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

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Primary CNF Tools Used: ABM contact aligner, SÜSS MA-6 contact aligner, MVD tool, Unaxis DRIE/RIE etcher, Anatech etcher, CVC sputtering tool, Tencor P-10 profilometer, Oxford 100 etcher, Trion etcher

Abstract:

It is becoming increasingly apparent that the physical properties of cells, including their size and deformability, play an important part in their function. One particular example is cell migration, which is crucial for physiological processes such as development and immune cell function, but also responsible for metastatic spreading in many cancers. To investigate how cells are able to squeeze through interstitial spaces smaller than the cross-section of the cell, we have developed microfluidic migration devices that provided 3D confinement and pore sizes mimicking physiological environments. Using these devices in combination with fluorescence time lapse-imaging, we have been able to confirm the role of nuclear deformability in confined migration, and also shown identified biological consequences of squeezing the nucleus through tight spaces, including transient nuclear envelope ruptures that result in DNA damage.

Summary of Research:

The ability of cells to migrate through tissues and interstitial spaces is an essential factor during development and tissue homeostasis, immune cell mobility, and in various human diseases. However, current methods to study the migration of cells in confining three-dimensional (3D) environments are limited by their imprecise control over the confinement, physiological relevance, and/or compatibility with high resolution imaging techniques.

We designed and built a polydimethylsiloxane (PDMS) microfluidic device composed of channels with precisely-defined constrictions mimicking physiological environments that enable high resolution imaging of live and fixed cells [1]. The device promotes easy cell loading and rapid, yet long-lasting (>24hrs) chemotactic gradient formation without the need for continuous perfusion, and is ideally suited for time-lapse imaging (Figure 1).

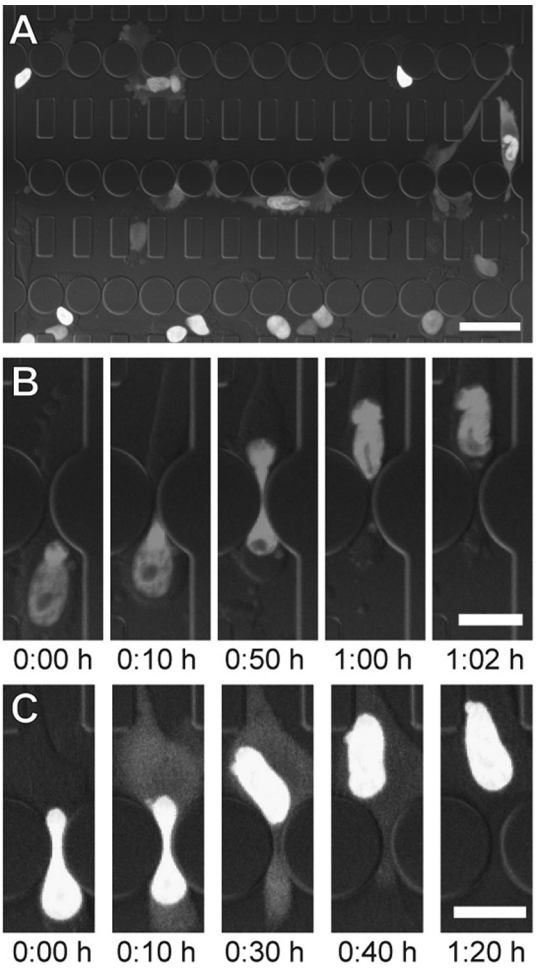

Figure 1: Cell migration through microfluidic constrictions. (A) Cells expressing NLS-GFP and H2B-tdTomato migrating through a microfluidic device. Scale bar: 50 µm. (B) Time series of a nucleus squeezing through a constriction. Scale: bar 20 µm. (C) Time series of a NE rupture event. NLS-GFP leaks into the cytoplasm upon NE rupture and is reimported into the nucleus as the NE is repaired. Scale bar: 20 µm. Figure reproduced from Elacqua, et al. PLoS one. 2018 [3]. See full color version on pages xxviii-xxix.

Using this device, we obtained detailed, quantitative measurements of dynamic nuclear deformation as cells migrate through tight spaces. We found that nuclear deformability, primarily governed by levels of lamin A/C, is a critical factor in determining the ability of cells to move through small constrictions. Furthermore, cells migrating through confined spaces incurred transient nuclear envelope rupture, nuclear fragmentation, and DNA damage, with ESCRT-III proteins playing an important role in restoring nuclear envelope integrity [2,3]. In addition, we showed that the exposure of genomic DNA to the cytoplasm during nuclear envelope rupture leads to activation of the cGAS/STING pathway, which promotes cancer metastasis [4].

The original device design was based on SU-8 soft photolithography [1]. While SU-8 was effective in creating these migration devices, this approach had several fabrication limitations. First, the reproducibility of the smallest SU-8 features (~1μm) was difficult to attain, which led to an over use of successful full wafer devices.

