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Extracellular matrix (ECM) patterning by atmospheric-pressure plasmas for arranging neuronal cells on silicon substrates

シリコン基板上への神経細胞配列のための
大気圧プラズマによる細胞外マトリックス（ECM)パターニング

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A new extracellular matrix (ECM) patterning technique has been developed with using the atmospheric pressure plasmas (APP) application. Arrangement of neuron cells in a desired pattern on a silicon (Si) chip is an important step for the development of neuron cell chips. In this study, ECM layers deposited on a Si substrate were patterned in 100~200 μm sizes by the APP etching and neuronal model cells were shown to grow on the patterned ECM. The results indicate that the process based on APP application for the ECM provides a simple mean of arranging neuron cells on a relatively large area of a Si surface.

1. Introduction

Control of cell adhesion to and proliferation on a specific surface connected with electrical circuits is a technology required for the development of next-generation biosensors/biochips. For example, the formation of a large-area neuron network on a silicon (Si) surface is considered to be a crucial step for the fabrication of cell chips for diagnosis of brain diseases such as Alzheimer's. The extracellular matrix (ECM) that consists of a various types of proteins, polysaccharides, and other polymers serves as a scaffold for cells. For the arrangement of living cells on a semiconductor substrate, therefore, the formation of patterned ECM layers on the semiconductor surface is an important first step. The goal of this study is to demonstrate that the atmospheric pressure plasma (APP) etching method for ECM layers can be applied to the arrangement of neuron cells on a Si chip. We have confirmed that Specific HEK293 cells (Human Embryo Kidney cells) proliferate only on specific fibronectins patterned by low-frequency APP jets (LF plasma jets) [1]. In this study, we shall examine the APP etching techniques for the growth of neuron cells on a Si chip, using PC12 cell (Rat adrenal pheochromocytoma cell line) as a neuronal model and Poly-L-Lysine (PLL) as their ECM.

2. Experimental set up

LF plasma jets were applied to a 15×15 mm^2 Si substrate coated with ECM and a stainless-steel stencil mask was directly placed on the ECM layer, as shown in Fig. 1. LF plasma jets were discharged in air by pulsed high voltages applied to an electrode that surrounds a glass tube inside which a helium (He) gas flows [1, 2]. In our experiments, the pulse frequency of power supply was 10 kHz, the inner diameter of the glass tube was 4 mm, and the distance between the sample substrate and the end of glass tube was 50 mm. The peak-to-peak voltage applied to the electrode, the flow rate of He gas, and plasma irradiation time were varied in the process of searching optimal conditions for ECM patterning.

Two different types of stencil masks were used in our experiments; one had three different sets of slits in area of 1 cm^2, as shown in Fig. 2 (a); (1) ten 100 μm-wide slits with a slit interval (i.e., the distance between two successive slits) of 100 μm, (2) ten 100 μm-wide slits with a slit interval of 200 μm, and (3) ten 200 μm,-wide slits with a slit interval of 100 μm, while the other had holes about 100 μm, in diameter with an interval of about 100 μm, in area of 1 cm^2, as shown in Fig. 2 (b).

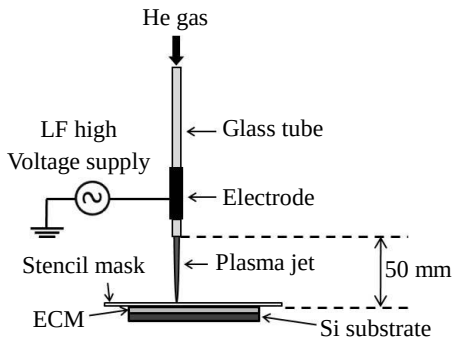

Fig. 1. Experimental set up for ECM patterning by LF plasma jets.

