

Spatial Patterning of Fibroblast Cells with Fabricating Holographic Patterning on the Photoresponsive Polymer

Jae Kyoo Lee^{1,3}, Eunje Jang², Sin-Doo Lee², and Sung June Kim^{1,2,3}

¹ Interdisciplinary Program of Biomedical Engineering, Seoul National University, Seoul, Korea

²School of Electrical Engineering, Seoul National University, Seoul, Korea

³Nano-Bioelectronics and Systems Research Center, Seoul National University, Seoul, Korea

Abstract—Micro topographic feature was obtained by laser holographic fabrication on the photoresponsive polymer. Surface feature was localized using photomask for developing two dimensional cellular patterning. Fibroblast cells were cultured and proliferated only on the patterned substrate. Obtained cellular patterning suggests that the laser fabricating with photoresponsive polymer would be applied to regenerating new tissue and developing biomedical device of living cells.

I. INTRODUCTION

Two dimensional cellular patterning attracts the intense interest for the development of cellular device and fundamental studies of cell-substrate interaction [1]. One of the methods for cellular patterning utilizes the biochemical patterning with micro contact printing [2]. By immobilizing cell-preferred biochemical to the substrate, the cellular growth and attachment is stimulated. The other methods utilize the topographic cue for cellular patterning. Each type of cells reacts to the micro and nano structure on the substrate and show the different behavior of growth [3].

The key to the successful cell patterning using topographic cue is to fabricate appropriate topographic feature for the cells and the fundamental and develop a structure showing large difference of cell behavior on the patterning. Azobenzene copolymer is one of the candidate for the fabricating the topographical patterning. Since the fabricating with laser holography on the photoresponsive azopolymer is easy and simple, there is no demand of conventional complex fabricating instruments [4]. The formation and characteristics of the azopolymer is reversible and repeatable [5]. Thus, the developed feature can be modulated by additional laser processes.

In the present paper, the evaluation of the holographic fabricating method for controlling cellular attachment and consequent two dimensional patterning of fibroblast cells were investigated for the development of successful foundation of

cellular micro device and tissue regeneration technology.

II. MATERIALS AND METHODS

A. Cell Culture

National Institutes of Health 3T3 fibroblast cells (Korean Cell Line Bank, Korea) were maintained at 37 and 5% CO₂ in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 units/ml penicillin, 100ug/ml streptomycin and 0.25ug/ml amphotericin B (Gibco). Experiments were performed ##h after the cells were seeded on the photoresponsive polymer at a low density and the fibroblast cells were periodically subcultured in trypsin-EDTA solution containing 0.25% trypsin and 1mM EDTA.

B. Photofabrication

Azobenzene copolymer, poly [(methylmethacrylate)-*co*- (disperse red 1 acrylate)] (57042-7, Sigma Aldrich) was used as a holographic photoresponsive polymer. It was dissolved in tetrahydrofuran at a concentration of 5% (w/w). The polymer film was formed by spin coating on a cover glass, which produced a coated film with a thickness of about 1 μm. The coated polymer was dried for 6 hours at 70°C to remove the solvent In order to form a holographic SRG, we used a 488-nm Ar⁺ ion laser and the classic Lloyd's mirror setup (Fig. 1). Light from the laser was expanded by a beam expander at an appropriate range and polarized. We formed an interference pattern by the superposition of two beams. One came directly from the laser and the other was reflected from a mirror. The incident beam from the laser was linearly polarized at an angle of +45° while the reflected beam from

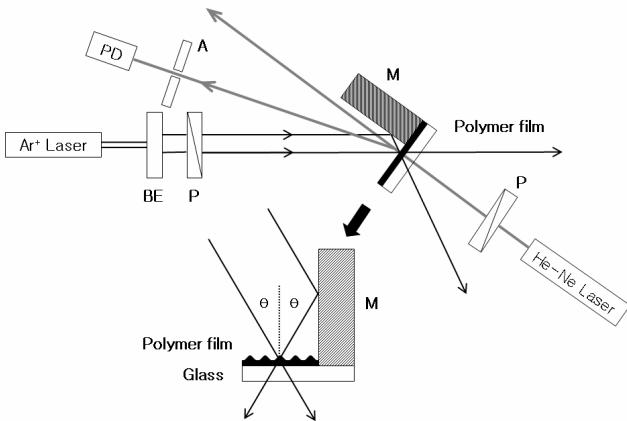


Fig. 1. Schematic view of the optic setup for the SRG formation. The laser beam was linearly polarized at an angle of $+45^\circ$ and the reflected beam on the mirror had an angle of -45° with respect to the substrate normal. Two orthogonal beams interfered on the surface of the polymer to form the groove structure. The period of grating pattern depended on the combination angle of θ . (PD: photo detector, BE: beam expander, P: polarizer, M: mirror, A: aperture)

