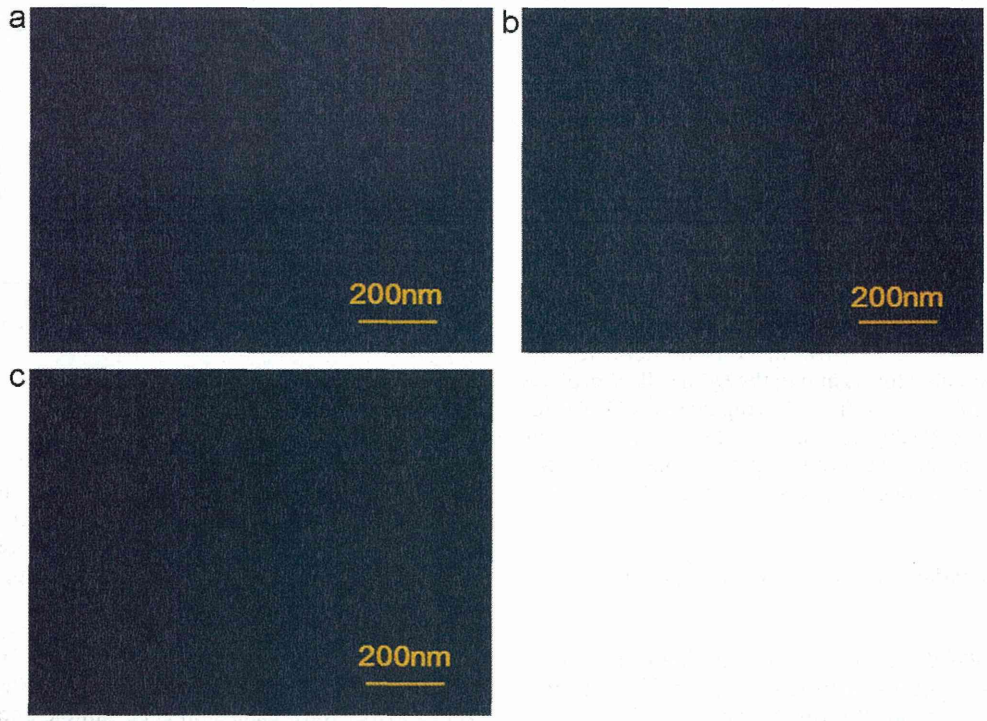

Fig. 3. SEM images of (a) Ti substrate, (b) PMDP–Ti substrate and (c) PMDP–Ti substrate after one month rinsing in PBS at 37 °C.

supported the presence of PMDP on the Ti substrate. As demonstrated in Table 1, the surface atomic concentration of nitrogen (N) and phosphorus (P) of PMDP coating remained at almost the same level during one month rinsing in PBS. This reveals that PMDP coating could function effectively for a long time even under long-term fluidic working conditions.

3.1.3. Surface morphology

As shown in Fig. 3, by SEM, the surface of PMDP–Ti substrate was uniform and consistent before and after one month rinsing in PBS at 37 °C.

These results prove that the PMDP covered the Ti substrate immediately and formed a uniform and stable coating layer.

3.2. In vitro hemocompatibility

The MPC polymers are widely used for improvement of biocompatibility, and they have excellent thrombogenicity and tissue compatibility [24–28].

3.2.1. Protein adsorption resistance of Ti substrate treated with PMDP

Resistance to protein adsorption is one of the most important properties of biomedical materials. Protein adsorption may induce the adhesion and activation of platelets and acute thrombus formation [3]. The effects of coating with PMDP were evaluated using the BSA solution [16]. BSA is the most highly concentrated protein in blood plasma, as shown in Fig. 4. According to the QCM signals, 530 ng/cm^2 of BSA was adsorbed on the original Ti substrate (30 Hz change in frequency), whereas after treatment with PMDP, no QCM signal because of BSA adsorption could be detected. These results indicate that the resistance to protein adsorption can be improved by coating with PMDP.

Table 1
Elemental analysis of each substrate.

Sample	Atomic composition (%)					*P*/*C* ratio
	C	N	O	P	Ti	
PMDP–Ti substrate	72.0	3.0	23.5	1.5	0	0.021
PMDP–Ti substrate after rinsing	68.0	3.9	25.4	1.7	0.9	0.025
Ti substrate	49.7	0	27.3	0	23.0	0

3.2.2. Platelet adhesion and morphology of adhered platelet

Platelets adhesion and activation are the main indicators to evaluate hemocompatability of the blood-contacting materials. Fig. 5 demonstrates the surface of substrate before and after platelet adhesion test. Before platelet adhesion test, each surface was clean and homogeneous. After platelet adhesion test, there were a lot of platelets adhered on both nontreated Ti substrate (Fig. 5c) and316L stainless steel (SS) (Fig. 5d). Moreover, some adherent platelets aggregated and took spread morphology with pseudopodium. On the PMDP–Ti substrate (Fig. 5e), a small number of platelets were