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 forgo SU-8 and instead etch the precise constrictions of our devices directly into the silicon substrate. We accomplished this "bottom-down" approach by using a negative photoresist mask and deep-reactive ion etching (DRIE). This approach enabled us to achieve the critical dimension features with consistent reproducibility (Figure 2), and it also cut the fabrication time in half.

Since our time-lapse studies identified nuclear deformability as a rate-limiting factor in confined migration, we set out to develop a microfluidic device to perform high throughput measurements of nuclear deformability, using the principle of micropipette aspiration.

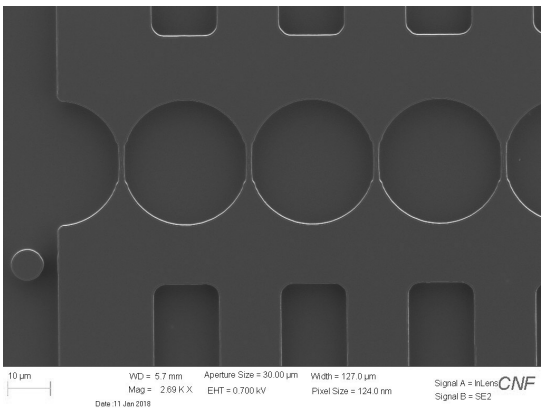

Figure 2: SEM image of a row of 1 µm constrictions created by etching 5 µm into silicon by deep-reactive ion etching.

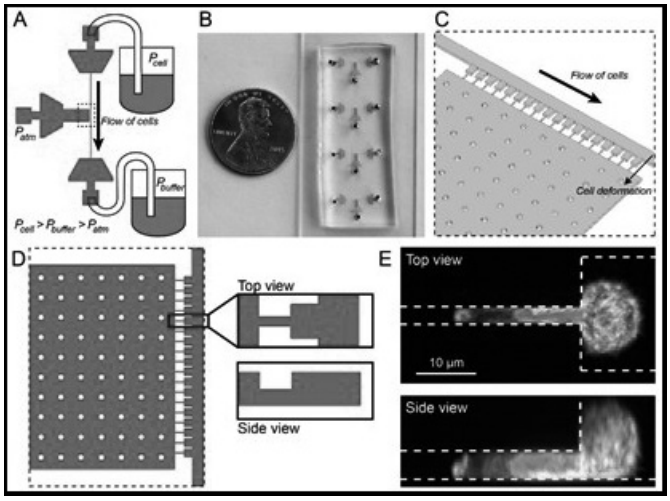

Figure 3: Overview of the microfluidic micropipette aspiration device. (A) Design overview, with applied external pressures indicated. (B) Image of four devices integrated onto a single glass slide. (C) Close-up of the design features containing the pockets and micropipette openings. (D) Top and side view of the same feature. (E) Confocal microscope images of a cell inside a pocket, with the nucleus (red) and cytoplasm (green) partially aspirated into the micropipette. See full color version on pages xxviii-xxix.

Cells in suspension are perfused into the device and segregated into single cells located in individual pockets, each equipped with a small micropipette-like opening (Figure 3). A large pressure gradient across the micropipette results in nuclear deformation (protrusion) into the micropipette, which can be quantified using automated image analysis and used to infer nuclear deformability.

In conclusion, the integration of our migration and micropipette aspiration devices with high resolution time-lapse imaging provides a powerful new approach to study intracellular mechanics and dynamics in a variety of physiologically relevant applications, ranging from cancer cell invasion to immune cell recruitment.

References:

[1] Davidson PM, Sliz J, Isermann P, Denais C, Lammerding J. Design of a microfluidic device to quantify dynamic intra-nuclear deformation during cell migration through confining environments. Integrative Biology. 7: 1534-1546, https://www.ncbi.nlm.nih.gov/pubmed/26549481 (2015).

[2] Denais CM, Gilbert RM, Isermann P, McGregor AL, te Lindert M, Weigelin B, Davidson PM, Friedl P, Wolf K, Lammerding J. Nuclear envelope rupture and repair during cancer cell migration. Science. 352(6283): 353-8, https://www.ncbi.nlm.nih.gov/pubmed/27013428 (2016).

[3] Elacqua JJ, McGregor AL, Lammerding J. Automated analysis of cell migration and nuclear envelope rupture in confined environments. PLoS One. 13(4): e0195664, https://www.ncbi.nlm.nih.gov/pubmed/29649271 (2018).

[4] Bakhoum S, Ngo B, et al. Chromosomal instability promotes metastasis through a cytosolic DNA response. Nature. 553(7689): 467-472, https://www.ncbi.nlm.nih.gov/pubmed/29342134 (2018).