

A STUDY OF AXONAL PROTEIN TRAFFICKING IN NEURONAL NETWORKS VIA THE MICROFLUIDIC PLATFORM

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ABSTRACT

This paper reports a microfluidic platform for culturing neuronal networks and the study of axonal protein trafficking. The device consists of cultural microchambers connected by microchannel array. Molecular biological methods were used to express fluorescent proteins in neurons. Preliminary results show that the neurons can be polarized with their soma and axons being compartmentalized into different fluidically isolated microenvironments. When chemical stimulation was applied to axonal chamber, anterograde migration of expressed fluorescent proteins could be observed. The platform will be useful in neuroscience research for studying the response of individual neurons to extracellular stimulation.

KEYWORDS

Neuron, protein trafficking, microfluidic, axonal protein

INTRODUCTION

Extracellular stimulation on different parts of a neuron commonly results in diverse effects on the intracellular trafficking of proteins between its production site (soma) and functional site (axon and synapse region) [1-3]. However, it is technically difficult to study the effect of extracellular stimulations with high spatial resolution in traditional neuron culture platforms [4-5]. However, microfluidic platform can be a suitable candidate to overcome this limitation [6]. Microfluidic platform provides a highly controllable fluidic environment, such that localized chemotaxis can be easily realized.

In this paper, a microfluidic neuron culture chip is designed and developed to compartmentalize the production site (soma, dendrite) and the functional site (axon) by using a microchannel array, which provides the function of precise fluidic control for highly localized chemical treatment and stimulation. Subsequently, the trafficking of axonal proteins is monitored to study the effect of the localized chemical treatment as shown in Fig. 1.

MICROFLUIDIC CHIP DESIGN AND FABRICATION PROCESS

Figure 2(a) shows a schematic illustration of the microchip. The device consists of two microchambers connected by a microchannel array. Each microchannel has a width of 8 μm , a length of 450 μm and a height of 5 μm as shown in Fig. 2(b). When neurons are seeded in the soma microchamber, the axons are able to grow through the microchannel array to the axon microchamber. The 5- μm height is the limiting dimension to avoid the soma ends of the neurons from reaching the axon microchamber. In addition, the microchannels prevent liquids in either side of the microchambers from flowing through due to the high hydrostatic resistance in the microchannels. Thus, the localized chemical treatment to either the soma or axon side can be achieved easily. To study protein trafficking in the neurons, somas in the microchamber are transfected with

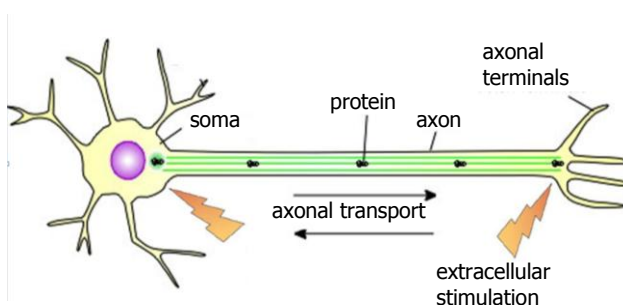
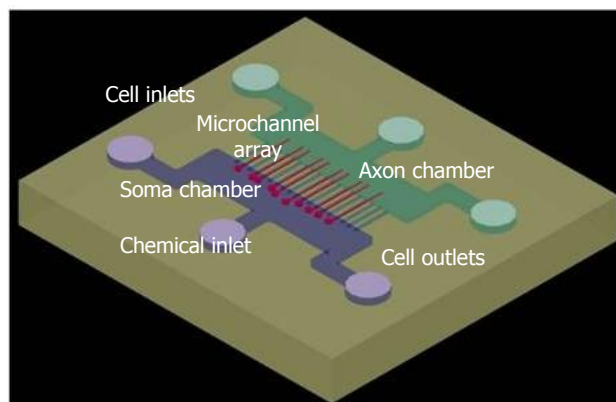


Figure 1: Illustration of axonal protein trafficking in a neuron being chemically stimulated externally.



(a)



(b)

Figure 2: (a) Schematic illustration of microfluidic neuron culture chip. (b) Cross-sectional view of the chip showing the 5- μm high microchannel.

16th International Conference on
Miniaturized Systems for Chemistry and Life Sciences
October 28 - November 1, 2012, Okinawa, Japan

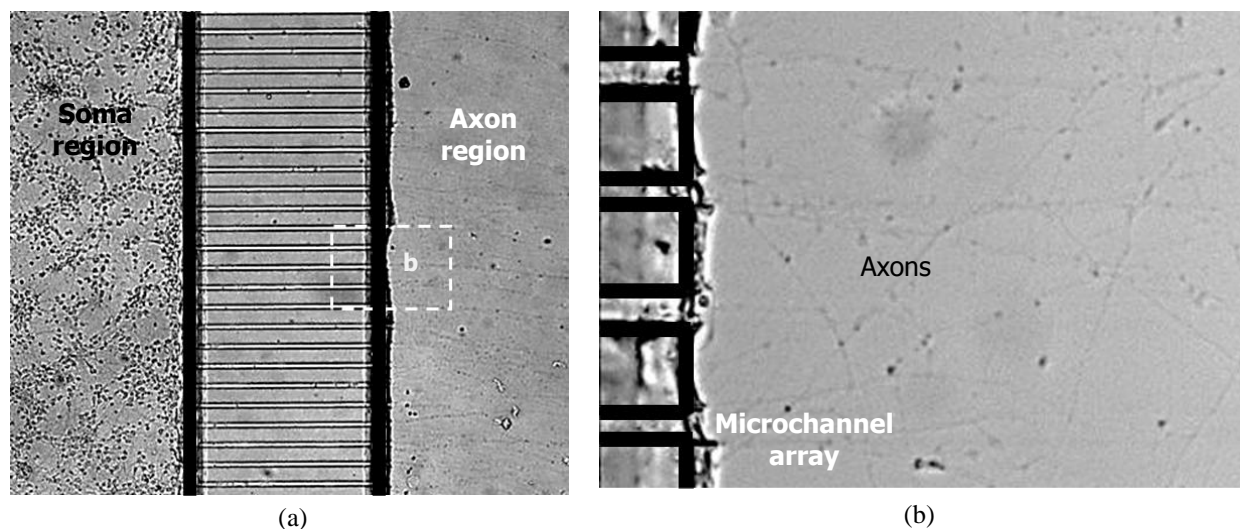


Figure 3: Rat cortical neurons cultured 14 days in the microfluidic device (a) overview of the neuron growth, and (b) close view of axon region.

