Ceric-Ion-Initiating Surface Graft Polymerization with Regional Control and Dimensional Precision

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ABSTRACT: This paper reports a surface modification method enabling graft copolymerization with regional control and dimensional precision. The method was based on dimensionally well-controlled photofixation of hydroxylated synthetic polymer such as poly(vinyl alcohol) or naturally occurring polysaccharides such as alginic acid and pectic acid, both of which were partially derivatized with a photoreactive *p*-azidophenyl group, followed by ceric-ion-initiating graft polymerization. ESCA measurements verified surface fixation of hydroxylated polymers and graft polymerization of vinyl polymers such as acrylamide and acrylic acid, which proceeded on ultraviolet (UV)-irradiated surface portions. Regional control of surface graft polymerization with dimensional precision was demonstrated by a patterned tissue produced by cells cultured on the microprocessed surface thus prepared.

Introduction

Advanced medical devices, assembled with different parts composed of different polymers have demanded differential designs of the bulk phase and surface region of the polymer components. The biocompatibility is determined by surface nature, composition, and structure, whereas gas or water permeability, mechanical properties, and durability are imparted by those at the bulk phase of the polymer. Thus, blood-contacting medical devices such as artificial hearts must have dual requirements: surface design and bulk phase design. We anticipate that the surface design may be achieved by surface modification after assembling of parts to fabricate a device. The surface may be modified by various methods, e.g., oxidation by frame, by corona discharge or plasma treatment, with chemical reagent, or with surface grafting. The requirements for surface modification of fabricated devices are that the surface can be modified at a desired portion of the device under a mild condition which cannot cause deterioration of the bulk phase of the polymer and shape or dimensional change of the devices, which is induced by an elevated temperature or an organic solvent.

A surface graft copolymerization technique has been used for providing surface functionality while still retaining the bulk properties of the matrix polymer. In general, surface radical graft copolymerization proceeds via two reactions; the first reaction is to create free radical species or radical-progenator groups at the outermost layer region of a surface, and the second is radical polymerization initiated from these surface-immobilized species. The first reaction can be accomplished with γ-ray irradiation,[1−3] plasma or glow-discharge treatment,[4−5] ultraviolet (UV) irradiation in the presence of photoreactive species such as benzophenone,[6] ozone gas treatment,[7] or surface immobilization of radical initiators such as 4,4′-azobis(4-cyanovaleric acid).[8]

In this paper, we prepared photoreactive hydroxylated polymers which were partially derivatized with the *p*-azidophenyl group (Figure 1: 1). Using these polymers, a graft polymerization technique with regional control and dimensional precision, which is especially suitable for surface functionalization of fabricated devices, was developed. The technique is based on two sequential steps (Figure 1: 2): (1) photochemically-driven surface immobilization of hydroxylated polymers which are partially derivatized with *p*-azidophenyl groups and (2) graft polymerization of acrylamide in the presence of the ceric ion (Ce^{IV}), which proceeds in water at room temperature.

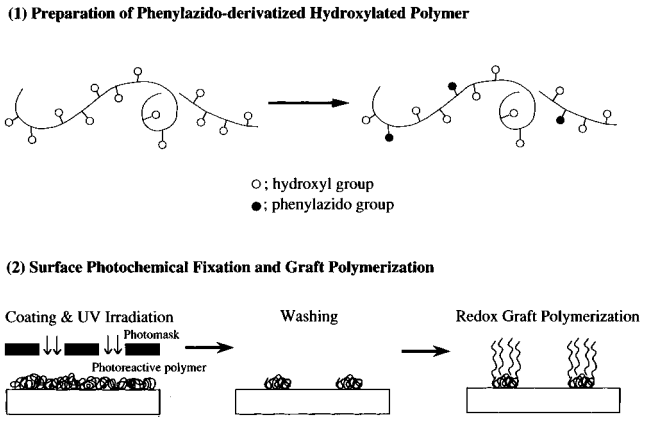

Figure 1. (1) Preparation of photoreactive hydroxylated polymer and (2) procedure of surface-microprocessed graft polymerization.

