PARALLEL DISCRETE CHEMICAL STIMULATIONS OF MATRIX-ARRAYED NEUROSPHERES USING A MICROHOLE ARRAY DEVICE

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ABSTRACT

We developed a microdevice for chemical stimulation of neurospheres. The device consists of 8 x 8 microwells for cell culture and 8 microchannels for transportation of stimulating solutions. The bottom of each well is made of a SiN membrane of 1 μm in thickness, and has 24 x 24 microholes of 2 μm in diameter which are used for diffusive release of stimulant molecules from the microchannel to the microwell. We succeeded in formation of a single neurosphere having average diameter of 160 μm in each well. Also, it was found that the stimulation using 20 % FBS (fetal bovine serum) highly enhanced the differentiation from NSCs (neural stem cells) to neurons.

KEYWORDS

Neurosphere, Neural stem cell, Microhole, Cell stimulation, Differentiation induction

INTRODUCTION

A neurosphere is a highly significant biological tool for basic researches of nerve regeneration. Several researchers have succeeded in development of microdevices for building a large number of uniform-sized 3D multicellular aggregates including neurospheres [1-4]. However, multiple chemical factors cannot be analyzed simultaneously on their single device because it doesn't allow a single aggregate to be stimulated individually. We present a novel technique for arraying multiple neurospheres, 3D multicellular aggregates of NSCs, in a matrix on a microdevice, and stimulating each row of neurospheres individually with a differentiation-inducing factor that is released from microhole arrays. This technique permits parallel discrete stimulations using several different factors on a single device, and, therefore, can be used for the high-content assay in differentiation induction of NSCs.

METHODS

The device consists of 8 x 8 microwells, 8 inlets, 8 air vents, and 8 microchannels (Fig. 1). The wells are arranged in a matrix, and each of the channels is located directly under a row of wells. The bottom of each well is made of a transparent thin SiN membrane, and has multiple microholes. Before starting the stimulation, a single neurosphere is formed in each of the wells (Fig. 2(a, b)). When solutions including differentiation-inducing factors are injected into the channels, the solutions flow up to the well bottoms by capillary force. Then, the factor molecules are released from the microholes, and delivered to the neurospheres by diffusion, which induces the cell differentiation. (Fig. 2(c)).

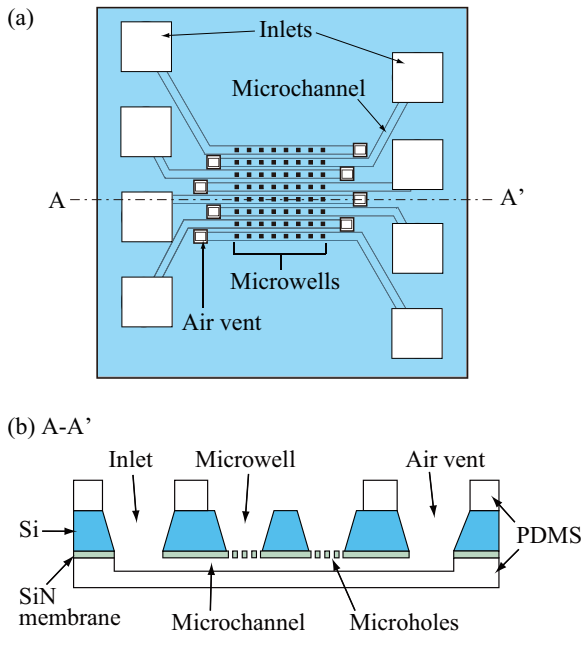

Figure 1. Schematic of a microhole array device.

