Cell Polarization on Circular Topography with Various Radii of Curvature

Peter Kim
Material Science and Engineering, Northwestern University

NNIN REU Site: NanoTech User Facility, University of Washington, Seattle, WA
NNIN REU Principal Investigator: Professor Deok-Ho Kim, Bioengineering, University of Washington
NNIN REU Mentors: Peter Kim and Kevin Mun, Bioengineering, University of Washington
Contact: peterkim2016@u.northwestern.edu, deokho@uw.edu, pkim40@uw.edu, kmuney@u.washington.edu

Abstract:

Recent studies have shown that cells orient along micro/nano-scale grooves that model the extracellular matrix (ECM) and provide a bidirectional cue for migrating cells [1]. Using ultraviolet (UV)-assisted capillary lithography techniques, sub-cellular sized arrays with varying radii of curvature were fabricated as a model system to probe cellular migration on curved substrata. Chinese hamster ovary (CHO) cells were cultured and plated on substrates with and without the micro-scale circular patterns. From time lapsed live cell imaging, the results demonstrated more explicitly that the cells polarized along the direction of topographic feature, and preliminary data showed that cell elongation was greater on the patterned surface verses cells on the flat surface.

Introduction:

The topographical and mechanical properties of the structure a cell adheres to are key factors in determining cellular response. These features are important for controlling the direction and speed of migration during cellular growth [2]. Better understanding of how cells sense and react to the physical features of their substrata can lead to optimized methods for tissue and muscular regeneration. With advances in fabrication techniques of biomaterials that has enabled stronger control over micromechanical and environmental properties such as local rigidity and curvature of the ECM components, micro-scale circular concentric ring patterns were constricted to model the natural curvatures in the ECM. We hypothesized that Chinese hamster ovary (CHO) cells will react to the micrometer range topographical features of the patterns by sensing the curvature and migrating along the grooves.

Experimental Procedure:

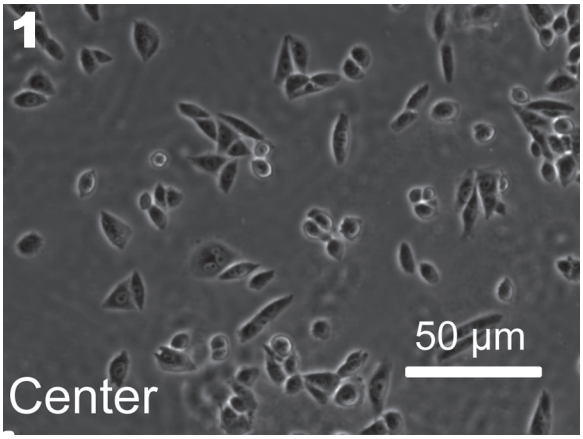
Liquid polyurethane acrylate (PUA, 301 MPa) was drop-dispensed onto treated cover glass (~ 10 μL/cm^2). A mold with the circular concentric patterns was pressed down on the PUA and rolled flat to form a uniform PUA layer on the cover glass. This combination was then exposed to UV light for 20 seconds, after which the mold was carefully peeled off from the cover glass, and an identical circular concentric array remained on the cover glass. The fabricated cover glasses were then placed under a UV lamp overnight to completely cure any remaining liquid PUA. Each cover glass was subsequently attached to the bottom of a cell culture device for cell plating.

Prior to plating the cells, each well of the cell culture device was coated with a 1.0 mL solution of Collagen Type I (50 μg/mL) for six hours. To detach the CHO cells from their culture dishes, 2.0 mL of trypsin base (0.05%) was pipetted into each dish for three minutes. The detached cells were washed down and added to 6.0 mL of Dulbecco's modified eagle medium (10.0% fetal bovine serum, 1.0% penicillin strip). One milliliter of this solution was added to each well of the cell culture device. The CHO cells were given 24 hours to adhere to the surface of the cover glass. Time-lapse image series were acquired from an electronic, inverted microscope (Nikon).

Results and Conclusions:

As seen in Figures 1 and 2, the cells on the flat substrate had random orientation while the cells on the patterned substrate aligned along the direction of the micro-scale grooves. After quantifying our data (Figure 3), the results confirmed that the cells on the flat substrata had a substantial amount of directional deviation from the curvature of a circle, while the cells that migrated on the patterned substrata had a very small average directional deviation from the curvature of a circle. Our protocol allowed us to successfully polarize cells along the grooves of our micro-patterned arrays.

