Dynamic topographical imprinting of cellular substrates

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Statement of Purpose: Cell cultures allow researchers to observe cell behavior in response to environmental cues (e.g. topographical features, chemical cues, and mechanical forces) leading to insights that enable better engineering design for biomedical applications such as tissue culture. Until recently, *in vitro* topographical cues have been limited to static substrates that do not subject cells to the highly dynamic conditions they experience *in vivo* when tissue remodels during development and wound healing.

An ability to deliver dynamic topographical cues to cultured cells can enable researchers to address long-standing questions about mechanisms of cell morphology changes. Resulting insights could enhance the development of wound-healing matrices and nerve guidance conduits by promoting migration of cells and providing directional guidance to cellular processes. Here we present an *in vitro* platform for investigating cell behavior in response to changing physical environments.

Methods: A substrate film comprised of proteins (gelatin and bovine serum albumin) and photosensitizers (rose bengal and eosin Y) was micro-3D (μ-3D) printed on cover glass through multiphoton photochemistry. This substrate was cultured with mouse embryonic fibroblast (NIH-3T3) cells. After cells adhered on the substrate, the surface was μ-3D imprinted with a grooved topography. Changes in cell morphology were tracked via brightfield microscopy. Intracellular organelles and imprinted surfaces of the substrate were characterized via confocal microscopy.

Results: Protein-based hydrogel substrates were remodeled in real time through localized contraction of the hydrogel induced by multiphoton photocrosslinking. Scanning the focal volume of a near-IR pulsed laser within the body of the substrate contracted the material in a user-defined pattern which imprinted new topographies on the surface without damaging cells. We found that cells transitioned from an initially stellate morphology to an elongated, bipolar morphology when the underlying substrate was imprinted with topographies of parallel grooves.

Cells plated on positive control surfaces with pre-imprinted grooves took on an elongated morphology parallel to the groove orientation (Figure 1 (a)). Cells plated on negative control surfaces without grooves maintained stellate morphology and random orientation. Cells plated on dynamic surfaces, which switched from ungrooved to grooved topography (at t=0 hr), transitioned from random stellate morphology to oriented bipolar morphology (Figure 1 (b)).

Actin filaments comprising the cytoskeleton of the cell also acquired an orientation parallel to imprinted grooves, confirming that imprinting of the substrate surface led to changes in the sub-cellular architecture of the cell.

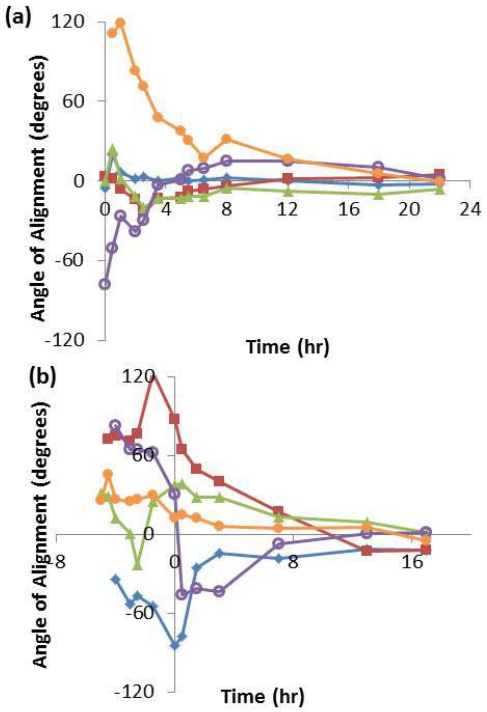

Figure 1. Line graphs track the alignment angles of cells on control (a) and dynamic (b) grooves.

Conclusions: These results demonstrate that cells respond to dynamic changes induced by real time topographical imprinting. In particular, cells reorganize their cytoskeleton in response to topographical remodeling of their underlying substrate, leading to observable changes in their morphology. These morphological changes occur over the course of 8-24 hours after topographical changes are introduced. Further studies may track the time course of actin reorganization within the cell, offering insights into the mechanisms of cell motility in response to dynamic environmental cues. This technique introduces a new tool to the repertoire of cell biologists for exploring the behavior of cells growing in a spatio-temporally dynamic environment, opening possibilities for studies of cell adhesion and cytoskeletal dynamics in conditions that may better reflect the environments that cells experience *in vivo*.

References:

Ritschdorff ET, et al. Lab Chip. 2012:12(5):867-71.