SUPPORTING INFORMATION

Nanopatterned Extracellular Matrices Enable Cell-Based Assays with a Mass Spectrometric Readout

Maria D. Cabezas[†], *Chad A. Mirkin*[†‡]* *and Milan Mrksich*[†‡§]*

[†]Department of Chemistry and International Institute for Nanotechnology, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, USA

[‡]Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, USA

[§]Department of Cell and Molecular Biology, Feinberg School of Medicine, 303 E. Chicago Avenue, Chicago, Illinois 60611, USA

Information on experimental methods and figures showing nanopattern arrangement across multiple length scales, optical micrograph of MHA patterned features after wet etching, and XPS analysis of monolayers.

Methods

Reagents. All reagents were obtained from the supplier and used as received. Phosphatase inhibitor I was purchased from Santa Cruz Biotechnology. Hexadecylphosphonic acid and 2,4,6-trihodroxyacetophenone were purchased from Sigma Aldrich. Amino acids and peptide synthesis reagents were obtained from Anaspec. All peptides were synthesized following standard solid phase peptide synthesis protocols as previously described.[1-3] A buffer comprising 20 mM Tris at pH 8.0 containing 0.5% triton was used for lysis and a protease inhibitor tablet obtained from Roche (cOmplete, Mini EDTA-free) was added to the lysis buffer prior use.

Preparation of SAMs. Glass slides were first cleaned by sonicating in ethanol for 30 min and dried under a stream of N_2. An electron beam evaporator was used to first deposit 5 nm of Ti onto the glass slides and subsequently vented to oxidize the Ti layer. Next, an aluminum mask having holes in a 384-well format was placed on top of the glass slide and an additional 5 nm of Ti were deposited followed by 35 nm of Au. The pen array was then immersed in a solution of MHA (10 mM in ethanol) for 1 min, dried with N_2, and mounted on a scanning probe instrument (Tera-Fab M series, TERA-print) where the humidity inside the chamber was fixed at 50%. A patterning routine was programmed in the instrument with tip-surface contact times of 1 s for 750 nm features. The Au-coated glass slides were soaked overnight at 4 ºC in an ethanolic solution containing a 1:4 ratio of an asymmetric disulfide terminated with a maleimide group and a tri(ethylene glycol) group and a symmetric disulfide terminated with tri(ethylene glycol) groups, with a 0.5 mM total disulfide concentration. The functionalized glass slides were rinsed with ethanol and then immersed in a 10 mM ethanolic solution of hexadecyl phosphonic acid for 10 min. After rinsing with ethanol and drying under air, a solution (3 µL) consisting of 40 µM phosphatase peptide substrate in 1X PBS at pH 7.5 was delivered onto each spot and incubated in a humidity chamber

for 1 h at 37 ºC. Surfaces were characterized by XPS using an electron spectroscopy for chemical analysis (ESCA) probe (Thermo Scientific EXCALAB 250 xi). Following peptide immobilization, the substrates were exposed to a solution of human plasma fibronectin (30 μg/mL in PBS) overnight at 4 ºC.

Cell-based assay for enzyme activity. HeLa cells were obtained from ATCC and cultured in DMEM medium supplemented with fetal bovine serum, glutamine, penicillin and streptomycin. All cells were cultured in a humidified incubator at 37 ºC and 5% CO_2. Cells were trypsinized and suspended in media, and the average number of cells per μL was estimated using a hemocytometer to seed the desired number of cells per spot. The volume of media per spot was 3 μL for all experiments. Cells were cultured on the monolayers presenting fibronectin and the phosphatase peptide substrate on glass slides for 2 h under standard growth conditions. Lysis buffer (1 μL) was delivered manually to each spot and the lysate was allowed to react with the monolayer for 1 h at 37 ºC in a humidity chamber. The surfaces were then rinsed with DI water and ethanol, and dried with air. A solution of 2,4,6-trihydroxyacetopehenone (THAP) in acetone (30 mg/mL) was delivered to each spot on the array and the surfaces were analyzed using an Applied Biosystems 5800 MALDI TOF/TOF instrument with a 20 kV accelerating voltage in positive reflector mode.

Lysis promotes interaction of PTPs with peptide substrate. A mild cell detachment reagent (TrypLE) was introduced to remove cells following culture on the patterned surfaces. This reagent (3 μL) was delivered to each spot and incubated for 5 min. The solution was then removed and the glass slide was rinsed with PBS, followed by DI water and dried with N_2. A matrix solution was applied prior to mass spectrometric analysis as described above.

Evaluation of PTP inhibition with SAMDI. Substrates that were nano-patterned with fibronectin islands surrounded by an immobilized phosphatase peptide substrate were generated using a Tera-Fab M series cantilever-free scanning probe instrument (TERA-print, IL) as described above. HeLa cells were seeded at 400 cells per spot on the monolayers. Following cell attachment and culture for 2 h, PTPI-I (1 µL solution in media) was added to each spot to achieve final concentrations ranging from 0 to 400 µM and incubated for 2 h. Following removal of the media, the lysis buffer with protease inhibitor was applied to each spot and incubated for 1 h at 37 ºC in a humidified chamber. The slide was then rinsed with water, ethanol and dried. Matrix was applied prior to analysis by mass spectrometry. All experiments were carried out at least 2 times, with 5 spots per condition each time. The dose-response data shows the averages and standard error of all spots and the IC50 was determined using Origin Pro8.

