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Surface Macromolecular Architectural Designs Using Photo-Graft Copolymerization Based on Photochemistry of Benzyl *N*,*N*-Diethyldithiocarbamate

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ABSTRACT: Surface macromolecular architectures with regional dimensional precision, control of the thickness of a graft layer, and blocks of graft chains were attempted using the surface photo-graft copolymerization method pioneered by Otsu et al. This is based on the photochemistry of benzyl *N*,*N*-diethyldithiocarbamate, which can be photolyzed into a radical pair (one radical can initiate radical polymerization and the other tends to recombine with the former radical). Ultraviolet light (UV) irradiation of a benzyl *N*,*N*-diethyldithiocarbamyl group-immobilized polymer surface in the presence of a vinyl monomer such as *N*,*N*-dimethylacrylamide, *N*-[3-(dimethylamino)propyl]acrylamide, methacrylic acid, or styrene at room temperature allowed precise control of the macromolecular architectures of the grafted surfaces. X-ray photoelectron spectroscopy (XPS) analyses and water contact angle measurements before and after UV irradiation in a monomer solution provided evidence that the graft copolymerization proceeded only during photoirradiation and at photoirradiated portions. Atomic force microscopic (AFM) observations showed that the thickness of the graft-copolymerized layers increased almost linearly with UV irradiation time. Patterned grafted surfaces, which were prepared using the ionic or nonionic hydrophilic monomers listed above under irradiation through a projection mask, were clearly visualized by dye-staining or cell-culturing. The graft copolymerization under irradiation through a projection mask with sequential monomer charges yielded surfaces with regionally dimensionally controlled macromolecular architectures such as di- and triblock graft-copolymerized surfaces. A graft thickness-gradient surface was obtained by using a gradient filter.

Introduction

Surface design has become increasingly important in various industrial fields such as printing, coating, fiber, membrane, and biomedical ones, where surface properties play a decisive role in function. Particularly in biomedical applications where the surface structure and properties at the outermost layers of a blood- or tissue-contacting surface play an important role in determining biological responses, surface design is crucial to whether artificial organs are accepted or rejected by the host due to activation of body defense mechanisms. In addition, dimensionally precise surface control is needed for the development of functional biomedical devices such as advanced artificial organs, micromachines, and microbiosensors.

Most polymer surface modifications are performed by chemical reaction in solution,[1] physical deposition or coating,[2] and γ-ray, ultraviolet light (UV), plasma, or ozone treatment and subsequent graft polymerization.[3−5] However, these existing methods are insufficiently precise for modifying only a given area of the surface of fabricated devices.

Recently, we reported a novel surface graft copolymerization method which allows surface modification with micron-order regional precision, which was based on surface photoprocessing leading to immobilization of radical initiators on a substrate surface and subsequent thermal radical copolymerization, allowing surface grafting in specific regions of a substrate.[6] Since radical polymerization proceeds with very reactive species which simultaneously cause various complex reactions such as transfer and termination, well-controlled surface graft polymerization in terms of molecular weight and its distribution or formation of blocked graft chains cannot in general be achieved using conventional radical polymerization techniques.

In the early 1980s, Otsu et al. reported that living-like radical polymerization of styrene (ST) and methyl methacrylate (MMA) in solution proceeds with new types of polymerization initiators classified as dithiocarbamate derivatives[7] and described them as photoiniferters, which mean that they act as an *ini*tiator, trans*fer* agent, and *ter*minator.[8] It has been reported that photolysis of the iniferter benzyl *N*,*N*-diethyldithiocarbamate by UV irradiation yields a pair of radicals (reaction I in Scheme 1): a reactive benzyl radical which can react with a vinyl monomer to yield a radical polymer end (reaction III) and a less- or non-reactive dithiocarbamyl radical which reacts weakly or not at all with a vinyl monomer but exclusively leads to termination reaction through recombination with a growing polymer chain (reaction IV). The dithiocarbamyl end-capped chain thus formed can be dissociated into a radical pair by UV irradiation (reaction I). When such photopolymerization occurs without coupling and/or disproportionation between growing polymer chain radical ends or between dithiocarbamate radicals, or chain transfer from a growing polymer chain radical end to a solvent molecule or another polymer chain, control of molecular weight and its distribution may be feasible. In addition, after cessation of UV irradiation and then changing of monomer species, UV reirradiation may induce formation of block copolymers. Otsu and colleagues[9] and others[10] have extensively studied photoinduced dithiocarbamate free-radical reactions and succeeded in preparing well-controlled block copolymers under appropriate experimental conditions in solution.

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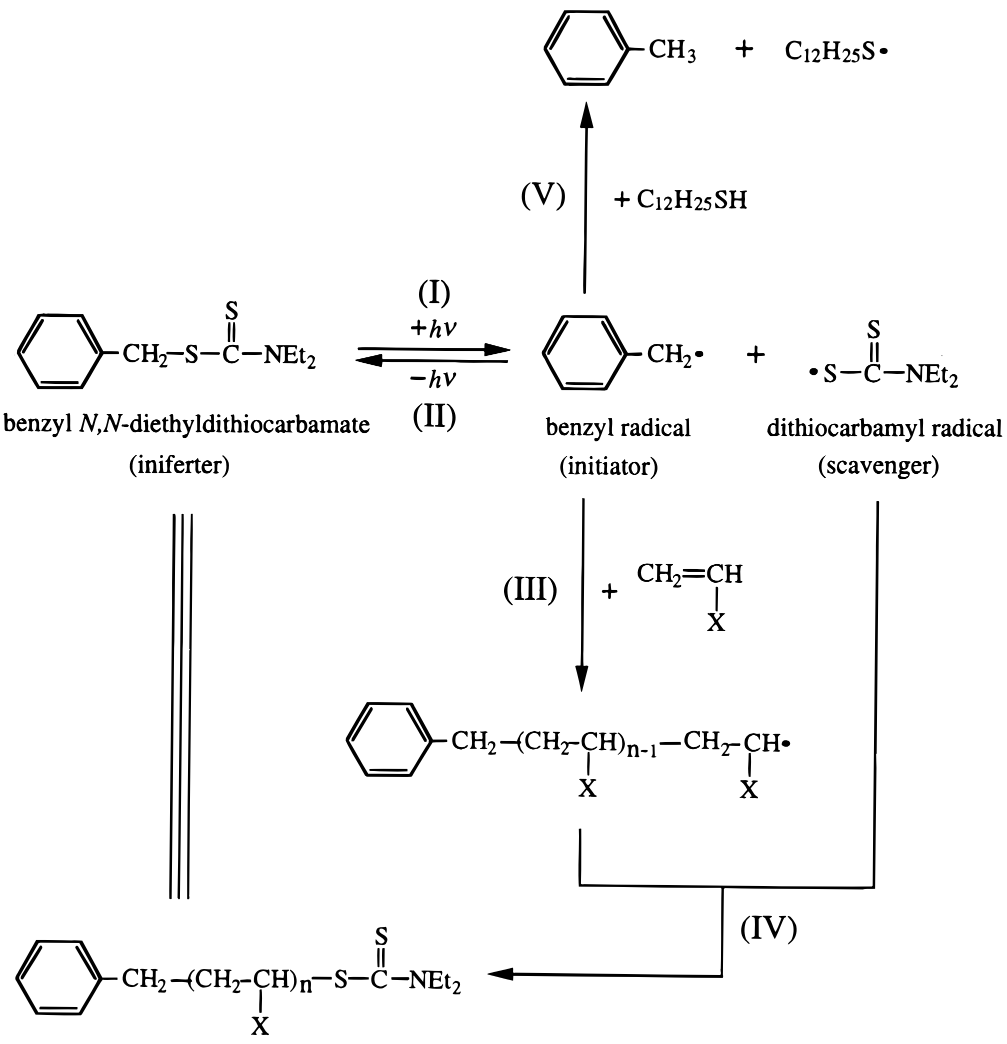
Scheme 1



