Electronic Supplementary Information

A lab-on-a-chip platform for studying the subcellular functional proteome of neuronal axons

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Supplementary Materials and Methods

Materials Pregnant Sprague–Dawley rats were obtained from BioLASCO Taiwan Company (Taipei, Taiwan). L-Glutamine, L-glutamic acid, cytosine-β-D-arabinofuranoside (ARC), HEPES, cysteine, Triton X-100, papain, DAPI (4', 6-diamidino-2-phenylindole), glucose, poly-L-lysine (70–150 kDa MW), bovine serum albumin (BSA) and DNaseI were purchased from Sigma (St. Louis, MO, USA). The primary antibodies against βIII-tubulin, MAP2 (microtubule binding protein 2) and GFAP (glial fibrillary acidic protein) were purchased from Chemicon (Temecula, CA, USA). The primary antibodies against GAP-43 (growth associated protein 43) and Tau (Tau1) were obtained from Millipore (Billerica, MA, USA). The antibody against synaptophysin was purchased from Leica Microsystems (Wetzlar, Germany). SMI312 antibody (the primary antibody against phosphoneurofilament) was purchased from Covance (Emeryville, CA, USA). Cy3-conjugated goat anti-rabbit IgG antibody was purchased from Jackson Immunoresearch Laboratories Inc. (West Grove, PA, USA). FITC-conjugated goat anti-mouse IgG and the antibody against calcium, calmodulin-dependent protein kinase II α-subunit (CaMKIIα) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Glutaraldehyde (25%) was purchased from Electron Microscopy Sciences (Fort Washington, PA, USA). B-27 serum-free supplement, Ca^{2+}-free, Mg^{2+}-free Hank's

balanced salt solution (HBSS), minimum essential medium (MEM), neurobasal medium and horse serum were purchased from Gibco (Frederick, MD, USA). Fetal bovine serum was obtained from Biological Industries (Kibbutz Beit Haemek, Israel). Prolong Gold Antifade reagent and Alexa Fluor546 phalloidin were purchased from Molecular Probes (Eugene, OR, USA). Pyrex #7740 glass wafers were obtained from S.I. Howard Glass (Worcester MA, USA). Polydimethylsiloxane (PDMS) prepolymer and the curing reagent (Sylgard 184 silicone elastomer kit) were obtained from Dow Corning (Valley Spring, CA, USA). Positive photoresist AZ4620 and DEP1000 developer were obtained from MicroChem (Newton, MA, USA). Steel blades were obtained from Nikken Kanisori (Japan). Non-linear pH 3-10 IGP strips were purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Other reagents were obtained from Merck-Schudardt (Darmstadt, Germany).

Fabrication of glass chips A glass wafer (Pyrex #7740, 0.76 mm in thickness) was spin-coated with the photoresist (AZ4620). Subsequently, on its surface, the Ti/Au-coated alignment keys for carrying out microcontact printing and for placing the stencil were made by conventional photolithography procedures using an E-Beam Evaporator (Fulintec, Taipei, Taiwan). Linear wedge-shaped grooves (100 μm wide at the opening and 0.46 mm deep) were cut by micromachining on the back of the slide, which was the side not covered by the Ti/Au-coated alignment keys. The wafer then

was finally cut into square chips (1.4 cm X 1.4 cm). The resultant glass chips were subject to sonication sequentially in acetone for 5 min and in 70% ethanol for 5 min. Before use, the glass chips were rinsed in distilled water three times and subject to autoclave sterilization.

