Development of a Photoresponsive Cell Culture Surface: Regional Enhancement of Living-Cell Adhesion Induced by Local Light Irradiation

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ABSTRACT: Using a functional polymer containing nitrospirobenzopyran residues and poly(ethylene glycol), we developed a photoresponsive cell culture surface (PRCS), at which the cell adhesion can be enhanced locally by UV light irradiation (350–400 nm, 35 mW/cm^2, 5 min) even on the region where living cells exist. Cell adhesion was evaluated by observing BALB/3T3 fibroblasts, which had been seeded on PRCS and then irradiated with UV light regionally, after 12 h incubation and subsequent washing to remove scarcely adhering cells. As a result, it was confirmed that the number of remaining cells in irradiated region was at most 2.5 times greater than that in nonirradiated regions, suggesting the implementation of a novel scheme to manipulate living cells individually by light irradiation in a parallel and simultaneous manner. Influence of the composition of the polymer material consisting the substrate surface was also investigated systematically. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 495–499, 2006

Key words: photo-response; cell adhesion; cell culture; nitrospirobenzopyran; light irradiation

INTRODUCTION

In recent years, various medical technologies, such as cell therapy and regeneration medicine, have been studied actively aiming at the clinical applications. In such situations, development of new methodology is needed to manipulate living cells and to analyze intercellular interaction.[1] Cell patterning cocultivation is one means of analyzing interactions between various types of cells. As methods to form micro cell patterns, various techniques, such as lithography employing electron beams or light, and microcontact printing have been examined and utilized in the former studies.[2–7] However, any method mentioned here has to be applied to make cell adhesion region prior to cell seeding, since the process in these methods result in physical shock that may damage cells seriously during pattern formation; patterns cannot be formed after cell seeding, in principle.

As a convenient method to form micro cell patterning, Nakayama et al. have reported the technique to enhance cell adhesion just by the preirradiation of UV light on a culture surface fabricated by coating a photoresponsive polymer onto polyethylene terephthalate film.[8] Although only the effect of UV irradiation before cell seeding was examined in their study, the experimental results suggested the possibility to append cell adhesion regions by UV irradiation even after cell seeding. However, short-wavelength UV light of 300 nm or lower, which cannot be irradiated onto living cells without giving them lethal damage, is required to enhance cell adhesion of the culture surface in this method. Therefore, this technique cannot be used to manipulate individual living cells.

In the current research, we developed functional polymer materials that respond to light having minimal effect on living cells (350–400 nm), and fabricated a novel photoresponsive cell culture surface (PRCS) that can locally and optimally enhance cell adhesion even on the region where living cells exist. With respect to this PRCS, we investigated, in detail, the characteristics of cell adhesion in response to light irradiation after cell seeding as a new technique to manipulate living cells. We also examined the influence of the composition of the polymer material consisting the substrate surface on the properties of PRCS.

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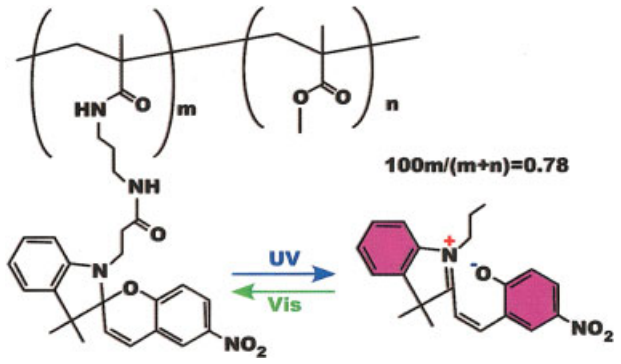

Figure 1 Structure and photoresponse of PMN. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

METHODS

Preparation of PRCS

A copolymer PMN, which is composed of methyl methacrylate and methacrylamide monomer with nitrospirobenzopyran (NSP) residue, was synthesized through radical polymerization (Fig. 1). The weight–average molecular weight of copolymer, estimated by gel permeation chromatography (GPC) analyses, was ~58,000. The content of NSP units in PMN was estimated to be 0.78 mol % by means of ^{1}H-nuclear magnetic resonance (NMR) spectroscopy. Poly(ethylene glycol) (PEG) was used as another component of the photoresponsive polymer material composing the substrate surface. Molecular weights (M_w) of PEG examined in this study were 50,000 (Wako Pure Chemical Industries Ltd., Osaka, Japan), 100,000, and 400,000 (Sigma-Aldrich Co., St. Louis, MO). The PRCS was prepared by dissolving PMN and PEG in 1,2-dichloroethane in a predetermined ratio and by spin-coating the solution onto a 25-mm-diameter circular glass substrate surface (Table I).