Considering these unique characteristics of photopolymerization, we devised a method of surface photograft copolymerization which allows production of surface macromolecular architecture with micron-order precision. Since the polymerization proceeds only during photoirradiation and at photoirradiated regions, the following types of surfaces were realized: regionally precise, microprocessed surfaces onto which different polymers were regionally grafted, surface graft block copolymers in which different polymer blocks were sequentially formed in graft copolymers, and a gradient surface in which the thickness of graft layer gradually varied in one direction.

Experimental Section

Materials. Vinylbenzyl chloride (*m* and *p* mixture) and chloromethyl ethyl ether were obtained from Tokyo Chemical Industry Ltd. (Tokyo, Japan). ST was purchased from Ohken Co., Ltd. (Tokyo, Japan). *N*-[3-(Dimethylamino)propyl]acrylamide (DMAPAAm) was obtained from Kohjin Co., Ltd. (Tokyo, Japan). Nitrogen methyl iodide of DMAPAAm (DMAPAAmMeI) was prepared by addition of methyl iodide to an ethanol solution of DMAPAAm.[11] 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized twice from methanol. Bis[*p*-(dimethylamino)phenyl]methylium hydroxide (malachite green carbinol base, Solvent Green 1, C.I. 42000B) was obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Solvents and other reagents, all of which are of special reagent grade, were obtained from Wako Pure Chemical Industry Ltd. (Osaka, Japan) and used after conventional purification. Poly(ethylene terephthalate) (PET) film was obtained from Bellco Glass Inc. (Vineland, NJ). PolyST (PST) film was obtained from Asahi Chemical Industry Co., Ltd. (Tokyo, Japan). Cross-linking of PST film was carried out by irradiation with γ-rays from a ^{60}Co source under an argon atmosphere at the Radiation Laboratory, the Institute of Scientific and Industrial Research, Osaka University (Osaka, Japan).

Preparation of Photoreactive Monomer (1) and Copolymer (2). The photoreactive monomer vinylbenzyl *N*,*N*-diethyldithiocarbamate (**1**) was prepared from vinylbenzyl chloride and sodium *N*,*N*-diethyldithiocarbamate.[7] A solution of sodium *N*,*N*-diethyldithiocarbamyl trihydrate (22.5 g, 0.1 mol) and ethanol (80 mL) was placed in a 200 mL three-necked flask equipped with a stirrer, a dropping funnel, and a reflux condenser. To this solution 20 mL of ethanol solution of vinylbenzyl chloride (12.7 g, 0.083 mol) was added dropwise over a period of 0.5 h at 0 °C. The solution was stirred for 24 h at room temperature. Then the reaction mixture was poured into a large amount of water and extracted with ether. The organic phase was washed with water, dried (Na_2SO_4), filtered, and evaporated under vacuum. The residue was recrystallized three times from methanol; yield of vinylbenzyl *N*,*N*-diethyldithiocarbamate (**1**): 20.1 g (91.3%); ^{1}H NMR (DMSO-d_6 with Me_4Si) δ 7.49−7.28 (m, 4H, C_6H_4), 6.71 (dd, $J = 11.0$ and 17.7 Hz, 1H, $CH{=}CH_2$), 5.81 (d, $J = 17.7$ Hz, 1H, $CH{=}CH_2$), 5.26 (d, $J = 10.4$ Hz, 1H, $CH{=}CH_2$), 4.51 (s, 2H, CH_2S), 3.98 (q, $J = 6.7$ Hz, 2H, NCH_2), 3.71 (q, $J = 6.7$ Hz, 2H NCH_2), 1.19 (t, $J = 7.0$ Hz, 6H CH_2CH_3).

The photoreactive copolymer (**2**) was prepared by radical copolymerization of vinylbenzyl *N*,*N*-diethyldithiocarbamate (**1**) with ST.[12] A glass tube containing a mixture of ST (0.83 g, 8.0 mmol), vinylbenzyl *N*,*N*-diethyldithiocarbamate (*m* and *p* mixture, 0.53 g, 2.0 mmol), AIBN ([monomer]/[initiator] =

100, molar ratio), and *N*,*N*-dimethylformamide (DMF) (13.7 mL) was sealed under reduced pressure after three freeze−pump−thaw cycles. The polymerization was carried out for 3 h at 60 °C. The polymer, precipitated by addition to a large amount of ether, was separated from the solution by filtration. Reprecipitation was carried out in a toluene−methanol system three times. The last precipitate was dried under vacuum and stored in a dark desiccator. The yield was 0.12 g (8.5%). The molecular weight of the polymer was estimated by gel permeation chromatography (GPC) analysis: $M_n = 51\,400$ (PST standard; eluent $CHCl_3$). The content of photoreactive moiety in the copolymer (**2**), which was determined by ^1H NMR spectroscopy from the integral ratios between the aromatic protons (δ 6.3−7.2) and the remaining *S*-methylene protons (δ 4.2−4.6) of the dithiocarbamyl moiety, was found to be 25.1 mol %.