Experiments

Preparation of *p*-Azidophenyl-Derivatized PVA. Poly(vinyl alcohol), PVA (2.0 g), and *p*-azidobenzoyl chloride (1.64 g, 0.20 equiv vs the hydroxyl group of PVA), prepared from the reaction of *p*-azidobenzoic acid (obtained from Tokyo Kasei, Co., Ltd., Tokyo, Japan) and thionyl chloride, were dissolved in anhydrous dimethyl sulfoxide (DMSO; 50 mL). The reaction mixture was heated at 70 °C with stirring. After 4 h, pyridine (10 mL) was added to the solution. The reaction was continued with stirring for an additional 3 h. A large volume of tetrahydrofuran (THF) was added. The resultant precipitant was thoroughly washed with THF. The yield was 1.95 g (82%).

Preparation of *p*-Azidophenyl-Derivatized Alginic Acid and Pectic Acid. Aqueous solutions of sodium salt alginic acid and sodium salt pectic acid, both of which were obtained from Nakarai Tesque Inc. (Kyoto, Japan), were passed through

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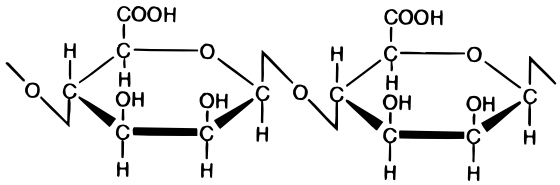
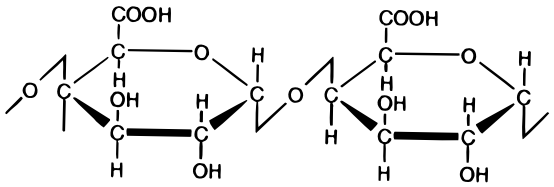


Alginic acid



Pectic acid

Figure 2. Repeating disaccharide units of alginic acid and pectic acid.

a minicolumn packed with an ion-exchange resin (Dowex 50W × 8). After triethylamine was added to the eluent, *N*,*N*-dimethylformamide (DMF) was added. Upon evaporation of water under reduced pressure, a transparent DMF solution was obtained. Aziodophenyl-derivatized alginic acid and pectic acid were prepared according to the same method as described above.

Degree of Substitution. The degree of substitution (DS) of the *p*-azidophenyl group for modified PVA was determined from the ^{1}H NMR spectrum (DMSO-d_6; ppm from $Si(CH_3)_4$) where the peak intensity ratio between phenyl protons at 7−8 ppm and methylene protons of the main chain at 1.8 ppm were used for calculation. The DS of *p*-azidophenyl-derivatized alginic acid, pectic acid, and PVA was also determined from UV absorbance at 275 nm (calibrated by (2-hydroxyethyl)-4-azidobenzoic acid).

Surface Graft Polymerization. A DMSO/methanol (8/2 v/v) mixed solution of the *p*-azidophenyl-derivatized PVA (0.5% w/v) was cast on PET film (18 × 18 mm, thickness 50 μm) using a spin coater. After air-drying, the sample was irradiated by ultraviolet (UV) light for 40 s by using a Toshiba UV lamp (H-400P; 400 W) at a distance of 30 cm (intensity; 2.2 mW/cm^2). After washing with DMSO and distilled water, the sample film was immersed into 0.5 N HNO_3 aqueous solution containing 5.0 wt % acrylamide in a glass tube. A predetermined weight of $Ce(NH_4)_2(NO_3)_6$ was added to the solution of 0 °C (the concentration was given in figure legends). The glass tube, degassed and sealed under cooling, was immersed in a bath at 40 °C for 2 h. After extensive washing with hot distilled water, samples were subjected to ESCA measurement. The graft polymerization of acrylic acid was performed in aqueous solutions (concentrations of the ceric ion and nitrate ion were shown in figure legends).

Surface Characterization. The surface chemical compositions were determined using as ESCA instrument (ESCA 750; Shimadzu Corp., Kyoto, Japan). The C1s spectrum was deconvoluted to subpeaks with the aid of computer-assisted curve software.

Cell Culture. Bovine endothelial cells, harvested from thoracic aorta, were cultured in Dulbecco's modified Eagle's medium supplemented with 15% of fetal bovine serum at 37 °C in a 5% CO_2 humidified incubator. The patterned cells were photographed using a phase-contrast microscope (Nikon Model III, Tokyo) with or without a Hofmann modulation apparatus.

Results

The surface graft polymerization method developed in this paper is based on photochemical surface fixation of hydroxylated synthetic polymer (PVA) or naturally occurring polysaccharide (alginic acid and pectic acid, structures of which are shown in Figure 2), all of which were partially derivatized with the *p*-azidophenyl group, and subsequent radical graft polymerization initiated by the Ce^{IV} ion.