Surface characterization

The absorbance spectra of the PMN thin layer formed on a flat glass plate were measured after sufficient irradiations with UV light in the wavelength of 365 nm, and with visible light in a wavelength range from 500 to 600 nm. To evaluate the difference in the surface condition between the UV-irradiated region and non-irradiated region of PRCS, the electron spectroscopy for chemical analysis (ESCA) was carried out for the PMN-PEG100k-20 (Table I), which had experienced the same processes as the experiments using cells and then dried completely. For the absorbance measurement and ESCA measurement, we used a UV–vis spectrometer V-560 (JASCO, Tokyo, Japan) and an electron spectrometer ESCA 5600 (ULVAC-PHI, Inc., Kanagawa, Japan), respectively.

TABLE I
Components of the Photoresponsive Polymer Materials

	PEG content (wt %)	M_w of PEG
PMN-PEG100k-10	9	100,000
PMN-PEG100k-20	4	100,000
PMN-PEG100k-50	2	100,000
PMN-PEG50k-20	4	50,000
PMN-PEG400k-20	4	400,000

Evaluation of photoresponsive cell adhesion

BALB/3T3 fibroblasts (Riken Bioresource Center, Tsukuba, Japan) were incubated in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 mg streptomycin, and glutamine. The PRCS was clamped at the base of a substrate holder to form a cell incubator for the experimental convenience. Cells were seeded onto the substrates at a density of 1.6×10^4 cells/cm^2. After the cell seeding, a part of PRCS was irradiated for 5 min with UV light (365 nm) at an intensity of 35 mW/cm^2. UV irradiation was carried out with a xenon light source (LC6, Hamamatsu Photonics, Hamamatsu, Japan) through glass filters (U-360, Kenko Co., Ltd., Tokyo, Japan; A7028−03, Hamamatsu Photonics) and a liquid fiber light guide. Then, cell incubation was carried out in the incubator at 37°C and 5% CO_2 for 12 h. After that, to remove the scarcely adhering cells, the PRCS was washed with 1 mL of phosphoric acid buffered solution (PBS) three times in a certain procedure. Samples

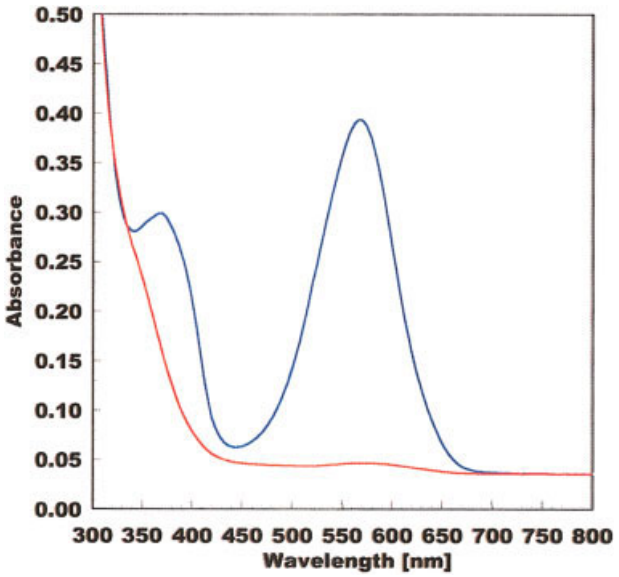

Figure 2 Absorbance spectrum of PMN thin film formed on glass plate after irradiation with visible light (red line) and UV light (blue line). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley-.com.]