the mirror had an angle of -45° with respect to the substrate normal. These two orthogonal beams exhibited polarization modulation on the polymer surface resulting in molecular migration to form regular sinusoidal SRG. The intensity of Ar^+ ion laser was about 300 mW/cm^2 . The width of grooves was determined by the combination angle between the two beams, and the depth by the duration of laser irradiation. The formation of the grating was monitored in real time by probing with a He-Ne laser (wavelength of 633 nm) onto the inscribed region. The probe beam was linearly polarized at 45° to the axis of grating and did not influence the fabrication process. Silicon photomask was fabricated by conventional SOI wafer process. A spatial patterning on photoresponsive polymer was conducted by inserting the photomask between light source and polymer film.

C. Analysis of Cell Morphology

Differential interference contrast (DIC) optical microscope was used to record the image of the cells with a CCD camera (Axiocam, Carl Zeiss, Germany) attached to a Zeiss LSM microscope. After inscribing SRG on the polymers, the exposed surfaces were investigated with a scanning electron microscope (SEM, XL30FEG, Philips) and an atomic force microscope (AFM, XE-150, PSI) in contact mode. Cell morphology was analyzed with a phase- and differential-contrast microscope. Rat hippocampal neurons were washed with phosphate-buffered saline (PBS) and fixed with 4% (v/v) paraformaldehyde for 10 min at room temperature. After fixation, cells were gently washed with PBS and dehydrated in

Deposition

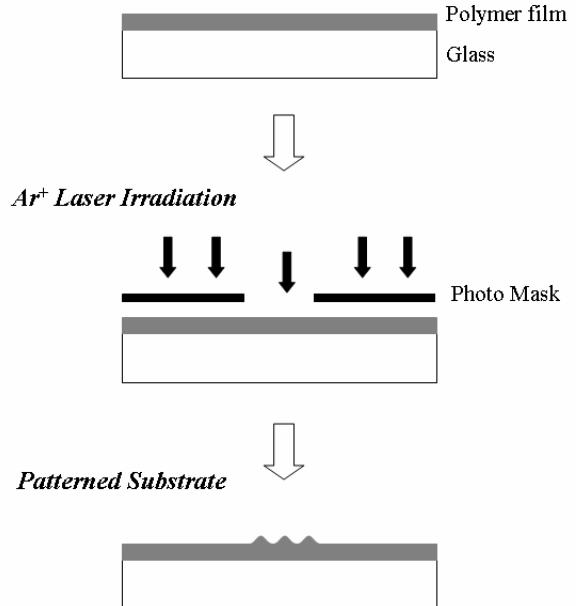


Fig. 2. Schematic procedure of photofabrication on photoresponsive polymer. SRG was formed in localized area of the polymer surface by photomask.

a series of ethanol solutions (70%, 80%, 90%, then 100% (v/v), each for 10 min) to ensure total dehydration. The samples were desiccated overnight, sputtered with gold, and analyzed by SEM. The fixed cells were gently rinsed three times with warm PBS. The DNA-selective Hoechst 33342 (Molecular Probes) was used to stain the nucleus of the cells. Hoechst 33342 was diluted in 2% (v/v) in PBS and treated to the fixed cells for 15 minutes at room temperature. After washed three times with PBS, the samples were analyzed with fluorescence microscope (Carl Zeiss) and CCD camera (Axiocam, Carl Zeiss).

III. RESULTS AND DISCUSSION

A. Fabricating Polymer Substrate

Figure 3 shows SEM images of microscale-grooved structures on the photoresponsive polymer. The SRG inscribed by laser holography manifested as a series of repeating, evenly spaced, sinusoidal grooves. The depths of the grooves were determined by the duration of laser irradiation, and the widths by the angle of the incident beam. The widths of the grooves produced by laser holography can be predicted by the classical theory of interference [16]. The He-Ne laser revealed that the intensity of the diffracted probe beam increased with the groove depth, and the end point of fabrication was estimated by measuring the relative intensity of the diffracted beam. Two dimensional

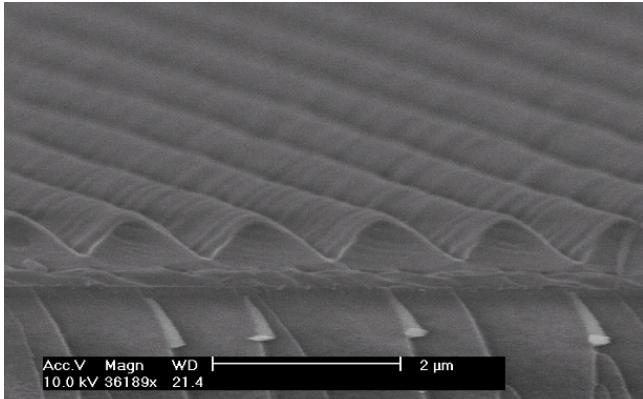


Fig. 3. Scanning electron microscope picture of photofabricated surface of azobenzene copolymer. The regular sinusoidal grooved structure had 1.3 μm periodicity and 400 nm modulation depth.