Preparation of Photoreactive Hydroxylated Polymers (Figure 1: 1). The condensation reaction of PVA, alginic acid, and pectic acid with *p*-azidobenzoyl chloride was carried out in anhydrous DMSO at 70 °C. Qualitative characterization was performed by infrared spectroscopy: irrespective of the polymer used, a peak ascribed to the azido group at about 2120 cm^{-1} and a peak ascribed to the ester group at around 1740 cm^{-1} were clearly identified, indicating that azidophenyl groups were derivatized to polymers via its hydroxyl groups. The degree of substitution (DS), determined by UV measurement at 275 nm, which is ascribed to the characteristic absorption peak of the azidophenyl group, was found to be 2.4 mol % for alginic acid, 3.7 mol % for pectic acid, and 6.0 mol % for PVA. The degree of substitution determined by ^{1}H NMR spectroscopy was found to be 6 mol % for PVA, which is in good agreement with that determined by UV spectroscopic measurement.

Surface Chemical Fixation and Graft Polymerization (Figure 1: 2). *p*-Azidophenyl-derivatized PVA, alginic acid, and pectic acid were cast on PET surfaces by a spin coater. After the sample was irradiated by UV light and subsequent vigorous washing with water, ESCA measurement was conducted to determine whether the coating was chemically fixed onto the surface. Figure 3 shows typical examples of ESCA spectra before and after treatment with *p*-azidophenyl-derivatized PVA. Upon UV irradiation, an increased subfraction of C−O (0.38) in the C1s peak and element ratio of O/C (0.47) were observed as compared with those of non-treated PET (0.25 and 0.42, respectively). Extensive washing with water did not cause any significant spectral change, indicating that PVA is covalently fixed on the PET surface.

The graft polymerization of acrylamide on the PVA-fixed PET surface was carried out in aqueous solution in the presence of $Ce(NH_4)_2(NO_3)_6$, which altered the surface chemical composition and water wettability. As can be clearly seen in Figure 3, the increased subfraction of C═O in the C1s peak and element ratio of N/C were noted: element ratios of N/C of 0.28 and O/C of 0.33 observed for a treated surface were very close to theoretical values (N/C = 0.33 and O/C = 0.33) for poly(acrylamide), PAm. Thus, it can be said that the graft polymerization indeed occurred to completely cover the PET surface with grafted PAm.

In general, ceric-ion-initiated radical polymerization is affected by several reaction variables such as ceric ion concentration,[9,10] monomer concentration,[11] and pH of the medium.[12] The ceric ion concentration dependence of surface composition upon graft polymerization of acrylamide on the PVA-fixed PET surface is shown in Figure 4. With an increase in the concentration, the element ratio of N/C at the surface layer increased. Excessive Ce^{IV} concentration seems to decrease the degree of surface grafting. The effects of monomer and nitrate concentrations on the surface graft polymerization of acrylic acid were examined in the presence of $Ce(NH_4)_2(NO_3)_6$ and nitric acid. At fixed concentrations of the ceric ion and nitrate ion, the ratios of peak intensities of C═O at 290 eV to C−O at 287 eV in the C1s spectrum increased linearly with an increase in the monomer concentration (Figure 5). At fixed concentrations of the monomer and ceric ion, little change in the