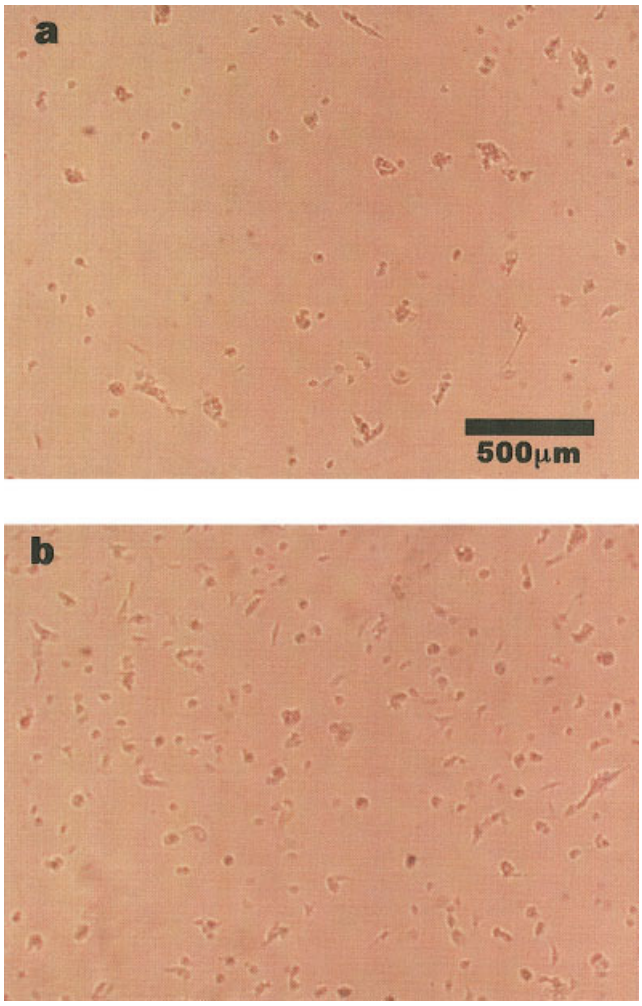

Figure 3 BALB/3T3 cells adhering to PRCS composed of PMN-PEG100k-20. (a) nonirradiated region; (b) irradiated region. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

were observed with an inverted microscope (IX70, Olympus Corp., Tokyo, Japan), and micrographs were taken for four visual fields in each sample with CCD camera, and the number of adhering cells per unit area was counted.

RESULTS AND DISCUSSION

The NSP residue introduced into PMN has basically two stable isomeric states; through irradiation with 350–400 nm UV light, it is isomerized to the twitterionic merocyanine form, which is highly polar and colored, while through irradiation with 500–600 nm visible light, it is isomerized to the nonionic spiro form, which is hydrophobic and almost colorless (Fig. 1).[9–11] Figure 2 shows the absorption spectra of a PMN solid thin film formed on a glass substrate after irradiation with visible light (500–600 nm) and UV light (350–400 nm). By irradiating colorless thin film of PMN with UV light, an absorbance peak at 566 nm increased indicating that isomerization from the spiro form to the merocyanine form proceeded. This absorbance band disappeared after visible light irradiation through the isomerization in the opposite direction.

Figure 3 shows micrographs of cells incubated on PMN-PEG100k-20 (Table I) after the substrate surface was washed with PBS. Compared with regions of nonirradiation where the number of adhered and extended cells is few, the number of adhered cells in irradiated regions is greater, and many of the cells were extended. Figure 4 shows the influence of UV irradiation and PEG content in the PRCS on the density of cells remaining on the PRCS after incubation and washing (M_w of PEG was fixed to be 100,000; Table I). As can be seen from Figure 3, cell adhesion was enhanced in light-irradiated regions compared with nonirradiated regions, in all cases. Figure 5 shows micrographs of cells, which remained on PRCS containing PEG of different M_w (PEG content was fixed to be 4 wt %, Table I) after cell incubation and washing with PBS. Although it was observed that UV irradiation enhanced cell adhesion for all M_w conditions of PEG, these images showed clearly that cell adhesion decreased along with increasing M_w of PEG whether UV light was irradiated or not.

