Curved Functionalized Microfluidic Channels for the Study of Cell Dynamics

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Abstract:

There have been studies in which physical environments are simulated by growing cells on different stiffness substrates to observe changes in migration speed, cell stiffness, etc [1]. However, these flat, two-dimensional systems do not accurately simulate *in vivo* environments in which cells exist. The vast majority of tissue in the human body exhibits at least some additional parameter, whether it is depth, curvature, etc. The objective of this study was to make observations on how curvature and stiffness affect cell proliferation, growth, and orientation preferences in channel-like structures. Polyacrylamide/bisacrylamide gels with different substrate stiffness were fabricated with cylindrical voids of varying channel diameters. Two-dimensional gels with similar substrate stiffness were also observed as a control for the system. Initial observations indicate curvature prompts fibroblasts to migrate along the lumen (length) of the channel as well as aggregate.

Introduction:

Many groups have simulated wound healing by performing scratch assays on a group of fully confluent cells on a flat surface and observing migration towards the wound. However, these substrates do not accurately simulate *in vivo* environments, as most tissues exhibit some additional parameter, whether it is depth, curvature, etc. Substrate stiffness has also been seen as a factor that influences cell migration velocities [2]. The aim of this study was to see how curvature affects cell migration on different substrate stiffness with the purpose of creating a curved wound-healing environment.

Experimental Procedure:

Substrates were made by polymerizing a mixture of 8 wt% and 4 wt% polyacrylamide with 0.3 wt% bisacrylimide solutions. 8 wt% / 0.3 wt% polyacrylamide/bisacrylamide gels yielded a Young's modulus of 25 kPa (stiff), while 4 wt% / 0.3 wt% polyacrylamide/bisacrylamide gels yielded a Young's modulus of 5 kPa (soft). Channels were made with diameters of 736 μm, 384 μm, and 114 μm. Flat substrates with the same composition served as the control. Gels were embedded with a synthesized GRGDSP acrylamide to promote cell adhesion to the substrate surface. Human foreskin fibroblasts from the BJ line were transfected with green fluorescent protein to observe migration patterns.

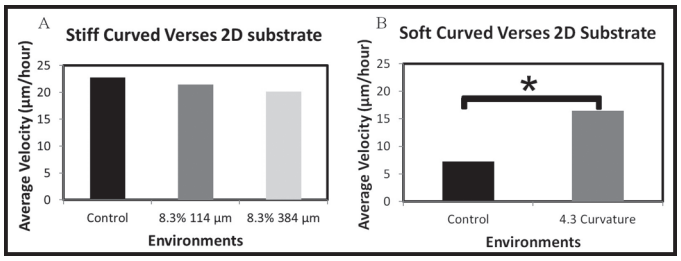

Figure 1: No significance was shown between curved environments verses flat substrates in the stiff gel (Figure 1A, n = 15, 27, p-value of control vs stiff 114 µm = 0.261, control vs. stiff 384 µm = 0.094, 114 µm vs 384 µm = 0319). Curved substrate resulted in cells having a significantly higher total velocity than that of the control (Figure 1B, n = 12, 13 p-value of 0.0019).

Migration was tracked using confocal microscopy with stacks starting and ending where green fluorescent protein detected. Projections were made along the Z axis in order to average the fluorescent signals for two-dimensional tracking. Migration was tracked using an ImageJ plugin, MtrackJ. Total average velocity calculated along with velocities in the X and Y vectors.

Results:

In looking at the migration patterns on the stiffer substrates, there was no statistically significant difference in total velocity between the control verses the curved environments. Additionally, there was no difference in total velocity between the 114 μm diameter channel and the 384 μm channels in the soft gel. However, cells that were seeded within the 768 μm channel in the softer gel exhibited a higher total velocity verses the cells on the two-dimensional substrate.

To better understand how the curvature of the substrate affects the migration patterns of the cells, the velocity along the major axis of the channel and around the curvature of the channel were measured. The total average velocities were broken down into their subsequent X and Y components. From our initial observations, fibroblasts within the 736 μm channel preferred

to move along the channel, having a higher average X velocity than average Y velocity. Fibroblasts within the 384 μm diameter channel did not have a preference for movement along one axis or the other. Within the 114 μm channel, fibroblasts had a higher X velocity, indicating faster movement along the channel.

Cells within curved channels had a tendency to aggregate and stay together, whereas fibroblasts that were seeded on the two-dimensional substrates exhibited little to no aggregation. During the course of the two-dimensional substrate experiment, fibroblasts that did come into contact with each other would instantaneously repel each other, whereas fibroblasts in the curved channel would aggregate together and stay together for the entire duration of the experiment.

Conclusions and Future Works:

There are indications that curvature has a minimal effect on the total velocity of fibroblasts. However, once these velocities have been broken down, there is a clear indication that fibroblasts grown on the curved channels have a preference to move along the major horizontal axis rather than moving around the curvature of the channel. In looking at the X and Y velocities in the stiff 384 μm channel, there was no difference between them. In qualitatively analyzing the migration patterns of the cells, the vast majority tend to also move along the major horizontal axis of the channel. This disparity may simply be attributed to having a small sample size, as there were only 49 samples measured.

Aggregation in the curved environments was quite unexpected, as fibroblasts are contact inhibitive cells. In future studies, we would like to see how the cells pull on their environment through traction force microscopy, as there are obvious differences in movement between both axes. We would also like to observe how cells pull on the substrate as well as each other on curved environments using traction force microscopy [3].

Figure 2: Cells were shown to have no preference migrating along the channel verses around the curvature in stiff 384 µm channels (n = 49, p-value = 0.127). (See full-color version on inside cover.)

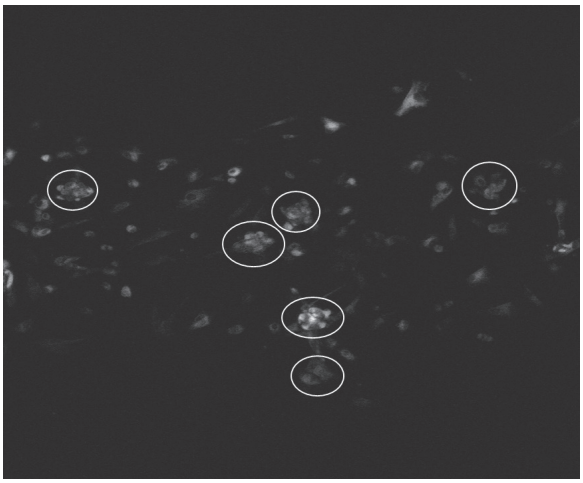

Figure 3: Fibroblasts grown on stiff two-dimensional substrates did not have a tendency to aggregate, whereas those in the curved channels did.

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References:

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