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# Microfabrication- and microfluidics-based patterning of cultured neuronal network

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***Abstract*— The cultured neuronal monolayer has been a promising model system for studying the neuronal dynamics, from single cell to network-wide level. Randomness in the reconstituted network structure has, however, hindered regulated signal transmissions from one neuron to another or from one neuronal population to another. Applying microfabrication-based cell patterning techniques is a promising approach to handling these problems. In the present study, we attempt to regulate the direction of axon development and the pathway of signal transmissions in cultured neuronal networks using micro-fabrication and –fluidic techniques. We created a PDMS-based culture device, which consisted of arrays of U-shaped cell trapping microwells, and placed it onto a chemically micropatterned glass substrate. After 6 days in vitro, we confirmed that cortical neurons extended neurites along the medium flow direction and the micropatterned regions.**

## I. Introduction

One of the difficulties in investigating brain function is its multiscale nature. Cultured neuronal networks, which consist of millions of neurons on a planar culture dish, have been a promising model system for studying the neuronal dynamics, from single cell to network-wide level. Randomness in the reconstituted network structure has, however, hindered regulated electrical signal transmission in the cultured neuronal networks, from a single neuron to another neuron or from neuronal population to another. Thus, the relationships between actual brain functions and electrical dynamics in the cultured neuronal networks are unclear.

Applying cell patterning techniques to neuronal culture is a promising approach to handling these problems. Historically, chemical patterning with cell-adhesive molecules [1] or physical patterning using the microfabrication technique [2] have been used for controlling general structures of cultured neuronal networks. However, single-neuron-level (synapse level) patterning has been difficult problem because neurons gradually migrated during development and extended neurites crisscross. Recently, microfluidic techniques have been used for neuronal studies, which can control cell environments and apply forces (such as laminar flow) persistently to cells [3]. Furthermore, Di Carlo et al. reported a microfluidic-based dynamics cell culture method, which could trap and fix cells in the desired region [4]. We hypothesize that the combination of cell patterning and microfluidic-based modulation techniques is useful for regulating the morphologies and functions (signal transmission pathways) of cultured neuronal networks.

In this paper, we attempt to fabricate a poly(dimethylsiloxane) (PDMS)-based microfludic neuronal culture chamber with U-shaped cell trapping structures and culture neurons within it. The PDMS microfluidic chamber was plated onto a glass substrate, which was previously patterned with cell-adhesive and -nonadhesive chemicals in order to regulate neuronal growth regions. These PDMS chamber and chemically patterned substrate were designed to connect neurons in a defined direction (Fig. 1).

## II. Materials and Methods

### A. Device fabrication

The master mold for the culture device was produced by patterning two layers of negative photoresist, SU-8 (3005, 3025; Microchem), on a silicon wafer (φ 75 mm, Ferrotec Silicon). A silicon wafer was previously cleaned with acetone (Wako) and isopropyl alcohol (IPA, Wako). Then, SU-8 3005 was spin-coated onto a silicon wafer for 60 s at 4000 rpm to make a thickness of 4 μm layer. The coated wafer was exposed

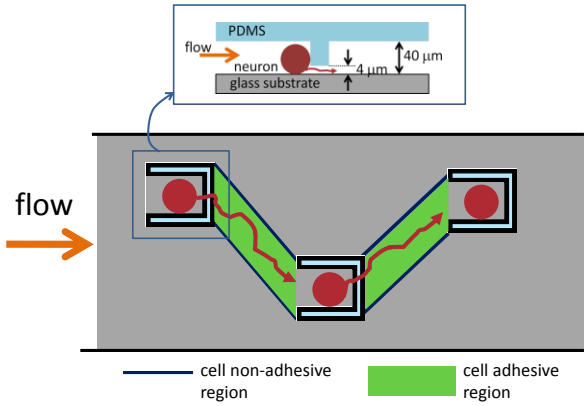


Fig. 1 The concept schematic of the microfluidic neuronal culture chamber. Cell trapping microwells were aligned to the chemical patterning.

