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CULTURED CELL PATTERNING FOR BIO-ELECTRONICS

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Abstract Adhesion and proliferation of cells on ion-implanted segmented polyurethane (SPU) surfaces was investigated. Cell adhesion and proliferation on non-implanted SPU surfaces was not observed. However, complete cell adhesion and proliferation was observed on ion-implanted SPU surfaces. Also, selective cell adhesion on the patterned organic substrates was controlled by ion implantation. A specific interaction between cells and micro-periodic structures produced by ion implantation is demostrated. It was concluded that the improvement of cell adhesion and proliferation was due to carbonized SPU surface. The obtained pattern of cultured cells may be useful for formation of neural networks and bio-electronics.

INTRODUCTION

In recent years, the advent of micro-patterned surface architectures has generated interest in new approaches to the study of selective cell adhesion and proliferation, intercellular communication and cell migration, or in controling the alignment of individual cells for formation of neural networks and fabrication of bio-electronic devices[1-4]. For example, the control of neurite outgrowth is possible with substrates having microgrooves and microsteps fabricated by conventional lithography[1]. Additionally, cultured cell patterns using organic self-assembled monolayers (SAMs) and photoresist method have been prepared[2]. Calvert and coworkers used a technique that involves direct modification of SAMs by deep UV exposure and controlled the adhesion and proliferation of cells on the modified substrates[3]. The technique developed by Matsuda and Inoue was the surface modifcation of photoreactive polymer by UV irradiation[4].

Ion implantation, which is a fundamentally new technique in this field (but important in the manufacturing process of semi-conductor devices), was used for direct modification of polyurethane, on which surface cells are not capable of cell adhesion and proliferation[5,6]. This technique has advantages over previous methods such as fewer processing steps and narrower width pattern which can be controlled by lithography.

In this paper we report the formation of cultured cell patterns on segmented polyurethane (SPU) substrates modified by ion-implantation. An elucidation of drastic improvements in cell adhesion and proliferation on ion-implanted SPU surfaces is presented here. Furthermore, formation of micro-periodic structures (MPS) produced by ion implantation technique are also presented.

EXPERIMENTAL

Materials

SPU was obtained from Kanegafuchi Chemical Industry Co. Ltd. (Osaka, Japan) and contains approximately 13 % poly(ethyleneoxide)-poly(dimethylsiloxane)-poly(ethyleneoxide). SPU solution was prepared as a 5 % THF solution. Polymer-coated substrates were prepared by coating the inner surface of a glass dish with SPU solution, then gently dried with N_2 gas.

Ion Implantation

Na^+ ions were implanted into a mask-covered SPU-coated surface, on which bovine aorta endothelial cells (BAECs) were not capable of proliferating (FIGURE 1a), at room temperature as shown in FIGURE 1b. Implantation was carried out with energies of 150 keV, fluences of 1×10^{15} ions/cm^2 and currents less than 0.5 μA/cm^2. Modified and carbon-deposited surfaces were characterized by Raman spectroscopy.

Cell Culture

BAECs were isolated from a descending thoracic aorta using 0.1 % collagenase by a method adapted from Jaffe et al.[7] and Schwartz[8]. BAECs (5×10^4 cells/ml) were suspended in RPMI-1640 medium (Nissui Pharm. Co., Japan) supplemented with 10 % fetal bovine serum (Gibco Lab., USA). Suspensions were poured onto the ion-implanted SPU surface of the dish and incubated for 5 days at 37 ℃ in a humidified atmosphere containing 5 % CO_2. BAECs were imaged with an IMT-2 phase contrast microscope (Olympus Co. Ltd., Japan).