Preparation of Dithiocarbamate-Derivatized PST Film (3). PST film (2 × 2 cm), which was cross-linked by irradiation with γ-rays from a ^{60}Co source, was immersed in 10 mL of 1,2-dichloroethane. To this solution chloromethyl ethyl ether (0.27 g, 2.9 mmol) and $ZnCl_2$ (15 mg, 0.11 mmol) were added at 0 °C. After the solution was shaken for 10 h at room temperature, the film was thoroughly washed with toluene. Of the ST units on the film surface, 36.8 mol % was chloromethylated, as determined by X-ray photoelectron spectroscopy (XPS) measurement. The chloromethylated PST film was then immersed in 10 mL of ethanol solution of potassium *N*,*N*-diethyldithiocarbamate trihydrate (0.65 g, 2.9 mmol). After the solution was shaken for 5 h at room temperature, the film was thoroughly washed with water and then dried under vacuum. The content of dithiocarbamyl groups was 30.5 mol % of the ST unit, as determined by XPS measurement.

Surface Photo-Graft Copolymerization. Surface photograft copolymerization was performed on two different surfaces: (1) a PET film which was precoated with the photoreactive copolymer (**2**) by casting a toluene solution (1 wt %) (thickness of coated layer: ∼10 μm), and (2) a PST film (**3**) whose surface was partially derivatized with *N*,*N*-diethyldithiocarbamyl groups.

Each polymer film was vertically mounted in 30 mL quartz cell containing 20 mL of vinyl monomer-containing methanol solution (0.5 mol/dm^3). A stream of dry nitrogen was introduced through a gas inlet to sweep the cell for 5 min or more. The film was then irradiated in an atmosphere of nitrogen from a 20 cm distance with a 200 W Hg−Xe lamp (L 2859-01, Hamamatsu Photonics Ltd., Shizuoka, Japan). The light intensity, measured with a photometer (UVR-1, Topcon, Tokyo, Japan), was adjusted to 5 mW/cm^2. The temperature of the polymerization samples was maintained at 20−25 °C. The treated film was washed to remove unreacted monomers and homopolymers and then dried under vacuum.

Two-Dimensional Microprocessing. Surface microprocessing was carried out using two types of projection metal mask: one is a lattice pattern of 60 μm width (Hirai Co., Ltd., Osaka, Japan) and the other is a stripe pattern arrayed with slits of 100 μm width (Nippon Filcon Co., Ltd., Tokyo, Japan). The photoreactive polymer film, which was PET film precoated with the photoreactive copolymer (**2**) or a dithiocarbamate-derivatized PST film (**3**), was tightly contacted to a projection mask and then immersed into a monomer solution. UV light was irradiated onto the film through the mask in the same manner as described above. Graft polymers were stained with a dilute hydrochloride solution of malachite green carbinol base (1.0 w/v %) for poly(methacrylic acid) (PMAA) and with a dilute aqueous solution of rose bengal (Acid Red 94, C.I. 45440) (1.0 w/v %) for poly(DMAPAAmMeI) for visualization under a light microscope with a Nikon Optiphot system (Tokyo, Japan).

Preparation of Graft Gradient Surface. The photoreactive polymer film was tightly contacted to a projection mask and a neutral-density filter (VND-U, Sigma Koki Co., Ltd., Tokyo, Japan) and then photoirradiated in a monomer solution. Surface graft polymers were stained with rose bengal for visualization under a fluorescence microscope with irradiation of 510−560 nm wavelength light. The images of the distribution of the fluorescence intensity from the stained grafted surfaces were recorded with an Argus-20 system (Hamamatsu Photonics) equipped with a CCD (charge-coupled device) video camera (C-2400-77i, Hamamatsu Photonics), which can be used to control the image acquisition and display. Three-dimensional representations of the fluorescence images were obtained using Hamamatsu Photonics software.

Cell Culture. Endothelial cells (ECs), harvested from bovine thoracic aorta by collagenase digestion, were cultured in Dulbecco's modified Eagle's medium (DMEM; Flow Laboratories, McLean, VA) supplemented with 15% fetal bovine serum (HyClone Laboratories, Inc., Logan, UT) at 37 °C in a water-saturated 5% CO_2/95% air atmosphere. ECs were seeded onto the polymer (**2**)-coated PET film partly graft-polymerized with *N*,*N*-dimethylacrylamide at a seeding density of 2×10^4 cells/well (1.8 cm^2) and then incubated at 37 °C in a humid atmosphere of 95% air and 5% CO_2. After 3 days of incubation, the treated film surface was observed under a phase-contrast microscope with a Nikon Optiphot system.

Physical Measurements. All ^1H NMR spectra were recorded in DMSO-d_6 solution using tetramethylsilane (0 ppm) as an internal standard with a 270 MHz NMR spectrometer (JEOL, GX-270, Tokyo, Japan) at 30 °C. GPC analyses in $CHCl_3$ were carried out with an HLC-8020 instrument (Tosoh, Tokyo, Japan) (column: Tosoh TSKgel G4000H_{XL} and G3000H_{XL}) at Osaka National Research Institute (Osaka, Japan). The columns were calibrated with narrow weight distribution PST standards. XPS spectra were taken with a Shimadzu ESCA 750 (Kyoto, Japan) using a magnesium anode (Mg Kα radiation) connected to an ESCAPAC-760 data processor at room temperature and 2×10^{-7} Torr (8 kV, 20 mA). The C 1s spectra were deconvoluted into subpeaks by computer-aided processing. Static advancing contact angles toward deionized water were measured with a contact angle meter (Kyowa Kaimen Kagaku Co., Ltd., Tokyo, Japan) at 25 °C by the sessile drop method. Atomic force microscopic (AFM) images were obtained on a NanoScope II (Digital Instruments, Inc., Santa Barbara, CA) using a Si_3N_4 cantilever with a spring constant of 0.12 N/m (reported by Digital Instruments, Inc.), which is V-shaped and 200 μm long, operating at −1.0 V and a scanning rate of 1.34 Hz. AFM images (400 × 400 pixels) were obtained using the "height mode", which kept the force constant, and visualized using the "surface mode". All data manipulations and image processing were carried out with Digital Instruments software.

