Supporting Information

Nanoporous Gold as a Neural Interface Coating: Effects of Topography, Surface Chemistry, and Feature Size

Christopher A. R. Chapman[a], Hao Chen[b], Marianna Stamou[b], Juergen Biener[c], Monika Biener[c], Pamela J. Lein[b], Erkin Seker[d]*

a) Department of Biomedical Engineering, University of California – Davis, Davis, CA 95616

b) Department of Molecular Biosciences, University of California – Davis, Davis, CA 95616

c) Lawrence Livermore National Laboratory, Livermore, CA 94551

d) Department of Electrical and Computer Engineering, University of California – Davis, Davis, CA 95616.

[*]Corresponding author e-mail: eseker@ucdavis.edu

1. Experimental Details

Chemicals and Materials: Thin (0.15 mm-thick) glass coverslips (12 mm-diameter), used as substrates for film deposition, were purchased from Electron Microscopy Sciences. Gold, silver, and chrome targets (99.95% pure) were obtained from Kurt J. Lesker. Polydimethylsiloxane (PDMS) sheets were obtained from B&J rubber products. Isopropyl alcohol, nitric acid (70%, used as received), boric acid, and borax were purchased from Sigma-Aldrich. Sulfuric acid (96%) and hydrogen peroxide (30%) were obstained from J. T. Baker. Piranha solution, for cleaning glass substrates consisted of 1:4 ratio (by volume) of hydrogen peroxide and sulfuric acid.

Sample Preparation and characterization: Glass coverslips were cleaned by oxygen plasma exposure (Harrick Plasma Cleaner) for 60 seconds at 10 W and subsequently immersed in freshly-prepared piranha solution for 10 minutes. The coverslips were then rinsed using deionized (DI) water and dried under nitrogen. PDMS stencil masks with 5 mm-diameter spots were prepared using a laser cutter (VersaLaser, Universal Laser System). The stencil masks were then cleaned with isopropyl alcohol and dried under nitrogen flow. Piranha cleaned coverslips were aligned over the stencil with the sample surface facing the stencil. The stencil was then mounted onto a 4-inch wafer using before being loaded into the sputtering machine (Kurt J. Lesker) for sequential deposition of metals. First, a 160 nm-thick chrome layer was sputtered at 300 W as an adhesive layer between the glass substrate and the subsequent metal layers. Next, 80 nm of gold was sputtered at 400 W as a seed layer to reinforce the porous coating. Finally, silver and gold were co-sputtered at 100 W and 200 W respectively. All depositions were performed under argon atmosphere at 10 mTorr. All films fabricated for this study presented a final stack

size of ~1 micrometer and an alloy thickness of ~600 nm. Prior to dealloying the precursor AuAg alloy to produce np-Au films, the samples were treated with oxygen plasma for 60 seconds at 10 W and then immersed in heated nitric acid for 15 minutes at 55 °C. Following dealloying, samples were rinsed with DI water three times and stored in DI water for one week while replacing the water every day. Before use, the samples were dried under nitrogen flow. In order to produce both a high silver content np-Au film with similar ligament widths and np-Au films with increased ligament width, both the dealloying time and nitric acid concentration were altered. Films were created using diluted nitric acid (50%) at 55°C while altering the dealloying times. The high silver np-Au film was produced from a 10 minute dealloying, and was characterized to have ~12% (atomic percent) silver after production. Longer dealloying times of 20 minutes, 40 minutes, and 24 hours unheated were used to produce np-Au films with 37%, 79%, and 97% increased ligament widths respectively. The bulk residual silver content was then analyzed using energy dispersive X-ray spectroscopy (EDS, Oxford INCA) and scanning electron microscopy (FEI Nova NanoSEM430) at 50 kX for each sample produced. Top view images of samples were analyzed using a custom ImageJ (NIH freeware) macro and a custom MATLAB script to quantify morphological differences between films through comparing average ligament width and pore diameter. Briefly, the gray-scale SEM images were segmented into monochrome images in order to define pore as black and ligaments as white. A MATLAB script was then used to calculate the average width of the black features (ligaments) and white features (pore diameters) by taking pixel intensity slices along nine lines through each image.

