Microfluidic culture platform for neuroscience research

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This protocol describes the fabrication and use of a microfluidic device to culture central nervous system (CNS) and peripheral nervous system neurons for neuroscience applications. This method uses replica-molded transparent polymer parts to create miniature multi-compartment cell culture platforms. The compartments are made of tiny channels with dimensions of tens to hundreds of micrometers that are large enough to culture a few thousand cells in well-controlled microenvironments. The compartments for axon and somata are separated by a physical partition that has a number of embedded micrometer-sized grooves. After 3–4 days *in vitro* (DIV), cells that are plated into the somal compartment have axons that extend across the barrier through the microgrooves. The culture platform is compatible with microscopy methods such as phase contrast, differential interference microscopy, fluorescence and confocal microscopy. Cells can be cultured for 2–3 weeks within the device, after which they can be fixed and stained for immunocytochemistry. Axonal and somal compartments can be maintained fluidically isolated from each other by using a small hydrostatic pressure difference; this feature can be used to localize soluble insults to one compartment for up to 20 h after each medium change. Fluidic isolation enables collection of pure axonal fraction and biochemical analysis by PCR. The microfluidic device provides a highly adaptable platform for neuroscience research and may find applications in modeling CNS injury and neurodegeneration. This protocol can be completed in 1–2 days.

INTRODUCTION

Microfluidics is a field of study that uses devices with small channels to control and manipulate minute amounts of fluids^{1,2}. Microfluidic devices were first developed to miniaturize chemical and biochemical analyses because they offered better sensitivity, faster speed and higher resolution of analyses over conventional methods^{2,3}. Recently, microfluidic devices fabricated in polymers (i.e., poly(dimethylsiloxane) (PDMS)) using soft lithography have found increasing applications in basic and applied biomedical research^{4–6}. Novel designs that exploit the advantages of miniaturization have resulted in devices for cell migration^{7–12}, drug screening^{13–15}, and long-term culture of stem cells¹⁶ and neurons^{17,18}. Microfluidic devices offer new capabilities for gaining biological insights owing to their ability to control and manipulate cellular microenvironments that have not been available in conventional macro-scale methods^{19–21}.

Neurons are unlike other cells in that they extend long processes over considerable distances and through varying extracellular microenvironments. Conventional in vitro culturing of neurons does not adequately model or offer the ability to control different neuronal microenvironments. Thus, it is difficult to investigate molecular and cellular mechanisms that differentially affect distinctive parts of the neuron which often occur in neuronal injury and neurodegenerative disease (e.g., spinal cord injury and Alzheimer disease). Compartmented chambers (i.e., Campenot and related chambers) have provided important tools for studying neuritic and axonal biology. Campenot chambers were first developed to study the influence of nerve growth factor applied to dorsal root ganglion (DRG) axons²². These chambers consist of a reusable Teflon piece, which has an applied grease layer placed on a Petri dish. Neurites of long projection neurons, such as DRGs and retinal ganglion neurons, are able to pierce the grease layer to enter an adjacent compartment where soluble factors may be added. The grease layer provides a hydrophobic barrier that prevents appreciable migration of soluble factors between the compartments. Campenot chambers have been success-fully used to study the transport of neurotrophic factors along axons^{23–25} and tubulin^{26,27}. Such devices have been used to biochemically isolate axons for PCR, Western blot analyses and thin-layer chromatography for lipid analyses^{28,29}. These chambers have also been used to study regenerative responses of sympathetic axons³⁰.

This protocol describes the fabrication and use of a microfluidic culture platform that compartmentalizes hippocampal and other CNS neurons. This novel device is fabricated using soft lithography in PDMS, a transparent and biocompatible polymer^{4–6,17}. Advantages of the microfluidic culture platform include (a) ability to culture hippocampal and other CNS neurons, (b) compatibility with high-resolution microscopy, including confocal and differential interference microscopy, (c) compatibility for long-term culture of both CNS and peripheral nervous system neurons for 2–3 weeks, allowing real-time live-cell time-lapse microscopy, (d) compatibility with immunocytochemistry and (e) robust reproducible devices that allow leak-free experiments.