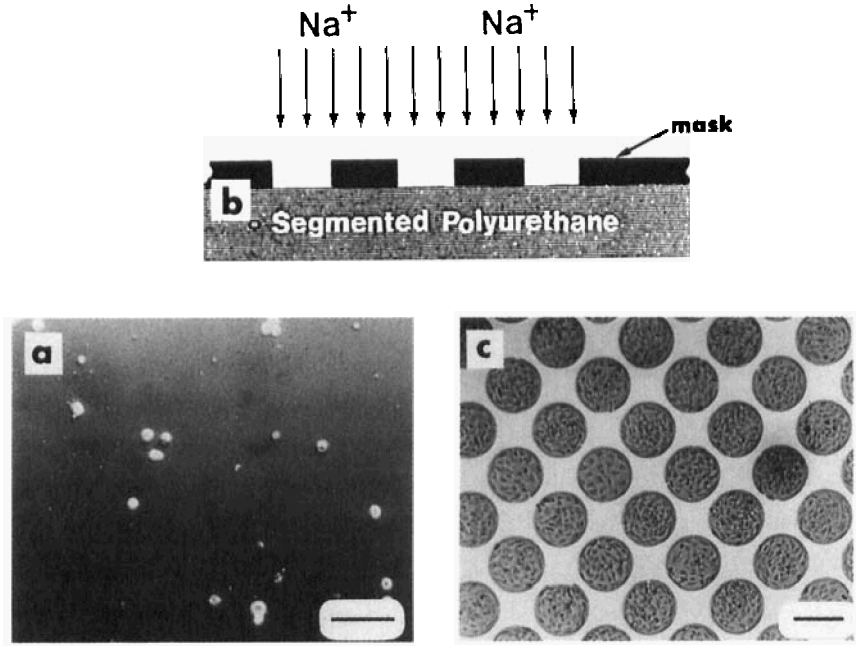

FIGURE 1 Fabrication of cultured cell patterns: (a) phase contrast micrograph of BAECs on SPU surface (bar=100 μm), (b) schematic diagram of surface modification by ion implantation and (c) phase contrast micrograph of BAECs on SPU surface patterned by ion implantation (bar=200 μm).

RESULTS AND DISCUSSION

As shown in FIGURE 1a, BAECs are not capable of proliferating on SPU. However, as FIGURE 1c depicts, BAECs have selectively adhered to and proliferated on circularly shaped ion-implanted SPU domains (FIGURE 1b). FIGURE 2 also shows a micro-pattern of cultured cells produced by ion implantation with different mask shapes. Cell-patterning is clearly observed on ion-implanted domains which have different shapes and sizes. This indicates that the micro-patterning of cultured cells is possible by ion implantation with masks.

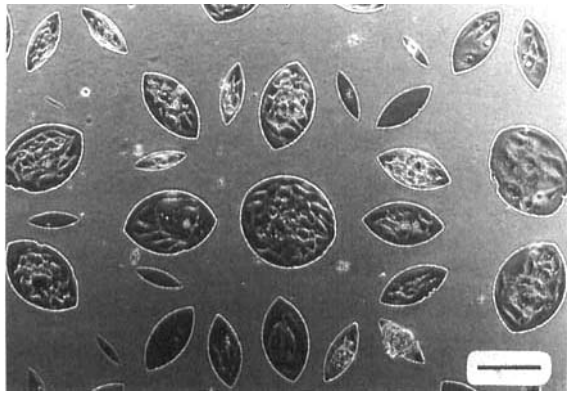

FIGURE 2 Micro-patterning of cultured BAECs with a different type mask (bar=100 μm).

SK-N-SH[9] cells, which are one of neuroblastoma, were used to check the possibility of neural patterning. The cells did not proliferate on non-implanted SPU surface and were capable of proliferating on ion-implanted SPU as shown in FIGURE 3. This suggests that micro-patterned surface architectures fabricated by ion implantation technique may be useful in forming neural networks from neuroblastomas with synapses, NG-108-15 cells[10] and dotsal root ganglion cells[11].

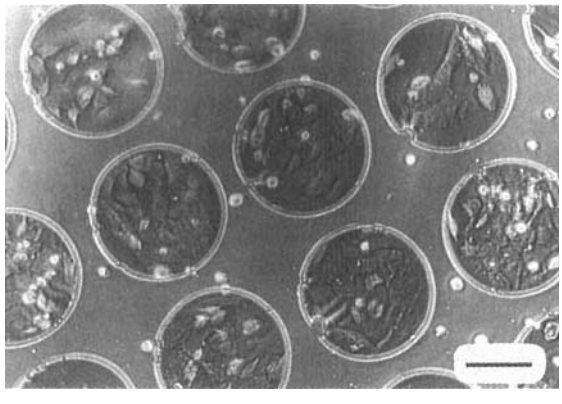

FIGURE 3 Micro-patterning of neuroblastoma SK-N-SH cells on ion-implanted SPU (bar=100 μm).