Sample preparation and imaging for cell culture: Previously prepared samples were treated with oxygen plasma exposure for 60 seconds at 10 W. Samples were then placed in 24 well plates

(Corning) and transferred to a sterile environment. 500 mL of 0.5 mg/mL poly-L-lysine (PLL) solution was pipetted onto each sample. This PLL solution consists of poly-L-lysine, boric acid, borax, and water adjusted to a final pH of 8.5 (Sigma Aldrich). Samples were incubated with the PLL solution for 4 hours at 37 °C and 5% CO_2 before being washed with deionized water 6 times for 1 minute each wash. After washing, 500 mL of plating media were pipetted onto the samples. Plating media consists of 2% B27 supplement, 1X Glutamax, 10% heat-inactivated horse serum, and 1M HEPES at pH 7.5 with Neurobasal A as the basal medium. Samples were incubated with plating medium for 12 hours at 37 °C in a 5% CO_2 incubator. Primary cortical rat cells were obtained from the laboratory of Dr. Pamela Lein at the University of California, Davis following a procedure seen in Wayman, *et al*.[34] Cells were counted and suspended into plating medium at the desired density. The plating medium was aspirated from the samples and the cells were plated onto the samples at a density of 50,000 cells/cm^2. Cells were incubated for 4 hours at 37 °C and 5% CO_2 with plating media in order to promote attachment. After 4 hours, the plating media was aspirated and replaced with 500 mL of a serum free growth media (growth medium consists of 2% B27 supplement and 1x Glutamax with Neurobasal A as the basal medium). The cultures were grown for 7 days *in vitro* while supplementing with fresh growth medium at day 4 *in vitro*. At day *in vitro* 7 the cultures were fixed by washing 3 times with phosphate buffered saline (PBS) and subsequent incubation with 4% paraformaldehyde in PBS for 30 minutes at room temperature. Samples were then washed 3 times with PBS and stored at 4 °C until further processed. Before staining, samples were washed twice for 5 minutes using 0.05% Tween20 in PBS containing calcium and magnesium ions (PBS[+]). The cultures were then permeabilized using 0.1% Triton X-100 in PBS[+] for 3 minutes and washed twice more with 0.05% Tween20 for 5 minutes each wash. After that, samples were blocked with 2% goat serum in PBS[+] for 30

minutes at room temperature. A primary antibody solution of 10 μg/mL of mouse anti-tubulin-βIII and 100 μg/mL of rabbit anti-GFAP was prepared with a base of blocking buffer (2% goat serum in PBS[+]). After aspirating the blocking buffer from the samples, 80 μL of primary antibody solution was transferred to the sample surface and incubated for 1 hour at room temperature. After 1 hour the primary antibody solution was removed and samples washed three times with 0.05% Tween20 in PBS[+], 5 minutes for the first two and 10 minutes for the final wash. Secondary antibody solution consisting of goat anti-mouse conjugated to AlexaFluor 555 and goat anti-rabbit conjugated to AlexaFluor 488 at dilutions of 20 μg/mL was prepared in a base of PBS[+]. After washing, 80 μL of the secondary antibody solution was transferred to the surface of each sample and incubated in the dark for 1 hour at room temperature. Samples were then washed three times using PBS[+] and 80 μL of 4',6-diamidino-2-phenylindole (DAPI; nuclear stain) solution at a dilution of 1:20000 was applied. Finally, samples were washed three times with 0.05% Tween20 in PBS[+], rinsed in TC grade water, and mounted to number 1.5 glass slides (24 mm x 60 mm, Ted Pella) using Fluoroshield mounting agent (Sigma). Samples were imaged using an inverted fluorescent microscope (Zeiss Observer D1). Images were taken using a 100x objective in five predetermined fields on each sample to account for variability in each culture. Analysis of neuron and astrocyte surface coverage (percent coverage of cells in unit surface area) was performed on co-cultures grown on glass, planar gold (pl-Au) and nanoporous gold, as well as aluminum oxide-coated planar gold and nanoporous gold. The images were analyzed using ImageJ (NIH freeware) to calculate cell coverage.