In addition to describing the fabrication and use of the microfluidic platform, we describe methods for fluidically isolating the axonal or somal compartment, axotomizing the axons and isolating RNA. Previous results have shown that the hydrostatic pressure difference can be used to maintain fluidic isolation in one compartment for approximately 20 h. Using radioactively labeled [³⁵S]methionine applied to the axonal side, only the background level of [³⁵S]methionine was detected in the somal compartment after 20 h. The microfluidic platform may also be used to model axonal injury, which is particularly relevant to the field of spinal cord regeneration. We also describe the method for isolating axonal mRNA in neurons without detectable somal or dendritic contamination.

The ability to direct the growth of neuronal processes and to control the microenvironments of CNS axons offers the chance of

overcoming several limitations in the investigation of axonal biology. The protocol describes a novel platform that enables the examination of multiple aspects of axonal biology, including axonal RNA localization, injury and regeneration.

· Propyleneglycolmethylether acetate (PGMEA) photoresist developer

Neuron culture media Neurobasal media supplemented with 2% B27

Borate buffer solution Prepare 0.1 M borate buffer solution (1.24 g boric acid,

PLL solution Dissolve 400 mg of PLL (1 mg ml^{-1}) in borate buffer solution by

stirring for 30 min. Sterilize the solution by filtration with 0.2 µm filter. PLL

(vol/vol), 0.25% Glutamax (vol/vol) and 1% penicillin-streptomycin.

1.9 g sodium tetraborate, 400 ml Nanopure water, pH 8.5).

• Transparency mask (CAD/Art service)

· Laboratory oven for curing PDMS

·Vacuum desiccator for degassing PDMS

Plasma cleaner (Harrick Scientific PDC-001)

solution can be stored at -20 °C for future use.

(cat. no. 537543, Aldrich)

· Silicon wafer (Silicon Inc.)

EQUIPMENT

· Digital balance

REAGENT SETUP

· Photoresists, SU-8 5 and SU-8 50 (Microchem)

MATERIALS REAGENTS

- Poly-L-lysine (PLL), MW 70,000–150,000, 1 g (cat. no. P1274, Sigma)
- Sodium tetraborate, 99%, 100 g (cat. no. 221732, Sigma)
- Boric acid, 99.5%, 100 g (cat. no. B7660, Sigma)
- PDMS (cat. no. Sylgard 184, Dow Corning)
- •Neurobasal media (cat. no. 21103-49, Invitrogen)
- B27 neubasal supplement (cat. no. 17504-044, Invitrogen)
- Penicillin–streptomycin (cat. no. 15140-122, Invitrogen)
- GlutaMAX (cat. no. 35050-061, Invitrogen)
- RNaqueous-Micro kit (cat. no. 1931, Ambion)
- Transcriptor First Strand cDNA Synthesis Kit (cat. no. 04379012001, Roche)
- Disposable plastic cups for mixing PDMS
- Disposable stir rods (plastic knife)
- Scotch tape (3M Scotch 471)
- Glass coverslips (Corning, no. 1, 24×40 mm)
- Razor blades
- · Sharpened stainless steel punch

PROCEDURE

Fabrication of master TIMING 3 h

1 Flood SU-8 5 photoresist on a cleaned 3-inch Si wafer and spin at 3,000 rpm for 60 s (3 µm thick) (Fig. 1).

2 Soft bake the wafer for 1 min at 100 °C in a leveled oven.

3| Expose the wafer through a high-resolution transparency mask (20,000 dpi, CAD/art service) containing a microgroove pattern and alignment marks for 30 s at 200 W.

4 After exposure, bake at 90 °C for 1 min on a hot plate or for 2–3 min in a drying oven.

- **5** Develop with PGMEA (photoresist developer) for 30 s.
- 6 Rinse two or three times with fresh PGMEA solution.
- 7 Dry using pressurized inert gas.

