Electronic Supplementary Information (ESI) for Lab on a Chip

Electronic Supplementary Information

Enhancement and control of neuron adhesion on polydimethylsiloxane for cell microengineering using a functionalized triblock polymer

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Abstract. This supplementary information provides all the additional information as mentioned in the text.

Materials and reagents

Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) block copolymers (Pluronic F127, average MW: 12,600), poly-D-lysine (PDL, MW: 150,000–300,000), fluorescein diacetate (FDA), propidium iodide (PI), and Hoechst 33258 were obtained from Sigma-Aldrich (MO, USA). PDMS prepolymer (RTV 615A) and curing agent (RTV 615B) were purchased from Momentive Performance Materials (Waterford, NY, USA). SU-8 2025 photoresist and developer were from Microchem (Newton, MA, USA). Primary antibodies of SMI-312 and MAP2 were purchased from Abcam (Cambridge, UK). Cell culture medium, fetal bovine serum (FBS), and Alexa Fluor-conjugated secondary antibodies were purchased from Thermo Fisher Scientific (Waltham, MA, USA). All solvents and other chemicals of analytical reagent grade were purchased from local commercial suppliers, unless otherwise stated. All solutions were prepared using ultra-purified water supplied by a Milli-Q system (Millipore®).

Synthesis of poly-D-lysine-conjugated Pluronic F127 (F127-PDL)

F127-PDL was synthesized using *p*-nitrophenyl chloroformate (*p*-NPC) mediated conjugation (Figure S1). Briefly, two terminal hydroxyl groups of Pluronic F127 (1.26 g, ~0.1 mmol) were preactivated with *p*-NPC (0.202 g, 1 mmol) in the presence of triethyleamine (TEA, 50 μL) in anhydrous dichloromethane (8 mL) under nitrogen atmosphere at room temperature (RT) for 12 h. The *p*-NPC activated F127 was precipitated three times in ice-cold ethyl ether for purification and dried under vacuum (yield: 71%). Next, the *p*-NPC activated F127 (65 mg, ~5.0 μmol) was placed in a round bottom flask and dissolved in dimethylsulfoxide (DMSO, 5 mL). The solution was stirred and PDL (10 mg, ~0.044 μmol) in DMSO (3 mL) was then slowly added into the flask in a dropwise manner. After the addition, the reaction mixture was stirred at RT for 36 h. The product was purified using extensive dialysis (MWCO: 300,000) against water and lyophilized (yield: 90%). 1H-nuclear magnetic resonance (NMR) spectra were recorded using a BrukerAvance DMX 500 spectrometer (Bruker, Billerica, MA, USA). ^{1}H-NMR (500MHz, D_2O,

ppm): 1.09 (PO, -CH_3); 1.24–1.64 (lysine, -CH_2-); 2.93 (lysine, -CH_2-N-); 3.42–3.56 (PO, -CH_2-/-CHMe-); 3.63 (EO, -CH_2-); 4.25 (lysine, -NCHR-CO-).[1,2]

Microfluidic device fabrication

The microfluidic device was fabricated using soft lithography with PDMS.[3–5] First, we designed patterns for the channels and chambers using AutoCAD software (Autodesk, San Rafael, CA, USA). Next, the channels and chambers were printed at a resolution of 20,000 dots per inch (DPI) on transparency film (MicroCAD Photomask Ltd., Shenzhen, China) to be used as the photomask. A fluidic mold was then fabricated in a single step under UV light using an SU8-2025 photoresist on a URE-2000 mask aligner (10 mW cm^{-2}, IOE, Chinese Academy of Sciences, China).

Before fabricating the microfluidic device, the mold was exposed to trimethylchlorosilane vapor for 3 min. A well-mixed PDMS prepolymer [RTV 615 A and B (10:1, w/w)] was poured onto the mold placed on a petri dish to yield a 3 mm thick fluidic layer. After degassing, the fluidic layer was cured in an 80 °C oven for 35 min and then peeled off the mold. Holes were punched using a hole puncher at the terminals of the channels. Next, the fluidic layer was placed on top of a glass slide, which was spin-coated with a thin PDMS film [RTV 615 A and B (5 : 1, w/w), 3000 rpm for 60 s, and cured at 80 °C for 10 min). The microfluidic device was then ready for use after baking at 80 °C for 48 h.

