SUPPORTING INFORMATION

Attachment of Cells to Islands Presenting Gradients of Adhesion Ligands

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Fabrication of PDMS with microchannels: Poly(dimethylsiloxane) (PDMS) devices were made from 184 Silicone Elastomer Kit (Dow Corning Sylgard Brand, 10:1 base:curing agent ratio). Channels of rectangular cross section (32 μm wide by 32 μm tall) with 32 μm spacing between channels were fabricated using rapid prototyping in PDMS (McDonald, 2002). Holes were punched to make inlets and outlets of the channels using a sharpened 19 Ga hollow needle.

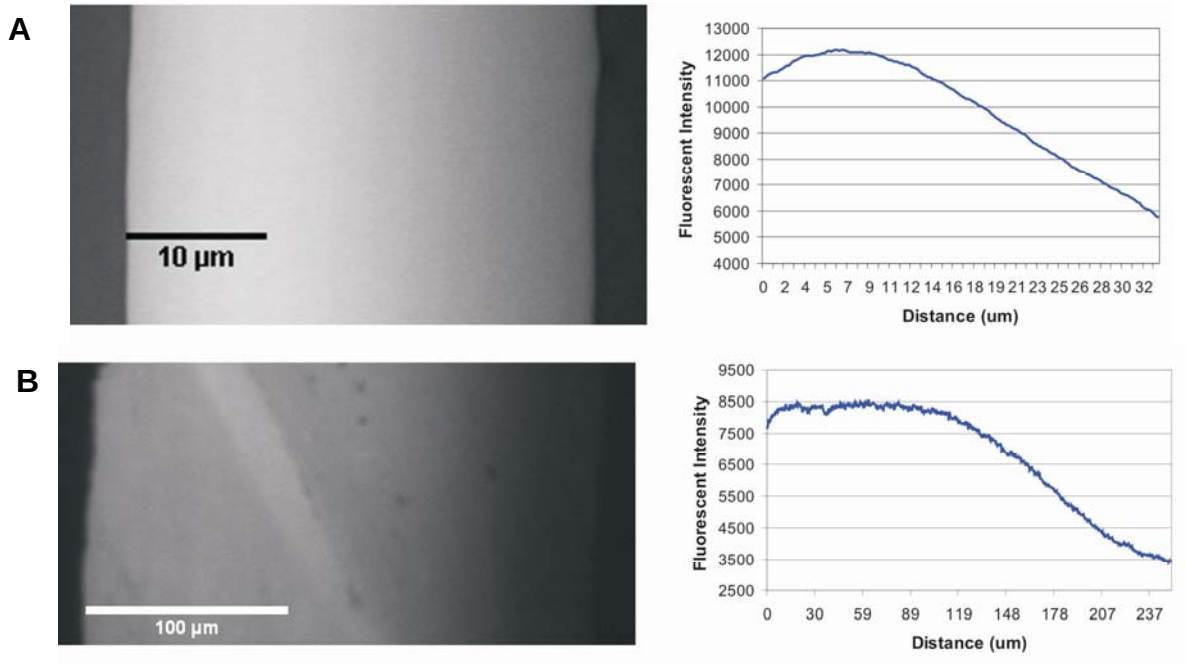
Synthesis of peptide substrates: All peptide substrates were prepared on Fmoc-Rink amide MBHA resin (AnaSpec. Inc., San Jose, CA) using standard Fmoc solid phase peptide synthesis techniques.

Preparation of self-assembled monolayers (SAMs): Monolayers of alkanethiolates on gold were prepared as described previously (Houseman, 2003). Briefly, gold substrates (prepared by evaporating 4 nm Ti and then 25 nm Au on #2 microscope cover glass) were immersed for 12 hours at room temperature in a 1 mM ethanolic solution of two disulfide species: a symmetric disulfide presenting tri(ethylene glycol) groups and an asymmetric disulfide presenting one maleimide group and one tri(ethylene glycol) group. The molar ratio of disulfides was 25:1 (EG3-EG3 : EG3-Mal) and gave surfaces having the maleimide group present at 2% relative to the total number alkanethiolate. The monolayers were rinsed with ethanol and dried under N_2 flow.