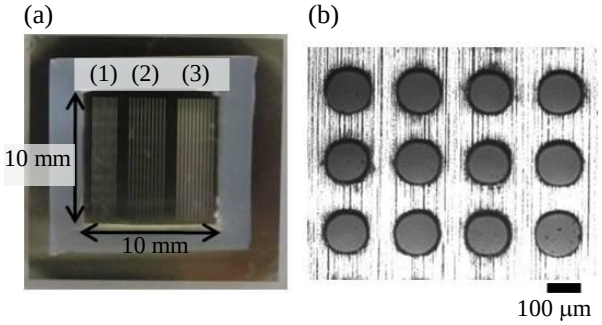

Fig. 2. Stainless-steel masks used in this study; (a) has three different sets of slits, (b) is the micrograph of mask which has holes.

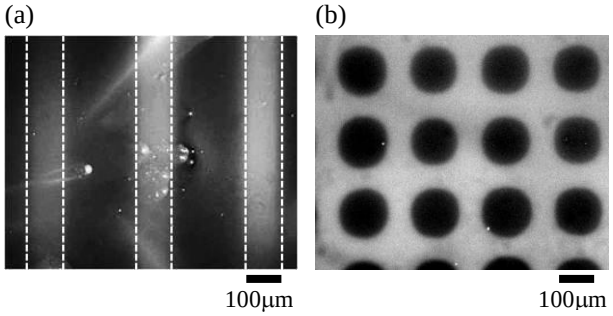

Fig. 3. Fluorescence micrographs of PLL patterns; (a) patterns in the area (3) of the mask shown in Fig. 2 (b), (b) patterns by using the mask shown in Fig.2 (b).

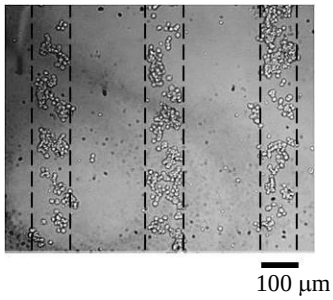

Fig. 4. A micrograph of cell arrangement patterns in the area (3) of Fig. 2 (a) on the Si substrate. The dashed black lines indicate the original mask slit patterns.

3. Experimental results

3.1 *Fluorescently-labeled Poly-L-Lysine patterning*

Si substrate which had been coated with a fluorescently-labeled PLL (FITC-PLL, SIGMA P3545, 500 μg/ml) layer was patterned by LF plasma jets. The substrate surfaces after patterning were observed by fluorescence microscopy. Figure 3 is a fluorescence micrographs of a Si surface. PLL remaining on the substrate is indicated in white in these micrographs. Figure 3 (a) shows the PLL patterns located in the area (3) of Fig. 2 (a) when LF plasma jets were applied for 15 s uniformly to the substrate with a peak-to-peak voltage of 7 kV and a gas flow rate of 4 L/min. The dashed white lines of Fig. 3 (a) delineate the original slit patterns of the stencil mask. Figure 3 (b) shows a fluorescence micrograph of a Si surface patterned with a mask of holes shown in Fig. 2 (b) after LF plasma jets were applied for 20 s uniformly to the substrate with a peak-to-peak voltage of 7 kV and a gas flow rate of 4.5 L/min. It has been observed that, if LF plasma jets were applied under right conditions for each mask, PLL deposited on the substrate was patterned more or less uniformly following the mask patterns.

3.2 *Cell culture on a patterned PLL*

PC12 cells were cultured on a PLL (SIGMA P4707, 0.01 % solution) layer patterned by LF plasma jets for the cellular adherence examination. The micrograph of area (3) in Fig. 2 (a) taken 24 hours after the cell seeding is shown in Fig. 4. Figure 4 shows that cells adhered to and proliferated along the patterned PLL strips although a small number of cells ran off the edge of the patterned PLL strips. Similar cell adhesion was also observed in the areas (1) and (2) of Fig. 2 (a), and on the patterned PLL with a mask of holes shown in Fig. 2(b) (data not shown).

4. Conclusions

It has been demonstrated that PC12 cells can be arranged and grown in a pattern of 100~200 μm size on a Si substrate by means of an APP etching for the ECM layer deposited on the substrate. The process based on APP etching for the ECM proposed in our study offers a cost effective means for neuron cell proliferation in a desired pattern on a relatively large area of a Si chip. Formation of neural networks by the growth of axons of neurons over the patterned ECM is the subject of a future study.

Acknowledgments

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