Effects of Cellular Architecture on MDA-MB-231 Breast Cancer Cell Motility

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Abstract:

Over 200,000 women living in the United States will be diagnosed with breast cancer this year. Of those whose cancer metastasizes, wherein the cells migrate away from the initial tumor, only 23% will survive [1]. Cellular mechanics and architecture drive a wide range of cellular behaviors, such as contractility and apoptosis [2, 3]. Here, we assessed the role of cellular architecture in breast cancer motility. MDA-MB-231 breast cancer cells were micropatterned into two sizes of four shapes. We explored cytocellular architecture, measured lamellipodia extension as a function of that architecture, and investigated cellular invasion into a collagen gel. Results suggest that cellular architecture plays a role in determining the likelihood of tumor cell metastasis.

Introduction:

Metastasis of a tumor to vital organs greatly decreases chances of survival [1]. The factors that drive tumor metastasis are poorly understood. Cellular architecture has been shown to dictate certain cellular processes indicative of migration intent [4]. Thus, we hypothesized that cellular architecture plays a role in tumor metastasis.

We studied the correlation between cellular architecture and breast cancer cell motility. MDA-MB-231 breast cancer cells were micropatterned into two sizes of four shapes each. Cells patterned at 100% area were patterned on fibronectin microfeatures, which displayed 100% of the cross-sectional area attributed to a single MDA-MB-231 cancer cell on an unpatterned substrate. Immunofluorescence confocal microscopy was used to examine cytocellular architecture. We also measured lamellipodia extension as a function of cytocellular architecture to determine cell motility with respect to cell shape. Finally, we assessed the effect of cytocellular architecture on cell migration into a collagen gel overlay.

Experimental Procedure:

As per Figure 1, casts for production of polydimethylsiloxane (PDMS) stamps were fabricated from SU-8 photoresist spun on a silicon wafer via standard photolithography technique. Casts were coated with Sylgard 184 polydimethylsiloxane (PDMS) (10:1 base: curing agent), degassed, and baked at 90°C for four hours.

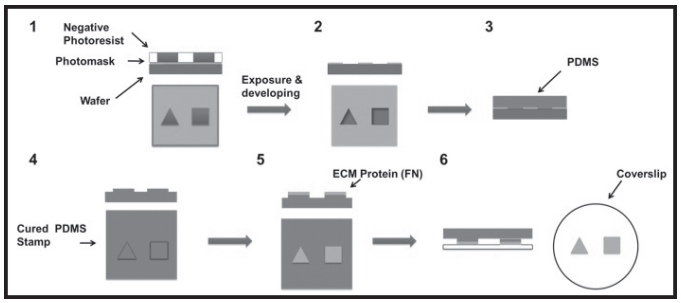

Figure 1: Photolithography and Microcontact Printing Methods.

1. Photomask aligned on SU-8 coated wafer.
2. Microfeatures developed on the wafer via selective exposure and a chemical etching process.
3. PDMS poured onto the wafer and heat-cured.
4. PDMS stamp with microfeatures is peeled off the wafer.
5. PDMS stamp coated with extracellular matrix (ECM) protein, fibronectin (FN).
6. FN coated PDMS stamp is stamped onto a coverslip transferring FN to the coverslip, providing guidance cues for cell shape.

Stamps were coated with fibronectin (50 mg/mL in H20), an extracellular matrix protein, and incubated for one hour. The fibronectin was then stamped onto PDMS-coated glass coverslips. A suspension of MDA-MB-231 human breast cancer cells (75,000 cells/mL) was seeded onto the coverslips. Overnight incubation at 37°C and 5% CO_2 allowed cells to settle and conform to the fibronectin microfeatures.

Micropatterned cells were fixed with 4% paraformaldehyde and permeablilized with 0.05%Triton-100 (in PBS at 37°C). Cells were stained with AlexaFlour 488 Phalloidin and 4′, 6-diamidino 2-phenylindole (DAPI) in PBS, then imaged with an Olympus IX81ZDC inverted confocal microscope.

Results and Discussion:

Microcontact printing reliably produced the confocal images shown in Figure 2.

