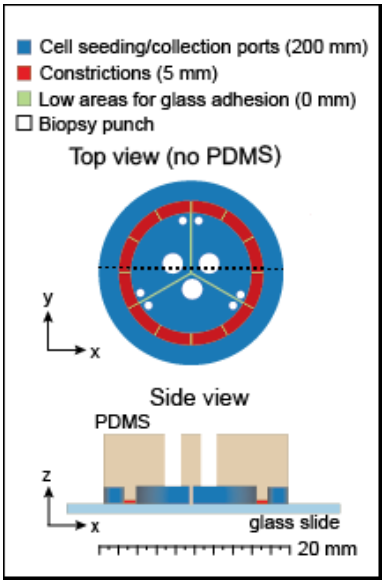

Figure 2: Schematic overview of the PDMS migration device. Top and side view of the device after bonding to glass slide to create a confined environment for cancer cell migration (red area). Figure adapted from manuscript submitted to Methods in Molecular Biology.

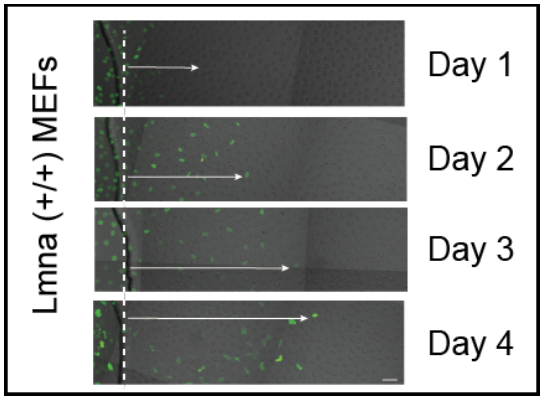

Figure 3: Cell migration in microfluidic device. Representative image series to show usage of microfluidic devices to determine migratory fitness as a function of distance traveled from seeding port into constriction area (white arrows) over four days. Figure adapted from manuscript submitted to Methods in Molecular Biology.

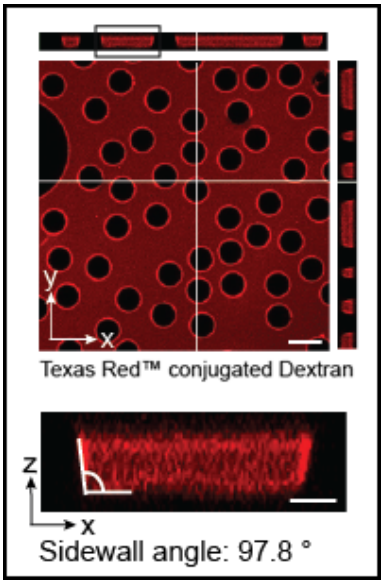

Figure 4: Confocal 3D reconstruction of confined migration area. The PDMS microfluidic device was bonded to a glass coverslip, filled with fluorescent TexasRed-conjugated Dextran, and imaged by confocal microscopy to create a 3D image stacks. Orthogonal projection used to measure sidewall angle, α, of 97.8°. Scale bars: 4 μm. Figure adapted from manuscript submitted to Methods in Molecular Biology.

(Figures 1 and 2). These new devices enable not only time-lapse microscopy, but also straightforward assessment of migratory fitness based on the distance traveled by the cells from the seeding port (Figure 3). These devices are also more amendable to enable collection of large numbers of cells following confined migration. Thus, the new devices present a high-throughput method for observing the short- and longer-term effects mechanically induced nuclear deformation and rupture has on the tumor cells.

In addition to the design, we have also improved the microfabrication methods of wafer used as the mold for the PDMS devices. Wafers for previous device generations were generated using either a thin layer of SU-8 photoresist or reactive ion etching (RIE) fabrication using the photonics etch in the Unaxis 770 deep silicon etcher. However, SU-8 lacked the fidelity to reproduce our fine features (1 μm), and repeated molding and removal of PDMS would, over time, weaken the SU-8/silicon substrate bond, eventually resulting in the delaminating of features. RIE etching in the Unaxis 770 enabled us to create our desired features but required time-consuming seasoning and re-seasoning of the chamber before and after the long photonics etch, as it is traditionally a deep reactive ion etching (DRIE) tool.

For the new devices, we shifted the nanofabrication process to etching using hydrogen bromide in the Oxford Cobra etcher, which has proven to be a highly efficient, reliable method to achieve vertical sidewalls (Figure 4). This revised approach has a faster etch rate than our previous RIE process and enabled us to improve the fidelity of our critical features, while also reducing the fabrication time and costs by more than two thirds.

We highly recommend etching using hydrogen bromide for the creation of PDMS microfluidic devices.

Future fabrication efforts will be focused on transitioning this process to a stepper, as this will enable us to create "taller" constrictions to serve as a vertically "unconfined control" (> 10 μm), which cannot currently be performed using HBr etching. The stepper approach will also enable additional modularity for device layout on wafers and allow us to produce our submicron features with greater ease, instead of the current "trial-and-error" exposure arrays required for the contact lithography process. Taken together, these examples illustrate new uses of the available nanofabrication technologies to create improved *in vitro* models to study cancer cell migration.

References:

[1] Davidson, P. M., Denais, C., Bakshi, M. C., and Lammerding, J. Nuclear deformability constitutes a rate-limiting step during cell migration in 3-D environments. Cell. Mol. Bioeng. 7, 293-306 (2014).

[2] Davidson, P. M., Sliz, J., Isermann, P., Denais, C., and Lammerding, J. Design of a microfluidic device to quantify dynamic intra-nuclear deformation during cell migration through confining environments, Integrative Biology, Volume 7, Issue 12, December 2015, Pages 1534-1546, https://doi.org/10.1039/c5ib00200a.

[3] Shah, P., et al. Nuclear Deformation Causes DNA Damage by Increasing Replication Stress. Curr. Biol. 31, 753-765.e6 (2021).

[4] Denais, C. M. et al. Nuclear envelope rupture and repair during cancer cell migration. Science Vol. 352, Issue 6283, pp. 353-358 (2016).