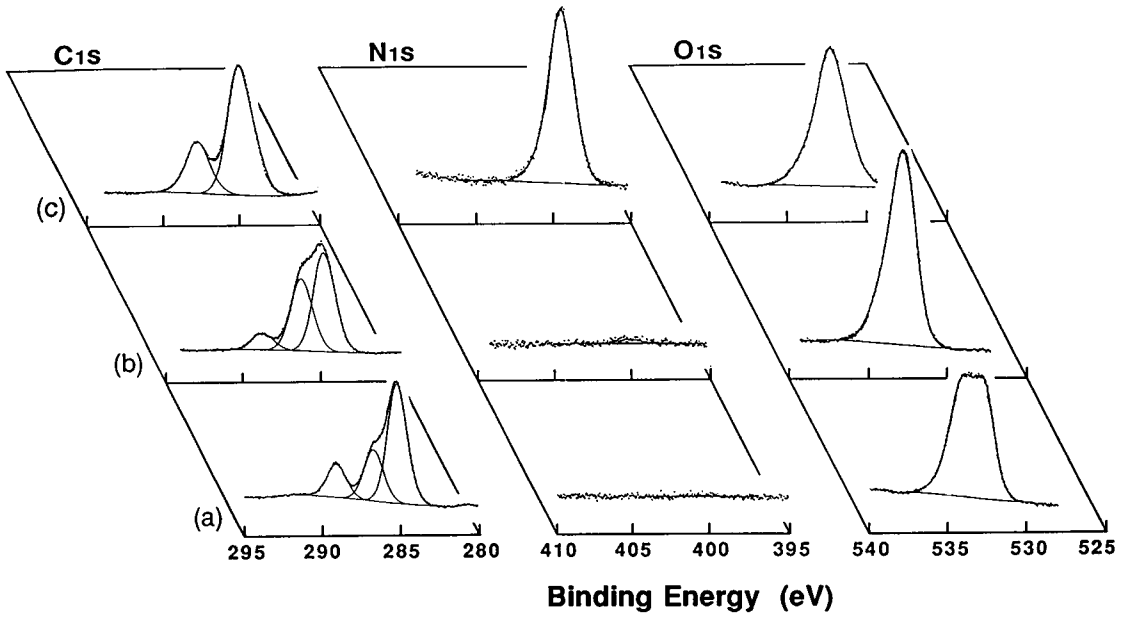


Figure 3. ESCA spectra of nontreated PET surface (a), *p*-azidophenyl-derivatized PVA-treated surface (b), and acrylamide-grafted surface (c). Conditions: [monomer] = 5 w/v %, [HNO_3] = 0.5 N, [Ce^{IV}] = 2.0 mM, temperature 40 °C, polymerization time 2 h.

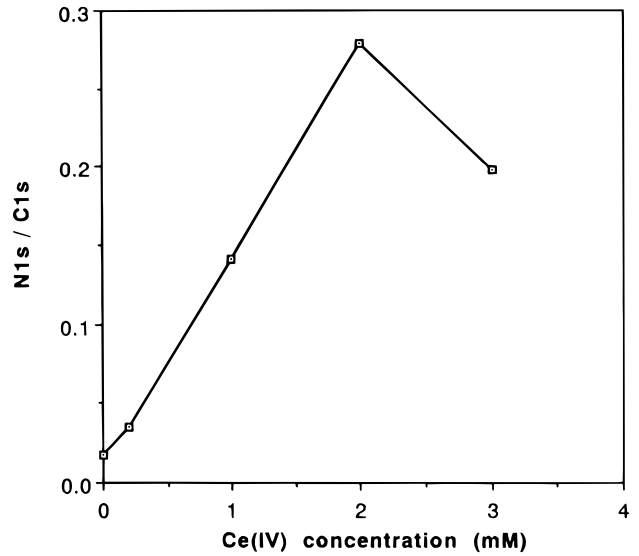


Figure 4. Surface element ratio dependence on the concentration of Ce^{IV}. (Reaction conditions were the same as those in Figure 3, except for Ce^{IV} concentration.)

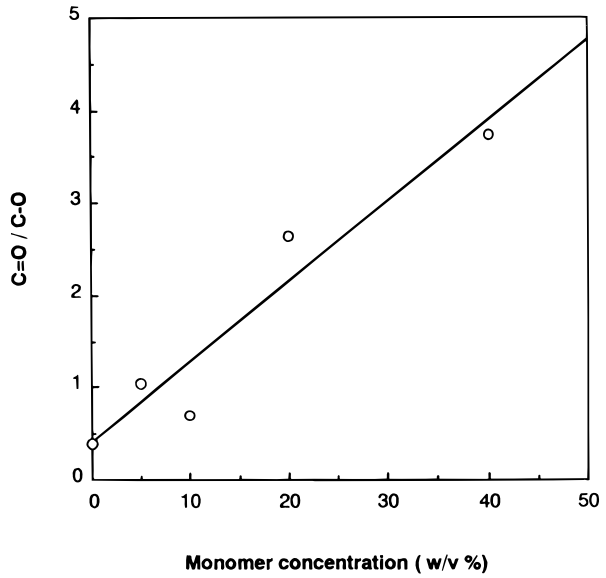


Figure 5. Effect of monomer concentrations on the surface graft polymerization of acrylic acid. Conditions: [HNO_3] = 0.025 mM, [Ce^{IV}] = 2.5 mM, at room temperature, polymerization time 6 h.

ratio was observed, irrespective of nitrate ion concentration, indicating that graft polymerization did not depend on the concentration of the nitrate ion under this particular experimental condition (Figure 6).