Atomic layer deposition on nanoporous gold samples: The np-Au samples were ALD coated with nanometer-thick Al_2O_3 films using the well-established trimethyl-aluminum ($AlMe_3/H_2O$) ALD process[48] in a warm wall reactor (wall temperature of 100 ^oC and stage temperature of 125

^{o}C). This process is ideally suited to coat high aspect ratio materials such as np-Au due to the high volatility of the $AlMe_3$ precursor. Static dosing conditions (during which the chamber was isolated from the vacuum line) and long exposure and purge times (200 seconds each) were used to ensure uniform coatings throughout the porous material. The growth rate per cycle was calculated from the measured mass gain and the known surface area of bulk np-Au. Typically we used 10 ALD cycles corresponding to a film thickness of a 2.5 nm.

2. Figures and Tables

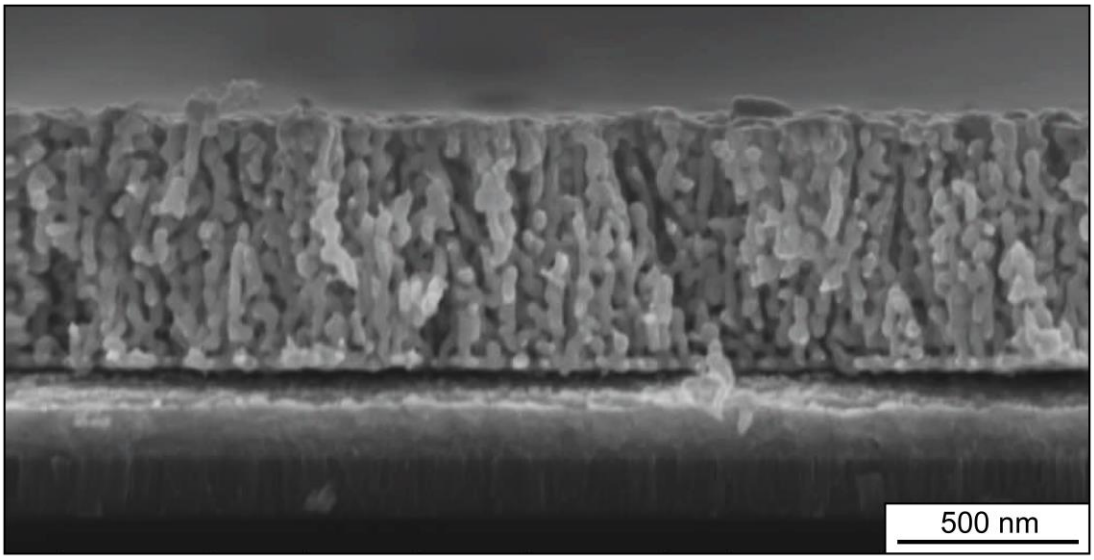

Figure S1. The cross-sectional image of np-Au reveals flat surface with minimal roughness.

