Adhesion Site Manipulation Using Multifaceted Micropatterned Surfaces Created with Laser Scanning Lithography

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Statement of Purpose: Integrin-mediated cell adhesion to the extracellular matrix (ECM) is an important regulator of intracellular signaling cascades. Many cell processes including capillary morphogenesis[1] and fibronectin (FN) fibrillogenesis[2] are integrin-specific and only occur in the presence of explicit ECM domains. In this work we are investigating the influence of α5β1 integrin ligation on directional cell motility. FN contains two distinct integrin binding domains, an RGD motif that binds αvβ3 integrins and a PHSRN sequence that ligates α5β1 integrins.[2] Surfaces presenting patterned arrays of either (1) RGD or (2) full-length FN allow for ligation of either αvβ3 or both αvβ3 and α5β1 integrins respectively and (3) multifaceted surfaces displaying interwoven patterned arrays of both RGD and FN, with each ligand confined to its own array, were created with Laser Scanning Lithography. These surfaces were used to assess the roles of biophysical cues and integrin ligation on focal adhesion formation, actin alignment, and cell migration.

Methods: Au surfaces were functionalized with a 4 mM ethanolic solution of oligo(ethylene glycol)-terminated alkanethiol (OEG) to create a passive background. A 1 mm^2 array of 1 by 8 μm elliptical patterns of the OEG SAM were thermally desorbed with a 532 nm laser focused through a 20X(NA0.8) objective at a fluence of 12.8 $nJ/\mu m^2$. The bare Au patterns were functionalized with either a 2 mM ethanolic solution of 1% GRGD-terminated alkanethiol in OEG or with FN at concentrations ranging of 0.025-25 μg/ml. Multifaceted surfaces displaying interwoven patterns of both ligands were created by functionalizing the 1st pattern array with 1% GRGD in OEG, thermally desorbing a 2nd array of the OEG orthogonal to the 1st, followed by exposure to FN. The surfaces were characterized with fluorescent microscopy, white-light interferometry, and X-ray photoelectron spectroscopy. HUVECs seeded on the surfaces were imaged with time-lapse differential interference contrast (TL DIC) microscopy, fixed, labeled for actin and vinculin, and imaged.

Results: ECs cultured on surfaces presenting ellipses of either RGD or FN formed vinculin-containing adhesions to the patterns, aligned their actin cytoskeleton to the long axis of the ellipses, and displayed directional motility parallel to the axis of alignment. Since intracellular force generation is governed by Rho-mediated actomyosin contraction, the observed directional motility is most likely due to an imbalance of the traction forces generated by the cells as dictated by the pattern geometry. Interestingly, HUVECs cultured on multifaceted surfaces presenting RGD ellipses orthogonally interwoven with FN ellipses formed adhesions to both the RGD and FN but the FN patterns dominated adhesion site placement, actin cytoskeletal alignment, and directional motility even when the FN surface density was orders of magnitude lower than the RGD density. This suggests that a biochemical influence rather than a biophysical influence was at play and we are currently testing the hypothesis that the FN patterns dominate motility through α5β1 ligation.

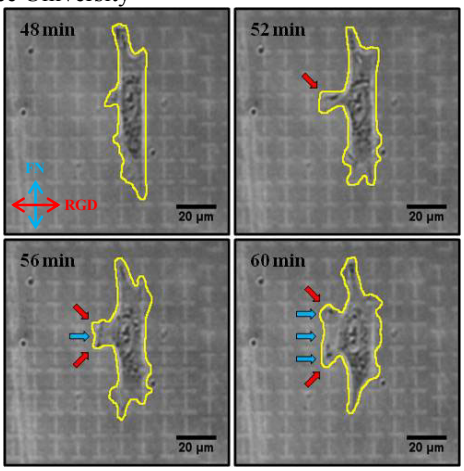

Figure1: Cell Motility on Multifaceted Patterned Surfaces ECs were cultured on multifaceted surfaces presenting interwoven elliptical patterns of GRGD-terminated alkanethiol (horizontal patterns) and FN (vertical patterns). Time-lapse DIC microscopy was used to monitor cell motility. ECs elongated to the long axis of the FN patterns but formed lamella (red arrows) on the RGD patterns that allowed for further binding to the FN patterns (blue arrows).

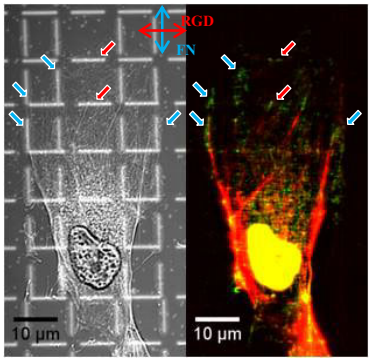

Figure 2: Cell Adhesion to Multifaceted Patterned Surfaces ECs on multifaceted surfaces bind to both RGD (red arrows) and FN patterns (blue arrows) as indicated by the vinculin-containing adhesions (green) but the actin (red) stress fiber alignment was typically dominated by the FN patterns.

Conclusions: Multifaceted patterned surfaces were implemented to elucidate the influences of biophysical and biochemical cues that regulate cell motility. The results suggest that biophysical cues (pattern geometry) can dictate directional motility regardless of the ligand present but that biochemical cues (specific integrin ligation) may be more influential in some cases.

References:

1. Liu & Senger. Faseb J. 2004;18: 457-468.
2. Vogel et al. Annu. Rev. Biomed. Eng. 2003;5: 441-463.