Curvature-induced Membrane Strain: A Possible Driving Force for Focal Adhesion Recruitment, Increased Phosphorylation, and Phenotypic Response

Jyotsnendu Giri[a b], Jessica A. Zimberlin[b], Michael C. Weiger[b], Marcus T. Cicerone[b]
[a]*Parffenbarg Research Center, American Dental Association Foundation, NIST*
[b]*National Institute of Standards and Technology, Gaithersburg, MD 20899*

Statement of Purpose: Recent *in vitro* experiments culturing cells on micron and nano sized channels, beads, fibers, and posts have shown that many cell types respond to their nano-topological environment. A common finding of these studies is that focal adhesions tend to align along the edges of highly curved topographical features. While the precise mechanisms for protein recruitment to regions of high curvature are still an area of active investigation, this spatial localization has potential to significantly impact a cell's functional response to nanotopology since protein clustering and recruitment often precedes signaling events. We propose that cells use curvature-induced localization of membrane proteins and subsequent localization of signaling events as a mechanism to sense and respond to topography.

Methods: Here we test our hypothesis that, similar to the behavior seen in model systems, curvature-induced localization of membrane proteins is active in live cells, and is furthermore a key mechanism by which cells sense and respond to topography. We test this hypothesis by performing a systematic study of the effect of curvature on localization of focal adhesion (FAs) and phosphorylation events, and enhanced gene expression of an osteogenic marker in both MC3T3-E1 preosteoblasts and human bone marrow stromal cells (hBMSCs). For protein recruitment studies the cells were cultured on flat tissue culture polystyrene (TCPS) surfaces that were sparsely coated with electrospun TCPS nanofibers of varying fiber diameter. The flat regions of the cell were used as a control, allowing us to quantitatively assess the impact of curvature on localization of FAs and phosphorylation events. For phenotype studies, cells were cultured either on flat TCPS surfaces or on thick fiber mats of TCPS of controlled diameter. The quantitative analysis we perform requires that cell membranes conform to the topological features we present to them. Literature reports show that cell membranes will conform to topological features ranging in height from 200 nm to 5000 nm under similar conditions, and we show by membrane bending energy calculations, and SEM imaging that the cells do conform to the surfaces presented.

Results: We show that the clustering of an adhesion protein such as vinculin (figure 1) and phosphorylation events depends in a non-monotonic way on the curvature of TCPS nanofibers, and that upregulation of an osteogentic differentiation marker mirrors this non-monotonic trend in fiber diameter dependency for both MC3T3-E1 cells and hBMSCs. Our results are consistent with our curvature-sensing hypothesis; the localization of FAs, enhanced phosphorylation, and upregulation of an osteogenic marker (ALP) all occur in a similar and characteristic, non-monotonic response to substrate curvature. FA and pY enhancement has been observed at sharp features in the past, although the mechanism for this recruitment has not been clearly demonstrated. Here we show that the recruitment is non-monotonic in surface feature diameter and consistent with a bending-induced increase in the membrane chemical potential. The enhancement is attributed to a reduction in bending energy as appropriate proteins diffuse into the highly bent regions and are thermodynamically trapped.

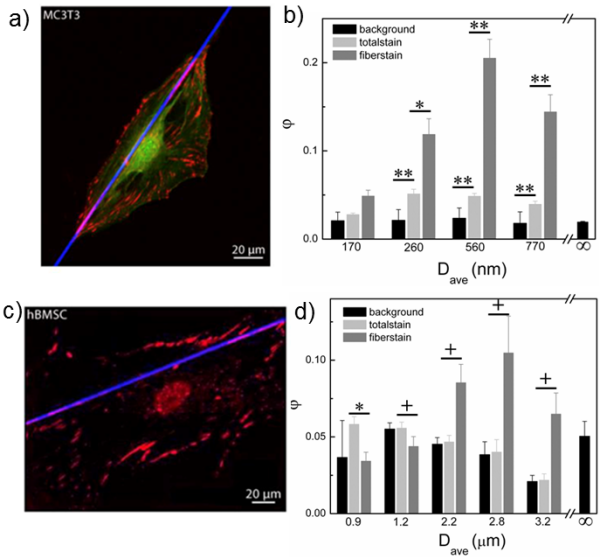

Figure1. Vinculin localization to fibers. (a) Representative image of an MC3T3-E1 cell, where blue corresponds to the fiber, red corresponds to vinculin, and green corresponds to the maleimide stain. (b) Area fraction graph of vinculin for MC3T3-E1 cells on fibers of different diameters. (c) Representative image of an hBMSC, where blue corresponds to the fiber and red corresponds to vinculin. (d) Area fraction graph of vinculin for hBMSCs on fibers of different diameters. Scale bar in images 20 μm. + denotes $p < 0.05$, * denotes $p < 0.005$, and ** denotes $p <<< 0.005$.

Conclusions: In this study, we used sparse coatings of nanofibers on TCPS and nanofiber mats to show that protein recruitment and clustering of early signaling events is guided by nanotopological substrate features, probably through modulation of the chemical potential of the cell membrane.

References: McMahon HT, Gallop JL. Nature. 2005;438:590-6. Flemming RG, Murphy CJ, Abrams GA, Goodman SL, Nealey PF. Biomaterials. 1999;20:573-88. Ro W, Wang, Z., Zhou, J., Lin, N.J., Cicerone, M.T., Soles, C., Lin-Gibson, S. Advanced Materials. 2011;23:421-5.