In all these experiments, we confirmed that the cells remaining on PRCS after UV irradiation and washing with PBS proliferated normally in the subsequent incubation. This observation indicated that BALB/3T3

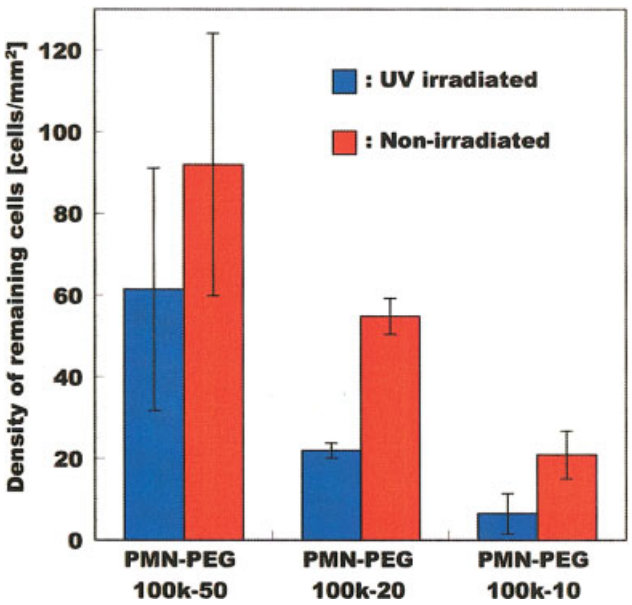

Figure 4 The influence of UV irradiation and PEG content in PRCS on the density of cells, which remained on PRCS after washing with PBS. Cell seeding density = 1.6×10^2 cells/mm^2 ($n = 4$). Significant difference at $P < 0.001$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