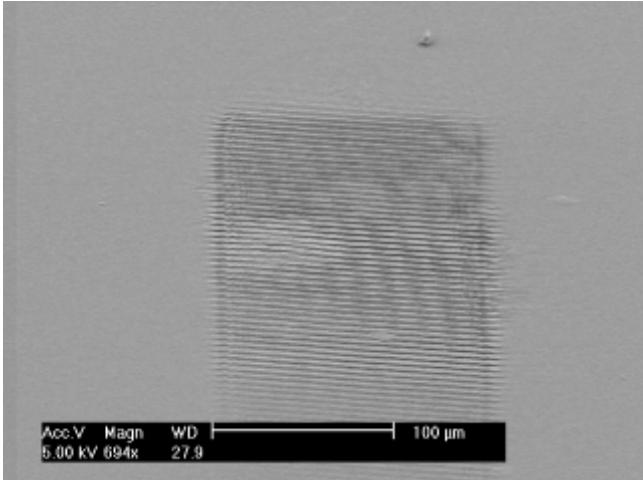


Fig. 4 Localized grooves pattern on photoresponsive polymer using photomask. The line pattern is 250 μm wide and 1mm long.

patterning of grooved structure is presented in Fig. 4. Along the boundaries of the patterning, the diffracted laser beam produced small scales of fringes on the patterned structure. These fringes were eliminated by reducing gap between photomask and polymer film.

B. 2D Patterning of Fibroblast Cells

Figure 5 shows the time-lapse images of fibroblast cells cultured on patterned polymer surface. After cells were seeded on the polymer, the cells initiated to attach to polymer surface. The cells behaviors on the grooved patterned region differed from the cells on plain surface. In Fig. 5(a), the cells on the patterned region formed stable contacts with the substrate, whereas the cells on the plain region were rounded. At 8 hours after seeding, the cells on grooved region produced ruffles around the cells and spread along the patterned region and cells on the plain region started to lose their contact to the substrate.

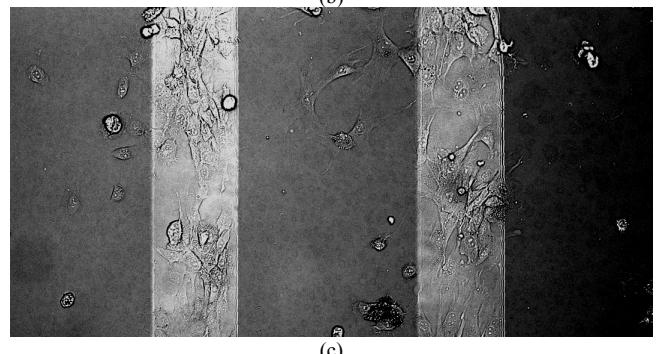
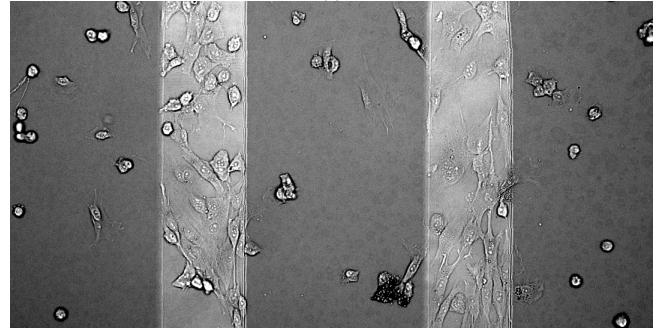
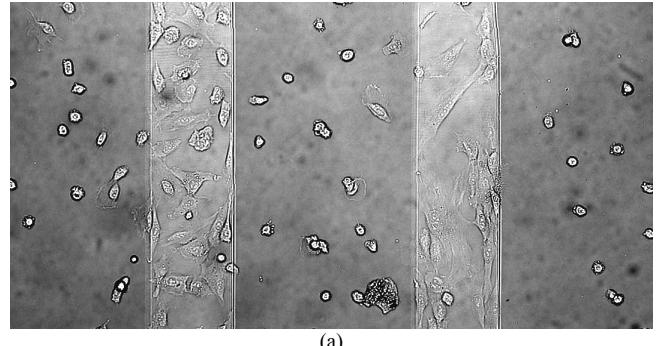


Fig. 5. Time-lapse images of two dimensional cellular patterning using Laser-fabricated photo-polymer, (a) after cell seeding, (b) after 8 hours, and (c) after 16 hours.