As shown in Figure 4, cell elongation was greater for the cells on the patterned surface compared to the cells on the flat surface. Our preliminary data also suggested that cell elongation slightly increased as distance from the center of the concentric rings increased. This may be due to factors involving focal adhesions to increasing degrees of curvature. Our results confirmed that the topographical features of the micropatterns increased the elongation of the cells that migrated on them, and the elongation of a cell may be dependent upon the degree of curvature of the substrata underneath.



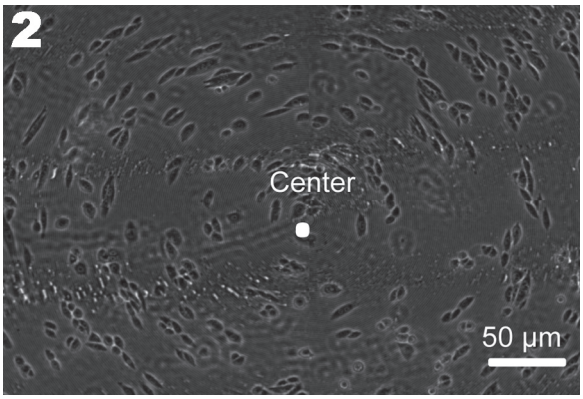


Figure 1, top: Cells on flat substrata after 24 hours of plating.

Figure 2, bottom: An entire micro-patterned array after 24 hours of plating. Cells appear to polarize along the concentric arrays.

Future Steps:

Studying initial adhesion processes, focal adhesions, and the attachment/detachment of cells will allow us to better understand how cells are able to sense the topographical features of the substrata they grow on. Observing cell skeletal fiber alignment and alpha-actin stress fiber distribution will help us better understand the mechanisms behind cellular migration.

Acknowledgements:

This research was supported by the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program and the National Science Foundation. I would like to thank the Kim Lab for their valuable guidance and the NanoTech User Facility at the University of Washington for providing the equipment and training necessary for this project.

References:

[1] D.-H. Kima, E.A. Lipkea, P. Kimc, R. Cheonga, S. Thompsona, M. Delannoyd, K.-Y. Suhc, L. Tunga, and A. Levchenkoa. Proceedings of National Academy of Sciences. J. 2010, 107, 565-570.

[2] D.-H. Kim, Kshitiz, R.R. Smith, P. Kim, E.H. Ahn, H.-N. Kim, E. Marba, K.-Y. Suhf, and A. Levchenko. Integrative Biology. J. 2012, 4, 963-1144.

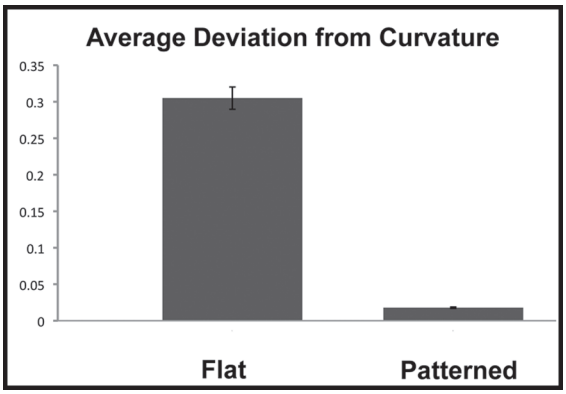


Figure 3: Cells on the concentric arrays showed much less directional deviation from curvature compared to the cells on flat substrata.

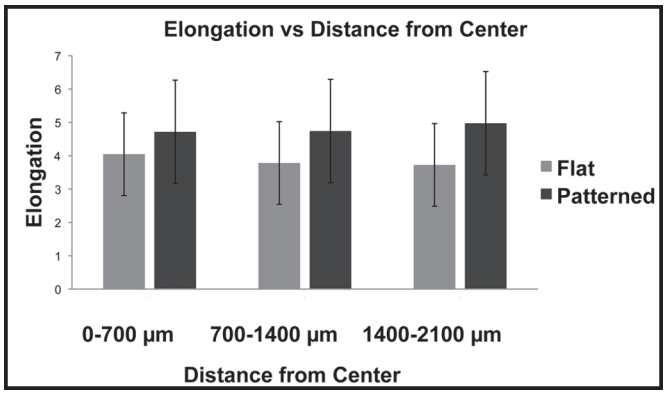


Figure 4: Cells on the concentric arrays had greater elongation than cells on flat substrata. Also, there seems to be a correlation between increasing distance from the center of the concentric arrays and increasing elongation.