THREE-DIMENSINAL NEURON CULTURE METHOD CONTROLLING THE DIRECTION OF NEURITE ELONGATION AND THE POSITION OF SOMA

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ABSTRACT

We have developed a 3D neuronal circuit culturing technique by controlling the position of nerve cell body and the direction of neurite elongation using Collagen fiber orientation techniques and poly-dimethylsiloxane (PDMS) micro-processing. Using these methods, we have constructed the 3D neuronal network model extending the neurite into one-way direction with multiple layers, and confirmed the function of it by electrical measurement using multi-electrode array system. These techniques can potentially be used for regenerative medicine and development of drug screening model, as well as research in basic neural biology.

KEYWORDS

3D neuronal circuit, Reconstruction, Direction control of neurite elongation, Collagen fiber orientation, Multi-electrode arrays (MEAs)

INTRODUCTION

Reconstruction techniques of an artificial tissue using dissociated cells were expected to become transplantation technology in regenerative medicine field and understand the meaning of tissue structure and cellular population size. Researches to create artificial tissue from the cells have been developed mainly to target tissues such as heart [1], liver [2], and blood vessel [3] using a lot of techniques such as cell sheet techniques and micro-fabrication techniques. However, 3D reconstruction techniques for neuronal tissue have not been developed a lot. One possible reason for the nerve tissue is complex; it is because it has a polarity such as axons and dendrites. Although random 3D culture techniques in collagen gel exist, three-dimensional culture technique to control the direction of neurites has not been developed.

In this study, we attempt to form a direction-controlled neurite in 3D cultured neuronal network, a fixed soma layers in 3D neuronal network. To do this, we fabricated a poly(-dimethylsiloxane) (PDMS) microchambers to fix soma layers and orientated collagen gel fiber to control the direction of neurites. We confirmed that fixed soma layers are constructed in 3D collagen gel and extend their neurites to the collagen fiber direction and that their reconstructed 3D neuronal networks have electrical functions.

EXPERIMENT

Figure1 shows the procedure of 3D culturing technique by controlling the position of nerve cell body and the direction of neurite elongation. The PDMS sheet with micro chambers fabricated was pasted on the culture dish with poly-d-lysine coated (Fig.1a). Hippocampal neurons from 18-day-old Wister rat embryos were mechanically triturated after digestion with 0.1 % trypsine-EDTA.