Fabrication of PDMS stamps 1 and 2 Two masters for making stamps 1 and 2 respectively with relief structures consisting of four rows of straight lines (each row containing 218 lines of 5 μm in width, 15 μm in depth and 850 μm in length, which were separated at 50 μm intervals) and four rectangular regions (0.25 mm in width, 12 mm in length and 175 μm in depth) were made using positive photoresist (AZ4620) on silicon wafers by conventional photolithographic methods. These masters also contained the relief structures of the alignment keys at the same positions and of the same shape as their Ti/Au-coated counterparts on the surface of glass chips. Parylene-C was finally deposited onto these masters. The stamps were made by pouring a mixture containing two commercially available components (Sylgard 184 silicone elastomer, Dow Coring) at 7:1 ratio by weight on the surface of the masters. After being degassed under vacuum for 1 hr, the PDMS on the masters was kept at room temperature overnight for curing. Subsequently, the PDMS layer was lifted from the master and cut into 1.4 cm×1.4 cm stamps.

Microcontact printing PDMS stamps were subject to sonication sequentially in

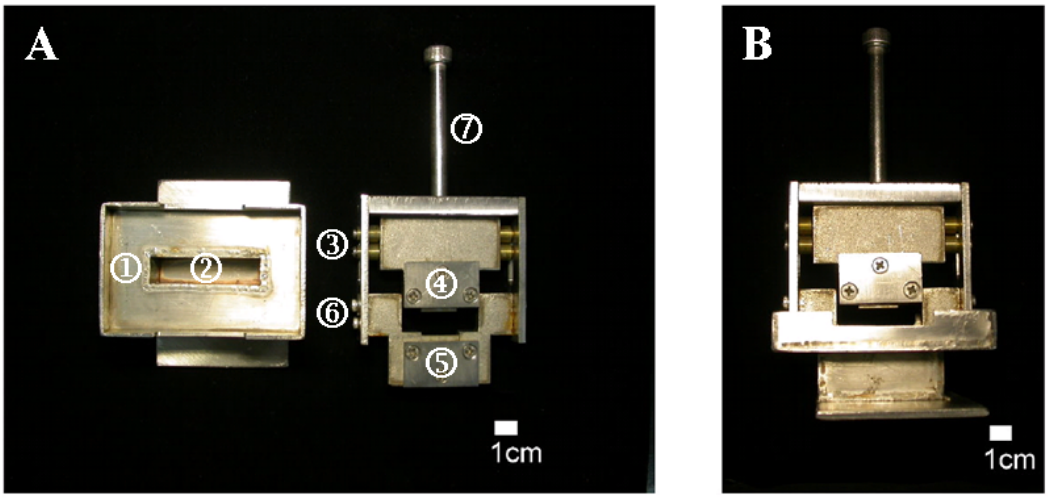
acetone for 5 min and in 70% ethanol for 5 min, rinsed in water twice and finally subject to autoclave sterilization. Prior to microcontact printing, the surface of PDMS stamps and glass chips were subject to air plasma (SEDE Soft Etching System, Japan) for 30 seconds. A drop of PLL solution (100 μg PLL/ml of 150 mM borate at pH 8.4) was applied to the stamp surface. Five min later, the excessive PLL solution on the stamp surface was removed by blotting with filter paper, and the stamp surface was then dried under a stream of N_2 gas. After matching the alignment keys of the stamp to the Ti/Au-coated ones on the glass chip with a home-made aligner, the stamp was pressed onto the surface of the glass chip at the pressure of 30 g/cm^2, and the stamp was removed 2 min later.

Fabrication of PDMS stencils The master for making stencils was made by sculpting an array of cuboidal features (2 mm in width, 12 mm in length and 1 mm in height) on one side of a Teflon block (3 cm in width, 3 cm in length and 0.8 cm in height) by machining (Scientific Instrument Center, National Tsing Hua University). To make the stencils, the Teflon master was placed in a Petri dish (100 mm in diameter) with the side containing the cuboidal features facing down, and a steel block of about 1 kg was then placed on top of the Teflon master. A mixture of two commercially available components (Sylgard 184 silicone elastomer) at 10:1 ratio by weight was poured into the dish. After curing at room temperature overnight, the Teflon master was removed.