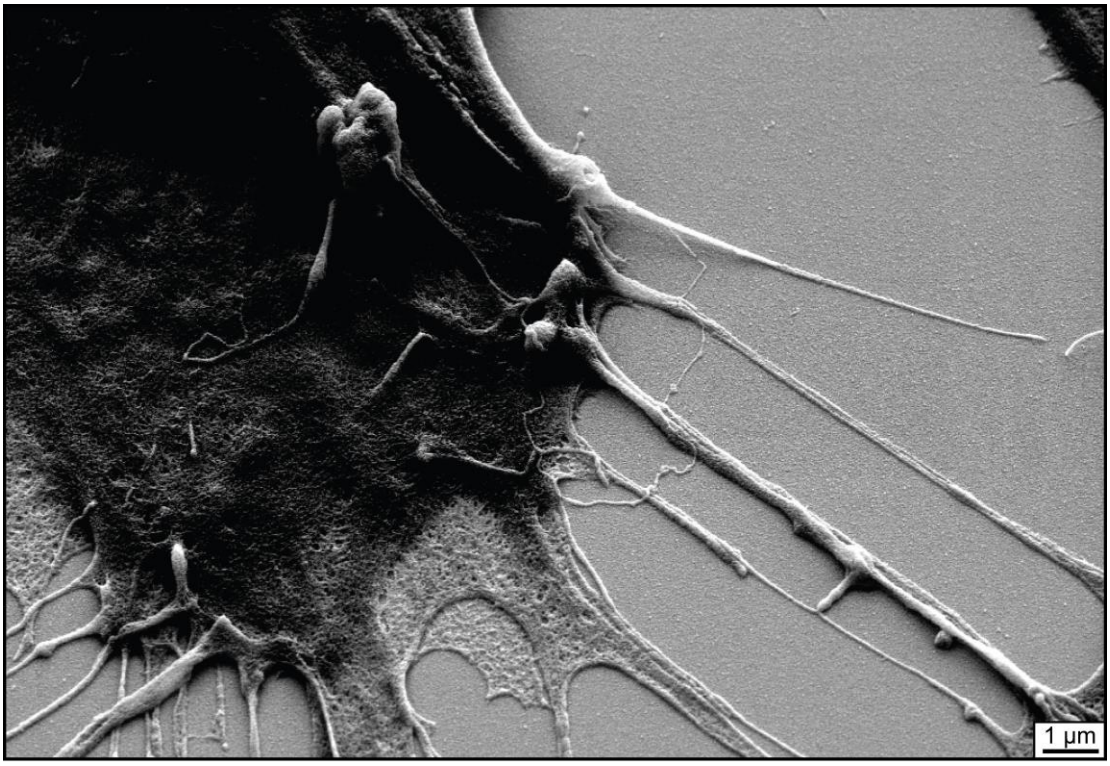

Figure S2. The SEM image illustrates typical astrocyte morphology on a planar gold surface, displaying characteristic thick and long processes.