The surface structure of ion-implanted SPU was analyzed by Raman spectroscopy. For comparison a carbon-deposited SPU was also analyzed. FIGURE 4 shows the Raman spectra of carbon-deposited SPU. The solid curve is a typical Raman spectrum of amorphous carbon. The Raman spectra of carbon-deposited SPU

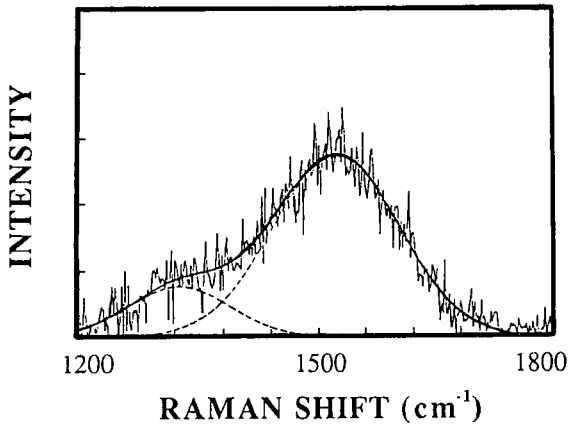

FIGURE 4 Raman spectra of deposited carbon on SPU surface.

surface have two peaks at about 1330 and 1520 cm^{-1} as shown two resolved curves in broken lines, which are characteristics of diamond and graphite-like bonds, respectively. The surfaces of organic polymers were carbonized by ion implantation at high fluence[12-15]. The carbon layers of ion-implanted SPU have a mixture of diamond and graphite-like bonds as shown by Raman spectroscopy[6]. The two Raman spectra obtained from ion-implanted and carbon-deposited SPU are very similiar as indicated in their spectra. Also, BAECs were cultured on the carbon-deposited SPU surface to observe cell adhesion and proliferation on the surface. Cell proliferation was observed on carbon-deposited SPU (FIGURE 5b) , whereas cell proliferation on SPU was not observed (FIGURE 5a). From these results, it is clear that remarkable improvements in cell adhesion and proliferation on an ion-implanted polymer surface are due to surface carbonization via ion implantation. A pattern of cultured cells by carbon deposition with mask could be formed, and the work is in progress now.

When a mask with wide area (1.5 cm x 1.5 cm) was used for ion implantation, MPS were found on SPU surfaces, whereas MPS did not appear if a mask with small holes (200 μm dia.) was used. FIGURE 6 shows MPS produced by ion implantation, as observed by phase contrast microscopy. Four kinds of structures are obtained by

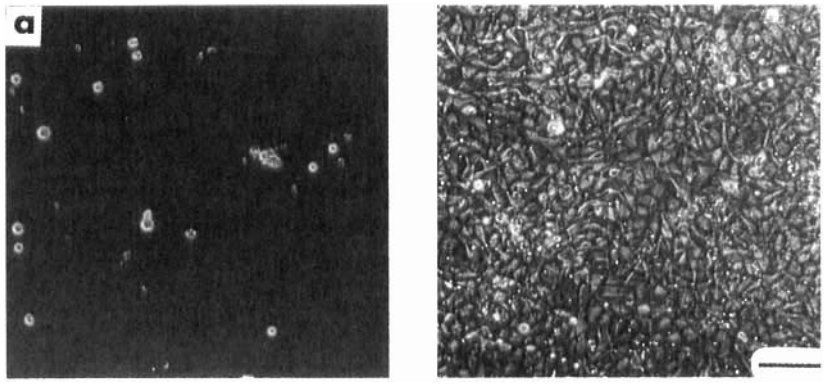

FIGURE 5 Cell culture on (a) SPU surface and (b) carbon-deposited SPU surface (bar=100 μm).