Neuronal cell preparation

Primary cortical neurons were obtained from embryonic C57BL/6 mouse pups following standard protocol.[6,7] Briefly, the mouse pups were sacrificed by cervical dislocation, and the cortex was separated from the brain and dissociated with trypsin for 20 minutes at 37 °C. Next, neurons were collected by centrifugation, and resuspended in neurobasal medium for use. The animal experiment was approved by Institutional Animal Care and Use Committee of Central South University.

Neuronal cell culture and patterning on PDMS surface

For neuronal cell culture, the PDMS substrate was placed on a 35-mm petri dishes (Corning), UV sterilized, and then modified using F127-PDL solution (100 μg mL^{-1}) for 2 h at RT followed by phosphate-buffered saline (PBS, 0.01 M, pH 7.4) rinsing. The primary cells (1.0×10^6 cells mL^{-1}, 2 mL) were seeded in the PDMS-contained dish and cultured using fresh neurobasal medium supplemented with 2% B27 (Gibco) at 37 °C in a humidified atmosphere with 5% CO_2. The cells were fed every third day by aspirating half of the medium from the dish and replacing it with fresh supplemented neurobasal medium.

For neuronal cell patterning, a PDMS fluidic layer with embedded microchannels was placed on top of teh PDMS substrate to make a temporary sealing. F127-PDL solution (100 μg mL^{-1}) was introduced into the channels and incubated to modify the substrate for 2 h at RT, followed by PBS rinsing. Next, the PDMS fluidic layer was peeled away, and the cell seeding and culture on the F127-PDL-patterned PDMS substrate were performed by the aforementioned operations.

Microfluidic neuronal cell culture

For microfluidic neuronal cell culture, the device was UV sterilized, gel-preloaded, and prepared for culture by coating the chamber with F127-PDL solution (100 μg mL^{-1}) for 2 h at RT, followed by PBS rinsing. The coated chamber was then equilibrated with fresh neurobasal medium. Neurons were loaded into the chamber by flowing the suspension for 5 min at a cell density of 2.0×10^6 cells mL^{-1}. The device was incubated at 37 °C for 2 h to allow cell adhesion. The loaded cells in the chamber were cultured using fresh supplemented neurobasal medium at 37 °C in a humidified atmosphere with 5% CO_2.

Cell staining

Cell viability was evaluated using a FDA/PI staining protocol. After removing the culture medium from cells and rinsing with PBS, the FDA/PI (10 μg mL^{-1} of each in PBS) staining solution was added, and the staining process was performed for 15 min at 37 °C. Afterwards, PBS was

introduced for 10 min as a final rinse.

For immunocytochemical analysis, neurons were fixed with 4% paraformaldehyde in PBS, permeabilized and blocked in PBS containing 0.3% Triton X-100 and 5% bovine serum albumin, and incubated with the primary antibody (SMI-312: axon-specific marker; MAP2: somatodendritic marker) in the same buffer, followed by secondary antibodies conjugated with Alexa Fluor 555 and Alexa Fluor 488 for visualization. Cell nuclei were counterstained using a Hoechst staining solution (H33258, 0.5 μg mL^{-1} in PBS).

Microscopy and image analysis

A confocal laser scanning microscope (Olympus, FV1000) and an inverted microscope (Olympus, CKX41) with a charge-coupled device camera (Olympus, DP72) and a mercury lamp (Olympus, U-RFLT50) were used to acquire phase contrast and fluorescence images. Image-Pro® Plus 6.0 (Media Cybernetics, Silver Spring, MD) was employed to conduct image analysis associated with the fluorescence intensity. ImageJ plugin NeuronJ (NIH) was used to trace the neuronal axons and quantify their lengths. SPSS 12.0 (SPSS Inc.) was employed to perform data statistical analysis. Values are represented as the means±SD, and statistical comparisons of means were made using one-way analysis of variance (ANOVA). For all statistical tests, ***P<0.001 was used as the criterion for statistical significance.