Preparation of gradient island arrays: A PDMS stamp with microchannels was sealed to the gold substrate and clamped between 2 pieces of ¼" thick polycarbonate using binder clips. Aqueous peptide solutions were introduced into the channels using syringes (Hamilton Co., Reno, NV) attached to the channel by 27 gauge Teflon tubing. Syringe pumps (Harvard Apparatus, Holliston, MA) were used to regulate the fluid flows. Immobilization reactions were performed by applying solutions of peptide (1 mM peptide in 10 mM Tris-HCl buffer, pH 7.0) to the monolayer for >1 hour at room temperature to ensure complete reaction. The inactive GGRDGSC peptide was first patterned into stripes with a fluid flow rate of 0.1 ul/min. The device was disassembled, rinsed with distilled water, dried under N_2 flow, and the Y-channel μFN was clamped to the substrate with the channels orthogonal to those of the first patterning step. Gradients in the Y-channel were generated using fluid flow rates of 0.5 ul/min for both peptide solutions (GRGDSC and GGRDGSC). The final patterning step was accomplished by immersing the entire chip in a 1 mM solution of GGRDGSC. The monolayers were rinsed with water and stored in PBS at 4 ºC until cells were applied.

Mass spectrometry: Monolayers on gold substrates were treated with a solution of 2,4,6-trihydroxyacetophenone (THAP, 10 mg/ml in an acetonitrile (50%) / water (50%) solution), dried and loaded into a Voyager DE-PRO Biospectrometry mass spectrometer (Applied Biosystems, Framingham, MA). A 337 nm nitrogen laser was used as the desorption / ionization source.

Gradient Profile:



We used fluorescence microscopy to characterize both the gradients of soluble reagents in the microfluidic device and the corresponding immobilized gradients of reagents on the monolayer. In these experiments we constructed counter gradients using the RGD peptide and thiol-derivatized fluorescein. Figure A shows an example of a confocal fluorescent image of the solution phase gradient profile at the median gradient steepness chosen for cell analysis (the experiment was performed as described in the manuscript). A line scan across the channel shows the distribution of fluorescein across the33 um channel. Note that the decrease in fluorescence on the left side of the channel is due to an edge effect, wherein the spot size of the light used to excite the fluorophore extends outside the channel and therefore averages regions with and without fluorescent reagent. Figure B shows a fluorescent image of the gradient bound to the monolayer, but this time using a wider channel. Because the gold film quenches the fluorescence of immobilized molecules, we transferred the monolayer to a PDMS substrate prior to microscopy, as described in an earlier report (Dillmore, 2004). The process used to 'peel' the monolayer from the gold substrate leads to slight artifacts, which are observed in the image. A line scan is again shown to the right of the image and verifies the presence of the immobilized gradient.

Cell culture: B16F10 cells (ATCC, Manassas, Va) were maintained in DMEM (Gibco, Gaithersburg, MD) supplemented with 10% FBS (Gibco, Gaithersburg, MD) and penicillin/streptomycin (Gibco, Gaithersburg, MD). Cells were used in passages 3-10 and seeded

onto 1000 μm^2 gradient islands at a density of 20,000 - 25,000 cells/cm^2 and allowed to spread for 5 hours before fixation.

Immunostaining: Mouse anti-vinculin primary antibodies (Sigma, St. Louis, MO), secondary AlexaFluor 488 anti-mouse, and Texas Red-X-phalloidin (Molecular Probes, Eugene, OR) were used at a dilution of 1:400. Hoescht 33258 (Molecular Probes, Eugene, OR) was used at a dilution of 1:1000.

Cells were fixed in 3.7% formaldehyde (Ted Pella, Redding, CA) at room temperature for 4 minutes and permeabilized in 0.3% Triton-X (Sigma-Aldrich, St. Louis, MO) for 1 minute. To help decrease non-specific nuclear staining, cells were incubated with Image iT-Signal Enhancer (Invitrogen, Carlsbad, CA) for 30 minutes at room temperature and then incubated with a blocking buffer containing 1% BSA (Sigma-Aldrich, St. Louis, MO), 0.1% Triton-X, and 5% goat serum (Gibco, Gaithersburg, MD) for 30 minutes. Primary staining and secondary antibody staining was done at room temperature for 2 hours and 1 hour, respectively. Slides were mounted in Aqua Poly/Mount (Polysciences, Inc., Warrington, PA).

Imaging and analysis: Slides were images with a Hamamatsu back-thinned EM-CCD camera on an Olympus IX81 spinning disc confocal microscope (Center Valley, PA) using a 100X oil-immersion objective. Images of cells were taken with identical exposure times to allow accurate comparison of fluorescence intensities between images. Cells were imaged using Slidebook (Intelligent Imaging Innovations, Inc., Denver, CO). Vinculin images were exported for analysis in ImageJ (NIH, Bethesda, MD), where they were aligned and stacked to generate an average grayscale heat map, which was then color-coded as a 16-bit tagged image file in PhotoShop (Adobe, San Jose, CA).

References

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Houseman, B.T.; Gawalt, E.S.; Mrksich, M. *Langmuir.* **2003**, *19*, 1522-1530.
Dillmore, W.S.; Yousaf, M.N.; Mrksich, M. *Langmuir*, **2004**, *20*, 7223-7231.