Supplementary Information

Patterned porous silicon photonic crystals with modular surface chemistry for spatial control of neural stem cell differentiation

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Experimental Section

Solid Phase Peptide Synthesis: Peptides were synthesized manually by standard Fmoc solid-phase methodology. N-terminal fluorenylmethyloxycarbonyl (Fmoc) protected rink amide resin was deprotected with 20% piperidine in N',N'-dimethylformamide (DMF) for 15 min. and washed 4 times with DMF. Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), and N-methylmorpholine in DMF was added to three equivalents of amino acid for 1 hour at room temperature. Each subsequent amino acid was reacted the same way. After building the peptide, capping was performed using a propargyl-PEG-NHS ester (Quanta Biodesign) followed by cleavage using 95% trifluoroacetic acid (TFA), 2.5% H2O, and 2.5% triisopropylsilane (TIS). The peptide was re-precipitated in cold diethyl ether 3 times and lyophilized overnight. Peptides were analyzed with low resolution electrospray ionization (ESI) (Waters Quattro II) and semipreparative reversed-phase high-performance liquid chromatography (RP-HPLC) (Perkin-Elmer Flexar). All peptides used were purified to >90% purity as assessed by HPLC.

Lithographic patterning of gold circuitry: Photolithography was performed using a Karl Suss MJB3 contact mask aligner. Patterns were designed in Autodesk and printed by Output City. Shipley 1813 was spun on a p++-type silicon wafer of 0.001-0.005Ω resistivity at 4500 rpm for 45 seconds. The wafer was than exposed under 365 nm UV for 100mJ/cm2 and then developed for a

minute using MF-321. A Temescal E-beam evaporater was used to deposit 5 nm of chrome or 5 nm of titanium as an adhesive layer, followed by 100 nm of gold. The resist layer was then removed using sonication in acetone for 5 minutes.

Porous Silicon Etching: After patterning gold on the silicon substrate, the substrate was immersed in an electrochemical cell containing 1:1 aqueous HF (48%) (Sigma-Aldrich) and ethanol (Decon), with a platinum wire electrode and steel electrode contacting the back of the patterned silicon chip. Distributed Bragg reflectors (DBRS) were etched using an alternating high current of 200mA/cm2 for 1.17 seconds and a low current of 150 mA/cm2 for 1.3 seconds for twenty layers using a SP-200 BioLogic Galvanostat. After etching porous silicon was washed in ethanol once and in hexane once, then dried under nitrogen.

PSi Chemical Modification: Hydrosilyation of freshly etched PSi was performed with neat undecenoic acid (Sigma-Aldrich), which was freeze-pump-thawed five times, under an argon atmosphere in a schlenck tube. Terminal carboxylic acids were activated using 0.1M ethyl (dimethylaminopropyl) carbooimide (EDC) (Sigma-Aldrich) with N-Hydroxysuccinimide (NHS) (Sigma- Aldrich) in distilled water for an hour. Amine conjugation was then carried out with 0.1M azido-PEG3-amine (Broadchem) in acetonitrile (Sigma-Aldrich). Click chemistry was performed by reacting alkyne terminated peptides with the azide terminated surfaces using the following reagents from Sigma-Aldrich unless noted otherwise; copper (II) sulfate pentahydrate (1 equivalent), sodium ascorbate (25 equivalent), tetramethylethylenediamine (1 equivalent).

Gold Chemical Modification: After modifying the PSi with peptides, gold regions were modified using self-assembled monolayer formation with alkanethiolates. Patterned surfaces were immersed in a 0.1 mM solution of 15 % 11-(2-{2-[2-(2-azido-ethoxy)-ethoxy]-ethoxy}:-ethoxy)-undecane-1-thiol (herein HS-C11-EG4-N3) (Prochimia) in triethylene glycol mono-11-mercaptoundecyl

ether (herein HS-C11-EG3) (Sigma Aldrich). Surfaces were rinsed with ethanol and peptides conjugated to the monolayers by incubation of click solution (above) and alkyne terminated peptide (1:1, 10μL) at room temperature for 12 hours.

Neural Stem Cell Culture: Rat neural stem cells (Millipore) were cultured on tissue culture plastic which was modified as follows: 10 μg/ml of poly-l-ornithine (Sigma) in 5 mL of PBS (Millipore) was added overnight onto the dishes. The liquid was then aspirated the next day and 5 μg/ml of laminin (Sigma) in 5 ml of PBS was added overnight. The NSCs were then cultured using rat neural expansion media from Millipore until confluent. After patterned porous silicon was functionalized, Rat neural stem cells (passage 2-4) were detached using Accutase (Millipore) and cultured using expansion media without FGF onto these substrates for 24 hours. This time was chosen because of previous literature demonstrating upregulation of GFAP/MAP2 after after 24 hours [59] [60].