After 16 hours later, it was observed that cell proliferated on the patterned region and a few cells survived on the plain region. These images indicated that patterned groove structure is an effective method to control the cell attachment to the substrate.

In Fig. 6, the fibroblast cells were stained with Hoechst 33342 that fluoresce bright blue binding to DNA. This staining was conducted to confirm the cell proliferation and aggregation of the cells. The stained image shows the proliferation of the cells on the patterned region.

Scanning electron microscopy was also used to assess cells attachment (Fig. 7). SEM observation revealed that the lamellapodia of fibroblast cells were mostly confined in the laser treated region.

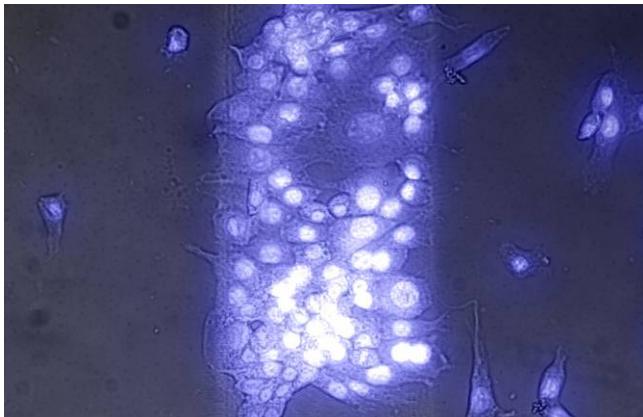


Fig. 6. Hoechst stained NIH3T3 fibroblast cells. The cells are localized only in the laser-treated area.

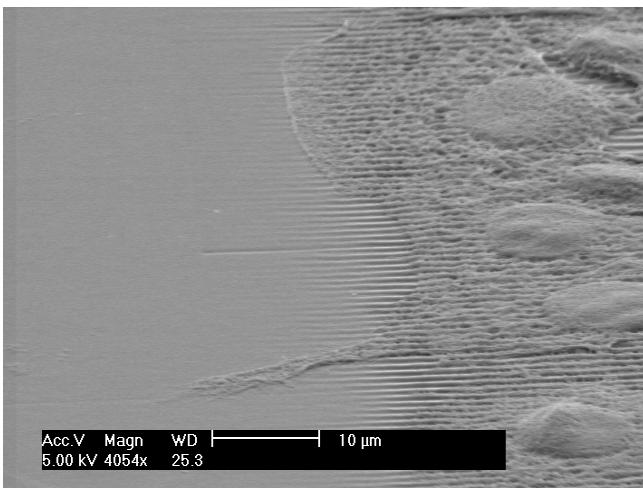


Fig. 7. Scanning electron microscope image of NIH3T3 attached to microgrooved surface.

REFERENCES

- [1] T. H. Park and M. L. Shuler, "Integration of Cell Culture and Microfabrication Technology," *Biotechnology Progress*, vol. 19, pp. 243-253, 2003.
- [2] M. Scholl, C. Sprossler, M. Denyer, M. Krause, K. Nakajima, A. Maelicke, W. Knoll, and A. Offenhausser, "Ordered networks of rat hippocampal neurons attached to silicon oxide surfaces," *J. Neurosci. Methods*, vol. 104 no. 1, pp. 65-75, Dec. 2000.
- [3] S.G. Curtis and C.D.W. Wilkinson, "Topographical control of cells," *Biomaterials*, vol. 18, pp. 1573-1583, 1997.
- [4] A. Natansohn and P. Rochon, "Photoinduced motions in azo-containing polymers," *Chem Rev.*, vol. 102, no. 11, pp. 4139-4175, Nov 2002.
- [5] X. L. Jiang, L. Li, J. Kumar, D. Y. Kim, and S. K. Tripathy, "Unusual polarization dependent optical erasure of surface relief gratings on azobenzene polymer films," *Appl. Phys. Lett.*, vol. 72, no. 20, pp. 2502-2504, May 1998.

IV. CONCLUSION

The patterned micro structure was produced by laser holographic fabrication on the photoresponsive polymer and photomask NIH3T3 fibroblast cells were cultured on the patterned substrate. The cells attached firmly to the patterned region and proliferated in the confined area. Two dimensional cellular patterning can be modulated further process since the photoresponsive polymer is reversible and repeatable. This result indicates that the laser holographic topography can be used to biomedical devices using living cells, tissue engineering, and cell biology.

ACKNOWLEDGMENT

This work was supported by Korea Science and Engineering Foundation (KOSEF) through Nano Bioelectronics and Systems Research Center (NBS-ERC) in Seoul National University.