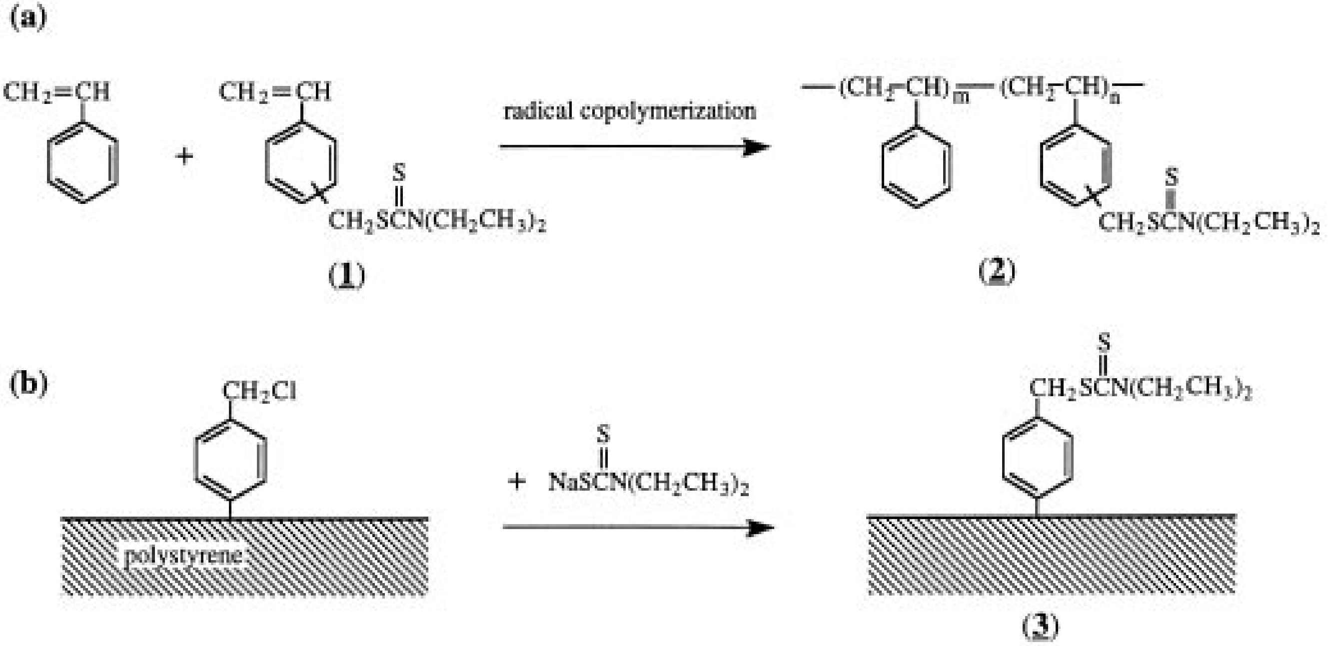
Results

Iniferter-Immobilized Surfaces. Surface photograft copolymerization was carried out on two different surfaces on which *N*,*N*-diethyldithiocarbamyl groups (iniferters) were immobilized (Scheme 2). One was a PET film coated with the photoreactive polymer (**2**) synthesized by radical copolymerization of styrene (ST) and vinylbenzyl *N*,*N*-diethyldithiocarbamate (**1**) (content of **1** in the polymer: 25.1 mol %). The other was a cross-linked chloromethylated poly(ST) (PST) surface which was partially derivatized with *N*,*N*-diethyldithiocarbamyl groups (dithiocarbamyl group immobilized on the surface: 30.5 mol %).

Surface Photo-Graft Copolymerization. A quartz cell containing the polymer (**2**)-coated PET film or the dithiocarbamate-derivatized PST film (**3**) in a vinyl monomer-containing methanol solution was UV irradiated under a nitrogen atmosphere. The vinyl monomers used were *N*,*N*-dimethylacrylamide (DMAAm), *N*-[3-(dimethylamino)propyl]acrylamide (DMAPAAm), *N*-methiodide of DMAPAAm (DMAPAAmMeI), methacrylic acid (MAA), and ST.

Figure 1 shows the XPS spectra of polymer (**2**)-coated PET samples before and after UV irradiation in the presence of DMAAm (recorded at a take-off angle of 90°, where the take-off angle is defined as the angle between the sample surface and the electron optics of the energy analyzer). The polymer (**2**)-coated film (Figure 1a)

Scheme 2



showed a high-intensity carbon peak at 285.0 eV, which was ascribed to hydrocarbon-like carbon. A small peak at 291.2 eV was assigned to a satellite peak derived from the benzene ring of styrene and benzyl *N*,*N*-diethyldithiocarbamate. In addition, N 1s and S 2p signals, both of which were derived from the dithiocarbamyl group, were observed. The deconvoluted peaks, which have almost equal intensity, of the S 2p signal were assigned to C−S−C (162.1 eV) and C=S (163.8 eV). The N/C ratio, determined from the respective peak areas of the C 1s and N 1s signals, was 0.019 (theoretical value: 0.026), irrespective of whether the take-off angle was 90 or 15°. This indicates that the dithiocarbamyl groups were homogeneously distributed in the surface region. Upon UV irradiation of the polymer (**2**)-coated PET films in a DMAAm methanol solution and subsequent washing with water, significant XPS spectral changes were noticed as shown in Figure 1b. The intensities of the nitrogen and oxygen peaks increased, and N/C and O/C ratios increased from 0.024 and 0.013 to 0.16 and 0.17, respectively. These values are close to the theoretical ones for poly(DMAAm) (N/C = 0.20 and O/C = 0.20). The relative contents of two higher-binding-energy components, which were assigned to C−N and C−S (286.0 eV), and C=O and C=S (287.6 eV), of the C 1s subpeaks increased. The satellite peak of C 1s disappeared. On the other hand, the S 2p peak was still observed even after 20 min of photoirradiation, although its intensity was reduced to about one-half of the initial value. This indicates that poly(DMAAm) fully covered the surface and that a considerable number of dithiocarbamyl groups remained at the outermost layers even after photopolymerization. No appreciable spectral change was observed after extensive washing with water.

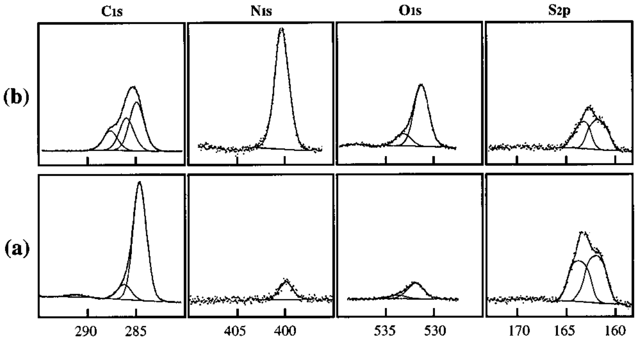


Figure 1. XPS spectra of the polymer (**2**)-coated PET film surfaces before (a) and after 10 min of UV irradiation in a DMAAm methanol solution (b).

Both advancing and receding contact angles decreased with increasing UV irradiation time (Figure 2). The receding water contact angle was approximately 90° for the nonirradiated surface and about 5° for the 20-min-irradiated surface, indicating that the hydrophobic surface had become hydrophilic. Considering these results together with the XPS results, it can be said that photo-graft copolymerization of DMAAm occurred on the polymer (**2**)-coated PET films. XPS and wettability studies revealed that surface photo-graft copolymerization of other vinyl monomers such as DMAPAAm, DMAPAAmMeI, MAA, and ST on both the polymer (**2**)-coated PET surface and the dithiocarbamate-derivatized PST surface (**3**) occurred as in the above case.

