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The Topographical Guidance of Neurons cultured on Holographic Photo-Responsive Polymer

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Abstract—**Neuronal cells to respond to submicron-scale groove structure. On the grooved structure of particular dimension, it has been reported that neuronal cells grew perpendicular to the groove direction. We used holographic photo-responsive polymer to form a submicron-scale surface relief grating structure. A sinusoidal groove pattern is built up by holographic interference of 488㎚ Ar ion laser beams. The primary hippocampal neurons cultured on the surface of the polymer film grew extending their neurites in a perpendicular orientation to the groove direction. This suggests that laser holography can be used to control the neurites orientation and growth. The holographic grating and photo-responsive polymer will raise the possibility of controlling neural network formation between living cells by light.**

Keyword—**cell growth, holography, neuron, polymer, topography**

Ⅰ. INTRODUCTION

Various techniques for cellular patterning have been developed for an application to cell-based biosensors, tissue engineering, and scientific studies of cell-surface interaction [1]. Microcontact printing based on chemical cues and microfabrication technique based on topographical cues have been widely used for guiding cellular growth or patterning cellular formation [2,3].

Microfabrication technologies using photolithographic and non-lithographic methods enable the diverse structures for cell substrata such as cliffs, grooves, spikes, tubes, mesh, and random roughness [4]. However, most of these substrata are limited by the fixed structures. Once it is fabricated, it is not allowed to modify the physical structures in cell culture environment for an additional purpose, for example, forming a new groove for changing the cell orientation.

Holographic formation of surface relief grating (SRG) is a promising approach for an easy fabrication of submicron-scale structure. Single-step irradiation on photo-responsive polymer makes the regular sinusoidal grating pattern with two interfered laser beams without complicated procedures. By this holographic manner, the surface pattern can be produced and removed on the polymer, and the depth and width of grooves can be readily modified with the light alignment [5].

The primary hippocampal neurons change their directions of neurites growth to reflect the preferred orientation. Neurites grow faster on the grooves in the preferred direction and aligned both perpendicular and parallel to groove direction. The frequency of the alignment and growth rate depended on the depth and width of the grooves. In a particular dimension of grooves, the response of hippocampal neurons significantly differed from the control. The neurites length was selectively increased in perpendicular direction to the grooves and decreased on parallel direction. The substrate of the dimension elicited the predominant (85±6%) orientation in the perpendicular direction [7]. This means that the hippocampal neurons can be guided by the contact guidance of the groove structure.

Previously, we reported that human astrocytes are preferentially attached onto holographic SRG region and also proliferated with specific orientation along the groove direction [6]. In this work, we investigate the behavior of primary hippocampal neuronal cells on holographic SRG considering the feasibility of SRG as a topographical guidance. We exploit a new methodology of the topographical control by laser holography. This will potentially enable the guidance of living cells by laser irradiation, manipulating neural network formation for neuroscientific studies and neural cell-based biosensors.

Ⅱ. METHODOLOGY

A. Holographic Photo-Responsive Polymer

Azobenzene copolymer, Poly [(methylmethacrylate)-*co*-(Disperse Red 1 acrylate)] (Sigma Aldrich, 57042-7) was used as a holographic photo-responsive polymer. It was dissolved in tetrahydrofuran at a concentration of 4% in weight. The polymer film was formed by spin coating on a cover glass. The coated polymer was dried for 6 hours at 70℃ to remove the solvent.

B. *Laser Holography*

In order to form a holographic SRG, we used 488㎚ Ar laser and classical 'Lloyd's mirror' setup (Fig. 1). We made an interference pattern by the superposition of two beams. One is irradiated directly from a laser source and the other is reflected from a mirror. The incident beam from laser source is linearly polarized to +45° while the reflected beam is of –45° polarization axis on the reflective mirror. These two orthogonal beams make a polarization modulation of the polymer surface resulting in molecular migration to form a regular sinusoidal surface relief grating. The SRG feature was controlled within less than 1㎛ in depth and a few ㎛ in width.

The intensity of laser is about a few hundreds of ㎽/㎠. The size of SRG pattern is several millimeters of diameter. Fig. 2 shows the scanning electron microscope image of the SRG pattern on the azobenzene copolymer. The width of grooves was determined by the combination angle between two beams, and the depth by the time of laser irradiation.

C. *Cell Culture*

Primary hippocampal neurons were prepared from embryonic day (E18) Sprague Dawley rat brain. The dissociated neurons were placed in modified Eagle's medium (MEM) supplemented with 20% Glucose, 1mM Sodium Pyruvate, 2mM L-Glutamine and penicillin-streptomycin (Invitrogen) for 3 hours and grown in neurobasal medium supplemented with B27 and 0.5mM L-glutamine.

