Surface Ligand Density Dependence of Collective Cell Migration on Photoactivatable Self Assembled Monolayers

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Abstract:

Cell migration is an important biological process that can be influenced by many extracellular factors. In this study, the effect of surface density of an extracellular matrix (ECM) ligand on collective migratory characteristics is explored using a tunable, photoactivatable substrate. The substrate exposes cyclic arginine-glycine-aspartic acid (cRGD) ligand at a given surface density upon photoirradiation. HeLa cervical cancer cells and Madin Darby canine kidney (MDCK) cells were photopatterned on circular regions (r = 75 μm) for 5 h and migration was induced by irradiating surrounding regions. Results show an increasing speed for MDCK cells as the cRGD ratio decreases ($p < 0.001$) and a constant migration speed for HeLa cells on all substrates.

Introduction:

Cell migration is an important component of cancer metastasis and the wound healing process, where the collectivity of migration can be influenced by many factors such as the composition of ECM, soluble factor gradients, etc. [1, 2]. Previous studies have also shown the dependence of patterned substrates on various cellular functions during *in vitro* experiments [3]. Here, the effect of cRGD surface density on collective migratory characteristics was explored in HeLa and MDCK cells using a photoactivatable substrate. As MDCK cells expressed a stronger cell-to-cell binding mechanism than HeLa cells (E-cadherin versus N-cadherin), it was expected that with varied concentrations of surface cRGD, a faster migration speed would be observed for HeLa cells in comparison to MDCK cells.

Experimental Procedure:

Polymer Synthesis. Photocleavable poly(ethylene glycol) (PCP) was synthesized through a 2:1 mixing of alpha-methoxy-omega-amino poly(ethylene glycol) (PEG, MW = 12K) and Bis(12-(4-(1-(succinimidyloxycarbonyloxy)ethyl)-2-methoxy-5-nitrophenoxy)disulfide (DSNBD) and reacted overnight at room temperature. Similarly, cyclic cRGD peptides were reacted in a 2:1 ratio with dithiobis (succinimidyl-3,6,9,12,15,18,21-heptaoxo-dotriacotanoate) (DSHODA) and reacted overnight at room temperature. Commercially available bis-1-(11-[2-{2-(2-[2-{2-(2-hydroxy-ethoxy)-ethoxy}-ethoxy]-ethoxy)-ethoxy}-ethoxy]-undecyl) disulfide (EG) was used as the diluting agent for cRGD. All disulfide compounds were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 50 μM.

Substrate Fabrication. Substrates were fabricated on quartz glass with a 5 nm titanium adhesion layer and 20 nm gold layer. EG was used to dilute cRGD to the studied concentrations and then each mixture of cRGD:EG was diluted in a 4:1 ratio of PCP:[cRGD:EG]. The final mixture of 4:1 PCP:[cRGD:EG] was mixed and 15 μL of the solution was pipetted onto the substrate. After an overnight incubation, the substrates were immersed in a DMSO bath and rinsed of unfunctionalized disulfides. Next, substrates were sterilized in 70% ethanol and prepared for photopatterning.

Cell Culture. HeLa and MDCK cells were seeded on circular photopatterned substrates (r = 75 μm) for 2 h in serum-free Dulbecco's modified eagle medium (DMEM) after which the media was exchanged to DMEM containing 10% fetal bovine serum (FBS). The cells were then incubated for 3 h at 37°C before being exposed to a secondary UV irradiation (Figure 1).

Results and Conclusions:

HeLa cells were cultured on a range of cRGD:EG concentrations (1:10 to 1:100,000,000) to test cell attachment and shape. Attachment was observed on concentrations ranging between 1:10 and 1:10,000 with the number of attached cells decreasing with lower concentrations of cRGD. Additionally, the shape

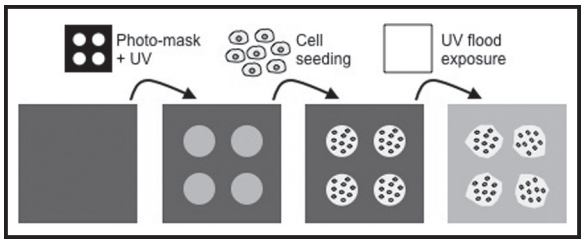

Figure 1: Outline of experimental procedure for culturing cells on circular patterns.

