SINGLE NEURAL CELLS ON MOBILE MICROPLATES FOR PRECISE NEURAL NETWORK ASSEMBLY

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ABSTRACT

This paper describes a method to assemble single neural cells into neural networks by utilizing microfabricated mobile microplates. Single neural cells were cultured on the mobile microplates. Directions of their neurite growth were controlled by guidepaths attached beside the microplates. We demonstrated that single neural cells with directed neurites were moved and assembled by mobile microplates into desired network structure, and also the network geometry was redefined. We believe this modular assembly method could be applied to precise patterning of neural networks, and also useful in basic studies of single-cell-level neural networks.

KEYWORDS: Single-cell-level neural network, Microplate, Modular assembly, Parylene

INTRODUCTION

Neural network construction *in vitro* has been an important method to investigate neural formation and activities [1]. They have been utilized as platforms of neural microcircuit analysis [2] and their pharmacological assays [3]. In order to understand their formation and activity in detail, it is required to construct a neural network at a single cell level.

Recent microtechnologies such as lithography have realized the single-cell-level neural networks by patterning single neural cells connected with their neurites on predefined geometries [1-4]. However, there was a problem that neural cells did not always adhere to the predefined areas and not extend their neurites to particular directions. Once neural networks were formed, they could not be moved and it was difficult to construct designed neural networks precisely.

Here, we propose a precise formation method of designed neural networks by placing single neural cells and guiding their neurites with microfabricated mobile microplates [5]. We cultured single neural cells on the mobile microplates, and controlled neurite growth directions by patterning polymers that block adhesion of cells and proteins (Fig. 1(a)). The microplates were designed to be movable and manipulatable in culture solution, thus we could locate them to aimed position (Fig. 1(b)). Using this method, we can also redefine neural network geometry.

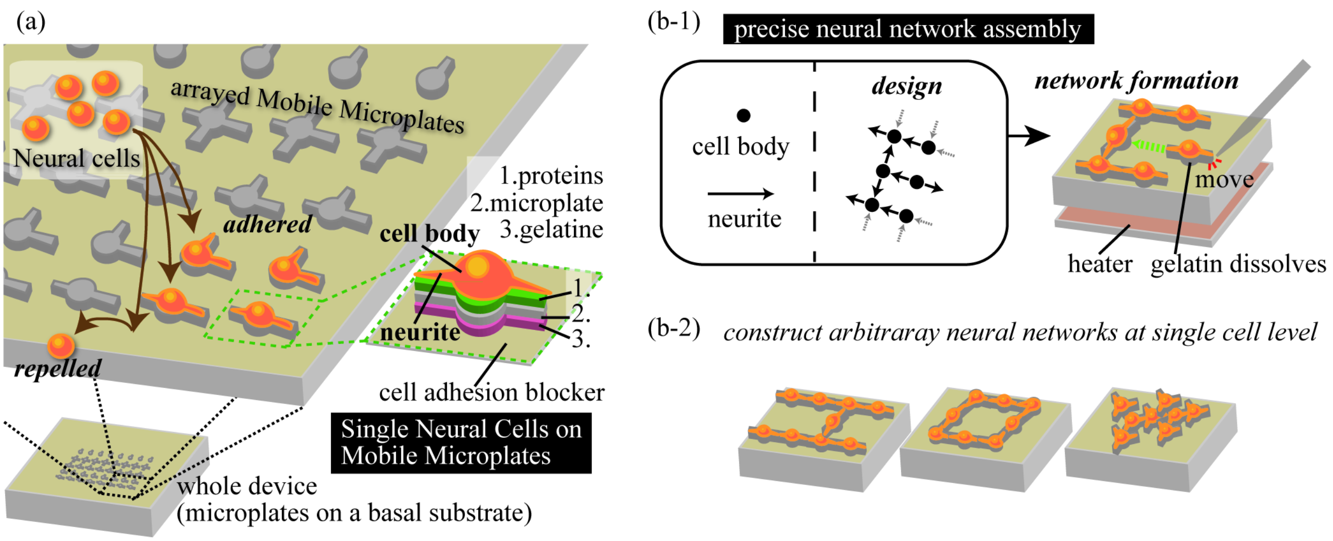

Figure 1. Conceptual illustration of modular neural network assembly method: assembling single neural cells into neural networks by utilizing mobile microplates. (a) Single neural cells selectively adhere onto microfabricated microplates. Neurites of each single neural cell are directed by guidepaths attached beside the microplates. (b-1) The microplates are detached from a basal substrate after dissolving gelatin by heat. They can be moved and assembled into desired network structure. Designed single-cell-level neural network is constructed by locating the cell bodies to defined areas and guiding their neurites toward particular directions. (b-2) Using this method, we could construct arbitrary neural networks precisely.