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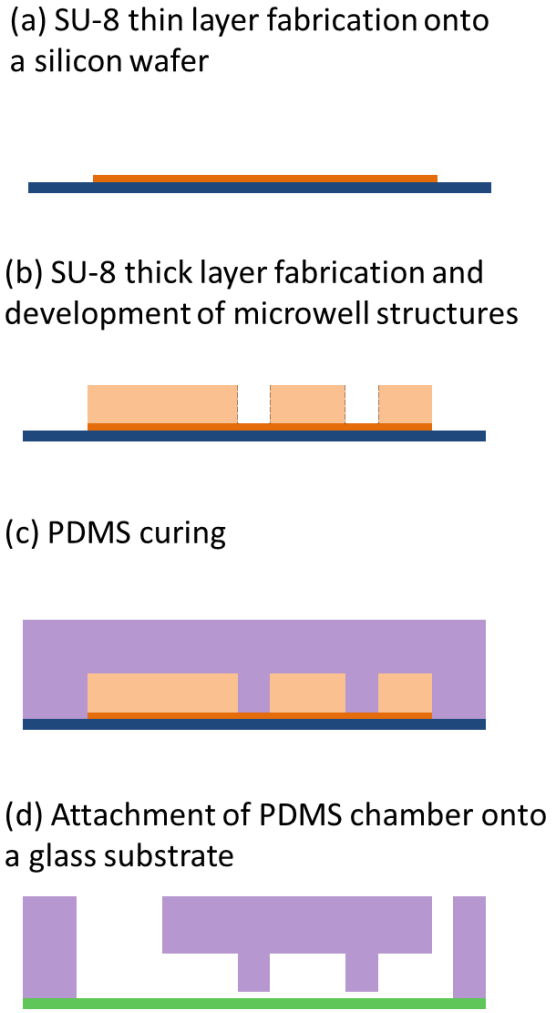


Fig. 2 Schematic of procedures for fabricating the microfluidic culture chamber.

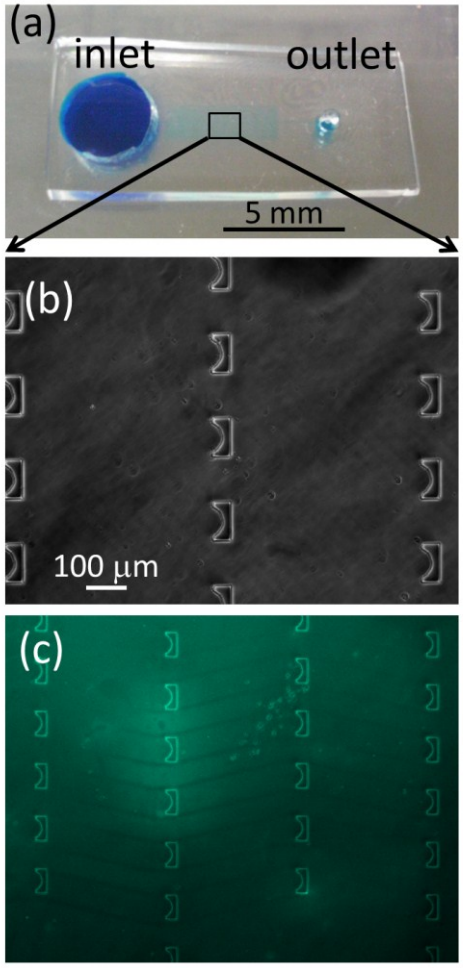


Fig. 3 Images of the microfluidic neuronal culture chamber. Cell trapping microwells were fabricated in array pattern (b). The microwells wrer aligned to the chemical micropatterning on a glass substrate.

to UV through a custom-made photomask. The first photomask featured an outer shape of a microchannel. Then, SU-8 3025 was coated for 60 s at 2000 rpm as a second layer with a thickness of 40 μm. The second photomask was aligned to the first patterns and used to fabricate the U-shaped microwell (50 × 100 μm) arrays [4]. For each exposure and development process, the coated wafer was soft-baked at 65 $^{o}$C for 1 min, followed by pre-baking at 95 $^{o}$C for 0.5 min/μm$_{(thickness)}$. The SU-8 coated wafer was exposed to UV light through the photomasks. This was followed by post-baking at 95 $^{o}$C for 10 min. After exposure and baking, the SU-8 patterns were developed with SU-8 developer (Microchem) and then rinsed with IPA. After development, the substrate was hard-baked at 200 $^{o}$C for 30 min to enhance the crosslinking of SU-8. The fabricated master was set in a polystyrene dish (φ 100 mm). The mixture of pre-polymer and catalyst (10:1 ratio, Silpot 184; Dow Corning) was poured over the master to a thickness of 4 mm. The dish containing the master mold and PDMS was placed on a hotplate at 80 $^{o}$C for 1 hour. After curing, the PDMS sheet was released from the master, trimmed it using a surgical knife and opened inlet and outlet for the microfluidic chamber using a punch. The PDMS microfluidic chamber was sealed onto a glass substrate (76 × 52 mm microslide glass; Matsunami Glass) pre-patterned with 0.1% polyethyleneimine (PEI, cell-adhesive; Sigma-Aldrich) and 1% pluronic F-127 (cell-nonadhesive; Sigma-Aldrich) solutions. The patterning of two chemical solutions was carried out by using the microcontact printing method [5]. To visualize the chemical patterning, 50 μg/ml FITC isomer 1 (Sigma-Aldrich) was added to the PEI solution. The schematic of the fabrication processes is shown in Fig. 2.