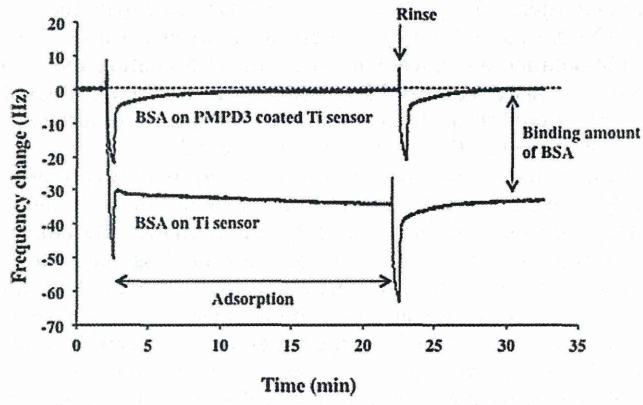

Fig. 4. Adsorption and detachment process of BSA on bare and PMDP-modified Ti-coated QCM sensor.

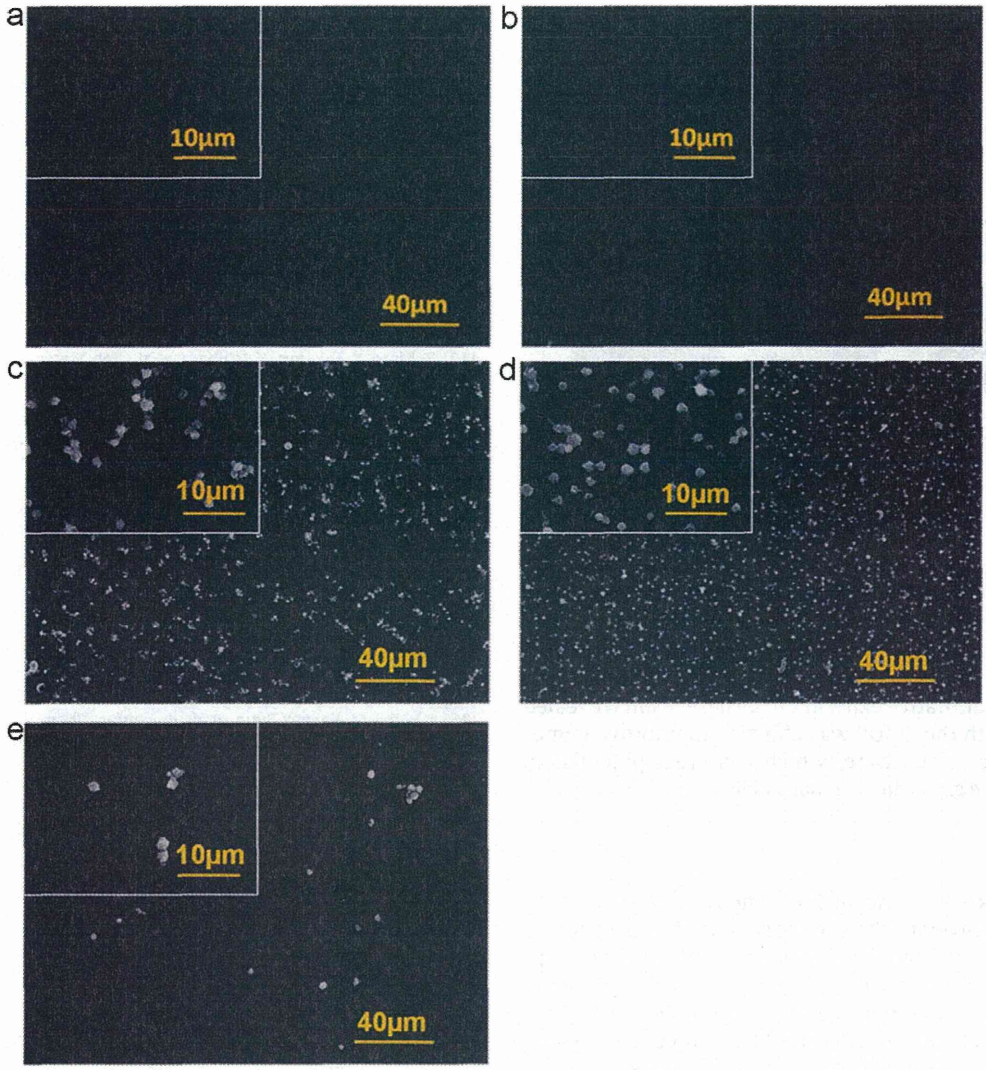

Fig. 5. Morphology of (a) Ti substrate, (b) PMDP–Ti substrate; morphology of adherent platelets (120 min incubation in PRP) on the surfaces of (c) Ti substrate, (d) 316L SS substrate and (e) PMDP–Ti substrate observed using SEM.

observed. And most adherent platelets remained spherical and separated without pseudopodium.