Effect of Hydroxylated Polymers on Surface Graft Polymerization. Three different types of hydroxylated polymers (PVA, alginic acid, and pectic acid) were surface-fixed, followed by graft polymerization of acrylamide in aqueous solutions under fixed reaction conditions. Figure 7 shows the surface elemental ratio (N/C) dependence on the polymerization time. Irrespective of the type of polymer used, a time-dependent increase in the N/C ratio was observed. The highest polymerization rate, as judged from the N/C ratio, was found for PVA, followed by alginic acid. The lowest was found for pectic acid.

Verification of Dimensional Precision: Patterned Tissue. Since a hydroxylated polymer was chemically fixed on only the UV-irradiated portion, the surface graft polymerization should proceed only on the fixed portion, as shown in Figure 1 (2). In order to demonstrate that this method allows grafting of PAm on a PET surface with micron-order dimensional precision, a honeycomb-like photomask (width of a spacer: 200 μm) was placed on a photoreactive PVA-coated PET surface during UV light irradiation. After surface graft polymerization of acrylamide was completed, bovine endothelial cells were seeded on the treated surface and cultured in the serum-containing medium. Figure 8 shows the photomask used and micrograph of endothelial cell pattern which was formed upon culturing. Cells adhered and grew well on only the nonirradiated PET surface, which is known as a cell-adhesive substrate, whereas little cell adhesion occurred on hydrophilic PAm-grafted regions. This eventually resulted in the formation of 2-dimensional (2-D) tissue. This is strong evidence that the method developed here can provide a

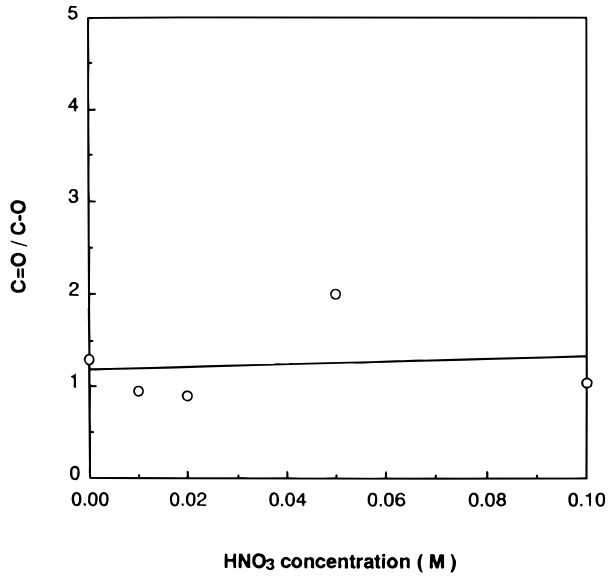


Figure 6. Effect of nitrate ion concentration on the surface graft polymerization of acrylic acid. Conditions: [monomer] = 5 w/v %, [Ce^{IV}] = 2.5 mM, at room temperature, polymerization time 6 h.

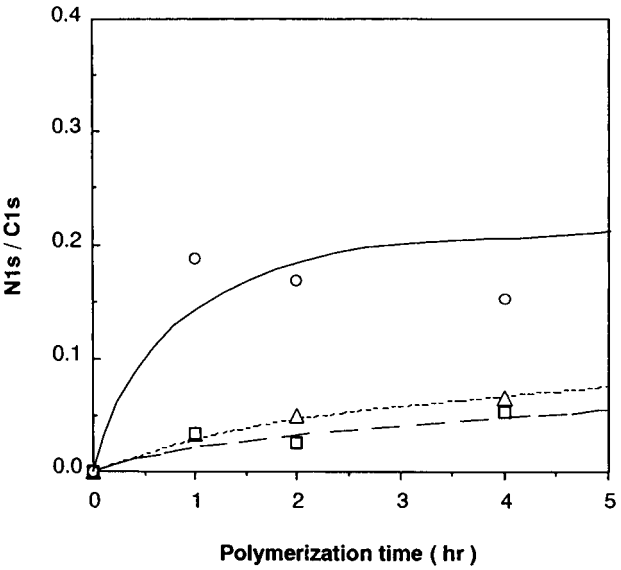


Figure 7. Surface element ratio dependence on the polymerization time. Conditions: [acrylamide] = 5 w/v %, [Ce^{IV}] = 2.5 mM, [HNO_3] = 0.025 mM, at room temperature, polymerization time 6 h. Key: (○) PVA; (△) alginic acid; (□) pectic acid.

unique feature in terms of regional control and dimensional precision, which cannot be attained by conventional surface graft polymerization methods.