plasmid encoding axonal proteins with green fluorescent protein tag. When chemicals inducing synapse activities was applied in either chamber, the trafficking of fluorescent proteins within the axons growing in the microchannels can be observed directly with a fluorescent microscope.

The microfluidic device is fabricated by using standard soft-lithography fabrication processes [7-9]. A two-step photolithographic technique is used in the fabrication process. First, a thin layer (5 μm) of SU-8 10 photoresist (MicroChem) was spin-coated on a wafer (CEE 200, Brewer Science). After soft baking, it was exposed with the first chrome mask, which defined the microchannel widths (8 μm) followed by the post exposure bake. Then, a second thick layer (100 μm) of SU-8 100 photoresist was spin-coated on the first layer and exposed with a second mask to fabricate the cell culture chamber. Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) prepolymer and curing agent (10:1) was poured over the master, degassed and baked 2 hours at 75 $^{\circ}\text{C}$ and then peeled off. The inlets and outlets holes were punched manually by using a Harris uni-core sampling puncher (5 mm). Then, the PDMS devices were exposed to air plasma for 15 s using a corona treater (BD-25, Electro-Technic Products) to bond it with a glass coverslip. To enhance cell attachment, a coating solution containing 800 $\mu\text{g}/\text{ml}$ of poly-D-lysine and 50 $\mu\text{g}/\text{ml}$ of fibronectin (Sigma) was injected into the PDMS chip and incubated overnight at 4 $^{\circ}\text{C}$. The coating solution was then removed and the microchannels were flushed 3 times with $1\times$ phosphate buffer saline.

EXPERIMENTAL RESULTS AND DISCUSSIONS

Rat cortical neuron was dissociated by using a papain dissociation kit (Worthington Inc.) based on the manufacturer's protocols. The dissociated cells were resuspended in a chemically defined medium of B27, glutamate, and glycine in neurobasal medium (Invitrogen) at a concentration of 1×10^7 cell/ml and injected into the microfluidic device. Neurons were

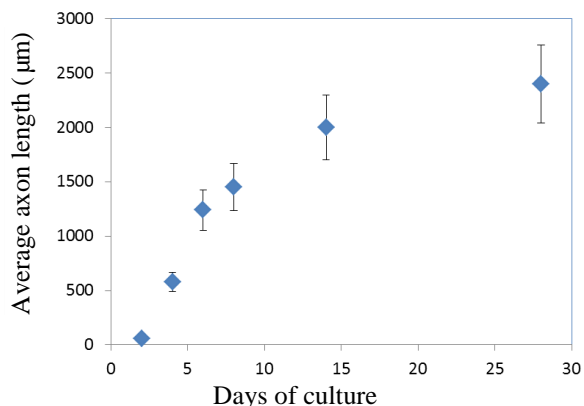


Figure 4: Length of axons in microgrooves as a function of days in culture.

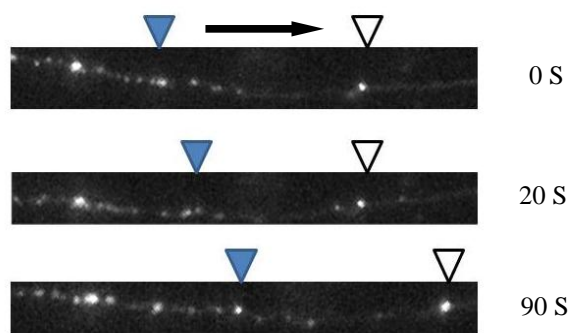


Figure 5: Expressions and migration of fluorescent proteins in axons.

fed once a week by refreshing half of the medium.

Figure 3(a) shows the rat cortical neurons in the microfluidic chip after 14-day *in vitro* culturing process. It can be seen that the soma ends of the neurons were limited to the left microchamber while the axons were able to grow through the channels to the right microchamber as shown in Fig. 3(b). The axon growing speed is monitored by measuring their length from high-resolution images captured once per day. The average axon length is illustrated in Fig. 4. The error bars show the standard deviation of the axon length of 10 neurons. Majority of the axons were able to grow through the 450- μ m microchannel to the axon microchamber within 7 days.

Figure 6 shows the migration of fluorescent proteins in axons. When synapse-activating chemicals were applied to axonal chamber, anterograde migration of the fluorescent proteins from soma side to distant axonal end could be observed in the microchannel.

CONCLUSIONS

In conclusion, we developed a microfluidic neuron culture system for the study of the axonal protein trafficking in neurons. Results show that the platform was able to polarize the soma and axons of cultured neurons into fluidically isolated environments and localized treatment on the axonal end of the neurons resulted in the anterograde migration of the fluorescent proteins. Future studies with different stimulation protocols and statistical analysis of the results will help to improve the understanding of the axonal protein trafficking.

ACKNOWLEDGEMENT

This work is supported by the Science & Engineering Research Council of Singapore (Research project with Grant No. SERC 102 152 0013 and 102 171 0159).

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