LDH assay was performed to quantitatively evaluate adhered platelets on the surfaces of the samples and the results are shown in Fig. 6. Adhesion of platelets on SS was around 50% of initial number of platelets. The Ti substrate reduced the platelet adhesion, while more effective reduction was observed after modification with PMDP. That is, comparing to nontreated Ti, the ratio of platelets adhered on the surfaces of PMDP significantly decreased from 25 to 17%.

When foreign bodies contact with blood, plasma protein adsorption occurs as the first phenomenon, which successively triggers platelet adhesion and activation, leading to thrombogenesis [3]. The nonfouling ability of the MPC polymer is tightly correlated with the hydration layer, because the tightly bound water layer forms a physical and energetic barrier to prevent protein adsorption and platelet adhesion on the surface [29–32]. As shown in Fig. 7, the PMDP gives a phosphorylcholine-group-arranged surface [33]. The phosphorylcholine group is electrically neutral and hydrated with free-water-like water molecules [34,35]. Thus, both electrostatic interaction and hydrophobic interaction are extremely weak and resistance to protein adsorption and platelet adhesion on the surface is improved [36].

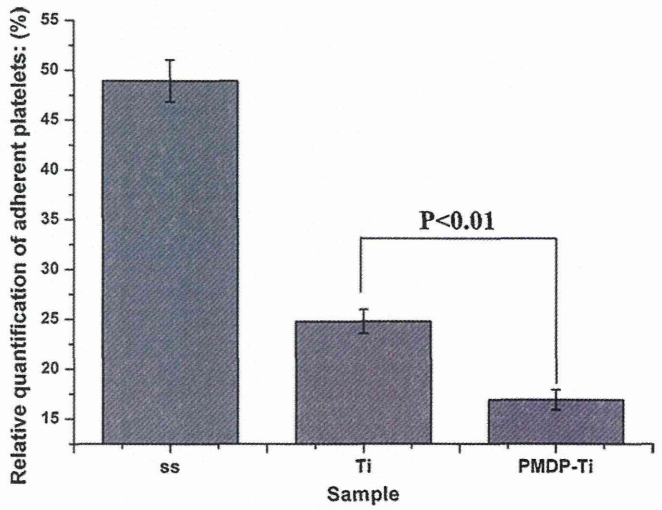

Fig. 6. Relative quantification of adherent platelets on Ti substrate, 316L SS substrate and PMDP–Ti substrate (results obtained by LDH assay, 45 min incubation in PRP, initial non-dilute concentration of platelets that used to make standard curve is taken as 100%). Data were analyzed using ANOVA, $P < 0.01$.

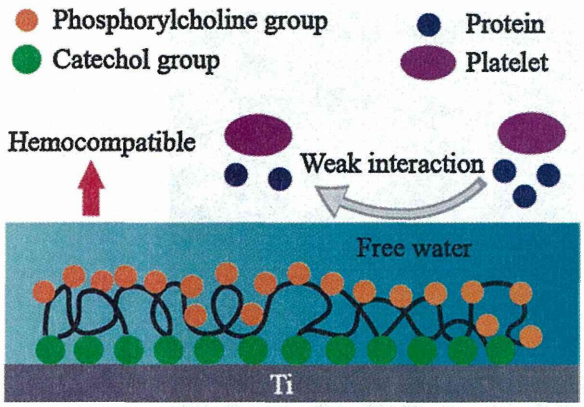

Fig. 7. Schematic representation of the mechanism of hemocompatibility observed on PMDP–Ti substrate.

4. Conclusions

To improve platelet adhesion-resistance on the Ti substrate, we applied mussel-inspired polymer with phosphorylcholine group. The polymer, PMDP could adhere on the Ti substrate spontaneously from an aqueous medium. We observed significant reduction of protein adhesion and platelet adhesion on the PMDP–Ti substrate by qualitative and quantitative experiments. These results revealed that modification with the PMDP was effective to improve hemocompatibility on the Ti substrate, which has great potential in medical application (*e.g.*, cardiovascular device).

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Cell adhesion control on photoreactive phospholipid polymer surfaces

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ABSTRACT

Non-invasive and effective cell recovery from culture substrates is important for the passage and characterization of cells. In this study, a photoreactive polymer surface, which uses UV-irradiation to control substrate cell adhesion, was prepared. The photoreactive phospholipid polymer (PMB-PL) reported herein, was composed of a both 2-methacryloyloxyethyl phosphorylcholine (MPC) unit as a cytocompatible unit and methacrylate bearing a photolabile nitrobenzyl group. The PMB-PL polymer was used to coat a cell culture substrate thus affording a photoreactive surface. Surface analysis of the PMB-PL coating indicated a strong photoresponse owing to the sensitivity of the PL unit. Before light exposure, the PMB-PL surface provided cell adhesion. Following UV-irradiation, the PMB-PL coating was converted to a neutral ζ-potential and hydrophilic surface. The photoreactive surface conversion process allowed for the detachment of adhered cells from the PMB-PL surface while maintaining cell viability. This study demonstrates the promise and significance of the PMB-PL photoreactive surface as a method to control cell attachment and detachment for cell function investigation.