### *B. Cell culture*

Cortical neurons from 18-day-old Wistar rat embryos were mechanically triturated after digestion with 0.1 % trypsin-EDTA (Invitrogen) in calcium- and magnesium-free Hank's balanced salt solution (HBSS; Invitrogen). A drop of cell suspension was applied into the inlet of the microfluidic chamber and then negative pressure was applied to the outlet using a vacuum pump. For a culture medium, neurobasal medium (Invitrogen) containing 2% B27 supplement

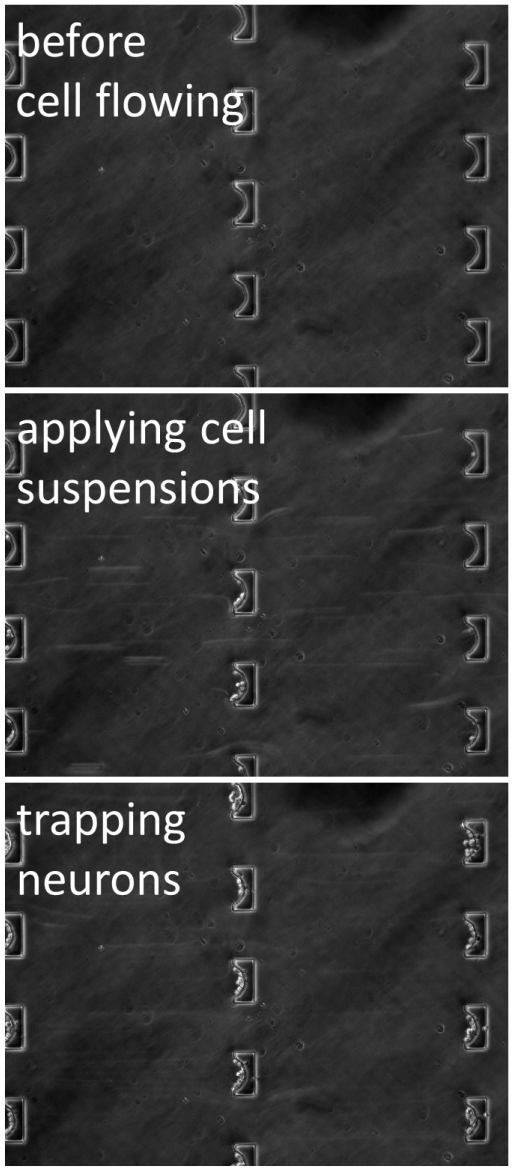


Fig. 4 Plating neurons to the U-shaped microwells in the microfluidic culture device. A negative pressure was applied to the right direction in this figure.

(Invitrogen) and 5 – 40 U/ml penicillin streptomycin, which was pre-incubated with rat astrocytes for 2 days, was used.

## III. Results and Discussions

Figure 3 showed an outline of the PDMS-based microfluidic chamber, a phase-contrast image of array of cell trapping microwells and a fluorescence image of chemical patterning. The bright region in the fluorescence image indicated the PEI coated cell-adhesive region. Contrary, the dark region indicated the pluronic F-127 coated cell-nonadhesive region. The fluorescent observation was also useful for alignment of the PDMS chamber and the patterned glass substrate. Cell suspension of rat cortical neurons was dropped in the inlet of the microchamber and then a negative