8 Spin a second layer of photoresist, SU-8 50, at 1,000 rpm for 60 s (100 μ m thick).

9 Soft bake for 30 min at 100 °C in a leveled oven.

10 Align the chamber mask to the grooves patterned on the wafer using the alignment marks. Expose for 2 min at 200 W.

11 After exposure, bake for 5 min at 90 °C.

12 Develop for 5–10 min as needed. Rinse three times with fresh developer solution. Dry using pressurized inert gas.

■ **PAUSE POINT** Several microfluidics foundries have been established to provide master molds for academic research groups.

http://thebigone.stanford.edu/foundry/maskdesignrules.htm Standford Microfluidics Foundry

http://www.kni.caltech.edu/foundry/

Caltech's Kavli Nanoscience Institute Microfluidic Foundry



Figure 1 | Schematic of master mold fabrication. The master mold for microfluidic device consists of two layers of photoresist structures on a flat silicon wafer substrate. Steps **a** and **b** show the patterning of a thin photoresist layer (3 μ m high and 10 μ m wide) that defines the microgrooves under the barrier that bridge the somal and axonal chambers. Steps **c** and **d** show the patterning of main channels (100 μ m high, 1.5 mm wide and 7 mm long) for soma and axon chambers where cells are plated and cultured. Microgrooves allow isolation of soma on one chamber and growth of axons across the barrier.

Fabrication of PDMS devices by replica molding • TIMING 5 h

13 Weigh out a 10:1 ratio of PDMS prepolymer to catalyst into a disposable container and mix thoroughly for 5–10 min. Follow the manufacturer's recommendations when mixing prepolymer with catalyst (10:1 for Dow Corning Sylgard 184). Different brands of PDMS have slightly different components and protocols (**Fig. 2**).

▲ CRITICAL STEP If PDMS is not mixed thoroughly or the amount of catalyst is not adequate, PDMS will not cure completely and will make the master mold unusable.

14 Place the master mold (i.e., patterned photoresist on Si wafer) in a Petri dish and slowly pour the PDMS mixture over the master. When using a master fabricated on a 3-inch wafer, place in a 100-mm Petri dish and use \sim 25 g of PDMS mixture (when using different-sized containers, aim for 5–7 mm thickness).

15 Place in a vacuum desiccator to remove bubbles trapped around the patterns in the master mold (\sim 30 min).

16 Remove the Petri dish from the desiccator and gently blow pressurized air or nitrogen over the remaining bubbles. Alternatively, use a clean razor blade to remove the remaining bubbles.

17 Place the Petri dish in a leveled laboratory oven and cure for >3 h at 70 °C. The PDMS mixture will solidify and become transparent when fully crosslinked or cured (**Fig. 2**).

▲ CRITICAL STEP Primary cortical and hippocampal neurons are more challenging to culture than other cell types. Trace amounts of chemicals left on the surface can leak out and adversely affect cell culture. Use a dedicated oven and dessicators for PDMS preparation. If sharing equipment, volatile organic chemicals should be kept away, as they can be absorbed by PDMS and released at a later time.

18 It is advised that the following steps be performed in a laminar flow hood to minimize dust particles adhering to PDMS and glass. Small particles remaining on the PDMS surface will result in incomplete sealing and cause leakage when bonding with a glass coverslip (see Step 23). Use a razor blade to cut out the cured PDMS from the master mold and separate the PDMS piece away from the master mold. Do not use excessive pressure when cutting. Masters fabricated on Si wafers are very fragile and may break. Trim the PDMS piece with a razor blade into individual pieces. When handling the PDMS piece, take care not to touch the bottom surface that contains the channels. To punch out the reservoirs, place the PDMS piece, channel side up, and use a sharpened stainless-steel punch (diameter = 8 mm).

19 Clean the surface by blowing off small particles with pressurized nitrogen gas. In addition or alternatively, use Scotch tape to remove any remaining debris.

▲ CRITICAL STEP Remove debris on the PDMS surface after punching holes. Small particles are detrimental when bonding PDMS to glass substrate and can potentially cause leaks. It is also important to handle devices with the channel side up. When PDMS is removed from the master, the side that contacts the mold contains microfluidic channels. This side is clean and should be kept particle free.