1. Introduction

Recently many researchers have shown interest in stimuli-responsive surfaces for cell engineering and other applications. The properties of such "smart surfaces" are effortlessly tuned using an external stimulus [1–5]. Control of cell attachment and detachment from a substrate with continued bioactivity is important for *in vitro* cell culture analysis. The stimuli-responsive surface properties that are of interest for cell engineering development include wettability, hydrophobicity, and hydrophilicity. Previously reported surfaces were responsive to electrical [6–9], temperature [10–12], pH [13–15], or light [16–18] external stimuli. Among the stimuli, light is regarded as ideal for increased spatial and temporal resolution control.

To achieve controlled cell attachment/detachment behavior under mild conditions, it is important to suppress non-specific biomolecule interactions. We have previously reported the 2-methacryloyloxyetyl phosphorylcholine (MPC) polymers that have excellent cytocompatibility due to the inhibition of non-specific biomolecule interactions [19]. These polymers have been widely applied in various fields within the life sciences, including the area of cell engineering materials [20–23]. The MPC polymers effectively suppress the typical inflammatory reaction of adhered cells [23]. Previously, a photo-functionalized MPC polymer bearing photoreactive moieties such as azidophenyl groups and photocleavable linkers were reported to prepare micropattern surfaces for cell adhesion control [24–27].

In this study, we prepared another photoreactive MPC polymer, which controls cell detachment using UV-irradiation. The 2-nitrobenzyl moiety is a typical photocleavable protective group for surface modification, which is cleaved by UV-irradiation ($\lambda = 365$ nm) using a mercury lamp [28]. Incorporating a photoreactive MPC polymer bearing a photocleavable (PL) monomer afforded the PMB-PL polymer. Upon UV-irradiation the cell adhesive molecules were converted at the PMB-PL surface and cell detachment was achieved. In this report, characterization of the PMB-PL polymer and cell attachment/detachment behavior at the surface were investigated.

2. Materials and methods

The MPC was purchased from NOF (Tokyo, Japan), which synthesized the product using the previously reported method [29]. Methacryloyl chloride was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The photolabile linker, 4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy]butyric acid was purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). Other organic reagents were purchased with the highest available purity and were used without further purification.

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HeLa (*Homo sapiens* epithelial cell line established from a uterine cervix carcinoma) and L929 cells (murine fibroblast cell line established from connective tissue) were purchased from Riken Cell Bank (Ibaraki, Japan). The cells were cultured in Dulbecco's Modified Eagle Medium, (DMEM Sigma, St. Louis, MO, USA) with 10% fetal bovine serum, (FBS, Invitrogen Life Technologies, Carlsbad, CA, USA).

2.1. Synthesis of the photocleavable monomer (PL)

The photocleavable monomer (PL) was synthesized under dark conditions using lightproof vials. The photolabile linker, 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy]butyric acid (1.0 mmol) was dissolved in dry dichloromethane (DCM), which had been purged with Ar gas. Both triethylamine (TEA, 3.0 mmol) and methacryloyl chloride (MC, 2.5 mmol), were dissolved in dry DCM and added dropwise to the photolabile linker solution at 0 °C. The solution was stirred overnight at room temperature (RT). The stirred solution was washed with sodium bicarbonate (5%, w/v *aq*), dilute hydrochloric acid (1%, v/v *aq*), and water. The washed solution was evaporated and the remaining liquid product was dissolved in aqueous acetone (50%, v/v *aq*). The reaction mixture was stirred overnight at RT and the liquid monomer was extracted using DCM. The DCM layer was collected, washed with dilute hydrochloric acid (1% v/v, *aq*) and water, dried over magnesium sulfate, and evaporated to yield the photocleavable methacrylate monomer referred to as PL monomer. The structure of the PL monomer was confirmed using ^{1}H NMR (300 MHz, JEOL, Japan). The ^{1}H NMR chart and FT-IR spectrum of PL monomer was shown in Figs. S1 and S2, respectively.

^{1}H NMR (300 MHz, DMSO-*d*6): δ 12.3 (br, $CH_2COO\mathbf{H}$), 7.55 (s, Aromatic-**H**), 7.10 (s, Aromatic-**H**), 6.39, 6.05 (d, d, $OC(dO)CCH_3dC\mathbf{H_2}$), 5.3 (q, Aromatic-C**H**$(CH_3)OC(dO)CCH_3dCH_2$), 4.1 (t, Aromatic-O**CH$_2$**CH_2CH_2COOH), 3.95 (s, Aromatic-O**CH$_3$**), 2.5 (t, Aromatic-$OCH_2CH_2\mathbf{CH_2}COOH$), 2.1 (s, $OC(dO)CHdCH_2C\mathbf{H_3}$), 1.9 (m, Aromatic-$OCH_2\mathbf{CH_2}CH_2COOH$), 1.55 (d, Aromatic-$CHC\mathbf{H_3}$).