Discussion

Although a variety of methods are available for surface graft polymerization, a limited number of methods enables graft polymerization at a given portion of a surface. Previously, we developed the method enabling the surface graft polymerization with micron-order dimensional precision.[8] The method was based on the sequential processes as follows: (1) photochemical fixation of poly(allylamine) partially derivatized with the *p*-azidophenyl group, (2) surface derivatization of a radical initiator, dicarboxylated azobis(isobutyronitrile), 4,4′-azobis(4-cyanovaleric acid), and (3) subsequent thermal decomposition of surface-fixed initiator in a monomer-containing solution, resulting in surface radical graft polymerization. The surface fixation was due to chemical bonding of highly reactive nitrene, which was generated upon UV irradiation of the *p*-azidophenyl group, onto a polymer surface. Since radical initiators were immobilized on UV-irradiated portions by a lithographic technique using a photomask, as commonly used for semiconductor fabrication, surface graft polymerization proceeded with micron-order precision, which was clearly observed under atomic force microscopy.[8]

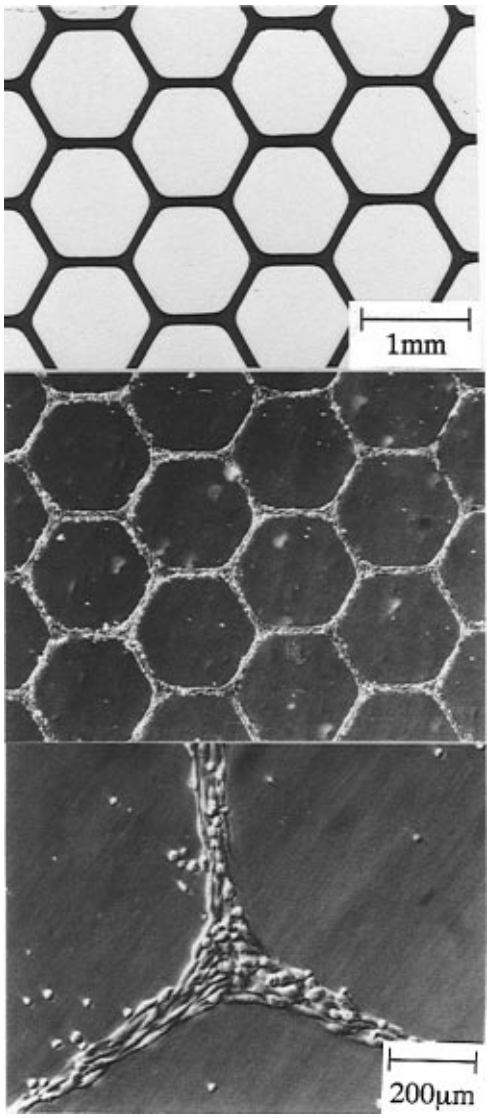
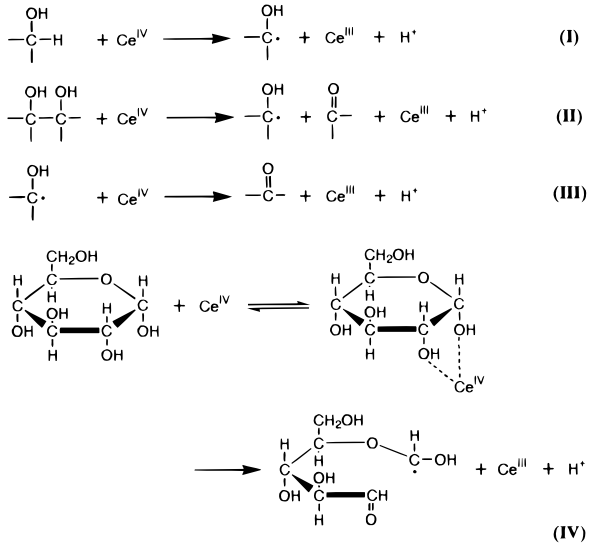


Figure 8. Photomask used for surface microprocessing (top) and resultant cell pattern: (center) phase-constrast microscopic image; (bottom) Hofmann-modulated contrast microscopic image.

