Supplementary Material (ESI) for Soft Matter

SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

Protein coating of silicon surfaces

The backside of 500 μm thick Si(100) wafers was scribed into 3x3 matrixes of 1x1 cm2 chips, which were then cracked into individual chips at the end of the fabrication process. First, the surface was cleaned with sonication in MilliQ water followed by O2 plasma treatment. The Si surface with its native oxide was activated by immersion into piranha solution (H2SO4/H2O2, 2:1, v/v) at room temperature for 15 min (Toxic, fuming solution). After extensive rinsing with MilliQ water, the surfaces were incubated in 2-3% APTES solution (3-aminopropyl triethoxy-silane SIGMA 3648, in acetone) for 15-20 min. The APTES treated surface was rinsed and dried first with nitrogen, then baked at 110oC for 10-15 min. The proteins, bovine serum albumin fraction V and fibronectin (BSA (BDH 9048-46-8) and FN (Sigma F2006)) were dissolved in universal buffer (150 mM NaCl, 5 mM Tris-HCl, 0.1% w/v NaN3, pH 7.6) and filtered through 0.2 μm Acrodisc syringe filters with HT-Tuffryn membrane. The APTES coated silicon surface was then incubated with 1% bovine serum albumin fraction V (BSA) for 2-3 hrs at room temperature, rinsed with MilliQ water and dried under nitrogen before electron beam exposure. After EBL, surfaces were backfilled with 0.05 mg/ml FN for 2-3 hrs at room temperature, rinsed with MilliQ water and dried under nitrogen.

Electron beam lithography

The BSA-coated chips were patterned by EBL using a Raith 150 Turnkey system with a high precision interferometric stage (Raith GmbH, Dortmund, Germany). The accelerating voltage was set to 5 kV and a 7.5 μm aperture gave a typical beam current of 10.5 pA, at a working distance of ~5.5mm. Patterns

were designed using the Raith 150 software in GDSII format. Point exposure doses ranging from 0.001 to 2.5 pC were tested. During electron beam exposure, chips were under a vacuum of ~$2x10^{-7}$ mBar for up to 12 hours.

Atomic force microscopy

Surfaces were inspected using a Multimode atomic force microscope with a J-scanner and NanoScopeIV controller (Veeco Metrology Group, Digital Instruments, Santa Barbara, CA). Contact mode imaging was performed with triangular cantilevers with sharp tips (nominal radius of curvature 10nm) and nominal spring constants of 0.03N/m (MSCT-AUHW). Images were processed with NanoScope Software v6.13r1 and ImageJ (NIH). In order to determine the radius of nanodots, the AFM height images were auto-thresholded and the "analyze particles" analysis was performed. For nanodots, a lower limit to the particle size was used to exclude the background particles. To examine the background, areas between the exposed nanodots were selected and all the particles in this area were counted. The mean spacing was calculated as follows: $\bar{d} \equiv \sqrt{\frac{area}{\#\ particles}}$

Immunofluorescence

The substrates were first stained for FN using FN specific primary antibodies produced in rabbit (SIGMA F3648, diluted 1:50), followed by TRITC conjugated anti-rabbit antibodies produced in goat (SIGMA T6778, diluted 1:100). Labeled substrates were mounted using glass coverslips and FluorSave (Calbiochem 345789). The samples were imaged using a Nikon epifluorescence microscope with a 100X oil immersion objective, a 12-bit cooled SPOT RT Monochrome CCD camera and SPOT Advanced image acquisition software (Diagnostics Instruments, Sterling Heights, MI).

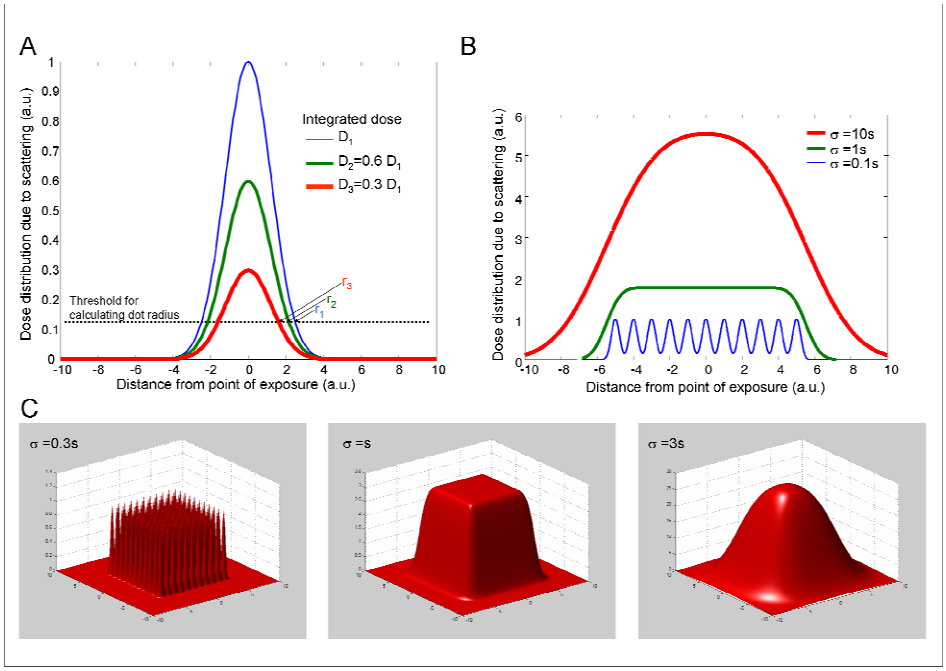
Cell culture

Swiss 3T3 fibroblasts were grown in DMEM (Gibco) with 10% fetal bovine (Atlanta biological) at 37^0C and 5% CO_2. Cells were passaged every 3 days with 0.25% trypsin-EDTA (Gibco).

For plating the cells were passaged and resuspended in serum free DMEM. Cell were then applied to the chips and kept at 37^0C and 5% CO_2 for 3 hours. Cells were then fixed and permeabilized with paraformaldehyde and triton X-100.

Immunocytochemistry and Microscopy

Cells were stained for FN with rabbit poly-clonal antibody to FN (ABCAM, AB299, Diluted 1:100) and goat anti rabbit antibody labeled with alexafluor 488 (Invitrogen, Diluted 1:100). Cy3 conjugated phalloidin (Jackson, Diluted 1:500) was used to stain the actin cytoskeleton. Vinculin was stained using a mouse anti-vinculin custom antibody with a Cy5 conjugated goat anti-mouse antibody (Jackson, Diluted 1:20). Samples were mounted onto glass coverslips in FluorSave. The samples were imaged using a Nikon epifluorescence microscope with a 60X oil immersion objective, a 12-bit cooled CCD camera (CoolSnap HQ, Photometrics, Tuscon, AZ), and image acquisition software (OpenLab, Improvision, Lexington, MA).



Exposures as a function of dose and step size. (A) As the dose is increased, a larger area is exposed to the electron beam. Nanodots, that could be identified using antibodies against fibronectin and immunofluorescence or atomic force microscopies, were created on areas that were exposed to a dose above the threshold value. (B) The edge quality of patterns depends on the step size(s) and the spreading radius (σ). (C) Exposure profile has steep edges and is uniform when step size equals the spreading radius. If the step size is larger, the exposure will not be uniform and if it is smaller, the edges will not be well-defined.