References

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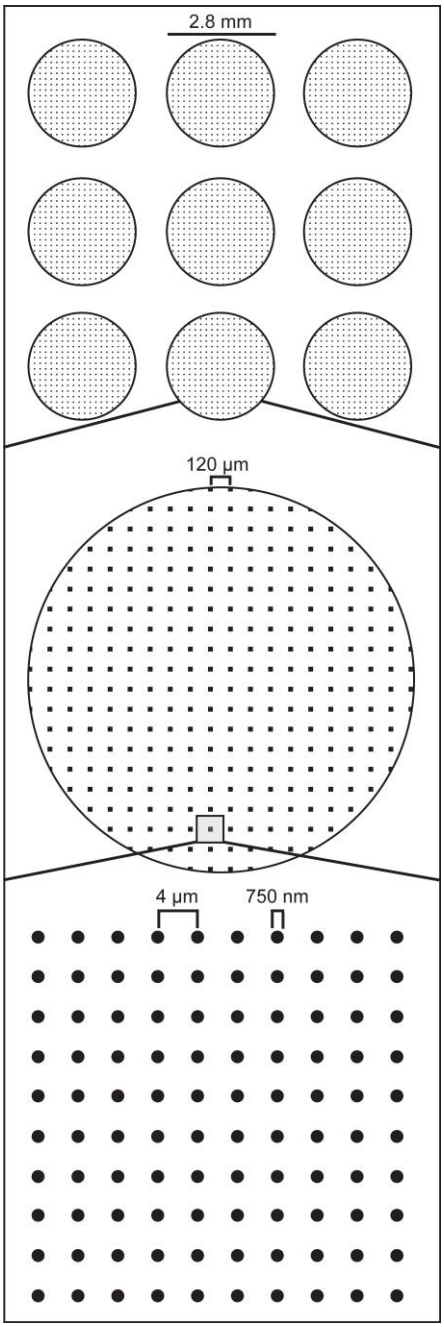

Figure S1. Pattern arrangement across multiple length scales. Nanoarrays were prepared on 384-well format gold islands, where each island was patterned using PPL to yield ~ 428 arrays of MHA features. Each array was patterned over a 40 x 40 μm^2 area having a total of 100 MHA features arranged in a 10 x 10 square matrix. The size of each individual MHA feature corresponds to ~ 750 nm.

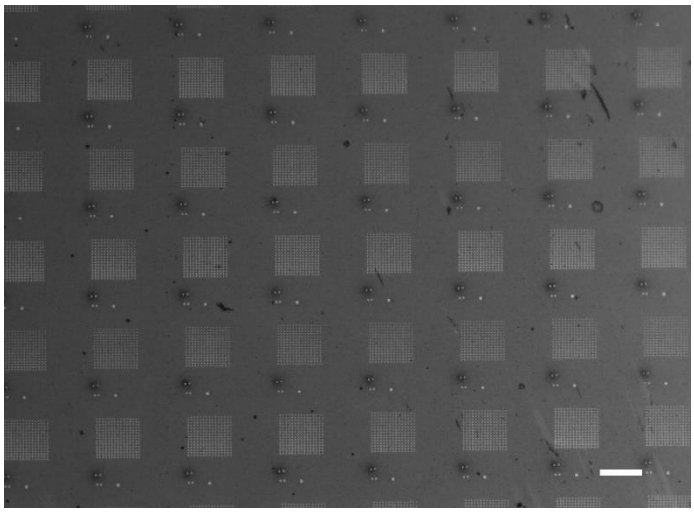

Figure S2. MHA features arranged in a square array patterned by polymer pen lithography (PPL). Optical micrograph of raised gold features ~1 μm in diameter made by chemical etching (with an aqueous solution of 13.3 mM $Fe(NO_3)_3$ and 20 mM thiourea) a portion of a glass slide having PPL-patterned mercaptohexadecanoic acid (MHA) features. The scale bar is 60 μm.

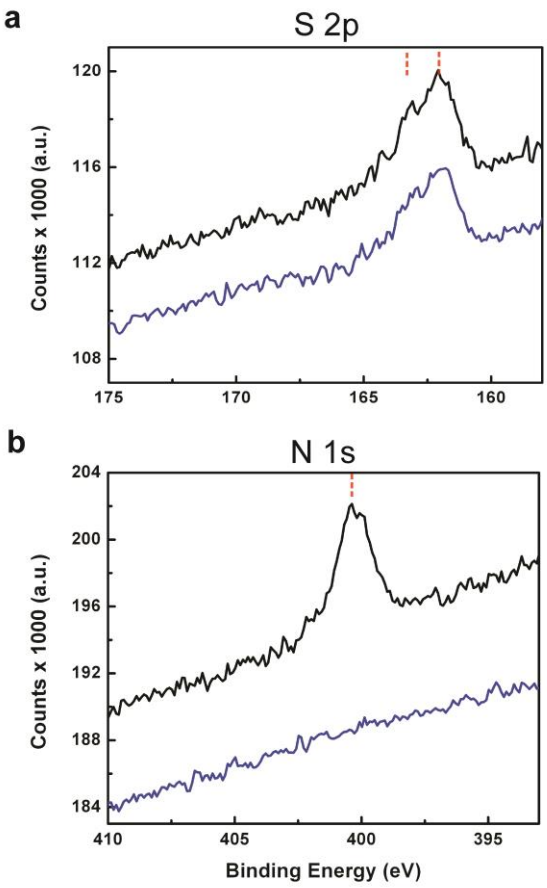

Figure S3. XPS spectra collected after peptide immobilization on Au regions that present a maleimide-terminated monolayer along with MHA nanoarrays. The presence of sulfur (a) and nitrogen (b) peaks indicate the availability of amide bonds and thiols on the surface (black trace), while a control surface consisting of a uniform MHA monolayer (blue trace) only shows presence of thiols. Dashed lines denote the N (1s) and S (2p) peak positions.