Design and Application of Microfluidic Devices to Study the Effect of the Physical Microenvironment on Cellular Function

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Primary CNF Tools Used: PT 770, Oxford Cobra, Heidelberg DWL 2000 Mask Writer, Karl Suss MA6, Anatech SCE-110-RF Resist Stripper, P-7 Profilometer, MVD 100, Oxford 81, Unaxis 700, EcoClean tool

Abstract:

The physical environment of cells directly modulates their function and fate. Using microfabricated devices that mimic the confined spaces within tissues, we previously demonstrated that cell migration through tight spaces is limited by the deformability of the large cell nucleus, and that the physical stress associated with such 'confined migration' can result in nuclear envelope rupture, DNA damage, and changes in chromatin organization [1-5]. As part of this project, we applied our microfluidic devices to study confined migration, identifying a novel mechanism by which cells squeeze their nucleus through small spaces [6], while also developing novel devices to enable the collection of large numbers of cells that have completed confined migration for genomic and transcriptomic analysis. Additionally, we developed a novel device to precisely confine cells to specific heights with micron resolution [7]. Expanding our mechanobiology research into another direction, we have begun developing microstructured surfaces that promote differentiation of cardiac and skeletal muscle stem cells into more mature muscle cells, thus enabling us to study the effect of disease-causing mutations on muscle function.

Research Summary:

During in vivo migration, 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 relatively rigid nucleus [1]. To study these processes in more detail, we have previously designed and fabricated polydimethyl siloxane (PDMS) microfluidic devices to

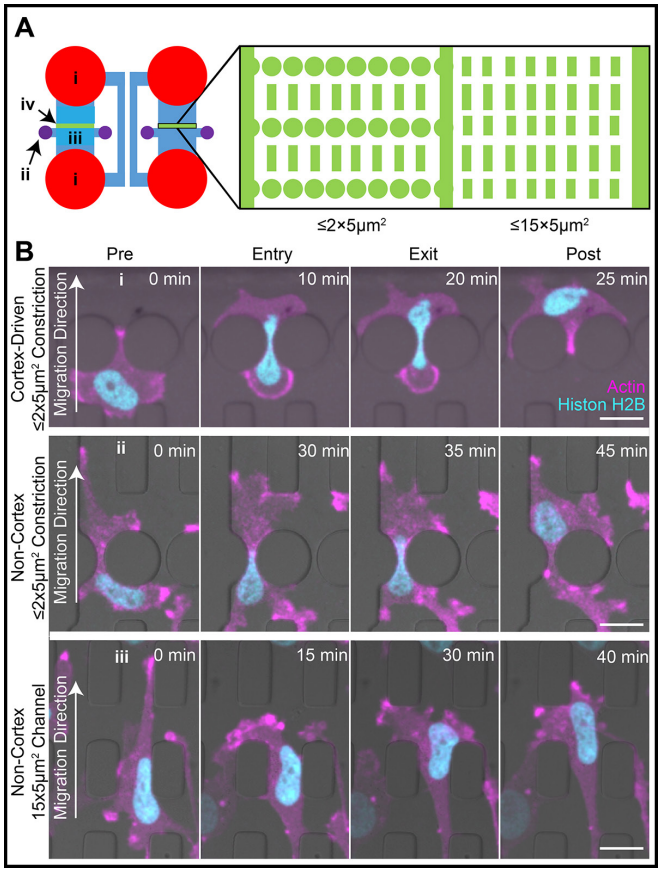

Figure 1: Microfluidic migration device to study confined migration mechanisms. (A) Overview of microfluidic migration device. (B) Time-lapse microscopy sequence of cells migrating through confined spaces using rear cortex contraction (top) or nuclear pulling (center and bottom) mechanism. Figure panels reproduced from Keys, et al. [7].

model the confined spaces that cancer cells may encounter during the metastatic process (Figure 1A) [8]. Using time-lapse imaging of cells expressing fluorescently labeled cytoskeletal components, we identified that cells can either use a 'nuclear pulling' mechanism, in which contraction of actomyosin fibers pull the nucleus through the tight space, or a 'rear cortex contraction' mechanism, in which contraction of the back of the cell generates a hydrostatic pressure gradient within the cell that drives the nucleus through the constriction (Figure 1B). We confirmed the biophysical mechanisms using laser ablation and fluorescent reporters. This work was recently published in the Journal of Cell Science [6].