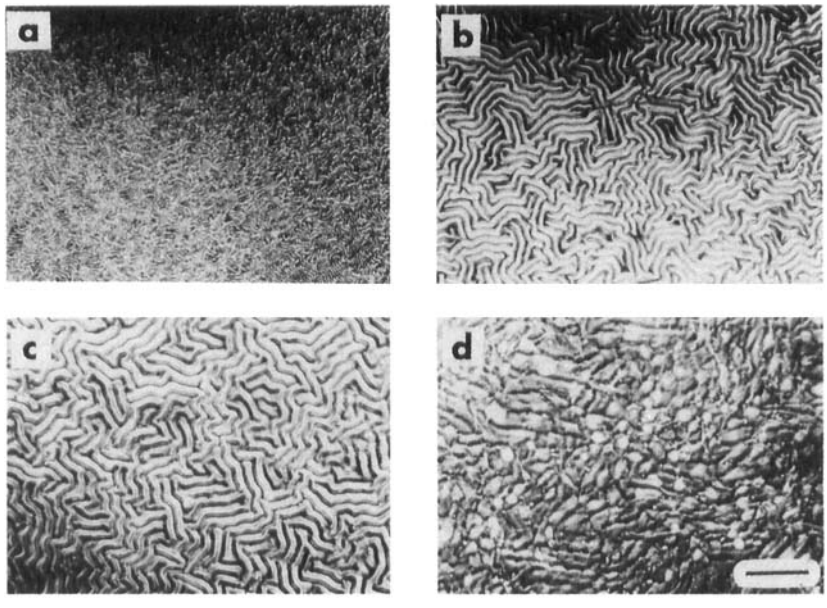

FIGURE 6 Formation of micro-periodic structures by ion implantation: with (a) 0.5 $\mu A/cm^2$, 50 keV and 1 x 10^{15} ions/cm2, (b) 0.5, 150 and 1 x 10^{15}, (c) 2, 150 and 1 x 10^{15} and (d) 0.5, 150 and 1 x 10^{17} (bar=100 μm).

different ion energies and current densities. Smaller microgrooves and microsteps are obtained from conditions using lower current densities and energies (FIGURE 6a). FIGURE 6c shows that larger microgrooves and microsteps are produced under more extreme conditions. FIGURE 6b and 6d were obtained by ion implantation with 0.5 $\mu A/cm^2$, 150 keV and at fluence of 1×10^{15} and 1×10^{17} ions/cm^2, respectively. This indicates that MPS are controlled by magnitude of energy and current density of ion implantation. It is interesting to note that some researchers have reported that MPS can also be obtained by excimer laser ablation[16,17]. The size and shape of MPS are different according to the polymers used and conditions of ablation.

Cell adhesion and proliferation on ion-implanted SPU surface with MPS, even though the surface was carbonized, was not observed. This is perhaps due to the fact that the width of the step is smaller than size of the cells (ca. 10-20 μm in short axis, FIGURE 6a-6c). On the contrary, cells were adhered and grown on wider steps (ca. 15-25 μm) which are produced by ion implantation at high fluence (FIGURE 6d). It is clear that the geometry of surface structures affects cell adhesion and growth. This result suggests available factors to design and prepare biomaterials related to cells. Additionally, these techniques may be useful for patterning of cultured cells with well-defined MPS. We are now attempting to elucidate the relationship between cell growth and MPS produced by using ion implantation and laser ablation techniques.

CONCLUSIONS

In conclusion, we have shown that the micro-patterning of cultured cells is possible by the newly developed ion implantation technique. Ion implantation has several advantages over conventional processes. Namely, dry processing and micro scale patterning with or without masks. Results from cell proliferation on carbon-deposited SPU and Raman spectra of deposited carbon show the carbonization of SPU surfaces was the primary factor for the improvement of cell adhesion and proliferation. Even though carbonized layers had covered on the surface, cell adhesion and proliferation depended strongly on the size of micro-periodic structure (MPS): if the width of the microstep is smaller than the cell size, cell adhesion and proliferation are not observed. Finally, this new patterning method may be available for preparing bio-devices consisting of synaptic junction between neuron cells and field-effect transistors[18].

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