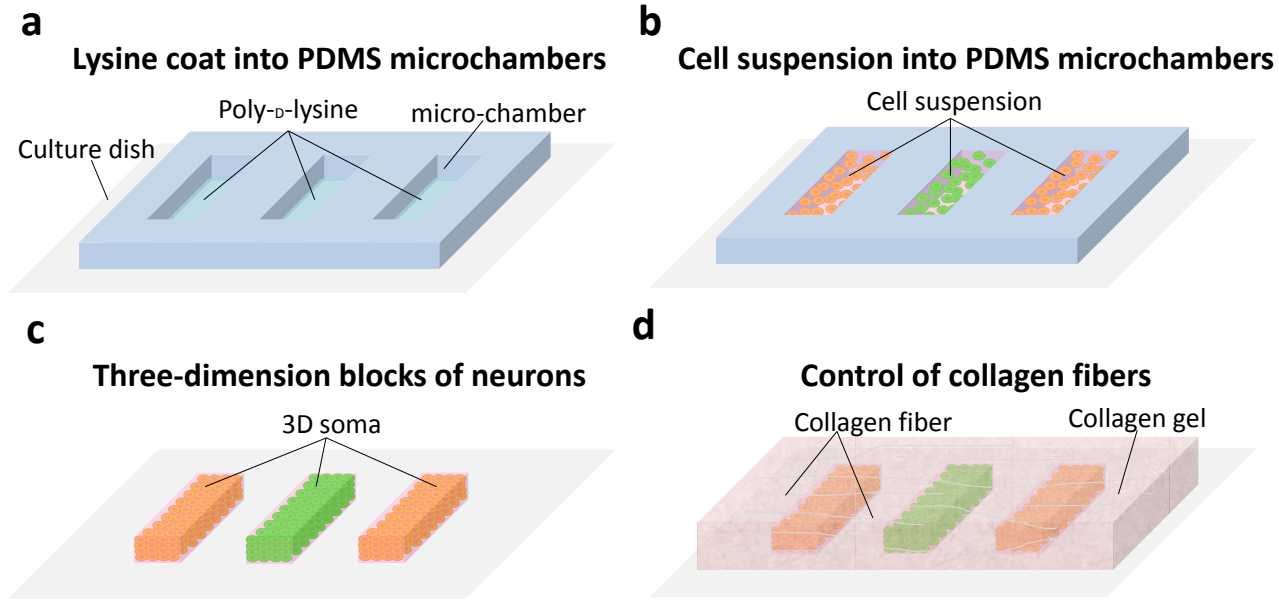

Fig.1 Procedure of 3D neuron culture method
Control of collagen fibers and nerve cell body position

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Then Rat hippocampal cell suspension was dropped into the micro chambers, and incubated at 5% CO^2, 37°C for 30 minutes (Fig.1b). The 3D cell blocks over two cell layers were constructed by removing PDMS sheet (Fig.1c). Orientation of collagen gel type I fiber was controlled on the 3D cell blocks, and then cells were cultured over 1 month. For a culture medium, neurobasal medium containing 2 % B27 supplement was used. To evaluate the structure of 3D neuronal network, we observed neurite and soma by immunofluorescence staining of the aldehyde fixed 3D cultures using fluorescence microscope.

As a result, we observed that the neurite grow into one-way direction along the collagen fiber with cell body positions fixed (Fig.2). In addition, by observing neurite configuration in Z axis using confocal microscope, we confirmed that neurite in 3D gel matrix are elongated in one way.

Furthermore, a multilayer structure that mimics the six-layer structure of the cerebral cortex was successfully created. It can be seen that neurite are elongated in one way among three cell body layers (Fig.3).