2.2. Synthesis of photocleavable phospholipid polymer (PMB-PL)

The photocleavable phospholipid polymer (PMB-PL) was synthesized via the conventional radical polymerization method using an α,α'-azobisisobutyronitrile (AIBN) initiator. The procedure was completed in a glass tube. The MPC (0.25 mol), BMA (0.50 mol), and photocleavable monomer (0.25 mol) were dissolved in a dioxane/ethanol mixture (1:1 by vol.) at a final concentration of 0.38 M. Thereafter, AIBN (0.38 mM) was added to the solution. The solution was purged with argon gas for 10 min and the glass tube was then sealed. The sealed tube was placed in an oil bath at 60 °C for 48 h. Following polymerization, the PMB-PL was precipitated from diethyl ether/chloroform (3:2 by vol.), and the solid product was collected. The PMB-PL solid was dried overnight, under reduced pressure.

The chemical structure of PMB-PL was confirmed using ^{1}H NMR (300 MHz, JEOL, Tokyo, Japan) and FT-IR (FT-IR 615, JASCO, Tokyo, Japan) spectroscopies. The molecular weight of PMB-PL was measured using a gel-permeation chromatography (GPC) system fitted with an OHpak SB-804HQ column (Shodex®, Showa Denko KK, Tokyo, Japan).

2.3. Surface characterization of PMB-PL

The glass cover slide (18 mm × 18 mm, thickness 0.12–0.17 mm, Matsunami, Tokyo, Japan) was cleaned using ultrasonication in hexane, ethanol, and chloroform solutions at RT for 20 min; then, the slide was treated with oxygen plasma. The glass slides were immersed in a 0.5% (w/v) PMB-PL ethanol solution, and were then dried under reduced pressure. To evaluate the photoreactive property of the PMB-PL surface, a UV-irradiation instrument (Spot-cure SP7, Ushio Inc., Tokyo, Japan) equipped with a 250-W UV lamp (UXM-Q256BY, Ushio Inc., Tokyo, Japan) was used. The power density of the UV source was 80 mW/cm^2.

Surface characterization of the PMB-PL coating was analyzed using Fourier transformed-infrared reflection adsorption spectroscopy (FT-IRRAS), X-ray photoelectron spectroscopy (XPS), static contact angle, ellipsometry, and surface ζ-potential measurement.

A FTIR-500 (JASCO, Tokyo, Japan) was used for the FT-IRRAS spectra measurement. The spectra were obtained under dry conditions at a resolution of 4 cm^{-1} and a scan number of 128.

The XPS spectra were measured using an AXIS-His instrument (Shimadzu/Kratos, Kyoto, Japan) equipped with a monochromatized, Mg-focused, X-ray source. High-resolution scans of C_{1s}, N_{1s}, O_{1s}, P_{2p}, and Si_{2p} were acquired at a photoelectron take-off angle of 90°. The energies in all spectra were corrected using the C_{1s} energy calibration peak at 285 eV.

Static water contact angle measurements were conducted at RT using a CA-W automatic contact-angle meter (Kyowa Interface Science, Tokyo, Japan). The water-in-air and air-in-water systems were applied in this study. In the water-in-air system, the typical protocol involved using a constant drop volume (200 μL) of ultra-pure water onto the surface. For the air-in-water system, the surfaces were horizontally submerged in ultra-pure water. Air bubbles were positioned on the undersides of the surfaces using a syringe equipped with a U-shaped needle. The water drops and air bubbles were monitored using a charge-coupled device (CCD) camera. The captured images were analyzed using FAMAS software (Kyowa Interface Science, Tokyo, Japan) to determine the static contact angle. The contact angle was calculated as the average of more than five values taken at different positions.

The thickness of the PMB-PL was measured under dry conditions using an ellipsometer (alpha-SE®, J.A. Woollam Co., Inc., Lincoln, NE, USA) with a He–Ne laser (632.8 nm) at a 70° incident angle. The refractive indices (n_r) of the Parylene C and poly(MPC) used in the measurement were 1.63 and 1.49, respectively, and both extinction coefficients (k_e) were 0.00. All measurements were conducted under RT air conditions. Data were collected at eight different locations from each sample.

The surface ζ-potential was measured in a 10 mM NaCl solution using a measurement unit (ELS-6000, Photal, Otsuka Electronics Co. Ltd., Osaka, Japan) with an ancillary flat plate cell (10 mm × 30 mm × 60 mm) coated with poly(acrylamide) at 25 °C. Polystyrene latex particles coated with hydroxypropyl cellulose were used as the mobility-monitoring particles.

2.4. Cell attachment/detachment at the PMB-PL surface

HeLa cells were cultured in a 100-mm cell culture dish at 37 °C in 5% CO_2 atmosphere using DMEM containing 10% FBS. After the cells reached sub-confluency, the old media was aspirated; the cells were rinsed with phosphate buffered saline (PBS) and then were exposed to trypsin (1 mL) for 2 min to detach the cells from the surface. The detached cells were added to fresh DMEM, and the cell suspension was centrifuged at 1000 rpm for 3 min. After centrifuging, the supernatant was aspirated and the HeLa cells were suspended in DMEM for the following experiments.