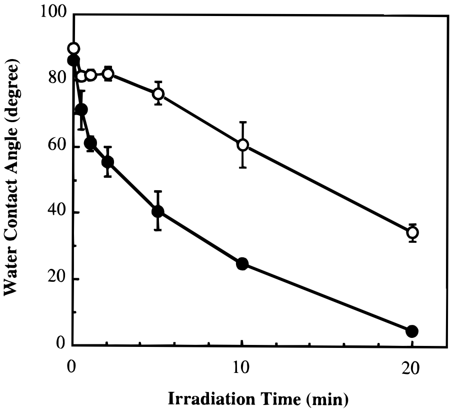


Figure 2. Water contact angle changes during UV irradiation of the polymer (**2**)-coated PET film surface in a DMAAm methanol solution. Symbols are (○) advancing water contact angle and (●) receding water contact angle.

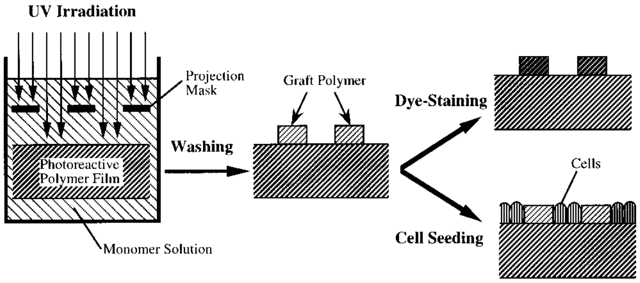


Figure 3. Preparation of two-dimensionally micropatterned surfaces by surface photo-graft copolymerization using the projection metal mask and their visualization.

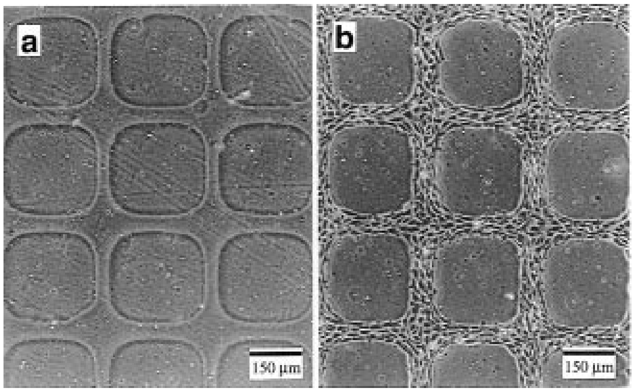


Figure 4. Phase-contrast micrographs of (a) the finely microprocessed PET surface where the hydrophilic domain consisted of the photo-graft-copolymerized poly(DMAAm) and the hydrophobic domain consisted of nontreated PET, which were prepared using the lattice-patterned projection metal mask, and (b) two-dimensional patterning of cultured endothelial cells formed on the patterned hydrophilic/hydrophobic surface.

Dimensionally Controlled Surface Photo-Graft Copolymerization. Since the surface photo-graft copolymerization proceeds at UV-irradiated regions, dimensional precision could be achieved. Figure 3 shows the method of pattern processing using a projection metal mask having a lattice or stripe pattern (widths of lattice and stripe were 60 and 100 μm, respectively) which was in contact with the polymer (**2**)-coated PET film. The film was UV irradiated in a DMAAm methanol solution through the lattice-patterned projection mask and then washed with water. Phase-microscopic observation clearly showed that a finely structured two-dimensional pattern composed of hydrophilic (poly-DMAAm-grafted surface) and hydrophobic (nontreated polymer (**2**)-coated PET surface) domains was formed (Figure 4a). When bovine endothelial cells were seeded and cultured on the patterned surface, the cells adhered and proliferated only on the nontreated hydrophobic domains, resulting in the formation of a two-dimensional pattern of cultured cells, as shown in Figure 4b.

When the polymer (**2**)-coated PET surface was UV irradiated in a MAA methanol solution through the stripe-patterned projection mask and then immersed into a dilute HCl solution of malachite green carbinol base, which is a dye for negatively charged ions, only the irradiated stripe regions were stained green (Figure 5a), indicating that carboxylated monomer was graft-copolymerized on photoirradiated regions. The observed width of the stained lines was 100 μm, which was same as the stripe width of the projection mask used. As shown in Figure 5b, UV irradiation in an aqueous solution of DMAPAAmMeI through the stripe-patterned projection mask and subsequent staining with rose bengal, which is a dye for positively charged ions, resulted in the formation of a pattern of red stripes.

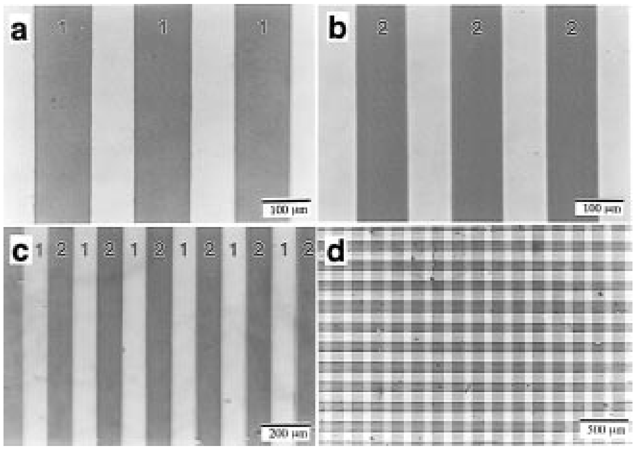


Figure 5. (a,b) Micrographs of two-dimensionally patterned surface upon photo-graft copolymerization using MAA or DMAPAAmMeI by UV irradiation through the stripe-patterned projection metal mask. Stripe lines 1 and 2 denote malachite green-stained poly(MAA) regions and rose bengal-stained poly(DMAPAAmMeI) ones, respectively. (c) Micrograph of alternately photo-graft-copolymerized patterned surface. (d) Micrograph of cross-hatched graft surface. The intersections of the lines represent poly(MAA-*b*-DMAPAAmMeI) photo-graft-copolymerized regions.

Surface regional patterning of two different graft polymers was carried out as follows. First, the polymer (**2**)-coated PET film was UV irradiated in an aqueous solution of the first monomer, DMAPAAmMeI, through the stripe-patterned projection mask. After 10 min of irradiation, a large amount of a free-radical chain transfer agent, 1-dodecanethiol, was added to the reaction solution under irradiation to convert the dithiocarbamyl group-linked polymer growing ends to alkyl group-endcapped polymer ends (reaction V in Scheme 1). After thoroughly washing with water, the treated film surface was irradiated in a methanol solution of the second monomer, MAA, without using a metal mask. When the film thus treated sequentially immersed in an aqueous malachite green carbinol base solution and an aqueous rose bengal solution, a pattern with alternating green and red lines was formed (Figure 5c), indicating that MAA and DMAPAAmMeI were alternately graft-copolymerized to form a microdomain surface.