20| Sterilize the devices by immersing in 70% ethanol. Dry in a laminar flow hood for >1 h before use. These steps must be performed in a sterile laminar flow hood to maintain sterility and minimize contamination. Alternatively, PDMS devices can be used after autoclaving. Exposure to high temperature may adversely affect bonding between PDMS and glass substrate.
 PAUSE POINT PDMS devices can be stored in a sterile container for up to 2 weeks before use.

Cleaning glass coverslips

TIMING 1 day

21 Clean glass coverslips in 95% ethanol (30 min) using an ultrasonic cleaner.

22 Remove from ethanol, rinse with deionized water and completely dry under a laminar flow hood.

■ **PAUSE POINT** Cleaned glass coverslips can be stored in a clean box and sealed with Parafilm until use.

23 The devices can be prepared for reversible bonding or irreversible



Figure 2 | Schematic of PDMS device fabrication by soft lithography. The microfluidic neuron culture device is formed by replica molding PDMS against the master mold.

bonding (see **Fig. 3**) depending on application. Use reversible bonding when a plasma machine is not available or when immunocytochemistry is required. Occasionally, reversibly bonded devices may have sealing problems (<5% of devices when using new PDMS devices). Irreversible permanent bonding yields reproducible results.

(A) Reversible bonding

PLL coating • TIMING 1 day

- (i) Place clean glass coverslips in PLL solution for 12 h.
- (ii) Immerse in DI water for at least 2 h. Wash twice with DI water.
- (iii) Dry glass coverslips overnight a in laminar flow hood under sterile conditions.
- (iv) Store coated glass coverslips in a 4 °C refrigerator until use.

PAUSE POINT PLL-coated glass coverslips can be used within 2 weeks.

Assembling the PDMS chamber with PLL-coated glass coverslips • TIMING 1 h

(v) Place the PDMS on the glass coverslip, channel side down, and lightly touch the PDMS until it is sealed to the glass. This type of conformal seal is reversible in contrast to irreversible seals formed when PDMS and glass are exposed to plasma and brought together (see Step 23B).

applications, reversibly bonded devices are adequate.

- ▲ CRITICAL STEP When sealing the PDMS to substrate, do not press around the barrier region near the grooves, as they can collapse and may become blocked when excessive pressure is applied.
- (vi) Use the assembled chambers within 1–2 h. For later use on the same day, fill the channels with DI water. Go to Step 24 before plating neurons.

▲ CRITICAL STEP Air bubbles may become trapped in the microgrooves if used after long storage. Avoid introducing bubbles. ? TROUBLESHOOTING

(B) Irreversible bonding

Irreversible bonding using a plasma cleaner • TIMING 0.5-1 h

- (i) Use a clean coverslip that is not coated with PLL. Place the PDMS device (microchannel side up) and glass coverslips in the plasma cleaner.
- (ii) Turn on the vacuum pump and wait until pressure drops below 200 mTorr or for 5–10 min (need tridifications for specific equipment available).
- (iii) Turn on the RF power to ignite the plasma. Bright violet-orange glow should be confirmed before starting the timer.
- (iv) After plasma treatment (1-2 min), immediately vent and place the PDMS on the glass to form irreversible bonding⁶. Other vacuum-based plasma machines in clean room facilities can also be used. Experimental conditions have to be optimized for each machine. In general, low power (<100 W) and short plasma treatment times (30-120 s) will result in reproducible bonding.

Coating assembled devices with PLL • TIMING 1 day

- (v) Add PLL solution to reservoirs and allow the entire device to be filled. In case the media do not completely fill the channel, use a pipette to force the solution into the empty channel with repeated withdrawal and injection. For other cell types, follow appropriate substrate coating protocols for collagen and fibronectin.
- (vi) Place the device overnight in a humidified incubator at 37 $^\circ$ C.
- (vii) Remove PLL solution and wash extensively (three times) with sterile water.

 CRITICAL STEP When rinsing, remove the solution in the reservoirs, but take care not to empty the main channels. Doing so will introduce air bubbles in the microgrooves that are difficult to remove.
- (viii) Fill the device with sterile water and place in the incubator for 2 h. Repeat rinse.
 - **PAUSE POINT** The devices filled with water can be stored in a humidified incubator for 2–3 days before use.