The PMB-PL coated cover-glass surfaces were placed into each well of a 24-well-plate cell-culture dish, sterilized with ethanol, and then washed with PBS. A cell suspension (2.0×10^4 cells/mL, 2 mL) was seeded on the PMB-PL surface and incubated under 5% CO_2 at 37 °C. After incubation for 4 h, unattached cells were washed off with warm fresh medium and the attached cells were observed

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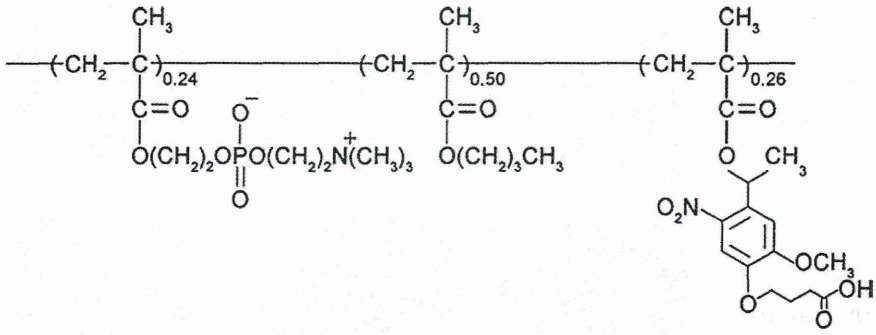

Fig. 1. Chemical structure of photoreactive phospholipid polymer (PMB-PL).

using a phase-contrast microscope. UV light (360 nm, 80 mW/cm^2) was administered to the PMB-PL surface for 60 s. Detached cells following UV-irradiation were recovered and calculated for cell density using a hemocytometer. After irradiation, the culture dish plate was washed with PBS, and the remaining cells were detached using the abovementioned trypsin method. Detached cells were counted and cell counts were converted to cell density per unit area (cells/cm^2).

3. Result and discussion

A photoreactive phospholipid polymer (PMB-PL) was synthesized with MPC, BMA, and PL monomer via the conventional radical polymerization technique. The chemical structure of PMB-PL is shown in Fig. 1. The monomer unit composition of the PMB-PL polymer was calculated by ^{1}H NMR measurement as MPC/BMA/PL = 0.24/0.50/0.26. The PMB-PL was soluble in organic solvents such as alcohol, dimethylsulfoxide, and dioxane. The molecular weight was $M_w = 1.43 \times 10^4$ and average molecular weight was calculated by GPC measurement based on poly(ethylene oxide) (PEO) standards to be M_w/M_n was 1.31.

To evaluate the photochemical activity, PMB-PL was dissolved in ethanol and its spectral change in response to UV light irradiation ($\lambda > 200$ nm) was examined. Before UV irradiation, the solution showed absorption transitions at 300 nm and 348 nm typical for a 3,4-dimethoxy-6-nitrophenyl group [30]. After UV irradiation, the spectrum showed a dose-dependent decrease at the 348-nm transition while two new adsorption transitions appeared at 265 nm and 375 nm. These new adsorption peaks belong to the 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoic acid photoproduct, which indicates that the PMB-PL in bulk solution undergoes a photochemical reaction that is characteristic of the 2-nitrobenzyl ester. The PMB-PL after photoirradiation can be soluble in methanol, ethanol, and dimethyl sulfoxide.

The surface of the quartz crystal glass was subjected to UV-ozone treatment, and the glass was immersed in a PMB-PL ethanol solution for several minutes. This process was repeated thrice. After UV irradiation ($\lambda > 200$ nm), the PMB-PL modified glass was washed with distilled water and the UV spectrum was measured. Before UV irradiation, the modified substrate surface showed a similar absorption spectrum between 250 and 400 nm (Fig. 2) as that of the PMB-PL in solution phase. After UV-irradiation, the transition intensity at 348 nm decreased similar to that of PMB-PL

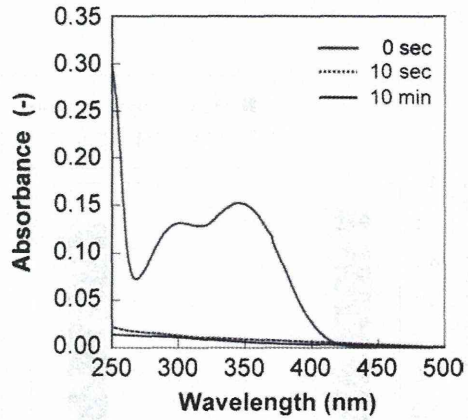

Fig. 2. Absorbance spectra of PMB-PL modified quartz glass surface, which were measured under varied UV-irradiation times.

in bulk solution. However, the transitions at 265 nm and 375 nm corresponding to the elimination of the 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoic acid photoproduct by washing, were not observed. These results indicate that the PMB-PL retained its photochemical activity.