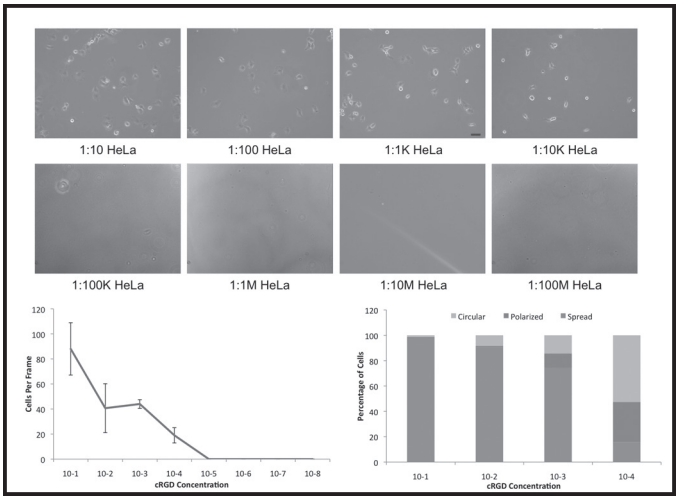

Figure 2: HeLa attachment and morphology on 8 cRGD concentrations.

of attached cells shifted from a spread to a more rounded morphology as cRGD density decreased (Figure 2).

Next, cell migration from the circular cluster was examined. After confinement cells were released via secondary UV irradiation and representative images of migration were collected every fifteen minutes for four hours (representative snap shots shown in Figure 3). MDCK and HeLa migration speeds were evaluated using time profiles of the average cluster radius change after confinement release and compared at each cRGD concentration. While MDCK cells show an inversely proportional relationship between cRGD density and speed ($p < 0.001$), HeLa cells show no significant difference at any cRGD concentration (Figure 4). When compared between cell lines, HeLa cells show a significantly higher migration speed at cRGD densities of 1:100 ($p < 0.001$) and 1:1,000 ($p < 0.05$), with no difference at 1:10,000.

In conclusion, it was found that HeLa cells selectively attach to a certain range of cRGD densities. After testing this range in MDCK and HeLa cells, an inversely proportional relationship between speed and cRGD density was observed in MDCK cells while no relationship was observed in HeLa cells. These results indicate a correlation between receptor type (E- versus

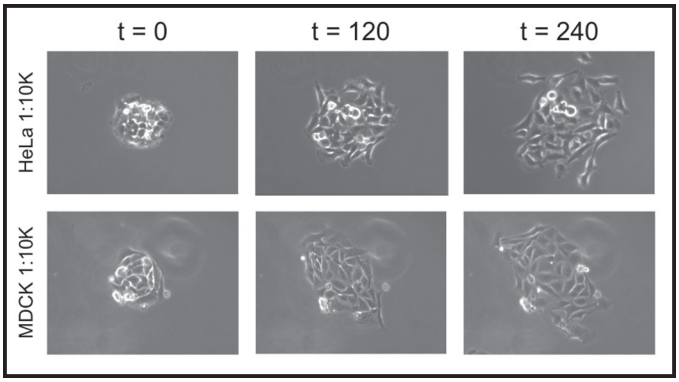

Figure 3: HeLa and MDCK cells seeded on circular (r = 75 μm) patterns at two and four hours after release.

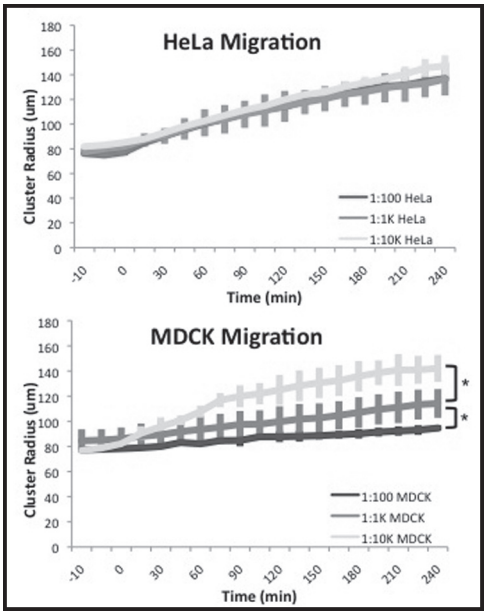

Figure 4: Migration speed profiles for three cRGD densities for the first four hours after release.

N-cadherin) and migration speed at high cRGD densities which could help predict migration characteristics in new cell lines based on cell-to-cell adhesion type.

Future Work:

In this study, a high throughput testing bed for cell migration was fabricated. By using this substrate for testing cell migration, the migratory behavior of cell lines can be determined at specific surface ligand concentrations. For future studies, cells will be fixed three hours after release and stained for E- and N-cadherin junctions. By quantifying cell-to-cell adhesion, the importance of receptor type on migration speed can be determined.

Acknowledgments:

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