Cell loading and culture • TIMING 4 h (Steps 24–30)

24 Fill the device with culture media before use. Devices should be incubated with culture media for at least 3 h before plating the cells.

PAUSE POINT The devices filled with media can be stored in an incubator until plating the cells.



be fixed and stained for immunohistochemistry. Irreversibly bonded devices provide more consistent

performance such as a lower chance of leakage during handling and tight fluidic sealing. For most

25| Prepare embryonic day (E) 18 rat cortical and/or hippocampal dissociated cell suspension with an approximate density of 3×10^6 cells ml⁻¹. Other neuronal cells such as embryonic DRG neurons, P1 DRG neurons, P6 DRG neurons, E18 septal neurons, P0 hippocampal neurons and cholinergic neurons have also been successfully cultured in the microfluidic device. Contaminating non-neuronal cells can be prevented from crossing across microgrooves by using longer barrier devices (900 µm long barrier). The width of the microgrooves can also be manipulated to prevent the contamination of non-neuronal cells (i.e., Schwann cells and fibroblasts). Use plasma bonded device for applications that require extracellular matrix to remain hydrated.

26 Aspirate all media from reservoirs but not from main channels and immediately add 20 μ l of cell suspension into one of the reservoirs (upper left reservoir in **Fig. 3**, somal chamber). Take care to add the cells next to the channel so that the cell suspension is drawn into the channel by capillary action. This will result in ~3,000 cells plated in the main channel (1.5 mm wide and 7 mm long). The rest of the cells will settle and attach in the reservoirs.

27 Place in a humidified incubator for 10 min to allow cells to attach.

28 Add 150 µl of Neurobasal media to the upper left reservoir.

29 Add additional media (150 μ l each) to three other reservoirs. The volume can vary with the size of the reservoir. Add enough media to completely fill the channels and reservoirs.

30 Place in a well-humidified incubator at 37 °C, 5% CO₂.

31| Check the level of the media in the reservoirs at least once a day. Add additional media as needed. ▲ CRITICAL STEP Excessive evaporation of culture media can induce osmotic imbalance and thus lower viability. Check the humidity level in the incubator regularly and, if possible, use a dedicated incubator that is used less frequently to minimize evaporation from door opening and closing.

32 Change media every 48–72 h. When performing media change, carefully remove the media in the reservoirs but do not remove the media in the main channels. Use a pipette or vacuum to gently withdraw the media by placing the suction tip as far away from the channel as possible. Add 150 μ l of new media to all reservoirs.

CRITICAL STEP To prevent damage to axons or cells, carefully remove the solution in the reservoirs but take care not to withdraw media from the main channels.

Fluidic isolation • TIMING 30 min

33 Fill all four reservoirs with the same volume (150 µl) of fresh media. Remove 15 µl from one side of the chamber (i.e., upper right reservoir, axonal side) and add it to the reservoir on the opposite compartment (i.e., upper left reservoir, somal side).

34 Repeat for the other reservoir (lower right reservoir to lower left reservoir).

▲ CRITICAL STEP To maintain a fluidically isolated microenvironment in the axonal compartment, we used a volume difference of 30 µl between the two compartments with lesser volume on the axonal side where the agent is to be localized (for molecules larger than MW. ~ 1 kDa). For agents with smaller MW, we used a volume difference of 50 µl. A small hydrostatic pressure difference results in slow but continuous flow across the microgrooves, which counterbalances diffusion of species from the axonal to the somal compartment. Effective fluidic



Figure 4 | Confocal micrograph of Tau (green) and MAP5 (red) immunostained neurons (**a**) and phase micrographs of 6 DIV neurons (**b**,**c**) cultured in the microfluidic device. Immunostain results indicate that all processes that cross the barrier are axons. Scale bars, 100 µm.

isolation can be verified by spiking the agent solution with fluorescence-tagged dextran and checking the fluorescence level in both the chambers.