EXPERIMENTAL

We designed microplates in a circular shape with pathways beside them. Figure 2(a) shows fabrication process of the microplates.

First, we fabricated mobile microplate devices by standard lithography. On a glass substrate, we spin-coated gelatin, then deposited poly-monochloro-paraxylylene (parylene C) and aluminum (Al). We patterned parylene and gelatine as a microplate by O_2 plasma with using Al as a mask. After patterning the materials, we spin-coated 2-methacryloyloxyethyl-phosphorylcholine (MPC) polymers [6] on Al and the bared glass substrate. We lifted off Al and MPC polymers over the microplates, and covered the whole surface with fibronectin. Eventually, MPC polymers on the glass substrate repelled fibronectin, and we achieved MPC-coated glass substrate and fibronectin-coated parylene microplates as a whole device (Fig. 2(b)).

Second, we immersed the microplate device into a cell culture solution, and seeded rat adrenal pheochromocytoma (PC12) cells which are commonly used in studies of neurite extension. After the cells adhered on the microplates, we injected nerve growth factor (NGF) into the culture medium to extend their neurites. The culture dish with the device was placed in a CO_2 incubator at 37 degrees C to culture cells and also to dissolve gelatine under the microplates by heat, which allowed the plates detachable from the basal substrate.

Finally, we pressed and moved the microplates with glass capillary under a microscope.

RESULTS AND DISCUSSION

Single PC12 cells selectively adhered on single microplates (Fig. 3(a)). The cells did not attached onto a basal glass substrate because MPC polymers blocked them to adhere. After injecting NGF, we observed their neurites grew along the guidepaths of the plate, and stopped at their edge (Fig. 3(a-1)). The neurites kept in the defined direction after culturing a few days (Fig.3(a-2)). In this way, we successfully acquired the network components: neurite-directed single neural cells. We investigated the optimal plate size and guidepath width which could realize maximum collection rate of the single cell components. We found that plate size and guidepath width should be in the range of 30 to 40 microns, and 10 to 20 microns, respectively.

We also prepared several kinds of microplates which had different neurite guidepath directions. We could control the neurites toward mutidirections (Fig. 3(b)). We have not always achieved forcing neurites grew along all directions: for instance, a neural cell on a 4-way-path-microplate sometimes extended their neurites less than 4 ways. We consider that this aspect could be overcome by patterning chemical cues which

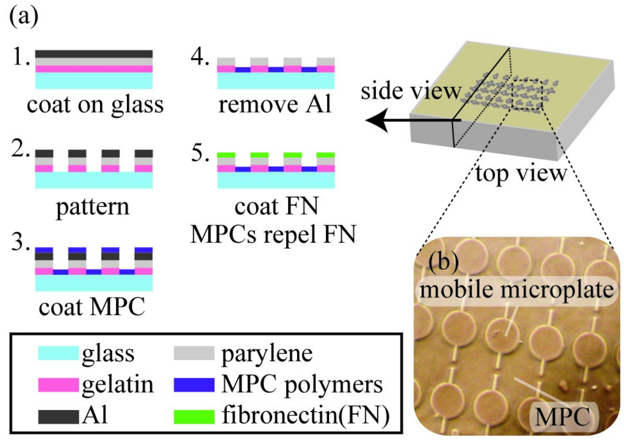

Figure 2. (a) Fabrication process of mobile microplates. Using standard lithography, we fabricated arrayed microplates made of gelatin, parylene, and fibronectin. (b) A photograph of fabricated mobile microplates.

