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Supporting information

Materials and Methods

Protein coating of silicon surfaces

We used 500 µm thick Si(100) wafers with a native surface oxide. The backside of the wafer was scribed into 3x3 matrixes of 1x1 cm2 chips. At the end of the patterning process the wafer was cracked into individual chips and used for surface and cell response analysis. Gold alignment marks were created on the silicon surface using UV lithography and a lift-off process as previously described[S1]. The surface was then cleaned and activated by immersion in piranha solution (H_2SO_4/H_2O_2, 2:1, v/v) at room temperature for 15 min (Toxic, fuming solution!). The silicon surface now bearing hydroxyl groups was extensively rinsed with MilliQ water before incubation 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. For initial protein coating and the backfilling, the proteins 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. APTES coated silicon surface was incubated in a 1% bovine serum albumin fraction V (BSA) or 0.05 mg/ml fibronectin (FN) (Sigma F2006) for 2-3 hrs at room temperature, rinsed with MilliQ water and dried under nitrogen before e-beam exposure.

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Electron beam lithography

The FN or BSA-coated chips were patterned by EBL using a Raith 150 Turnkey system with a high precision interferometric stage (Raith GmbH, Dortmund, Germany). An accelerating voltage of 5 kV with a 60 µm aperture corresponding to a typical beam current of 0.7 nA was used. The step size was 32 nm, and the area dose was 125 µC/cm2. Areas with widths ranging from 10 µm to 50 µm were exposed to electron irradiation. During exposure chips were under a vacuum of ~2x10-7 mBar for up to 12 hours. The surfaces were biofunctional, even after 12 hours in vacuum. 12 hours corresponds to an overnight, preprogrammed exposure, with several hundred unique patterns in 100 um x 100 um fields.

Atomic force microscopy

Surfaces were analyzed using a Multimode atomic force microscope with a J-scanner and NanoScopeIV controller (Veeco Metrology Group, Digital Instruments, Santa Barbara, CA). The substrates were imaged under ambient conditions. For contact mode and tapping mode imaging, triangular cantilevers with oxide sharpened tips and nominal spring constants of 0.03N/m, and 150 µm diving board cantilevers of 300 kHz were respectively used (Veeco Metrology Group, Digital Instruments, Santa Barbara, CA). Images were processed with NanoScope Software v6.13r1.

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Immunofluorescence

The templates were 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). Finally, templates were mounted on glass coverslips using FluorSave (Calbiochem 345789).

Cell Culture

Embryonic cortex was dissected in Hanks' balanced salt solution (HBSS, Invitrogen) from timed-pregnant Sprague-Dawley rats (B&K) on embryonic day 15 (E15; E1 was defined as the day of copulatory plug). The tissue was gently triturated and larger cell clumps were allowed to sediment for 10 minutes, after which the cell suspension was centrifuged. The pellet was resuspended in DMEM/F12 (Invitrogen) supplemented with B27 (1:50), Hepes, Penicillin/Streptomycin (Invitrogen) and 10 ng/ml FGF2 (PeproTech) and plated directly on silicon chips with FN lines at a concentration of 200 000 cells/ml. The following day the chips were washed two times with media to remove cells that were not attached to the surface. The cells were then left to differentiate in media without FGF2 for 7 days. During this time the media was changed every second day. The cells were examined and photographed in culture using differential interference contrast (DIC) microscopy (Nikon).

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Immunocytochemistry

Neural cells grown on silicon chips were fixed in 4% paraformaldehyde (PFA) at day 7 after FGF2 withdrawal. Cells were permeabilized with 0.2% Triton X-100 in PBS and incubated in blocking solution with 20% normal goat serum (Dako) in PBS, for 1 hour at room temperature. Double immunostainings were performed using the primary antibodies: mouse III-tubulin (BabCO, Nordic Biosite AB 1:200) and rabbit FN (AbCam, 1:100), and the secondary antibodies ALEXA Flour 488 and ALEXA Fluor 546 (Invitrogen). Incubation with primary antibodies was performed at 4°C over night and incubation with secondary antibodies at room temperature for 1 hour. The cell nucleus was labeled with DAPI (4'-6-Diamidino-2-phenylindole) (Molecular Probes).

Reference:

S1 Rundqvist, J.; Mendoza, B.; Werbin, J. L.; Heinz, W. F.; Lemmon, C.; Romer, L. H.; Haviland, D. B.; Hoh, J. H. Journal of American Chemical Society 2006, 129, 59-67.