Topographic images of a graft-copolymerized surface were recorded by scanning the surface with the sharp tip of an atomic force microscope (AFM). Figure 6 shows AFM images, including a top view and a cross-sectional view, of the micropatterned surface which was obtained by graft copolymerization of ST on the polymer surface (**3**) using the lattice-patterned projection mask. The nontreated surface (**3**) was relatively flat (Figure 6a): the surface roughness was estimated to be within 10−20 nm over a horizontal distance of 40 000 nm. On the other hand, the grafted region of the treated surface showed a large undulation densely covered by graft polymers (Figure 6b,c,d). The boundary between the PST-grafted and nontreated regions was a very sharp edge on the micron order over the horizontal distance of 40 000 nm, indicating that this surface photo-graft polymerization allows a considerable degree of control of the thickness of graft-copolymerized layers. The dependence of the average thickness of the grafted layer on UV irradiation time is shown in Figure 7. The average thickness, which was measured from cross-

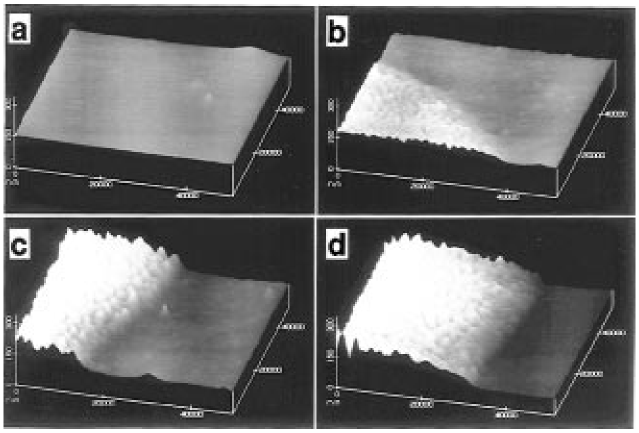


Figure 6. AFM images, projected at 30° for viewing, of nontreated photoreactive polymer film (**3**) surface (a) and the PST-grafted surface by UV irradiation through the lattice-patterned projection mask for 5 (b), 10 (c), and 20 min (d).

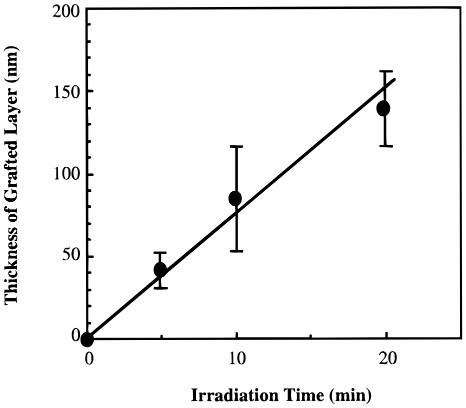


Figure 7. Relationship between the average thickness of the PST-grafted layers and UV irradiation time.

sectional images obtained by AFM, almost linearly increased with irradiation time, suggesting that the length of the graft polymer chains increased with irradiation time. After 20 min of UV irradiation, the measured thickness was approximately 140 ± 20 nm.

Surface Block Graft Copolymerization. If the surface photo-graft copolymerization sequentially proceeds well in a grafted chain, a block graft copolymer can be formed on a surface. Surface block graft copolymerization using two different monomers (DMAPAAm and ST, **A** and **B**, respectively) was performed as follows: first **A** (first monomer) was surface photografted on the polymer (**2**)-coated PET film. After cessation of irradiation, the **A** solution was replaced with the **B** (second monomer) solution. Then, the **A**-grafted surface was irradiated. XPS measurements (Figure 8a) and water contact angle measurements (Figure 8b) showed that, upon the surface graft copolymerization of **A**, the N/C ratio increased from 0.02 to 0.16 and the receding water contact angle decreased from about 60° to about 30°, indicating that DMAPAAm was graft-copolymerization on the surface. Subsequently, upon irradiation of the treated film in the **B** solution, the N/C ratio decreased to 0.04 and the receding water contact angle increased to about 55°, indicating that ST was graft-copolymerized on the DMAPAAm graft-copolymerized surface, thus producing **A**−**B**-type surface block graft polymer chains. Subsequent graft polymerization in the **A** (third monomer) solution resulted in an increased N/C ratio (0.13) and a decreased water contact angle (about 30°). These results indicated that a surface **A**−**B**−**A**-type block copolymer was prepared by repeated UV irradiation in alternative immersion into two different monomer solutions.

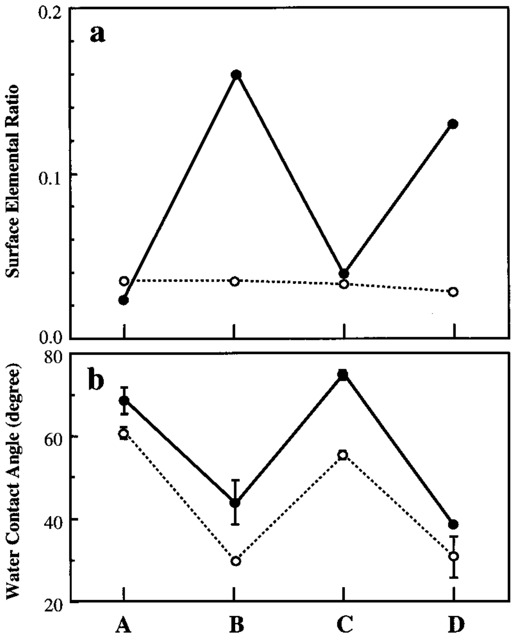


Figure 8. XPS elemental ratio ((a) ●, N/C ratio; ○, S/C ratio) and water contact angle ((b) ●, advancing water contact angle; ○, receding water contact angle) changes during stepwise photo-graft copolymerization: (A) the nontreated polymer (**2**)-coated PET film surface; (B) after photo-graft copolymerization with the first monomer, DMAPAAm; (C) after photo-graft copolymerization with the second monomer, ST; (D) after photo-graft copolymerization with the third monomer, DMAPAAm.