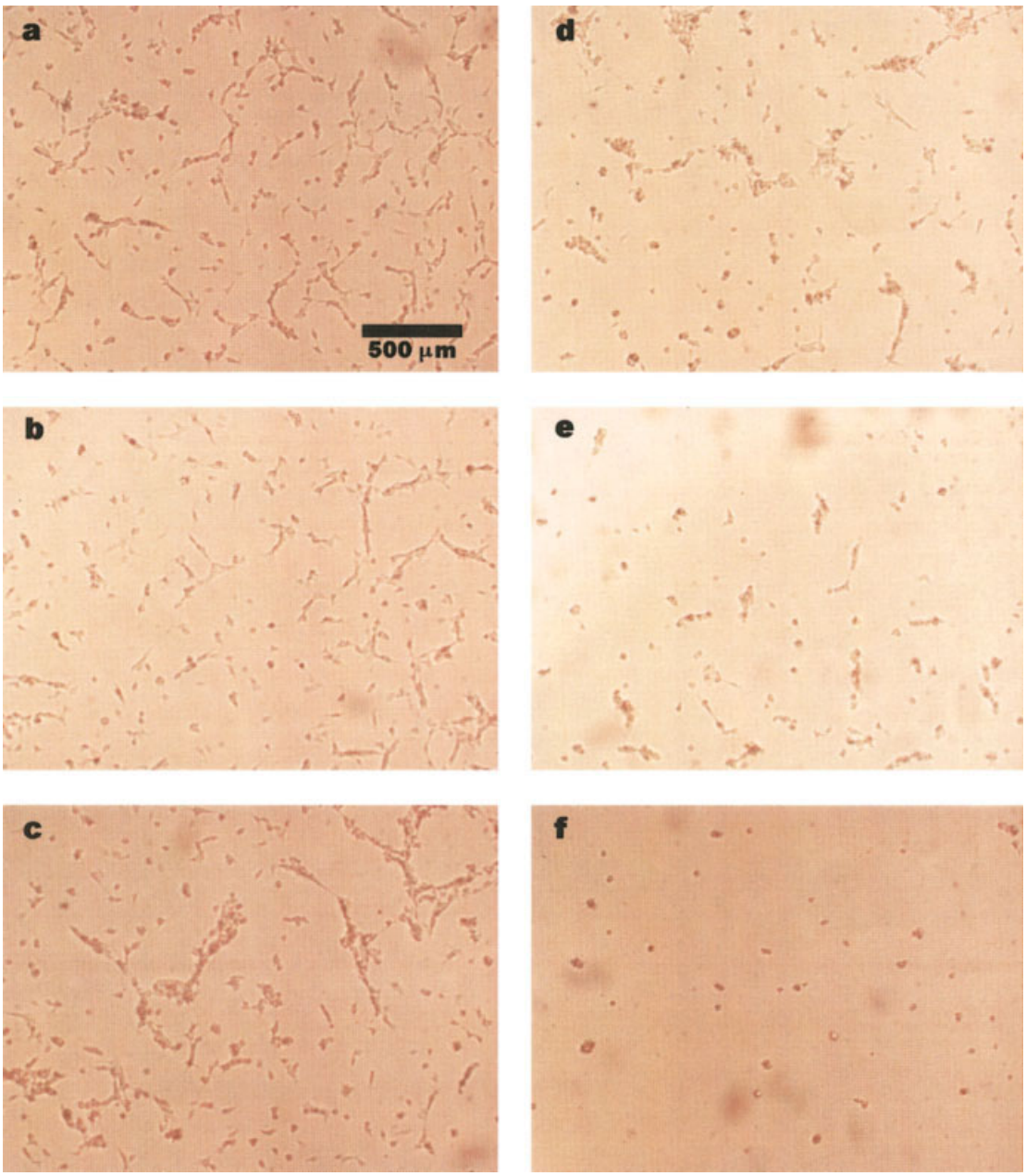

Figure 5 Micrographs of cells adhering to PRCS after 12 h incubation and washing with PBS. (a–c) irradiated regions; (d–f) nonirradiated regions; (a, d) PRCS containing PEG 50k; (b, e) containing PEG 100k; (c, f) containing PEG 400k. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cells were not damaged fatally in the cell manipulating process developed in this study. Further, the cell adhesion of PRCS, which had been once enhanced by UV irradiation, was not reset to the initial low state by visible light irradiation or annealing. Considering this irreversibility in photoresponse of PRCS and the influence of the content and M_w of PEG mentioned earlier, the following mechanism was suggested for this photo-induced enhancement of cell adhesion; initially the PRCS developed in this study had contained PEG, which has been reported to have strong hindering effect on cell adhesion through steric repulsion to adhesive proteins and cells.[12–15] At the substrate surface immersed in culture medium, a part of amphiphilic PEG chains had been anchored in matrix of PMN, of which the hydration is relatively low, while another part had stretched out in culture medium. Accordingly, the cell adhesion of PRCS had been considerably suppressed in the initial state. When the NSP residues were isomerized into the twitter-ionic merocyanine form by UV irradiation, however, the ability to retain PEG of PMN was weakened, and the PEG, which had hindered cell adhesion at the substrate surface, was lost through dissolution into culture me-

TABLE II
Elementary Compositions of the PRCS

Sample	C (%)	O (%)	N (%)
Nonirradiated region	69.80	29.25	0.95
Irradiated region	71.52	27.08	1.40

dium. As a result, cell adhesion of PRCS was enhanced greatly only in irradiated regions.

Also, the result of ESCA measurement supported the above scheme supposing that the dissolution of the PEG from the PRCS was triggered by the light irradiation. The elementary compositions of the PRCS in the irradiated and nonirradiated regions are shown in Table II. The compositions of C and N in the irradiated region were greater than those in the nonirradiated region. Since the PMN contains C and N more than the PEG, this result is corresponding to the increase of PMN fraction at the very surface. Especially, the apparent increase of N, which is contained only in PMN, suggested strongly that a part of the PEG, which had been at the substrate surface and had hindered cell adhesion, was removed in response to the light irradiation.

CONCLUSIONS

A newly developed photoresponsive polymer was used to create a novel cell culture surface, of which the cell adhesion is locally enhanced by light irradiation. The results of systematic experiments showed that this culture surface clearly enhanced cell adhesion through light irradiation even on the cell adherent region, and that the cell adhesion was varied by the content and M_w of PEG in the substrate surface. Since the condition of light irradiation was mild and photo-induced enhancement of cell adhesion was brought even for the adhering cells, this photoresponsive culture surface is applicable not only to cell patterning, but also to cell fractionation; adhesion of individual cells can be enhanced after observing seeded cells and getting hold of their each properties. In contrast to the existing method such as laser trapping, this method has an advantage of parallel processing; adhesion of all the cells on PRCS can be controlled individually in a parallel and simultaneous manner. This new scheme to manipulate cells proposed in this study is expected to provide a useful and effective method in the research areas of cell biology and tissue engineering.

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