Time-lapse photography acquisition every five minutes for one hour of micropatterned cells allowed for quantification of cell area change. The lamellipodia protrusion parameter (LPP), which is the standard deviation of the normalized area measurements spanning this hour, was used to quantify lamellipodia extension.

Figure 3 indicates a relatively large LPP for the 100% triangle cell. This was attributed to the high concentration of actin filaments found at the cellular periphery (Figure 1). It was noted that, for all but the triangle, the smaller cell size produced a higher LPP. However, further data acquisition is required to validate the results.

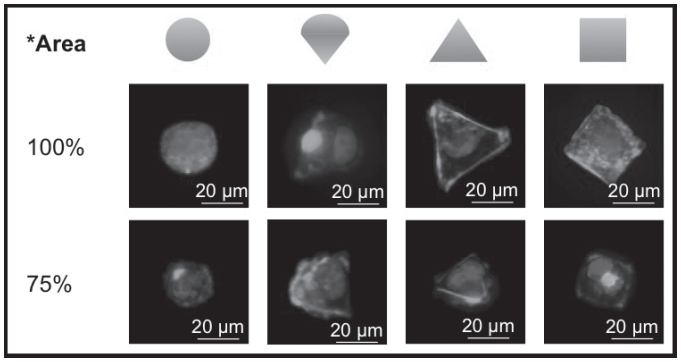

*Figure 2: Fluorescent images of micropatterned MDA-MB-231 cancer cells. Blue: nucleus Green: actin * Percent area is defined as the percentage of the total area of an MDA-MB-231 cell on an unpatterned substrate. Shapes are circle, teardrop, triangle, and square from left to right. The top row of shapes was patterned at 100%; the bottom row was patterned at 75%. Shapes were selected based on number of corner regions to constrain lamellipodia extension as explained in [4].*

Future Work:

Attempts at a collagen invasion assay adapted from previous work by Goswami et al. [5] were made. Methods for the assay can be found in Figure 4. Imaging techniques are yet to be perfected. We aim to solidify a protocol and imaging technique that allows us to quantify degrees of invasion with respect to cellular shape.

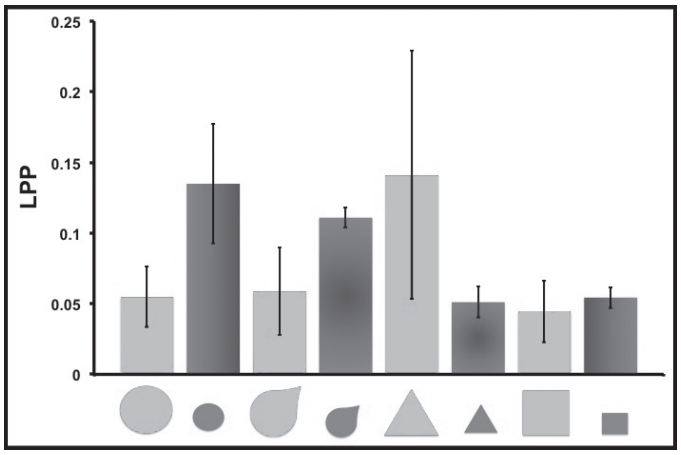

Figure 3: Lamellipodia protrusion parameter (LPP). The LPP is the standard deviation of the normalized area. The mean LPP and standard deviation error bars are given for each shape and size (N = 3, 3, 5, 3, 4, 7, 4, 3 from left to right, respectively).

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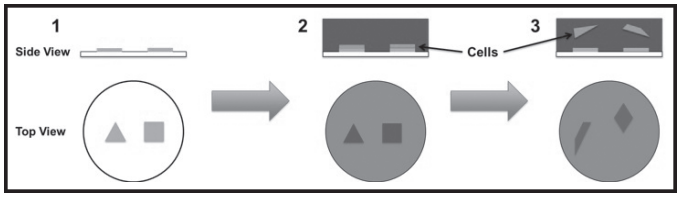

Figure 4: Collagen invasion assay methods. 1. Seed micropatterned coverslip with MDA-MB-231 breast cancer cells. 2. Overlay with 2.5 mg/mL rat tail collagen. Allow to cure at room temperature for 30 minutes. 3. Cells invade matrix for eight hours.