Immunofluorescence: To assess adherent cell counts, neural stem cells were stained with Hoechst dye (1:2000 in 1xPBS) after culture for two hours. For other immunofluorescence studies, neural stem cells were fixed after 24 hours in 4% PFA in PBS for 20 minutes. Cells were then permeabilized with Trition-X 100 for 30 minutes followed by primary antibody staining overnight at room temperature using the neural stem cell characterization kit purchased from Millipore. Briefly, samples that were stained with mouse anti-nestin (1:200) were also stained with rabbit anti-GFAP (1:250), and samples stained with Rabbit anti-SOX2 (1:1000) were also stained with Mouse anti-MAP2 (1:200). Secondary staining was done for 20 minutes at 37oC using 4',6-diamidino-2-phenylindole (DAPI; 1:2000), Anti-Mouse 647 (1:200) and Anti-Rabbit tetramethylrhodamine (1:200). Cells were marked positive for markers when they were

thresholded to be above average intensity of all samples. Immunofluorescence images were analyzed using ImageJ (NIH).

UV-Vis spectra: The fixed samples were mounted on glass slides and the reflectivity data was collected by a Zeiss Axio Observer D1 inverted microscope with a 10X objective lens. The measurements were spatially resolved and the spot size used was 0.5 mm. The spectra of six adjacent spots on each sample were collected and these spots were coincident with regions where cell were attached.

Characterization Instrumentation: A Thermo Nicolet Nexus 670 was used for diffuse reflectance Fourier transform infrared (DRIFTS) measurements on samples. Reflectivity data was gathered using a Zeiss Axio Observer D1 inverted microscope in Beckman. NSCs were images using an Olympus IX81 Inverted Cell Culture Microscope System with integrated high resolution three mega pixel camera.

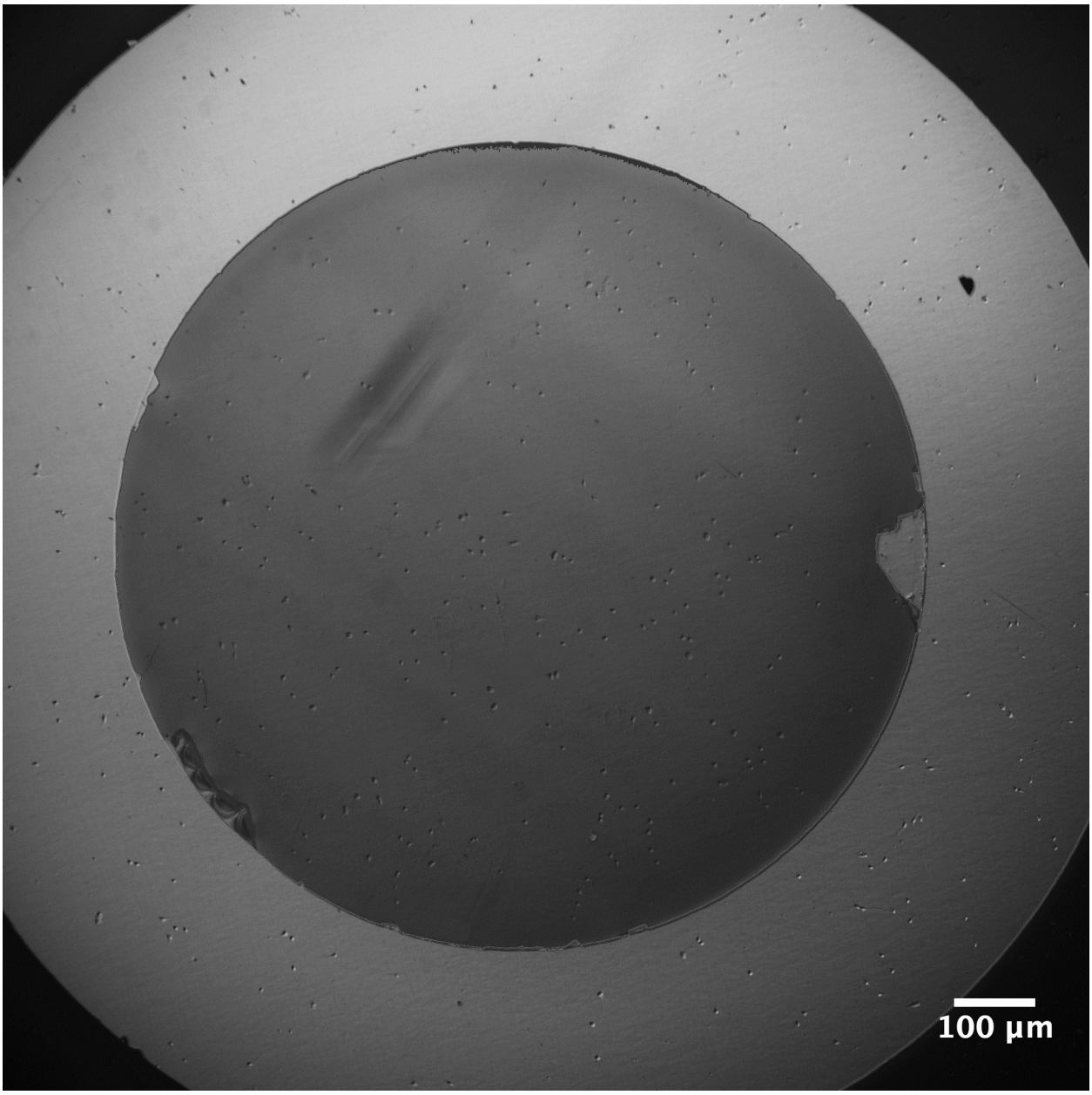

Figure S1. Phase contrast image of RGD modified gold ring (light) and IKVAV modified porous silicon (dark) with adherent neural stem cells.