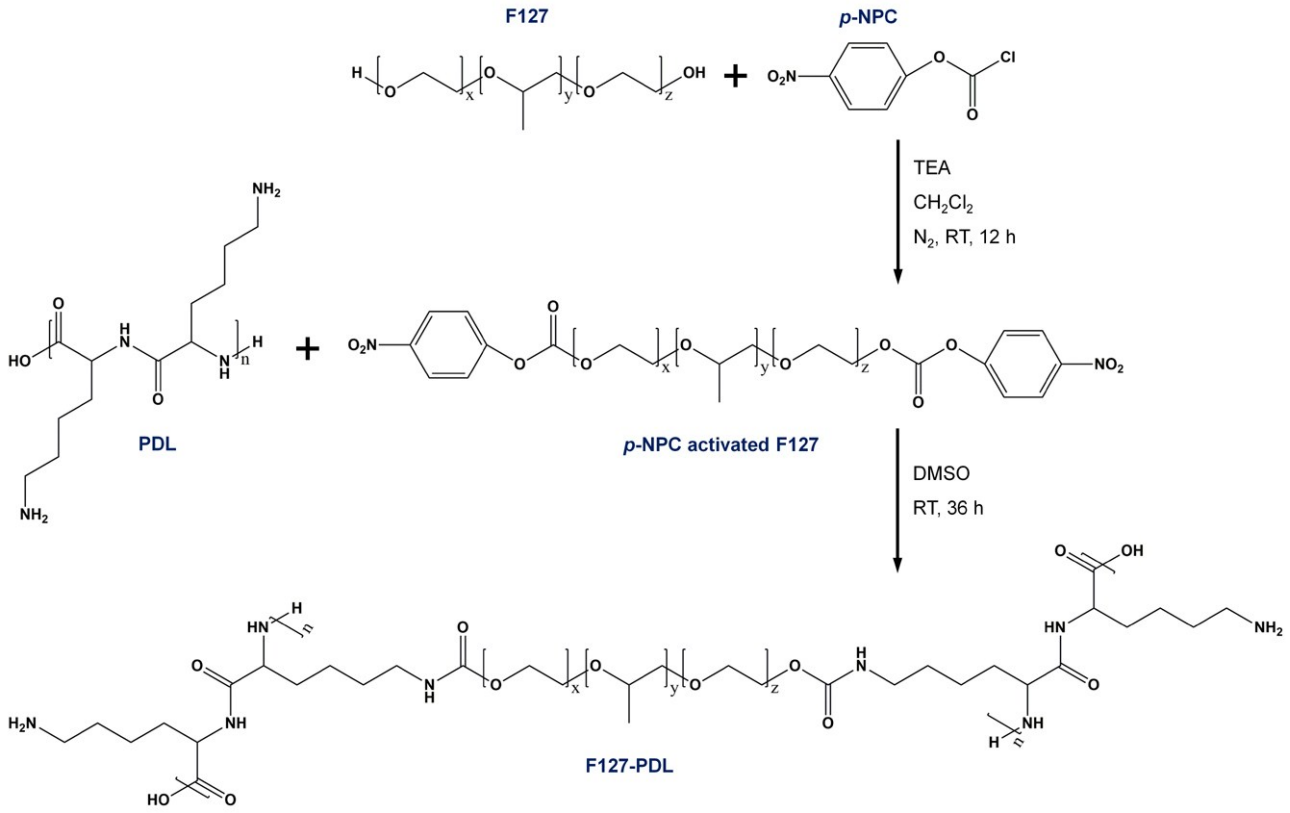

Fig. S1. Synthesis of poly-D-lysine-conjugated Pluronic F127 (F127-PDL)