After an initial stripe grafted region was created, another stripe grafted pattern perpendicular to and overlaying the first grafted surface was created. The cross-hatched graft surface regions, which are the intersection of the two grafted layers, consist of block graft copolymers. This was prepared by sequential steps: first, a stripe pattern of poly(DMAPAAmMeI) was prepared using the stripe-patterned projection mask and then stained with rose bengal. Subsequently, a second stripe pattern of poly(MAA), which was formed crosswise to the first stripe pattern, was prepared by using the mask placed on the surface after rotating it 90° compared with its orientation in the former case and then stained with malachite green carbinol base. The surface thus prepared was composed of four types of regions: nongrafted regions (square pattern), poly(DMAPAAmMeI)-grafted regions (red-colored stripe pattern), poly(MAA)-grafted regions (green-colored stripe pattern), and poly(DMAPAAmMeI-*b*-MAA)-grafted regions (mixed colored stripe intersections) (Figure 5d). A topographic image of the stripe intersection portions prepared in Figure 5d was obtained by AFM observation (Figure 9). The thicknesses of the poly(DMAPAAmMeI)-grafted layer, the poly(MAA)-grafted layer, and the poly(DMAPAAmMeI-b-MAA)-grafted layer, determined from the cross-sectional AFM images, were about 120, 330, and 420 nm, respectively. This indicated that the poly(MAA) graft layer was lamellarly formed on the poly(DMAPAAmMeI)-grafted layer at the intersection portion.

Graft Thickness-Gradient Surface. A graft thickness-gradient surface in which graft yields or possibly graft chain length changed unidirectionally was pre-

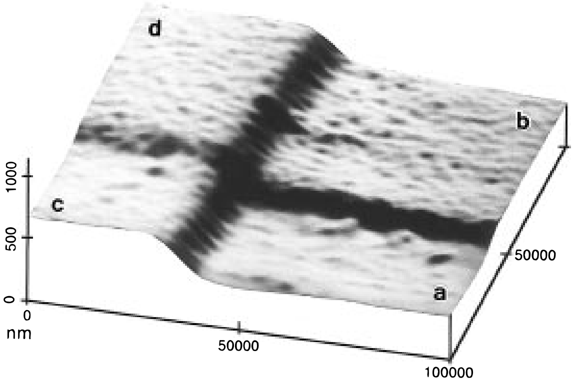


Figure 9. AFM image, projected at 30° for viewing, of the cross-hatched graft surface shown in Figure 5d: (a) nontreated photoreactive polymer film (**3**) surface; (b) poly(DMAPAAm-MeI)-grafted reigon; (c) poly(MAA)-grafted region; (d) poly-(DMAPAAmMeI-*b*-MAA)-grafted region.

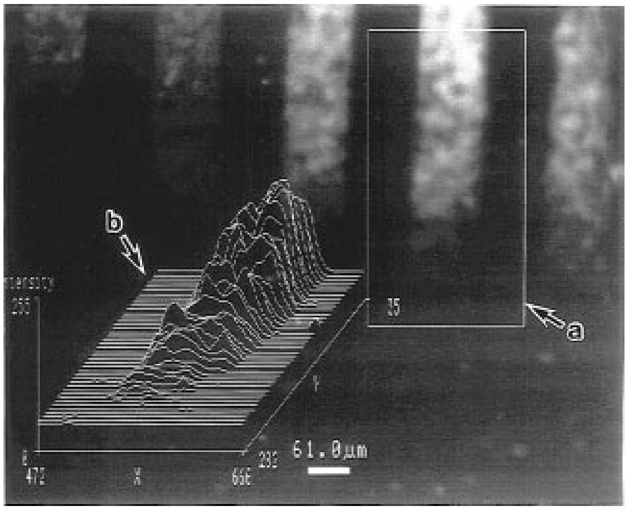


Figure 10. Fluorescence micrograph of surface photo-graft-copolymerized with DMAPAAmMeI by UV irradiation through the stripe-patterned projection metal mask and the neutral-density filter and subsequently stained with rose bengal, and the three-dimensional image (b) of the distribution of the fluorescence intensity in the area shown in (a).

pared using the stripe-patterned projection mask coupled with the gradient neutral-density filter which is a cut filter with a unidirectional continuous change in light intensity. The polymer (**2**)-coated PET film was UV irradiated in a DMAPAAmMeI methanol solution through the mask and the filter, both of which were tightly contacted to the film. The treated surface, stained with rose bengal, which fluoresces a light orange color upon exposure of light of wavelength 510−560 nm, was observed under a fluorescent microscope. The image of the distribution of the fluorescence intensity from the stained grafted surface was recorded with an Argus-20 system via a video camera to obtain a three-dimensional representation of the fluorescence intensity image. Figure 10 shows a fluorescence micrograph of the treated surface where the distribution of the fluorescence intensity derived from rose bengal in the square area shown in Figure 10a was superposed on the micrograph. The fluorescence intensity (Figure 10b), which can be converted to graft yields, decreased gradually from the top to the bottom of the micrograph. This is in good agreement with the gradient light transmittance of the filter used and indicates that the graft yields of poly(DMAPAAmMeI) gradually increased with the distance from the nonirradiated end of the film.

Discussion

Surface graft copolymerization alters the composition and properties of a polymer surface and thus affects its biocompatibility or printability. Surfaces grafted with nonionic water-soluble polymers such as poly(acrylamide) and poly[methoxypoly(ethylene glycol) methacrylate][5] provide a highly swollen diffuse layer at the water/substrate interface, which imparts a high antithrombogenic potential to blood-contacting devices. Surface graft copolymerization is usually achieved via the formation of highly reactive radical-generating species such as peroxide groups by γ-ray irradiation,[4] UV irradiation,[3] plasma discharge, glow discharge, or ozone treatment,[5] and subsequent graft copolymerization at an elevated temperature.

Due to the fact that during radical polymerization highly reactive radicals participate in various reactions such as coupling and/or disproportionation, control of graft chain length and its distribution is very difficult. Two different approaches to designing radical chain ends, which enable prevention of adverse reactions and possibly precise control of molecular weight, have been proposed. Solomon et al.[13] and Georges et al.[14] have been working with a stable free radical, 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), which reacts with a growing polymer chain radical to form a relatively stable C−N covalent bond, acting as a potent polymerization inhibitor. At a relatively high temperature, this bond dissociates to yield a polymer radical and a TEMPO molecule. During the dissociation phase, polymer chain growth occurs. They have reported that the molecular weight of the polymer increased with reaction time and that its molecular weight distribution was much narrower than that formed by conventional radical polymerizations. The other approach pioneered by Otsu et al. involves a photochemically driven process in which photochemical properties of dithiocarbamate compounds are utilized, as described in the Introduction.[8] Irrespective of whether a thermal or photochemical approach is used, the essential feature of living-like radical polymerization is to enforce the recombination reaction between a polymer end radical and a relatively stable counter radical as much as possible and to minimize adverse reactions such as chain transfer to solvent molecules, monomer addition to a counter radical, and diffusion of a counter radical from a neighboring growing chain end.