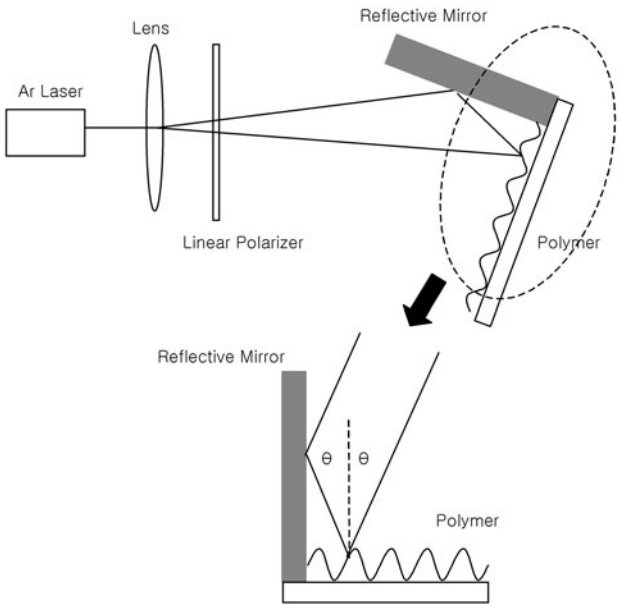


Fig. 1. Schematic view of optical setup for SRG formation. Laser beam is linearly polarized to +45°. The reflected beam by mirror has -45° polarization axis. Two orthogonal beams interfere on the surface of the polymer to form the groove structure. The period of grating pattern depends on the combination angle θ.

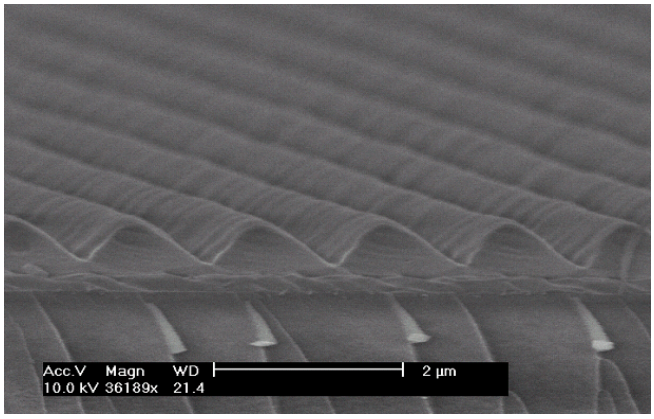


Fig. 2. SEM image of SRG on azo-dye copolymer (1.3㎛ wide, 400㎚ deep).

For reducing glial proliferation, we treated Ara-C after 3 days. Once a week, we removed half the volume of medium and replaced it with fresh maintenance medium. Prior to cell seeding, cover glasses were treated with poly-D-lysine coating to enhance the adhesion of the neuronal cells on the polymer substrate.

Ⅲ. RESULTS AND DISCUSSION

A. *Cell Viability*

The primary hippocampal neurons proliferated on the poly-D-lysine coated with the photo-responsive polymer. The viability of neural cells on the polymer was investigated by optical microscopy. The morphology of the cells ensures the healthy status of the cells, the lack of any symptoms of cell deterioration such as granularity, cytoplasmic vacuolation, and rounding of the cells on the surface of polymer. The cultured cells survived for over 6 weeks and the degeneration of the polymer has not occurred during experiment.

B. *Neurites Orientation*

The primary hippocampal neurons were cultured on the photo-responsive polymer with SRG pattern. Prior to the cell seeding, the holographic SRG pattern (1㎛ width, 600 ㎚ depth) was formed on the polymer. It is known that the neurites were aligned in perpendicular orientation to the groove with 1㎛ in width and several hundreds of ㎚ in depth [7]. We used the same dimension of grooves for contact guidance of the neurons. However, the groove structure here has a sinusoidal shape, which is different from the conventional rectangular shape. This may provide some degree of discrepancy in cell reaction on topography.