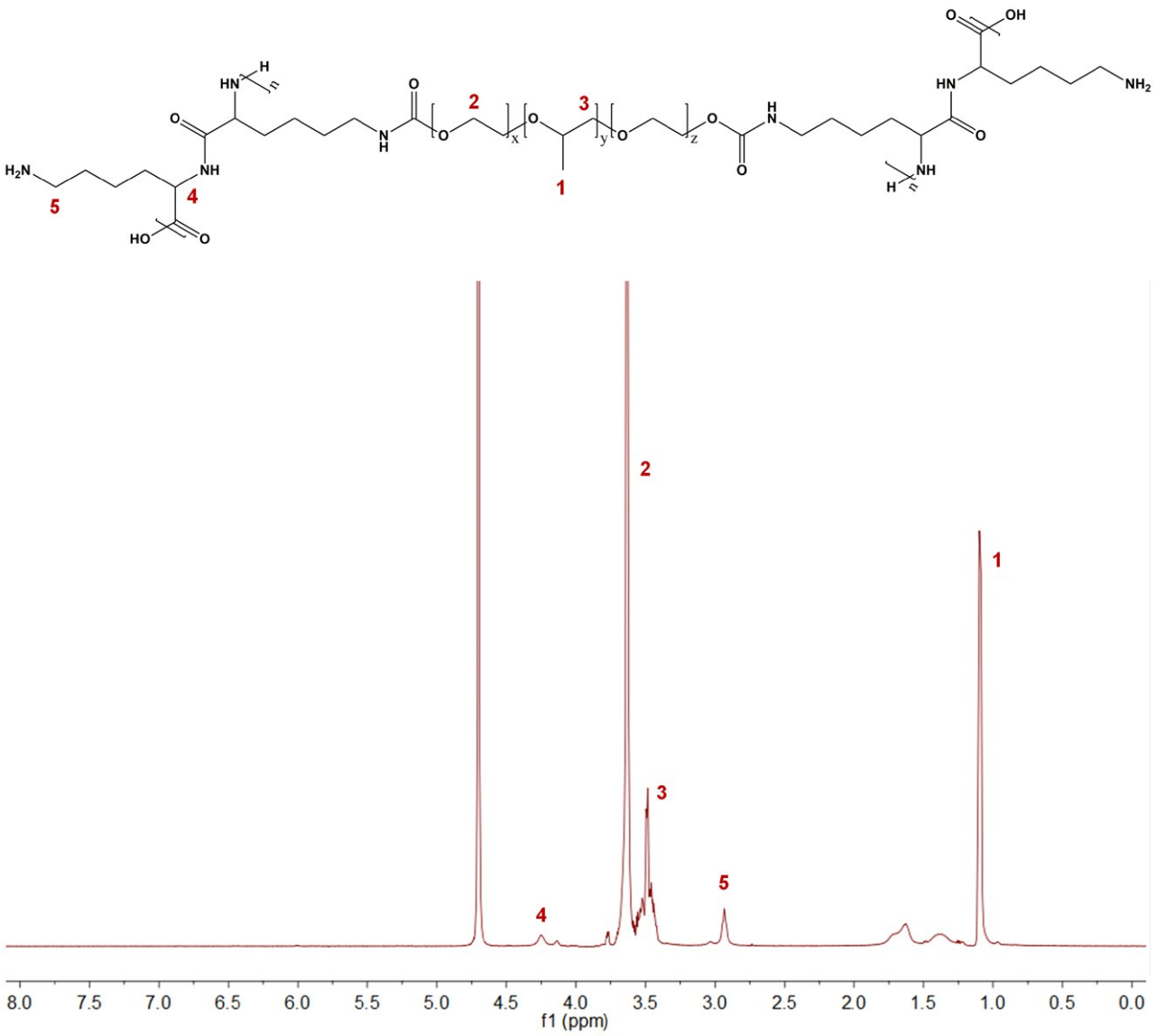

Fig. S2. ^{1}H-NMR (500 MHz) of F127-PDL in D_2O.