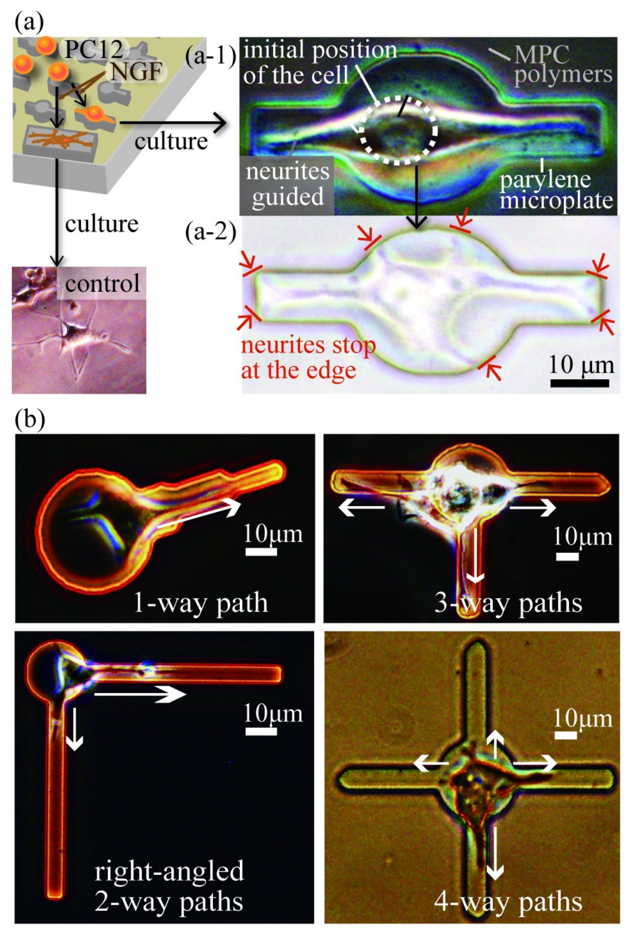

Figure 3. (a) Microimages of a single neural cell cultured on a mobile microplate. Neurites grew along the guidepaths and stopped at the edge. (b) Multidirectional guidance was also achieved. Neurites grew toward the given directions.

induce neurite growth on microplates.

To show the capability of constructing neural networks of arbitrary geometry, we moved and assembled single neural cells on the mobile microplates into network structures (Fig. 4). In a CO_2 incubator at 37 degrees C, PC12 cells on microplates were successfully cultured and a gelatine layer under them dissolved, resulting the microplates movable. Microplates were touched softly and handled easily by the glass capillary (Fig. 4(a)), then directly brought into contact with each other (Fig. 4(b)). Not only did we assembled microplates, but also we rearranged network geometry which was once defined in the culture dish. Redefinition of network geometry (Fig. 4(c)) could allow precise patterning of aimed neural network.

Using this method of modular network formation , we could construct single-cell-level neural networks in arbitrary structures. We consider it will be also possible to apply this method to constructing heterogeneous-neural-cell networks by assembling network components with different types of neural cells.

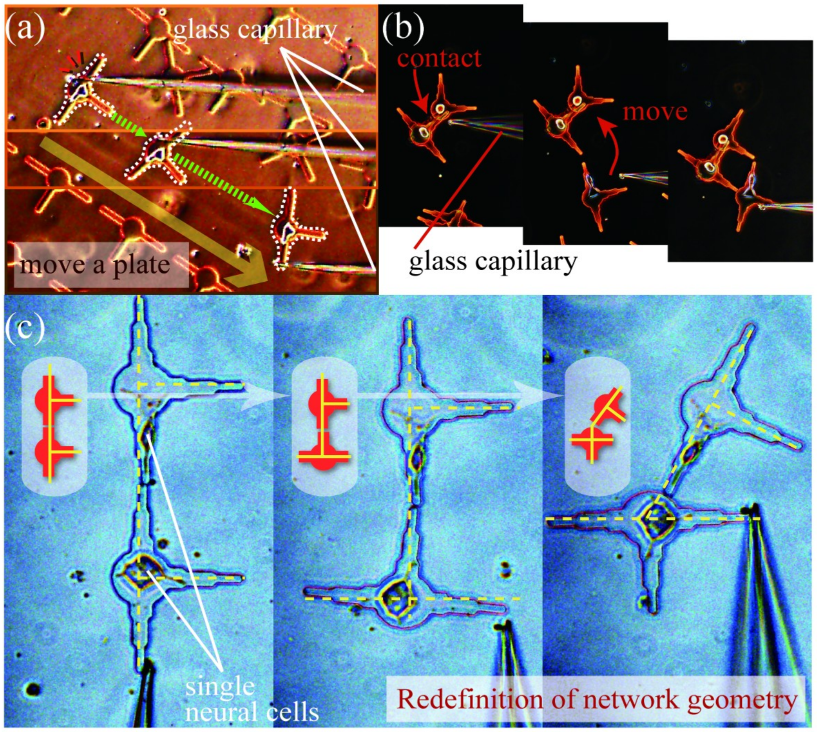

Figure 4. (a) Manipulation of a microplate that has a single neural cell. Microplates can be easily pressed and moved with a glass capillary. (b) Time lapse images of microplates with single neural cells assembled into a network structure. (c) The network geometry once defined can be altered by rearranging microplates.

CONCLUSION

We patterned single neural cells, controlled their neurite growth directions, and made them movable by utilizing microfabricated mobile microplates. Each of the neurite-directed single neural cells can be regarded as a network component, and we can assemble them into arbitrary network structures, or also redefine their network geometry. We believe this simple method will be useful in constructing single-cell-level neural network *in vitro* since neural cells can be patterned precisely. It would also be possible to make heterogeneous-cell network which is found in native brain. We consider this method will newly contribute to the basic study of neural networks such as analysis of neural coding in a certain network structure.

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