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Microfabricated Cell Array Device for Screening of Metastatic Potential

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Abstract:

Metastasis is a complex cell migration process where a cancer cell leaves its primary tumor site to establish a secondary tumor site, causing greater than 90% of cancer related deaths. Traditionally, metastatic potentials have been quantified by individually tracking the migration of cells plated on a dish [1]. However, this method is low-throughput and requires costly live microscopy chambers. Here, we develop a high-throughput cell migration assay by employing microfabrication techniques to develop a method to capture single cells and place them in an organized array. We quantify cell migratory behavior by quantifying the disorder of the initial organized array. Migration of cancerous cells depends on the interactions between the cells and their microenvironments. Thus we validate our device by characterizing the migration of cells on substrates of varying stiffness. Upon completion of this project, the device will be usable as a diagnostic tool for rapid high-throughput analysis of the metastatic potential of biopsied tumor cells.

Experimental Procedure:

Classic Cell Migration Assay. We plated 3T3 fibroblast cells on Sylgard 184 polydimethylsiloxane (PDMS)-coated coverslips of four different substrate moduli: 100 kPa, 300 kPa, 500 kPa, and 1000 kPa. Each substrate was then coated with fibronectin using microcontact printing [2]. The 3T3 cells were seeded onto each substrate (100 μl; 100,000 cells/μl) and incubated over night at 37°C. Cells were then tracked using an Olympus IX81ZDC inverted confocal microscope by manually locating the position of cells, and obtaining images at 10 minute intervals, over 90 minutes. Celltracker [3] was used to determine the mean squared displacement of each cell, which is how far a cell has migrated from its original position.

Cell Array Device Assay. The design for the microfabricated cell array device (MCAD) was based on the work of DiCarlo, et al. [4] and was fabricated using standard soft photolithography techniques [5]. Masters were fabricated from SU-8 3025 photoresist spun on silicon wafers. PDMS (10:1 base:curing agent) was poured over the master and baked at 90°C for three hours. The MCAD was placed in conformal contact to a substrate, identical to the substrate used in the traditional cell migration assay. The MCAD (Figure 1) functioned as a microfluidic device designed with an array of cell traps (12 μm in diameter), and bumpers used to direct cells into the traps.

A 3T3 fibroblast suspension (1 ml; 100,000 cell/ml) was flowed through the device using negative pressure, so that each trap became filled with one cell. The device was then incubated for one hour at 37°C and 5% CO_2, allowing trapped cells to attach to the fibronectin coated substrate in an organized array. Following device removal, the substrate was placed back into the incubator for three additional hours to allow for cell migration. The cells were then fixed using 4% paraformaldehyde and stained with 4',6-diamidino-2-phenylindole. Images of the cell arrays were obtained using an Olympus IX81ZDC microscope.

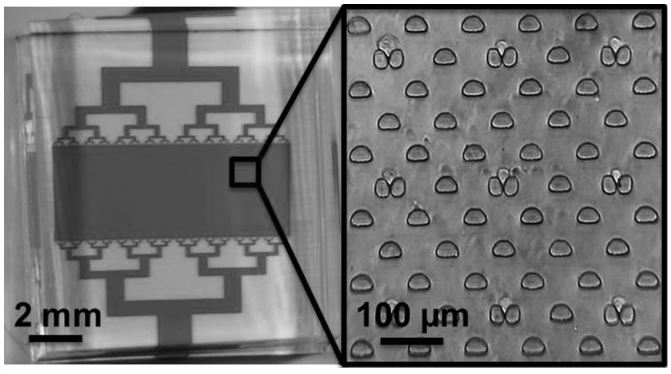

Figure 1: Microfabricated cell array device (MCAD) is a silicon microfluidic device containing an array of cell traps and bumpers.

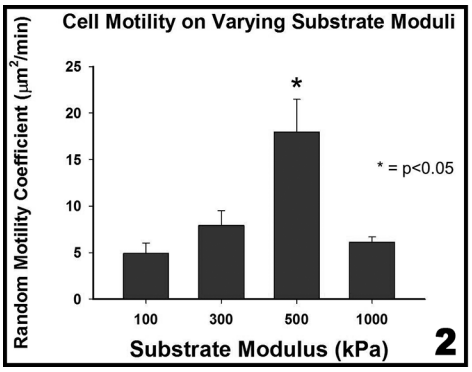

Figure 2: The traditional cell migration assay confirms that 3T3 motility on substrates of varying moduli show biphasic behavior.

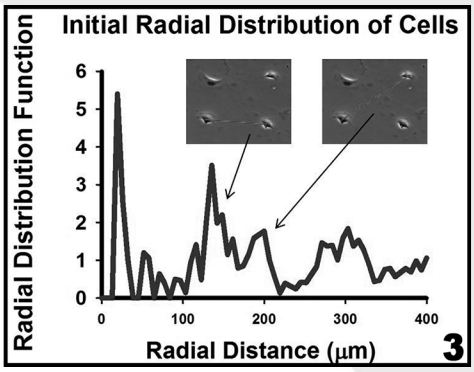

Figure 3: The radial distribution function of cells fixed immediately after MCAD removal.

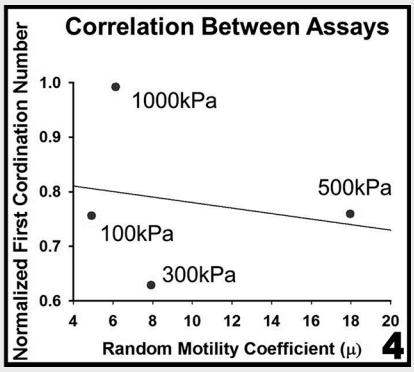

Figure 4: Correlation between the MCAD assay and the traditional cell migration assay.

Results and Conclusions:

Substrate Modulus Affects Migration. By tracking the mean squared displacement of a population of cells as a function of time, a random motility coefficient was determined for each substrate modulus by fitting a linear line through the MSD vs. time plot. The slope of the line is indicative of how migratory a population of cells is. The cells migrated significantly more on the substrate modulus of 500 kPa, shown in Figure 2. This biphasic result is consistent with previous experiments [6].

MCAD Assay. Images of cell arrays were analyzed using a custom MATLAB code to calculate a radial distribution function, shown in Figure 3. This is a way of characterizing the order of a system by calculating how the density of cells varies as a function of distance from a reference cell. The area under the second curve of the radial distribution function, corresponding to the 120 μm distance between each cell trap, is the first coordination number. A normalized first coordination number was determined for each MCAD substrate modulus and is a quantification of cell migration.

Correlation Between Assays. The results of the MCAD experiment were compared to the results of the traditional cell migration assay, as shown in Figure 4. A negative correlation between the assays would indicate agreement, as a low first coordination number corresponds to high cell migration. We see the trend that validates our device, but this data is very preliminary and inconclusive until this experiment can be repeated.

Future Work:

With further study, this device could be usable as a diagnostic tool for rapidly measuring cancer cell metastatic potential. Future work will include repeating this experiment so that a significant correlation can be obtained, and further optimizing the device.

Acknowledgements:

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