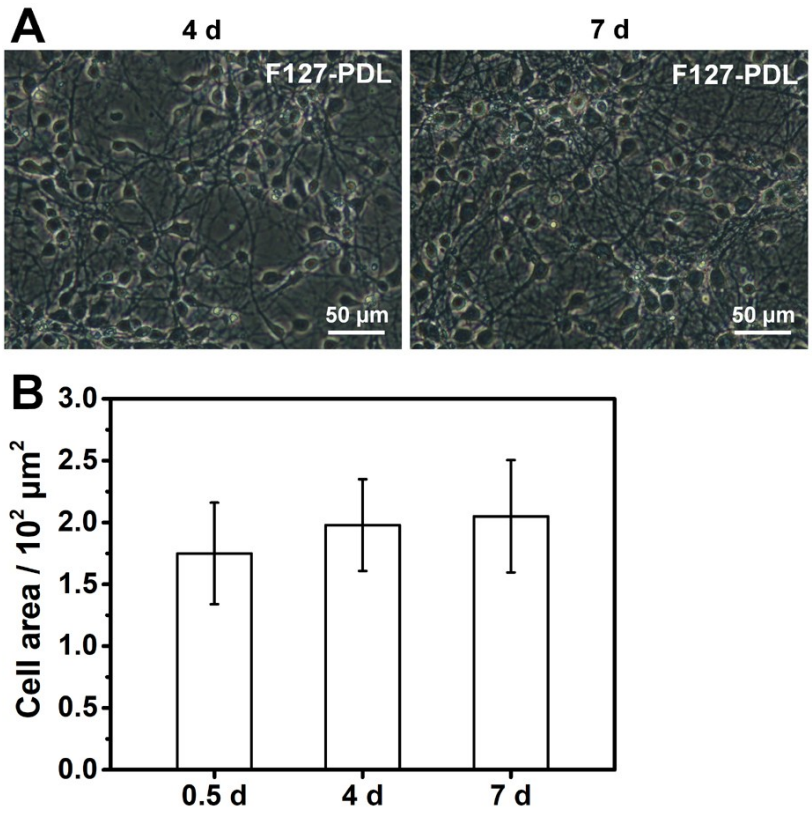

Fig. S3. Neuron adhesion on F127-PDL-modified PDMS surface with sustained cultivation (7 days). (A) Optical images of primary neuronal cell adhesion on the F127-PDL-modified PDMS after 4 d and 7 d in culture. (B) Adhesive cell area on the F127-PDL-modified PDMS after 0.5 d, 4 d, and 7 d in culture.

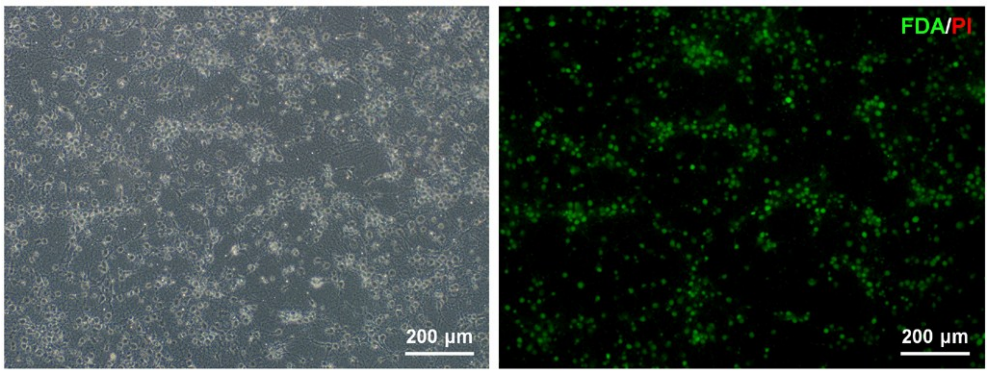

Fig. S4. Optical (left) and fluorescent (right) images of neurons after 7 days in culture. Live/dead (green/red) cells were visualized by FDA/PI staining.

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