35 Prepare 2 μ l of concentrated agent solution (~ 300 times the desired concentration) and gently add 1 μ l to each reservoir on the same side (i.e., axonal compartment) containing lesser volume. Gently mix by pipeting up and down. If highly concentrated agent is not available or possible, prepare the desired concentration of agent and use it to completely replace the media. Follow the same procedure for replacing media, as described earlier in Step 32, by removing the media in reservoirs. If localized application of the agent to the axonal chamber is desired, first add normal culture media (165 μ l) to the reservoirs on the somal side. Immediately, add the agent containing solution (slightly less volume, 135 μ l) to each of the reservoirs on the axonal side. This will set up hydrostatic pressure imbalance corresponding to a 30 µl difference across the barrier. Fluidic isolation of the axonal side (stopping diffusion of agents applied to axon to somal chamber) results from slow but steady flow from the somal chamber to the axonal chamber. Owing to high fluidic resistance of the microgrooves, the fluid level will take more than 24 h to equilibrate. There will be slight dilution



Figure 5 | Schematic of axotomy procedure in the microfluidic device. Rat E18 cortical neurons are cultured for >7 days before cutting them by aspiration.

(<10%) of the agent on the axonal side after 24 h. The approximate total volume of liquid that flows across the barrier is a fraction $(<30 \ \mu l)$ of the total volume (300 μl). If a longer isolation period is desired, hydrostatic pressure difference can be set up every 24 h.

Axotomy • TIMING 30 min

36 Use a glass Pasteur pipette connected to laboratory vacuum. Place the pipette close to the channel that leads to the axonal compartment for 5–10 s. Axons are severed when a bubble passes through the channel. Aspirate until all media from the opposite reservoir are removed and the main channel is emptied (**Figs. 4** and **5**).

CRITICAL STEP Owing to high fluidic resistance of the microgrooves, cells in the somal compartment are not disturbed by gentle vacuum suction in the axonal compartment. However, be careful, as excessive suction applied continuously for a longer period can damage cells in the somal compartment.

37 Quickly add 150 µl of media to the empty reservoirs. If the channel is not completely filled, use a pipette to force media flow with repeated withdrawal and injection.

RNA isolation • TIMING 30 min

38 | To isolate pure axonal RNA, aspirate and remove cells from the somal chamber until completely empty. Continue aspirating the somal chamber until the procedure is complete. This will minimize contamination with the somal fraction (Fig. 6). ▲ CRITICAL STEP To avoid

contamination from soma RNA, cells in the somal compartment can be completely removed before the procedure or be immobilized in agarose.

39 Remove most, but not all, of the media from both reservoirs on the axonal side.



Figure 6 | Schematic procedure for isolating axonal RNA.

40 Add 100 μl of Ambion RNAqueous-Micro lysis solution to the axonal chamber. Keep aspiration to block somal and dendritic contamination.

41 After 30 s, collect the axonal lysate with a micropipette from both reservoirs and place the solution in a 1.5 ml Eppendorf tube containing 50 µl of lysis solution on ice. Because the lysis solution is diluted as it mixes with the media on the axonal side, the excess lysis solution prevents further degradation of RNA quality. Pool the lysates from two to three devices to obtain larger sample.

42 Isolate RNA using the RNaqueous-Micro kit (Ambion) following Ambion's protocol.

43| To isolate somal RNA, remove the PDMS chamber (use reversibly bonded device; see Step 23A). Scrape off and discard the cells within the reservoir area. Add 100 µl of cell lysis solution to the remaining neurons that were in the main channel.
 ▲ CRITICAL STEP For isolation of somal RNA after axotomy, cells attached in the reservoirs that were not axotomized were scraped off before collecting cells that were attached in the main channel.

PAUSE POINT Store purified RNA at -20 °C overnight and at -80 °C for longer storage.

RT-PCR for detection of axonal RNA • TIMING 3 h

44 Set up cDNA synthesis reaction. To avoid RNA degradation, it is best to perform cDNA synthesis on the same day as RNA isolation.

