Generating Microfluidic Devices to Study Confined Migration of Cancer Cells

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Primary CNF Tools Used: Heidelberg mask writer - DWL2000, Hamatech-Steag HMP900 mask processor, SÜSS MA6-BA6 contact aligner, Hamatech hot piranha, MVD 100, Anatech plasma asher, Unaxis 770 deep silicon etcher / Oxford Cobra ICP etcher

Abstract:

During metastasis, cancer cells spread from a primary tumor to distal sites. As they travel through interstitial environments, they compress their nucleus to fit through confined areas smaller than themselves. Nuclear deformation can put stress on the cells and have consequences such as nuclear envelope rupture and DNA damage. To study changes induced in cancer cells due to confined migration, polydimethylsiloxane (PDMS) microfluidic devices can simulate the intermittent confinement of an *in vivo* environment. The goal of this research was to create devices which allow for time-lapse imaging and enable collection of cells that had successfully performed confined migration for other characterization. We fabricated devices with constriction areas consisting of a field of heterogeneously spaced pillars by using photolithography techniques to create a wafer with the features for the devices, then creating devices from a PDMS casting of the wafer. We seeded HT1080 fibrosarcoma cells in the devices and allowed them to migrate through the constrictions for up to six days. A study of the cells migrating through the devices over 12 hours showed successful migration. However, over six days, the progress of the cells stalled, possibly due to limited nutrient supply in the confined environment. Overall, the devices successfully model cancer cell migration in a heterogeneous environment, and will be useful for time-lapse microscopy and short-term studies.

Summary of Research:

Cancer cells spread from primary tumors to distal sites during metastasis, which contributes to up to 90% of all cancer-related deaths [1]. As they travel through extracellular matrices, they often must compress their nucleus to fit through tight interstitial spaces [2]. The force required to squeeze through the confined environments can have various consequences for the cells. These effects can include DNA damage from either nuclear deformation that causes increased replication stress [3] or nuclear envelope rupture that allows contents of the cytoplasm and nucleus to mix uncontrollably, leading to greater instability and causing the cancer to advance [4]. However, the full extent that the cells are affected by confined migration is still poorly understood.

To better understand the consequences of confined migration on cancer cells, we designed and fabricated polydimethylsiloxane (PDMS) microfluidic devices with precisely defined confined areas. Previous microfluidic device designs had two main limitations: (1) successive constrictions did not resemble the heterogeneous confinement of tissues, and (2) the small constriction area limited the number of cells for analysis. Our new device designs included the following improvements: (1) the constriction area consisted of a "heterogeneous field" of pillars with varying spacing, which is more physiological (Figure 1), and (2) the constriction area expanded by

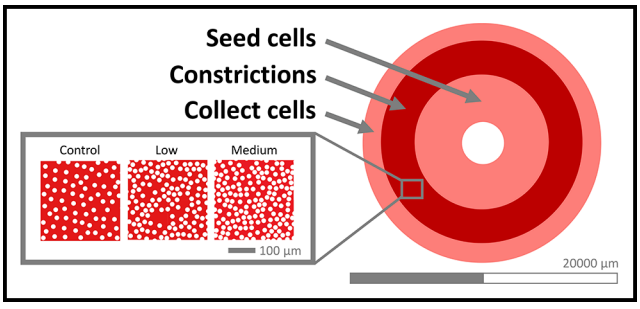

Figure 1: Randomized pillar device design showing the seeding, constriction, and collection areas. Pillars were 5 µm tall and 15 µm in diameter. Modified from Richa Agrawal, submitted to Methods in Molecular Biology.

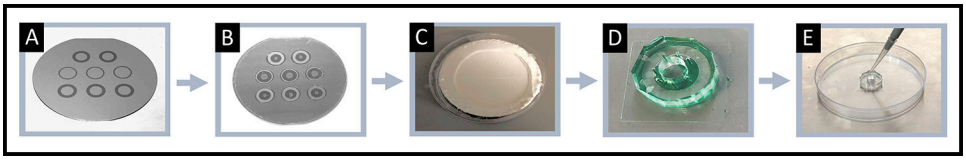

Figure 2: Fabrication workflow for microfluidic devices. (A) First layer of wafer: features for constrictions. (B) Second layer of wafer: features for seeding and collection areas. (C) PDMS casting from plastic mold created from wafer. (D) Devices and seeding ports cut out, then bonded to glass. (E) HT1080 fibrosarcoma cells seeded into device.