The changes in the surface features before and after UV-irradiation are summarized in Table 1. XPS analysis indicated that the PMB-PL modified surface had a phosphorus peak, a nitrogen peak, an oxygen peak, a silicon peak, and a strong carbon peak. After a 10 min UV-irradiation period, the *P*/*C* ratio on the PMB-PL modified surface increased and the N/C, O/C ratios decreased. These results support the notion that the ester groups on the PMB-PL surface are photocleaved under UV-light.

Ellipsometric measurement revealed that the thickness of the PMB-PL surfaces were 25 ± 7 nm under dry conditions. In addition, from atomic force microscopy (AFM, Nihon Veeco, Tokyo, Japan) observations, the root mean square roughness (RMS) of the PMB-PL surface was calculated as 0.826, which suggests a smooth surface obtained by spin coating (data not shown). The static wettability of the PMB-PL surface was estimated for the air-in-water and water-in-air systems (Table 1). During UV-irradiation of the air-in-water system, the water contact angle ($\beta = 180° - \theta$) was changed from 48° to 34°, which suggests a more hydrophilic surface was observed after 10 min of photolysis. This result indicates that the

Table 1
Changes in PMB-PL surface features before and after UV-irradiation.

PMB-PL surface	Ellipsometric thickness (nm)	Contact angle (°)		*P*/*C* (-)	ζ-potential (mV)
		Water-in-air	Air-in-water		
Before irradiation	25 ± 7	88	48	0.088	−44.3
After irradiation	25 ± 7	111	34	0.145	−2.1

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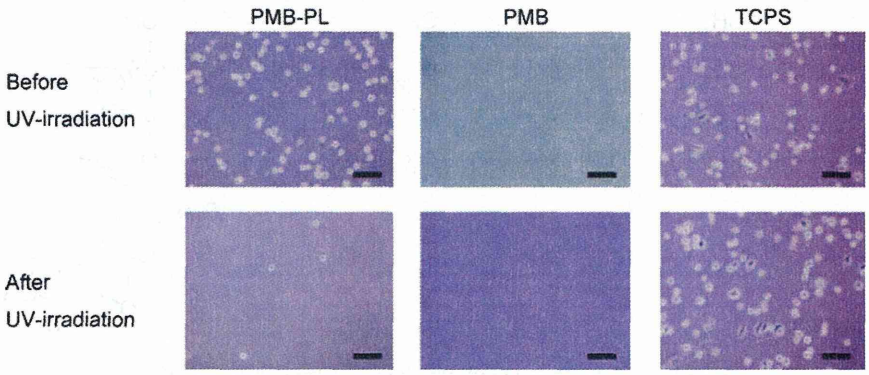

Fig. 3. Phase contrast microscope images (scale = 100 μm) displaying HeLa cells on PMB-PL, PMB, and TCPS surfaces. The upper images were taken before UV irradiation and the lower images were taken following irradiation.

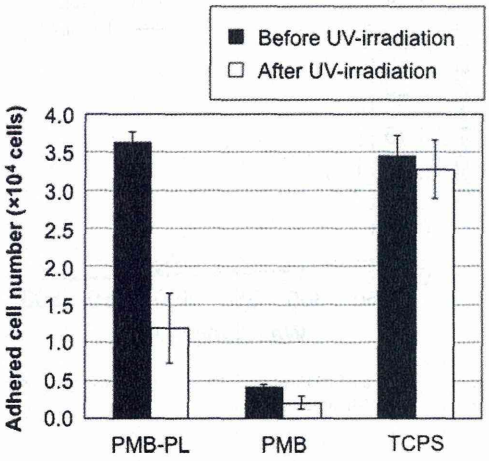

Fig. 4. Adhered cell number on PMB-PL, PMB, and TCPS surfaces shown on left side and detached cell number after UV-irradiation for respective surfaces on right.

surface was mostly converted to the hydrophilic phosphorylcholine (PC) groups, which results from removing the photocleavable PL groups from the substrate. In contrast, for the water-in-air system, the surface static contact angle changed from 88° to 101° which indicates a more hydrophobic surface was obtained after 10 min of light exposure. These results occur because under dry conditions, the PC groups migrate into the inner area and, consequently, leave the hydrophobic butyl methacrylate (BMA) units covered at the uppermost substrate surface. This indicates that surface chemical composition and surface wettability can be controlled using an external UV-light stimulus.

The surface ζ-potential of the PMB-PL surface was −44.3 mV, which is strongly negative. During UV irradiation, the surface ζ-potential changed to −2.1 mV (Table 1), which is a result of an increase in the composition of the MPC unit in the PMB-PL. This increase is attributed to an increase in PL unit photocleavage. It is well reported that the zwitterionic PC groups in PMB-PL surface form an inner salt and thus the electrostatic effects diminish [31–34]. When the composition of the MPC units increased, the surface ζ-potential of the PMB-PL surface was close to zero. This result was in agreement with the results of the static contact angle measurement. From the contact angle measurement and surface ζ-potential measurement, it is concluded that the negatively charged hydrophilic PMB-PL surface is changed to a neutrally charged more hydrophilic surface during UV-photolysis.