Mix 1	
Anchored oligo(dT) ₁₈ primer (50 pmol μ l ⁻¹)	1 µl
Random hexamer primers (600 pmol μ l ⁻¹)	2 µl
Template RNA (Steps 42–47)	10 µl
Total volume	13 µl
Mix 2	
Deoxynucleotide mix	2 µl
RNase inhibitor (40 U μ l $^{-1}$)	0.5 μl
RT reaction buffer, $5 \times$	4 μl
Reverse transcriptase (20 U μl^{-1})	0.5 μl
Total volume	7 µl

45| For first-strand synthesis, incubate Mix 1 at 65 °C for 10 min and place the tube immediately on ice to denature the template-primer mixture.

- 46 Add Mix 2 to the Mix 1 tube.
- 47 Incubate the RT reaction for 10 min at 25 °C and then for 30 min at 55 °C.
- **48** Inactivate the enzyme by heating to 85 °C for 5 min.
- **49** Set up in a sterile microtube on ice.

dNTP-Mix	2
Gene-specific primers (1 μ M)	2 μι 8 μl
Template cDNA	5 μl
$10 \times$ PCR buffer	5 µl
Taq DNA polymerase	2 µl
H ₂ 0	28 µl
Final volume	50 μl

50 Amplify cDNA according to the following program:

Cycle number	Denaturation	Annealing	Polymerization	Hold
1	2 min at 94 $^\circ$ C			
2–41	1 min at 94 $^\circ$ C	30 s at 60 $^\circ\mathrm{C}$	1 min at 72 $^\circ C$	
42			7 min at 72 $^\circ C$	
43				4 °C

51 Store the RT-PCR product at -20 °C before gel electrophoresis.

• TIMING

Steps 1–12: Mold fabrication, 3 h Steps 13–20: PDMS device fabrication, 5 h Steps 21–22: Glass cleaning, 1 day Steps 23A(i–iv): PLL coating, 1 day Steps 23A(v–vi): Assembling device, 1 h Steps 24–32: Cell loading and culture, 4 h Steps 33–35: Fluidic isolation, 30 min Steps 36–37: Axotomy, 30 min Steps 38–43: RNA isolation, 30 min Steps 44–51: RT–PCR of axonal RNA, 3 h

Figure 7 | RNA blot of samples isolated from somal and axonal chambers. RNA encoding the presynaptic vesicle protein synaptophysin but not postsynaptic

(CaMKII α) mRNAs is



localized to axons. Single-chamber RT–PCR analyses of the somatic compartment and the axonal compartments indicate that β -actin and synaptophysin mRNAs are localized to axons. Histone H1 and dendritically localized CaMKII α are not detected in the axonal chamber.

? TROUBLESHOOTING

Troubleshooting advice can be found in Table 1.

TABLE 1 | Troubleshooting table.

Step	Problem	Possible reason	Solution
23A(vi)	Air bubbles are trapped in the grooves when adding media into the device	PDMS chamber and glass surfaces are hydrophobic	Use plasma treatment to make the surfaces hydrophilic. For devices that are reversibly bonded, use 0.02% Triton X-100 solution and wait until all
			sterile water once before filling with media

ANTICIPATED RESULTS

The microfluidic neuron culture device provides a new method to direct, isolate, lesion and biochemically analyze CNS axons. Soft lithography uses replica molding to fabricate devices, resulting in reproducible experiments. Another advantage of the microfluidic device is that it uses hydrostatic pressure to fluidically isolate one side of the chamber.

This microfluidic culture device can serve as an *in vitro* model for axonal injury and regeneration within the CNS and can be used to test soluble factors involved in injury and regeneration. For example, we investigated the influence of axonally localized neurotrophin treatment on regeneration. The isolated axons in neurotrophin-treated chambers had a dramatic increase in axonal branching and growth versus control-treated chambers¹⁸. This device also provides a unique method to investigate axonal mRNA in developing neurons without detectable somal or dendritic contamination. Using the microfluidic device, mammalian axonal mRNAs were isolated from the axonal compartment of 6 DIV neurons. Specific RNAs were detected by RT–PCR analysis (**Fig. 7**).

COMPETING INTERESTS STATEMENT The authors declare competing financial interests (See the HTML version of this article for details).

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