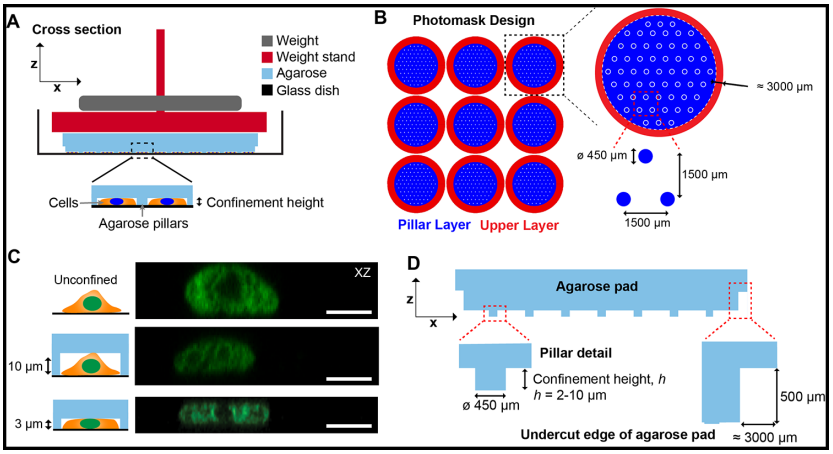

Figure 2: Agarose-based cell confinement device. (A) Cross section (left) schematic of the confinement device assembly. Assembly includes a glass bottomed dish (black), agarose device (blue), weight holder (red), and weight (gray). (B) Overlay of the lower- and upper-layer photomask designs showing the array of micropillars for one confinement device. (C) Representative images of breast cancer cells with nuclei fluorescently labeled with histone 2B-mNeonGreen under either unconfined, confined to 10 µm, or confined to 3 µm conditions. Scale bars: 10 µm. (D) Schematic depiction of a single agarose device. Figure adapted from Elpers, et al. [5].

Since the previous generation of microfluidic devices could not collect sufficient numbers of cells that have migrated through the confined spaces to perform biochemical or sequencing-based analyses, we designed novel migration devices that enable collection of 1000's of cells post confined migration. We optimized several fabrication steps for these devices, including etching with hydrogen bromide in the Oxford Cobra etcher to achieve vertical device features in the mold for the PDMS microfluidic devices. We are currently in the process of applying these devices to analyze the effect of confined migration on gene expression and changes in chromatin organization in cancer cells.

In addition to the migration devices, we also developed a cell confinement device (Figure 2), in which we can precisely confine cells using an agarose pad containing micro-pillars fabricated to the height of interest (i.e., 3-15 μm). To make the device, agarose is cast onto a mold consisting of a silicon wafer fabricated to contain the negatives of the desired pillar features, and the agarose device is cut out and placed onto cells seeded on a glass-bottomed dish. The silicon wafer is fabricated using photolithography with SU-8 photoresist. We have fabricated devices with various heights to induce different degrees of confinement. The devices are suitable for short- and long-term confinement studies and compatible with imaging of both live and fixed samples. This work was recently published in Current Protocols in Cell Biology [5]. We are currently applying these devices to assess the effect of confinement on polarization and gene expression in primary bone marrow-derived macrophages.

Lastly, we recently designed microstructured substrates consisting of approximate 800 nm x 800 nm (W x H) ridges that promote alignment and maturation of muscle cells grown on them. To fabricate the structures, OiR620-7i photoresist was spun onto silicon wafers and exposed using the i-line stepper. Next, the Oxford 81 etcher was used to perform a descum process to prepare the wafer for deep silicon etching. The Unaxis 700 Deep Silicon Etcher was used to etch ca. 800 nm into the silicon. Excess photoresist is stripped using the EcoClean tool, and a FOTS coating is applied using the MVD 100 so that a PDMS cast can be made of the wafer, which then serves as the actual microstructured substrate for the cells. The transparent and flexible PDMS allows for imaging cells through the substrate, and also for applying uniaxial strain to the substrate, mimicking muscle contraction.

Taken together, these examples illustrate new uses of the available nanofabrication technologies to create improved in vitro models to study confined cell migration and mechanobiology.

References:

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