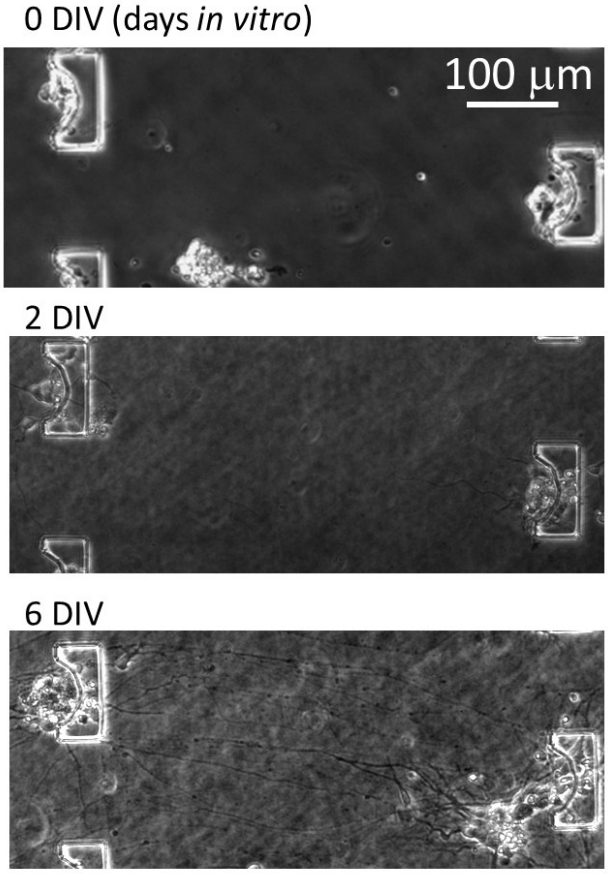


Fig. 5 Neuronal culture in the microfluidic culture chamber. Neurons mostly extended their neurites toward right direction.

pressure was applied to the outlet. Cortical neurons flowed from the inlet to the outlet and were trapped in the microwells as shown in Fig. 4. The U-shaped structure and the offset of 4 μm from the substrate were useful for trapping cells [4] and also worked to pass neurites to a defined direction. Cortical neurons trapped in the microwells were cultured over 1 week after planting. Images of cortical neurons 2 and 6 days in culture were shown in Fig. 5. Neurons trapped in the microwells gradually extended their neurites to other neurons trapped in a next microwell and morphologically connected. Neurites mostly grew within the cell-adhesive region. Thus, we could regulate the network structures. Based on the previous reports that axons in cultured neurons extend more rapidly and longer [6] than dendrites, we hypothesized that signal transmissions in the cultured networks formed as same direction as the medium flow in the microchannel. To confirm this, immunofluorescent identification of axon growth and synapse formation, and electrical recording of the constructed cultured networks will be required.

## IV. Conclusion

We fabricated the novel PDMS-based microfluidic neuronal culture chamber, which consisted of the array of U-shaped cell trapping microwells and chemically patterned

surface. We cultured rat cortical neurons within it and neuronal growth along the microwells and chemical patterning. This neuronal culture device would be a promising tool to produce the morphologically and functionally regulated cultured neuronal networks.

## ACKNOWLEDGMENT

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## REFERENCES

[1] A. K. Vogt, G. J. Brewer, T. Decker, S. Böcker-Meffert, V. Jacobsen, M. Kreiter, W. Knoll and A. Offenhäuser, "Independent of synaptic specificity from neuritic guidance", Neuroscience, Vol. 134, pp. 783-790, 2005.

[2] G. Zeck and P. Fromherz, "Noninvasive neuroelectronic interfacing with synaptically connected snail neurons immobilized on a semiconductor chip", Pro. Natl. Acad. Sci., Vol. 98, pp. 10457-10462, 2001.

[3] P. G. Gross, E. P. Kartalov, A. Scherer and L. P. Weiner, "Applications of microfluidics for neuronal studies", J. Neurol. Sci., Vol. 252, pp. 135-143, 2007.

[4] D. Di Carlo, L. Y. Wu and L. P. Lee, "Dynamic single cell culture array", Lab Chip, Vol. 6, pp. 1445-1449, 2006.

[5] T. Kaufmann and B. J. Ravoo, "Stamps, inks and substrates: polymers in microcontact printing", Polymer Chem., Vol. 1, pp. 371-387, 2008.

[6] C. G. Dotti, C. A. Sulliyan and G. A. Banker, "The establishment of polarity by hippocampal neurons in culture", J. Neurosci., Vol. 8, pp. 1454-1468, 1988.