Recapitulating the Architecture of Cells of Interest Using Cell-Derived, Biomimetic Patterning

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Statement of Purpose

Micropatterns that regulate cell shape or spreading have provided much insight into tension-mediated control over cell fate. All of the patterns implemented have been derived from simple shapes (circles, squares, triangles, etc.). Although these simple patterns allow for control over cellular tension they do not provide a means to recapitulate the cellular architecture or tension state of a specific cell of interest (COI). To address this limitation we developed Image-Guided Laser Scanning Lithography[1] (IGLSL) to fabricate cell-derived, biomimetic patterns. Two pattern configurations were derived from each COI: one consisted of a continuous, pattern derived from each COI's morphology (insets in Fig. 1e-h) and the other a discontinuous configuration composed of many subcellular-sized patterns derived from an image of each COI's adhesions (insets in Fig. 1i-l). Cells cultured on both pattern configurations displayed an architecture (morphology, adhesive state, cytoskeletal organization, and nuclear properties) that quantitatively recapitulated the COIs that defined the patterns. Slight modifications to pattern design allowed for suppression of user-chosen, specific actin stress fibers and modulation of adhesion site dynamics. This approach to patterning provides a strategy to decouple the influences of cytoskeletal structure, adhesion dynamics, and intracellular tension on mechanotransduction processes and potentially the ability to recapitulate the adhesion site signaling state and phenotype of user-chosen COIs.

Methods

Human umbilical vein endothelial cells (HUVECs) were cultured on fibronectin (FN) functionalized glass coverslips for 16 hr, fixed, immunolabeled, and imaged. Four COIs were chosen and their adhesion sites (red in Fig. 1a-d) or morphology were used to create adhesion- (insets in Fig. 1i-l) or morphology-derived (insets in Fig. 1e-h) patterns. The COI images were processed in MATLAB to approximate the image features as a mosaic of quadrilaterals. The quadrilateral coordinates were exported as an overlay file to define regions of interest where an oligo(ethylene glycol) (OEG) terminated self-assembled monolayer was thermally desorbed from a platinum coated glass coverslip by raster scanning a 532 nm laser focused through a 63X(NA1.4) oil immersion objective operating at ~8.08 nJ/μm^2 using a Zeiss 5Live confocal microscope.[1-2] The patterned surfaces were functionalized with FN and HUVECs were cultured on the surfaces for 16 hr, fixed, fluorescently immunolabled and imaged.

Results

Implementing a multimetric, hierarchical clustering analysis we demonstrate that HUVECs cultured on both cell-derived pattern configurations formed a cellular architecture (morphology, adhesive state, cytoskeletal organization, and nuclear properties) that quantitatively recapitulated the COI used to define the patterns. While both pattern configurations induce similar architectures, the adhesion-derived pattern allowed for direct control over adhesion site placement and growth and induced a more static adhesive state with an average adhesion site lifetime of 26 min compared to only 13 min for cells on morphology-derived patterns as determined via time-lapse imaging of GFP-talin. Additionally, since IGLSL utilizes digital, virtual masks rather than physical masters required for most patterning techniques we demonstrate the ability to suppress the formation of specific, user-chosen actin stress fibers using "on the fly" pattern modifications.

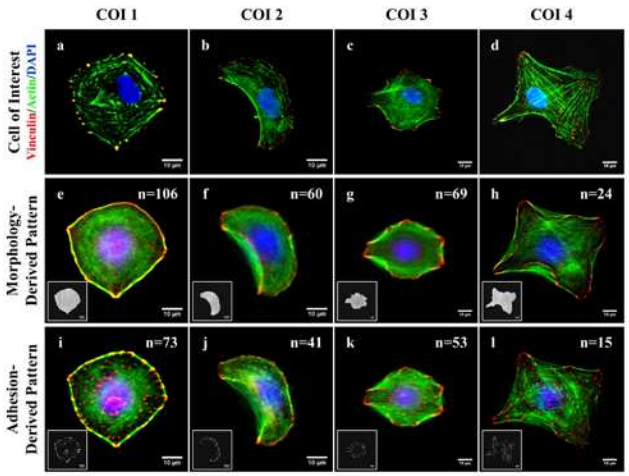

Fig. 1: Recapitulating the Cellular Architecture of COIs Using Cell-Derived, Biomimetic Patterns. (a-d) COIs were immunolabeled for vinculin (red) and actin (green), and counterstained with DAPI (blue). (e-h) Cells cultured on patterns derived from the morphology or (i-l) adhesion sites of COIs adopt a cellular architecture that mimicked the COIs that defined the patterns. (e-l) Insets display the pattern. The number in the top right of each image indicates the number of cells imaged and overlaid to create the "average cell" image.

Conclusions

We demonstrated the ability to fabricate high resolution, single-cell biomimetic patterns derived from the morphology or adhesion sites of user-chosen COIs and that ability to induce cells to adopt an architecture that quantitatively resembled the COI that defined the patterns. This approach to patterning could potentially be used to recapitulate the adhesion site tension and signaling state of COIs in order to control cell phenotype.

References

1. Slater, JH. et al. Methods Cell Biol. 2014:193-217.
2. Slater, JH. et al. Adv. Funct. Mater. 2011:2876–2888.