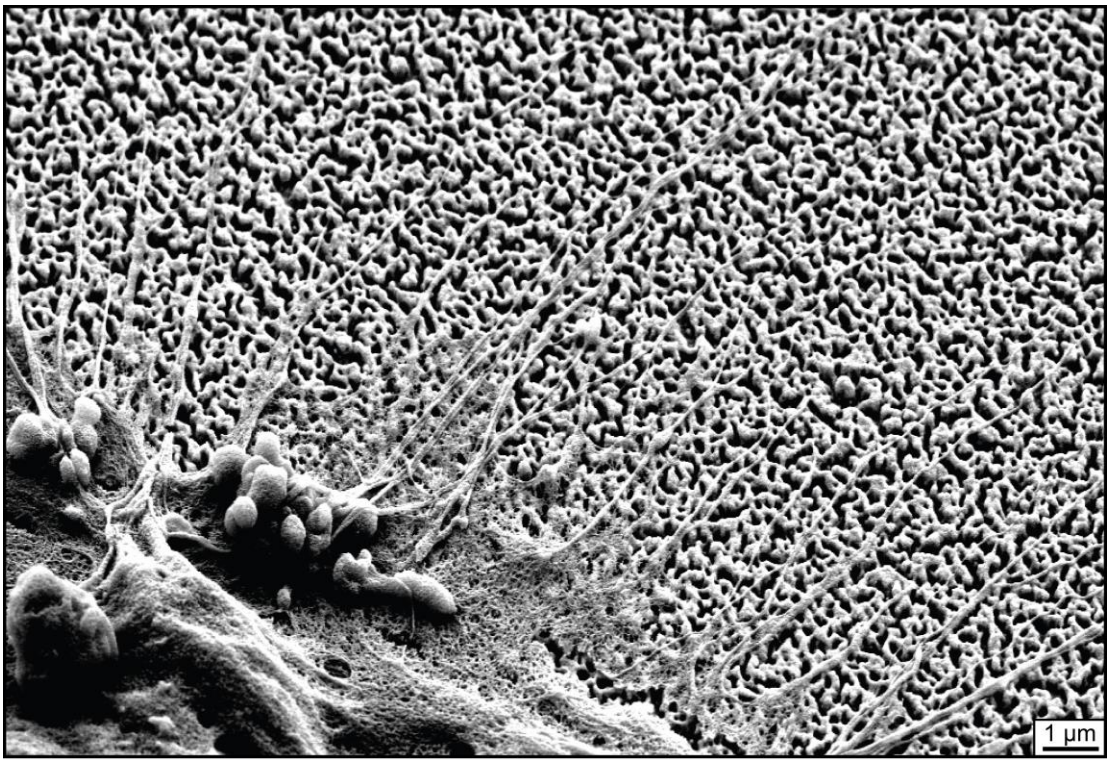

Figure S3. The SEM image illustrates that np-Au topography limits astrocytic morphology to the extension of fine and short processes spreading on continuous paths of ligaments.

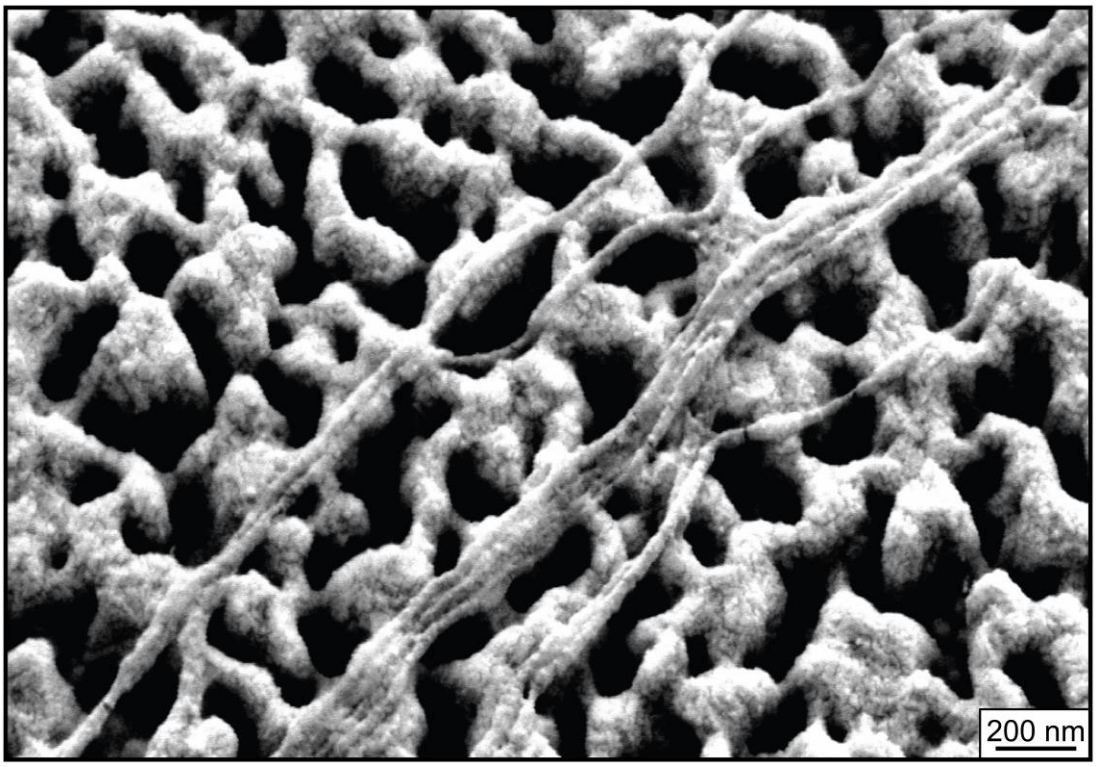

Figure S4. The SEM image illustrates the continuous path of ligaments that guide the growth of fine cellular processes.

Table S1. Average and Standard Deviations for Ligament Widths and Pore Diameters of Fabricated np-Au Films

Film	*Ligament Width (nm)*	*Pore Diameter (nm)*
Morphology 1	*30.62±18.94*	*87.11±65.55*
Morphology 2	*44.54±29.10*	*88.64±66.39*
Morphology 3	*70.65±52.34*	*149.20±104.07*
Morphology 4	*88.61±69.26*	*149.24±101.73*