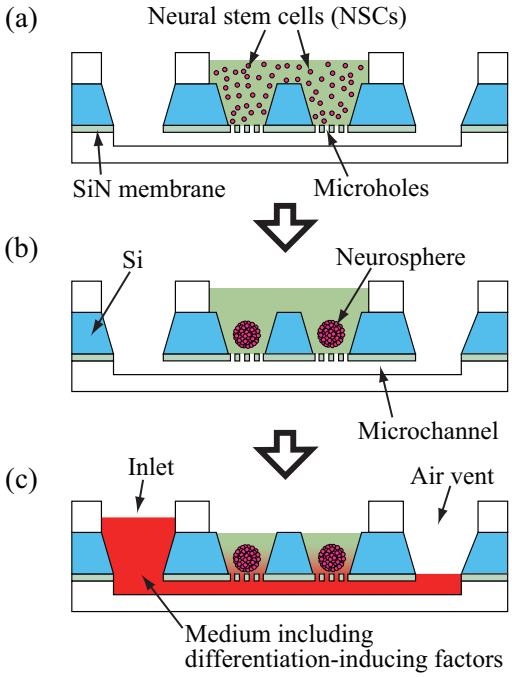

Figure 2. Procedure of neurosphere formation and stimulation.

EXPERIMENTS

Figure 3 shows the photographs of the fabricated device. The microwells were formed by anisotropic etching of a Si wafer. The well bottom measuring 350 μm x 350 μm was made of a 1 μm thick SiN membrane deposited by plasma CVD. The 24 x 24 microholes of 2 μm in diameter were opened in the membrane of each well bottom by dry etching with CF_4 plasma. This microwell plate was sandwiched between two plates made of PDMS (polydimethylsiloxane); one includes 8 channels, and the other is used to make a cell-culture chamber surrounding the microwell array.

We carried out experiments for formation and stimulation of neurospheres on the fabricated device. First, in order to prevent NSCs from adhering to a SiN surface, it was coated with PEG (polyethylene glycol) molecules. Next, NSCs were disseminated onto the microwell array in an optimal cell density of 2300 cells/well. Within a day, NSCs aggregated and formed a few neurospheres in each well. Then, they merged with one another, and the total number of neurospheres on the device decreased with time. Eventually a single neurosphere having an average diameter of 160 μm was formed in each well in 5 days (Fig. 4, 5). Also, the standard deviation of neurosphere diameter was highly improved during a single neurosphere formation (Fig. 6).

Next, we stimulated the neurospheres using medium containing FBS (fetal bovine serum) which is a differentiation-inducing factor of NSCs. After the stimulation for three days, β-tubulin III which is a microtubule element found almost exclusively in neurons was fluorescently stained in order to evaluate differentiation from NSCs to neurons (Fig. 7). Experiments using FBS of eight concentrations of 0.1 to 40 % indicated that 20 % FBS most highly enhanced the differentiation (Fig. 8).

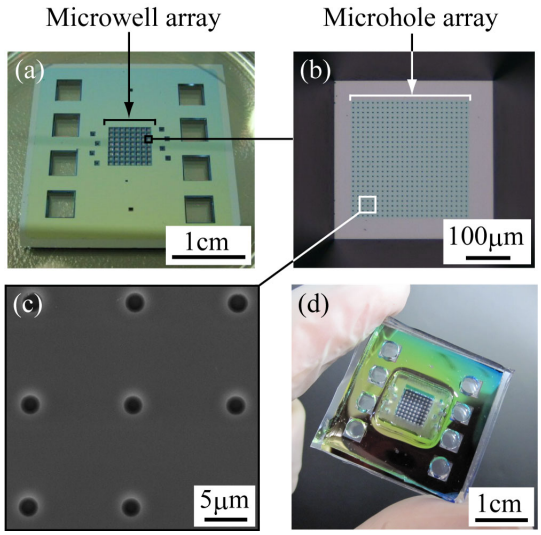

Figure 3. Photographs of the mcrowell array (a), the microhole array (b, c), and the entire device (d).

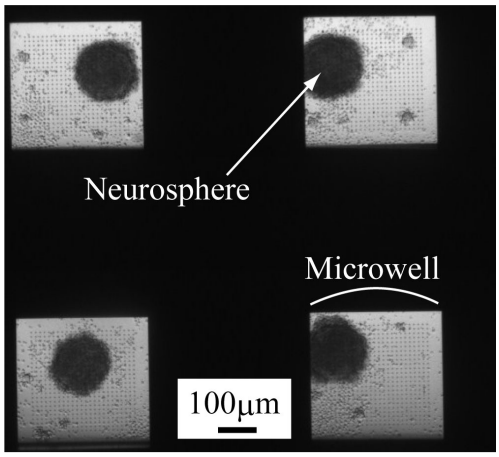

Figure 4. Single neurosphere formed in each microwell.