100-fold so more cells can be collected after migrating. In addition, the devices have randomized pillars with varying densities, including a relatively "low density" control, to simulate increasing degrees of confinement (Figure 1).

Fabricating microfluidic devices involved making a silicon master mold with two layers. The first layer used reactive ion etching in the Oxford Cobra etcher to create a 5 μm tall constriction area, and the second layer consisted of SU-8 photoresist for 250 μm tall seeding and constriction areas. Microfluidic devices were cut out from a PDMS casting of the wafer and bonded to glass (Figure 2).

Time-lapse imaging of the devices over 12 hours showed that the cells migrate through the constrictions and travel the fastest in the devices with lower pillar densities (Figure 3). Migration was also tracked over several days in another set of devices, and the cells' progress was measured by studying the leading edge of cells. The cells advanced through all of the devices over the first five days, but the average distance from the seeding area decreased on the sixth day, likely due to insufficient nutrient supply that caused some cell death (Figure 4).

However, overall, the devices successfully model migration through a heterogeneous environment and are suitable for time-lapse microscopy and short-term endpoint studies (< four days).

Future Steps:

Decreased migration through the devices after several days most likely indicated insufficient culture media supply or stress from prolonged vertical confinement. Current efforts are focused on creating a version of the devices with a smaller constriction area (< 500 μm) to help with nutrient supply issues, and increased PDMS adhesion area to ensure sufficient bonding to the glass coverslip. We have also created another device with an increased vertical height from 5 μm to 10 μm to eliminate the possibility that cell death was due to prolonged vertical confinement.

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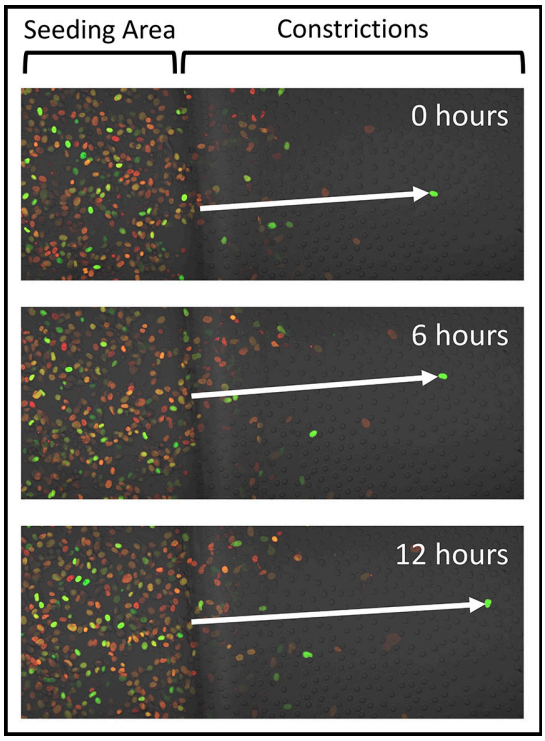

Figure 3: HT1080 fibrosarcoma cells, fluorescently labeled with NLS-GFP (green) and 53BP1-mCherry (red), in a device with a control pillar density region imaged over 12 hours. Migration distance of a single cell inside the constrictions indicated by arrow.

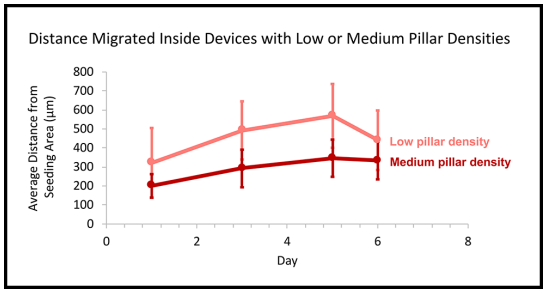

Figure 4: Distance migrated by HT1080 fibrosarcoma cells inside devices with low or medium pillar densities. n=1, 50 cells per timepoint.