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

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Primary CNF Tools Used: PT 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, SU-8 Lithography Room (Spinners, Hot Plates, etc.)

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 fabricated polydimethyl siloxane (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).

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 [5] (Figures 1,2). We have created two different geometries of these devices optimized for (1) rapid, easy assessment of migratory fitness as a function of distance traveled, and (2) collection of cells that have successfully completed confined migration through precisely defined constrictions.

One type of device (Figure 2a) features a large constriction area that is compatible with time-lapse microscopy and also enables assessment of migratory fitness from single images captured on multiple consecutive days (Figure 3). Another device design (Figure 2b) features a shorter pillared distance (i.e., fewer constrictions) and is well suited for easy collection of large numbers of cells following confined migration. In ongoing studies, we are combining these devices with the use of the LEGO-optical barcoding system, which utilizes combinations of red, green, and blue fluorescent markers to monitor individual cells based on their distinguishable and heritable colors. This enables us to track cells and their progeny over days and weeks and to quantify the varying abilities of different cells to perform confined migration and to tolerate the associated physical stress. This experimental pipeline presents a high-throughput method for observing the short- and longer-term effects

of mechanically induced nuclear deformation and rupture on cancer cell function and survival.

In order to microfabricate the constriction layer of these designs, we etch silicon using hydrogen bromide in the Oxford Cobra etcher, which has proven to be a highly efficient, reliable method to achieve vertical sidewalls necessary for PDMS devices (Figure 4). We have found this to be advantageous over other etching methods or SU-8 lithography because it provides sufficient resolution of the fine (1 µm) features, and is more reliable and practical than using the photonics etch on the Unaxis 770 and seasoning/re-seasoning the chamber. We continue to use SU-8 photolithography for rapid prototyping and versions of the device which do not require very closely spaced features. Eventually, we will transition this process to a stepper, which will enable us to create "taller" constrictions to serve as a vertically "unconfined control" (>10 µm), which cannot currently be performed using HBr etching. Taken together, these examples illustrate new uses of the available nanofabrication technologies to create improved *in vitro* models to study cancer cell migration.

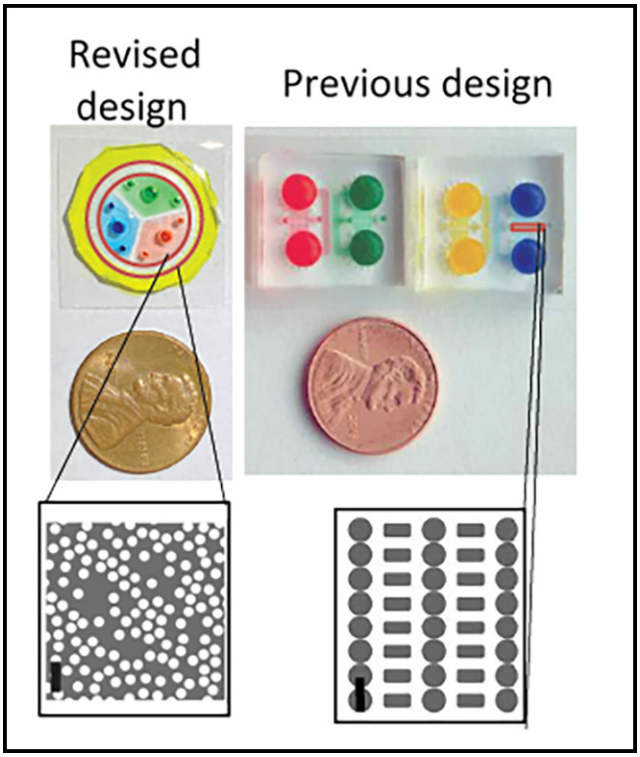

Figure 1: Overview of cancer cell migration device. Top: Partial figure reproduced from Davidson, et al. [2]. Previous PDMS microfluidic devices bonded on glass coverslips and filled with food coloring dye. Bottom: New design of "random pillar" microfluidic devices also bonded to glass coverslip and 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.

References:

[1] Davidson, P. M., et al. 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., et al. Design of a microfluidic device to quantify dynamic intra-nuclear deformation during cell migration through confining environments.

[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 (80-.). 352, 353-358 (2016).

[5] Agrawal, R., et al. Assembly and Use of a Microfluidic Device to Study Nuclear Mechanobiology During Confined Migration. Methods Mol Biol. 329-349 (2022).

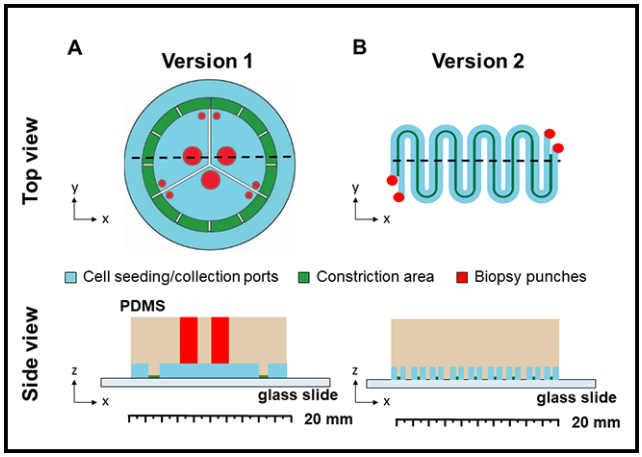

Figure 2: Schematic overview of PDMS migration device geometries. (A) Device optimized for long-term imaging and easy analysis of confined migration efficiency. (B) Device optimized for collection of cells that have completed confined migration. Devices are created by casting PDMS (tan) from wafer and bonded to glass slide to create a confined environment for cancer cell migration (green), after which cells are seeded through biopsy punches (red).

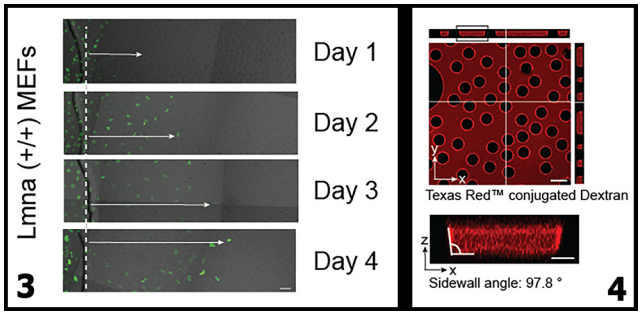

Figure 3, left: Cell migration in microfluidic device. Representative image series to show usage of microfluidic devices to determine migratory fitness as a function of distance traveled from seeding port into constriction area (white arrows) over four days. Figure adapted from manuscript submitted to Methods in Molecular Biology. Figure 4, right: 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.