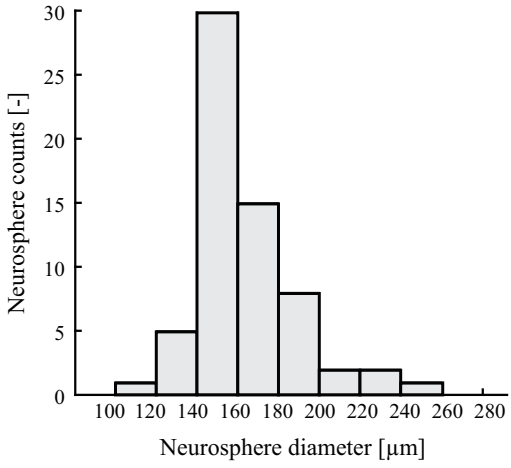

Figure 5. Neurosphere diameter distribution.

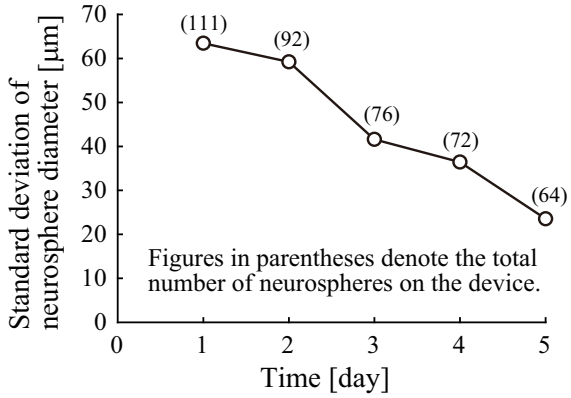

Figure 6. Decrease in standard deviation of neurosphere diameter.

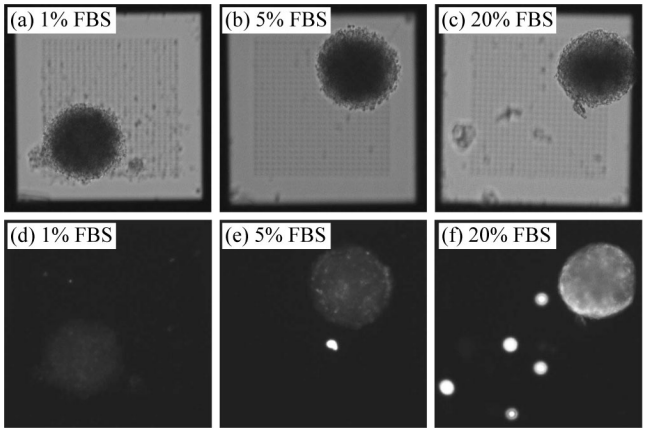

Figure 7. Bright-field images (a-c) and fluorescence images (d-f) after FBS stimulations.

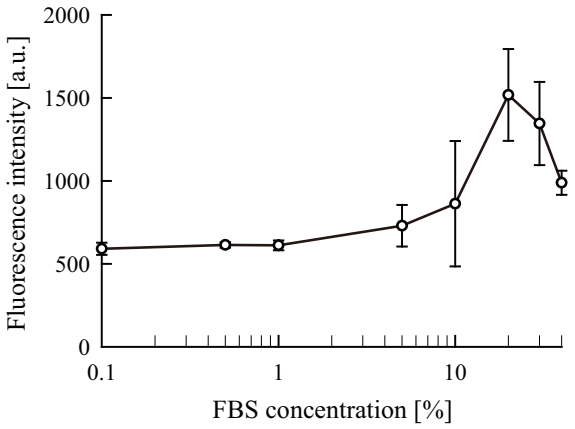

Figure 8. Fluorescence intensity of neurospheres differentiated by FBS stimulation.

CONCLUSIONS

The fabricated device succeeded in forming a single neurosphere in each microwell and stimulating a row of neurospheres individually through the microholes opened in the microwell bottoms. As a result, it was found that a neurosphere was most rapidly differentiated by 20 % FBS stimulation. In the near future, we will evaluate differentiation and morphological change of neurospheres after injecting other different factors into microchannels on a single device.

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