We extended our study to the development of the ceric-ion-initiating surface graft polymerization method for fabricated devices. Since potential application of surface graft polymerization developed here is directed at upgrading blood compatibility of complex-shaped, fabricated, cardiovascular devices, surface graft polymerization under milder conditions than those *via* thermal decomposition of surface-immobilized radical initiators is essential. For example, artificial hearts are composed of elastomers. Thermal treatment results in the deformation of shape of fabricated devices. We envisaged that ceric-ion-inducing graft polymerization, which occurs at room temperature, is a better choice for surface modification of fabricated devices.

Scheme 1. Schemes of Reactions of Hydroxylated Substances with Ceric Ion[a]



[a] I, II, and III are cited from the literature (Storey and Goff).[17]

In this study, we utilized the photoreactivity of the *p*-azidophenyl group for chemical fixation of hydroxylated polymers, similarly as in our previolus studies.[8,13−16] Upon UV irradiation, covalent fixation of photoreactive hydroxylated polymers on a substrate surface and crosslinking between them take place simultaneously. ESCA analysis (Figure 3) showed that a durable hydroxylated polymer layer was formed on the PET surface.

The oxidative initiation of radical polymerization by Ce^{IV} ions on hydroxylated polymers such as PVA and cellulose has been extensively investigated over the years.[9−12] In particular, in their recent article, Storey and Goff[17] summarized the complexity of mechanisms as follows. According to the well-accepted mechanism for ceric-ion-inducing graft polymerization on PVA, there are two pathways involved in the formation of a free radical. One is the unimolecular disproportionation of the hydroxyl group complex with Ce^{IV} to yield the cerous ion (Ce^{III}), a proton, and a free radical on the alcohol substance (reaction path I in Scheme 1). The other is that a coordination complex between Ce^{IV} and 1,2-diols, produced by occasional head-to-head monomer incorporation (reported to occur to the extent of 1−2 mol % along the backbone), leads to the oxidative cleavage of diols (reaction path II). That is, the carbon−carbon bond between the hydroxyls cleaves to yield an aldehyde or ketone and a free radical at the fragment end of PVA. Ceric ions participate not only in the formation of radical species but also in the inactivation of the free radical generated or termination of growing chains (reaction path III). This suggests that, with excess Ce^{IV}, there is more opportunity for earlier termination, which would decrease the polymerization rate as well as the length of grafted chains. The ceric-ion-induced grafting thus emerges as a complex process in which vinyl polymerization of grafted chains occurs simultaneously with the degradation of the PVA backbone.

The factors which control the polymerization rate have been identified as (1) Ce^{IV} concentration, (2) monomer concentration, (3) pH of the aqueous solution, and (4) vicinal structure of the hydroxyl group.[17−19] The Ce^{IV} concentration dependence on surface graft polymerization, in which an optimal Ce^{IV} concentration appears to exist (Figure 4), may be explained by a balanced effect of Ce^{IV} on initiation and termination reactions. As expected, the increase in monomer concentration enhanced the polymerization rate of acrylamide (Figure 5). Generally, the pH of the aqueous solution affects the extent of dissociation of the cerium salt; a lower pH is conducive to dissociation, resulting in a higher polymerization rate. Since the pH of the polymerization media of the graft polymerization of acrylic acid is low enough to accelerate the polymerization, we did not observe pH dependence (Figure 6). As for the effect of the vicinal structure of the hydroxyl group on polymerizability, the following order in the polymerization rate has been reported:[18,19] 1,2-glycol > primary hydroxyl > secondary hydroxyl > tertiary hydroxyl.

When polysaccharides are used as a grafted matrix, as contrasted with PVA, little degradation reaction of polymer backbone occurs theoretically. In addition, the vicinal 1,2-diols present at every sugar unit may facilitate the formation of a coordination complex between Ce^{IV} and vicinal hydroxyl groups, which is the initial step of ceric-ion-inducing graft polymerization. The cis-vicinal diol is much more conductive to complex formation than the trans one.[17−19] In fact, it has been reported that the oxidation rate for *cis*-glycol was found to be 200-fold higher than that for *trans*-glycol.[20] As shown in Figure 2, alginic acid has two pairs of cis-vicinal diols in every repeating disaccharide unit, whereas pectic acid has two pairs of trans-vicinal diols. We attempted to compare the ceric-ion-initiating graft polymerizability of acrylamide on PVA-, alginic acid-, and pectic acid-fixed surfaces (Figure 7). We anticipated that the highest polymerization rate is achieved by alginic acid, followed by pectic acid and that the lowest one is PVA at an equimolar basis of the amount of coating. However, our results appear to show that the highest polymerization rate was found for PVA, followed by alginic acid and pectic acid. The polymerization rate with alginic acid was slightly higher than that with pectic acid. This may result from the difference in the amounts of chemically fixed hydroxylated polymers on PET surfaces.