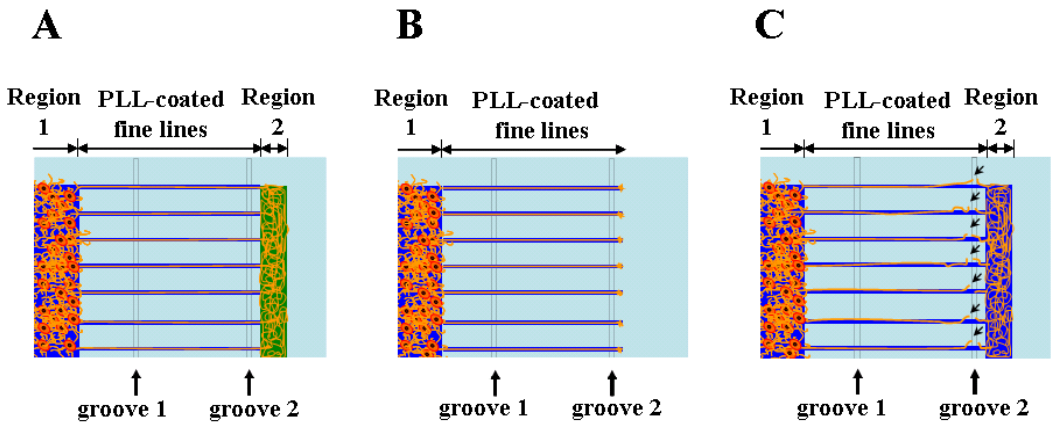
The PDMS sheet left in the Petri dish was lifted and cut into 1.4 cm×1.4 cm square pieces by a scalpel. The PDMS stencil was placed on top of the glass plate by aligning the top and bottom edges of the rectangular openings of the stencil to the corresponding alignment keys on the chip surface.

PLL-coating PLL solution (100 μg PLL/ml of 150 mM borate at pH 8.4) was applied to the openings over the Region 1 areas on the chip as defined by the stencil. After being kept in a vertical laminar flow hood overnight, excessive PLL solution was removed, and the openings were rinsed three times with distilled water before use.

Supplementary Figures



Supplementary Figure 1 The chip cutter. (A) Photographs of the upper part (left, top view) and lower part (right, front view) of the cutter. The major components are: (1) medium holding chamber, (2) insertion slot for accommodating the insertion block of upper part, (3) blade guiding wheels, (4) upper blade holder and steel blade, (5) lower blade holder, steel blade and insertion block to the lower part slot (2), (6) blade adjustment screws and (7) pressing handle. (B) Photograph of the assembled cutter from front. The cutter was made by the Scientific Instrument Center of National Tsing Hua University.



Supplementary Figure 2 The chip device can be modified to address different issues affecting neuronal axon. (A) A chip device is fabricated according to the procedures as described in Fig. 1A except that the Region 2 areas are coated with various molecules (in color green), such as the axonal guidance cues, which attract or repel growing axons, and cell adhesion molecules, which induce differentiation of axons into presynaptic terminals, other than PLL (in color blue), which is used to coat the Region 1 areas and the fine lines connecting the Region 1 and Region 2 areas. This chip device could be useful for the study of how various extrinsic molecules affect the biochemistry of the axon. (B) A chip device is fabricated according to the procedures as described in Fig. 1A except that the step for printing the Region 2 area with PLL is omitted. Axons growing along the PLL-coated fine lines will thus stop at the ends of these lines. Cleaving the chip along grooves 1 and 2 will produce fragments that respectively containing the top 50 μm segments of the axon, consisting primarily of the growth cones (finger-like structures at the ends of axons), and the

shaft region of the axon of ~400 μm in length. These substructures of the axons could then be harvested separately and subject to biochemical analysis. (C) The axons growing along the PLL-coated fine lines on chip surface could be easily severed (indicated by arrows). Biochemical changes in the distal and proximal axon segments from the injury site could be studied by analyzing the tissues harvested from the chip fragments generated by cleaving the chip along grooves 1 and 2.