Fig. 3 shows the cells grown on the polymer. The neuri-

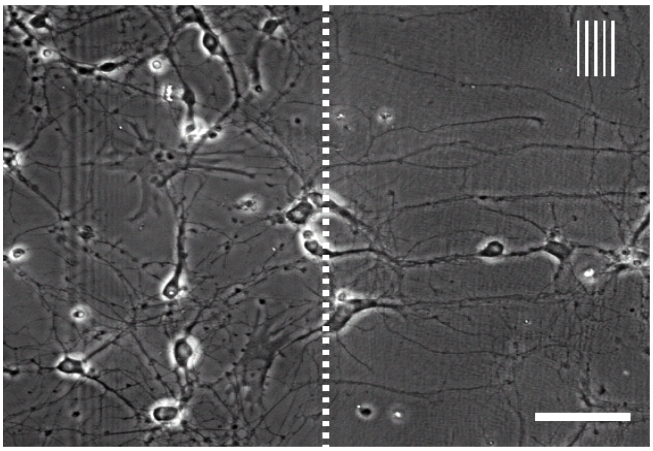


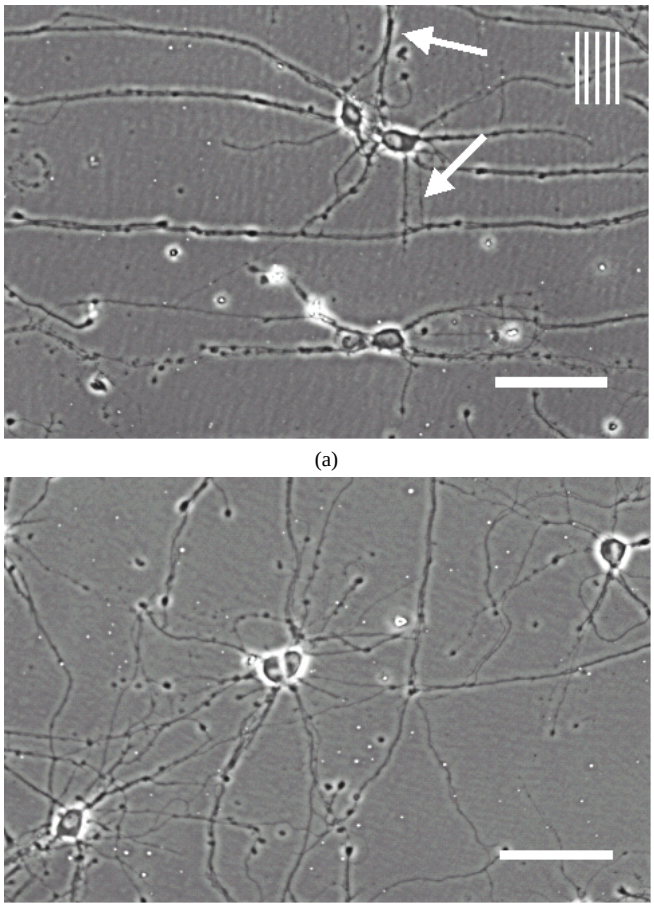
Fig. 3. Phase contrast image of primary hippocampal neurons after 6 days in culture. SRG on the right side of white dotted lines and no grating pattern on the left side. Narrow solid lines on the upper right indicate the direction of grooves. (1.2㎛ width, 600㎚ depth). Scale bar=50㎛.

tes of neurons grow perpendicular to the direction of the grooves (on the right side of the white dotted lines) while the neurites extends in random direction on the area with no grating pattern (on the left side of the white dotted lines). This contact guidance of sinusoidal grooves make the neurites turn perpendicular to the grooves. The neurites change the direction as they enter the SRG area.

The depth of the grooves becomes deeper from the white dotted line to the SRG region of right half side. The neurites turn towards the perpendicular direction to the grooves correcting their pathways in neural pathfinding.

In Fig. 4(a), the neurites orientation appears both parallel and perpendicular to groove direction compared to the control in Fig. 4(b). On the smooth surface without the groove, the neurites expanded in random direction. On the groove-patterned surface, the neurites mostly grow in perpendicular orientation while some neurites extends in parallel orientation with relatively short length. This shows that the contact guidance of neurons can steer the bi-directional growth of neurites.

However, the neurites with perpendicular orientation are predominant in frequency and length. This result agrees that on the grooved quartz the frequency of perpendicular orientation was about 70%, while parallel orientation was about 20% and the neurites grew faster in the perpendicular orientation on the grooves [7]. On the grooves with 4㎛ in width and 1㎛ in depth, the parallel orientation exceeds the perpendicular orientation in frequency. This means that the wider grooves play a role of the parallel contact guidance on the neural cells. The frequencies of the neurites orientation may be also significantly influenced by the width and depth of the grooves [7,8].



(a)

(b)

Fig. 4(a) Hippocampal neurons after 6 days in culture on SRG pattern. The neurites grow perpendicular to groove direction (Narrow lines on upper right corner). White arrows indicate parallel growth of neurites. (b) Hippocampal neurons after 6 days in culture on azobenzene copolymer without SRG pattern. The neurites grow in random direction. Scale bar=50 ㎛.

Ⅳ. Conclusion

The hippocampal neuronal cells proliferate well on the photo-responsive polymer substrate coated with poly-D-lysine. The neurites of the cells expand in random direction on the polymer. On the regular sinusoidal grooves of the polymer, the axons grow both perpendicular and parallel to the grooves. The frequency of perpendicular alignment is much higher than that of parallel alignment. This suggests that the cell growth can be controlled by the groove dimension and direction. We expect that the growth of neurites may be guided in desired direction by intentional formation of the grating on their pathways. This contact guidance may play a role of topographical cue for artificial neural network formation.

The photo-responsive property of the polymer has the crucial advantages over conventional substrate. The grating pattern is fabricated directly on the surface of the polymer

by laser holography without complicated procedures. Then, by modulating the alignment of the light, we will be able to guide the living neuronal cells in a noninvasive manner, leading to a cultured neural network formation.

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