Otsu et al. reported that photopolymerization of ST or MMA occurs in solution in a living-like radical mechanism under appropriate experimental conditions and that poly(ST-*b*-MMA) and poly(ST-*b*-MMA-*b*-ST) can be prepared by sequential monomer charges. Their research was extended to surface graft copolymerization in which dithiocarbamyl groups were attached to PST microspheres via an ester bond as a hydrolyzable spacer.[15] The results of photopolymerization using ST or MMA and subsequent hydrolysis showed that both the polymer yield and the number-average molecular weight of the resultant polymer increased with UV irradiation time, indicating that the surface graft copolymerization proceeded in a manner similar to the living-like radical mechanism in solution. In addition, they have shown evidence that the block chain lengths of the diblock and triblock copolymers grafted on the surfaces were precisely controlled. Other researchers utilized this surface photo-graft copolymerization technique to modify a poly(dimethylsiloxane) film whose surface was partially substituted with dithiocarbamyl groups.[16] Photo-graft copolymerization of hydrophilic vinyl monomers, e.g. 2-hydroxyethyl methacrylate, acrylamide, and MAA, changed the surface from a very hydrophobic to a very hydrophilic one. Graft yields increased with UV irradiaton time, as revealed by attenuated total reflection infrared (ATR-IR) spectroscopy and water contact angle measurement.

Using the knowledge mentioned above, we initiated the study on photochemically driven surface photo-graft copolymerization as a fundamental technique leading to well-controlled surface molecular engineering for biomedical applications. The potential features of dithiocarbamate-based surface photopolymerization were as follows: (1) Under appropriate reaction conditions, living-like radical polymerization may be attained, which results in better molecular weight control than in conventional graft copolymerization. (2) Radical polymerization proceeds only during photoirradiation and at photoirradiated regions. (3) These may lead to regional and dimensional control of surface graft copolymerization such as block graft surfaces, microdomain surfaces, and gradient surfaces.

In this study, using surface-immobilized benzyl *N*,*N*-diethyldithiocarbamyl groups as exemplified by the polymer (**2**)-coated surface and the polymer surface (**3**), surface graft copolymerization was carried out, which eventually yielded very fine surface designs as follows. In the surface graft copolymerization, vinyl monomers such as DMAAm, DMAPAAm, DMAPAAmMeI, MAA, and ST were graft-copolymerized on the surfaces, as determined from the results of XPS measurements and wettability studies (Figures 1 and 2). The results of our XPS study, which revealed an appreciable content of S in photo-graft-copolymerized surfaces even after 20 min of UV irradiation as shown in Figure 1, may imply that growing chain ends preferentially recombine with dithiocarbamyl radicals. AFM cross-sectional analyses of graft-polymerized surfaces indicated that the thickness of graft layer increased almost linearly with UV irradiation time. These results highly suggested that the photo-graft polymerization proceeded in a living-like radical polymerization mechanism within at least 20 min of irradiation time. We have also found, using a quartz crystal microbalance (QCM) system, that the weight increase on the electrode surface due to surface graft polymerization occurred linearly with irradiation time. In addition, the measurement of the molecular weight of grafted chains, which was isolated from graft-copolymerized surfaces by hydrolysis, provided additional evidence that living-like polymerization occurs under these appropriate conditions (both results will be reported in the near future).

The resultant dithiocarbamate-terminated graft polymer initiated block copolymerization upon UV irradiation in the presence of the second monomer, producing an **A**—**B**-type block-graft-copolymerized surface. Upon repeating the cycle of graft copolymerization, an **A**—**B**—**A**-type block-graft-copolymerized surface was prepared. The changes in surface composition and wettability reflected well the change in the top layer of the grafts at each graft polymerization step (Figure 8).

Regional control of surface photo-graft copolymerization was attained using a projection mask. As shown in Figures 4 and 5 (a and b), the dimensions of the patterned formation on the surface were in agreement with those of the projection mask used. AFM images showed that the border between the graft-polymerized layers, whose thickness increased almost linearly with UV irradiation time, and the nontreated portion was a very sharp edge on micron order (Figures 6 and 7). The differential cellular response was clearly observed on microdomains composed of the two different surface regions; the nonionic graft polymer regions served as noncell adhesive regions and the nongrafted regions as cell-adhesive regions (Figure 4). Details on the cell adhesiveness dependence on substrate properties are discussed elsewhere.[17]

The photo-graft copolymerization was effectively terminated by the addition of a large amount of 1-dodecanethiol as a chain transfer agent during irradiation (reaction V in Scheme 1). Surface microdomain structures where two different polymer chains were grafted separately were prepared using the sequential steps of photo-graft copolymerization of one monomer through a projection mask, irradiation in the presence of 1-dodecanethiol, and graft copolymerization of the other monomer. The parallel lamellar patterning of the surface composed of positively and negatively charged graft polymers is shown in Figure 5c. In addition, AFM observation clearly demonstrated that the hierarchically lamellar layering in the block-graft-copolymerized surface was formed by combination of the surface photo-graft copolymerization using a projection mask and sequential monomer charges (Figure 9).

Simultaneous use of a projection mask and a gradient neutral-density filter during irradiation allowed simultaneous control of the grafted region and the thickness of the graft polymer layers. The surface thus prepared was found to be thickness-gradient one with regional precision, which was revealed by a three-dimensional fluorescence visualization (Figure 10). As for a gradient surface where the surface density of the graft chain varied continuously from one end to the other end, Lee et al. reported that gradual exposure of polymer surfaces to radio frequency plasma discharge or corona discharge and subsequent radical graft copolymerization produced a gradient surface where the surface chemical composition varied gradually from one end to the other.[18] On the other hand, the results of the present study demonstrated that the thickness of the graft layers varied gradually unidirectionally.

In conclusion, surface photo-graft copolymerization via benzyl *N*,*N*-diethyldithiocarbamate free-radical chemistry appears to be a powerful tool for well-controlled surface macromolecular engineering for biomedical applications. The photoinduced polymerization mechanism based on dithiocarbamate chemistry, involving reversible dissociation of a radical pair, may be useful for preparing functional surfaces under appropriate reaction conditions. It is anticipated that the dithiocarbamate-initiated surface photo-graft copolymerization is the first method that permits dimensionally precise control of surface graft copolymerization in terms of region control and the complexity of the graft architecture. The method will be useful for functional surface design for artificial organs, micromachies, and microbiosensors. It was reported that this method was used for surface heparinization on commercially available catheters via surface-grafted poly[2-(dimethylamino)ethyl methacrylate].[19] In a series of our study on dithiocarbamate-derived surface microarchitectural design, the scope and limitation of precise control of the photochemical driven process in conjunction with mechanistic aspects as well as biomedical application will be reported in the near future.

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