Future study on ceric-ion-inducing surface graft polymerization under an optimally designed reaction condition may allow its application to surface modification of blood-contacting surfaces of cardiovascular devices. As exemplified above, the surface graft copolymerization aiming at the formation of a diffuse layer composed of nonionic hydrophilic polymer such as PAm proceeds in water at room temperature, which is best-suited for surface modification of complex-shaped, fabricated devices. The other feature of this surface process technology is its regional control and dimensional precision. As pointed out earlier, radical graft polymerization proceeded only on a hydroxylated polymer-fixed PET surface. Upon cell seeding, cell adhesion and growth were limited only at nongrafted, nontreated PET regions. The resultant tissue had a dimensional precision (Figure 8). This demonstrates that our surface graft polymerization method developed here has the unique feature of regional control and dimensionality which cannot be attained by the conventional graft polymerization method. Since the minimal limit of dimensionality of photochemical reactions is typically micron order, a very precise microprocessed surface graft polymerization can be attained. In addition, ceric-ion-inducing polymerization proceeds at room temperature. This is an another advantage for fabrication of elastomer-based devices such as artificial hearts, which

prevents shape and surface damage upon thermal treatment.

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References and Notes

(1) Suzuki, M.; Tamada, Y.; Iwata, H.; Ikada, Y. In *Physicochemical Aspects of Polymer Surfaces*; Andrade, J. D., Ed.; Plenum Press: New York, 1983; Vol. 2, Chapter 18.
(2) Omichi, H.; Okamoto, J. *J. Polym. Sci., Chem. Ed.* **1982**, *20*, 521, 1559.
(3) Chapiro, A.; Jendrychowska-Bonamour, A. M.; Mizrahi, S. *Eur. Polym. J.* **1976**, *12*, 773.
(4) Yasuda, H. *Plasma Polymerization*; Academic Press: New York, 1986.
(5) Onishi, M.; Shimura, K.; Seita, Y.; Yamashita, S.; Takahashi, A.; Masuoka, T. *Radiat. Phys. Chem.* **1992**, *39*, 569.
(6) Oster, G.; Shibata, O. *J. Polym. Sci.* **1957**, *26*, 233.
(7) Korshark, V. V.; et al. *J. Polym. Sci., Part C* **1964**, 753.
(8) Sugawara, T.; Matsuda, T. *Macromolecules* **1994**, *27*, 7809.
(9) Hunang, R. Y. M.; Chandramouli, P. *J. Appl. Polym. Sci.* **1968**, *12*, 2549.
(10) Storey, R. F.; Sudhakar, D.; Goff, L. J. *J. Macromol. Sci., Chem.* **1987**, *A24*, 1051.
(11) Schwab, E.; Stannett, V.; et al. *Tappi.* **1962**, *45*, 390.
(12) Haworth, S.; Holker, J. R. *J. Soc. Dyer. Color.* **1966**, *84*, 257.
(13) Matsuda, T.; Inoue, K. *Trans. Am. Artif. Intern. Organs.* **1990**, *36*, 161.
(14) Matsuda, T.; Inoue, K.; Sugawara, T. *Trans. Am. Artif. Intern. Organs.* **1989**, *35*, 195.
(15) Matsuda, T.; Inoue, K.; Ozeki, E.; Akutsu, T. *Int. J. Artif. Organs.* **1990**, *14*, 193.
(16) Matsuda, T.; Inoue, K.; Sugawara, T.; Akutsu, T. In *Artificial Heart-3*; Akutsu, T., Ed.; Springer-Verlag: New York, 1991; p 65.
(17) Storey, R. F.; Goff, L. J. *Macromolecules* **1989**, *22*, 1058.
(18) Iwakura, Y.; Kurosaki, T.; Imai, Y. *J. Polym. Sci.* **1965**, 1185.
(19) Wallace, R.; Young, D. G. *J. Polym. Sci.* **1966**, *4*, 1179.
(20) Pottenger, C. R.; Johnson, D. C. *J. Polym. Sci. Polym. Chem. Ed.* **1970**, *8*.

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