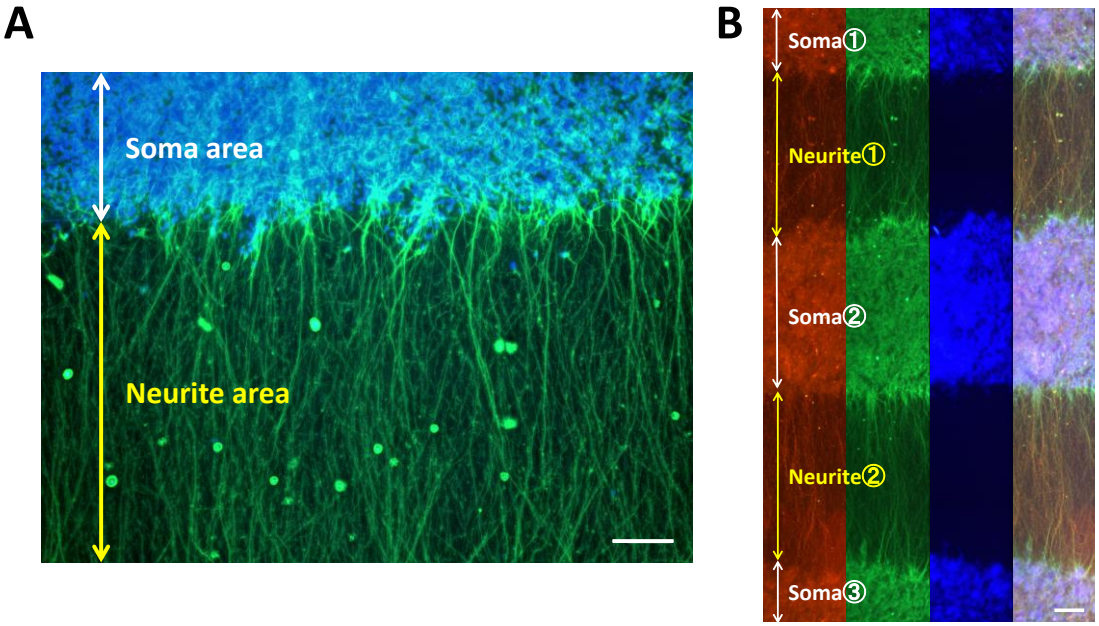

Fig.2 Reconstructed 3D neuronal circuits
(Blue: Hoechst 33258, Green: MAP2, Red: Tau-1), Scale bars=100μm

Finally, to confirm the function of a 3D reconstructed neuronal circuit, we measured the propagation of action potentials between one and another cell body layer using planer multi-electrode arrays (MEAs) chip. MEAs have been developed as typical tools for noninvasive and long-term electrical measurement, which made it possible to observe the spatiotemporal activities of dissociated cultures for days and weeks. We constructed two layered 3D neuronal network on MEAs chip (Fig.4A). As a result, in spontaneous activity and evoked responses, the delay time of propagation of action potential between A and B cell body layer was observed. As shown in figure 4B, the delay time was 15 ms. This result suggests that the interlayer propagations were caused by chemical synapse.

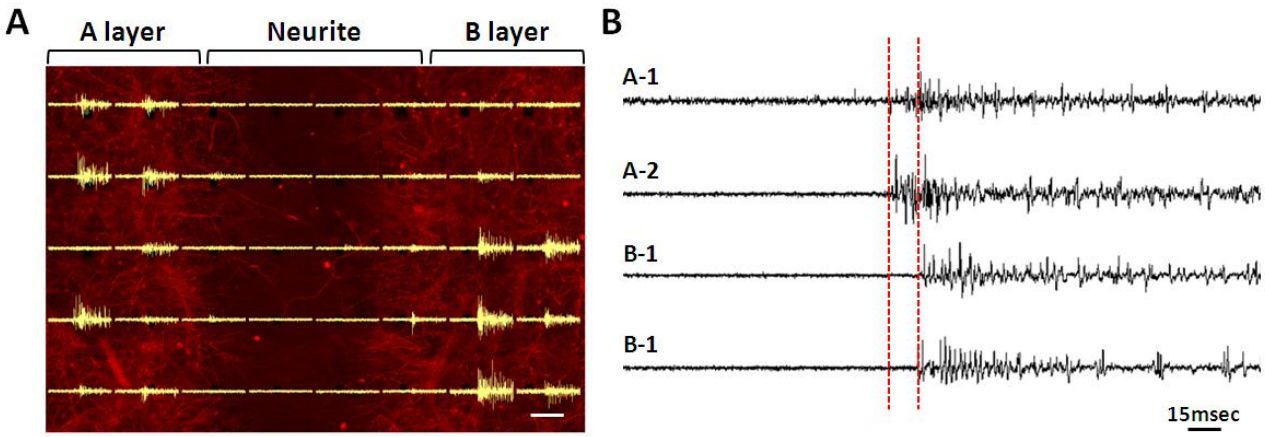

Fig.4 Propagation of action potentials between A and B layers
(A) 3D neuronal networks cultured on multi-electrode array chip, (B) Waveforms of the action potentials (Red: Tau-1), Scale bar=100μm

In this work we have developed a 3D reconstructed method of the neuronal circuit controlling the position of cell body and the direction of neurite elongation. These results show the potential for reconstruction of the six-layer structure of the cerebral cortex and for use in biological/medical field for investigating the properties of 3D neuronal dynamics and neurological disorder in laboratories. These results also suggest that our method will be useful in drug evaluation models and regenerative medicine in future. Furthermore, our methods may be useful in other biological tissues possessing the property of orientation.

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