Cell attachment and detachment on the PMB-PL surface with UV-irradiation were also examined. In this experiment we used the photoreactive PMB-PL surface, a PMB surface that did not contain the photocleavable PL moiety, and the conventional tissue culture treated polystyrene (TCPS). Fig. 3 shows the phase-contrast

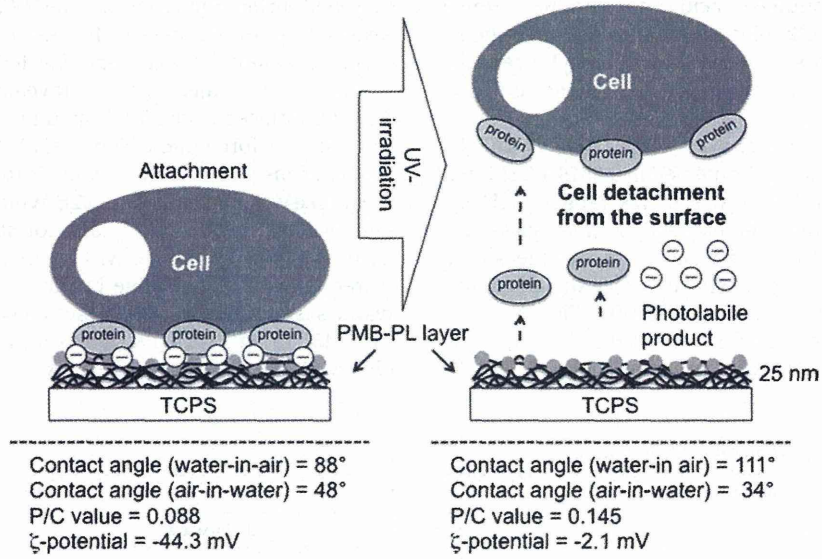

Fig. 5. Schematic of cell attachment/detachment at PMB-PL surface based on alteration of surface properties following UV-irradiation.

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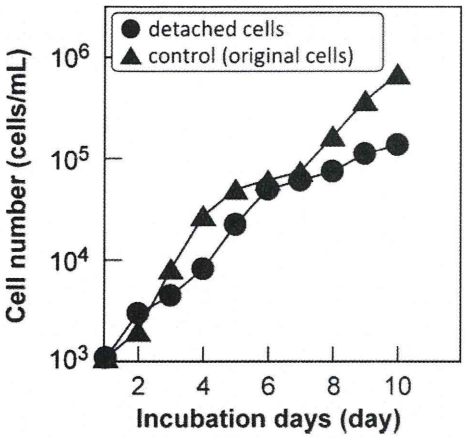

Fig. 6. Cell number dependency on incubation time. The cells were detached from the PMB-PL surface using UV-irradiation.

microscope images of the HeLa cells before and after UV irradiation, and Fig. 4 shows the cell density on each surface before and after irradiation. From the calculations, it was concluded that more than 90% of the seeded cells were attached onto the PMB-PL surface, and 67% of attached cells were detached by photo-irradiation. In the case of the PMB-only surface and the TCPS, less than 5% of the seeded cells were detached following UV-irradiation. This result indicates that UV-exposure induces detachment of attached cells. The proposed mechanism by which this occurs is as follows: the cells are initially bound to the cell-adhesive proteins via the photocleavable PL unit; the PL units are cleaved following the photochemical reaction and the non-biofouling surface of phosphorylcholine groups remain intact at the substrate surface. In generally, the cells were adhered through the adsorbed protein on the substrate. In this study, the cell adhesive experiment was performed in the serum containing medium. Under the condition, it is considered the protein adsorption from the medium is considered to have occurred prior to the cell adhesion. Fig. 5 shows a schematic of cell attachment/detachment processes at the PMB-PL surface based on alteration of the surface properties using UV-irradiation. These results demonstrate the selective detachment of cells at the PMB-PL modified surface, which was related to the photocleavage of the PL unit using UV-irradiation.

Cell attachment and detachment behavior at the PMB-PL surface was also observed using fibroblast cells, L929 (data not shown). These results indicate that the mechanism of cell attachment and detachment on the PMB-PL surface was a consequence of the change in surface properties due to the photocleavage of the PL unit.

We also examined cell proliferation activity after the photoinduced detachment. The detached cells were cultured under usual culture conditions. Fig. 6 shows the cell proliferation of the detached HeLa cells from the PMB-PL surface. The detached cells from PMB-PL after photoirradiation proliferated at the same rate as the normal (original) cells cultured under usual conditions. The PMB-PL detached cells maintained their physiological properties, indicating that the UV-irradiation process did not affect the cell viability, and the PMB-PL surface non-invasively recovered the attached cells.

4. Conclusions

A photoreactive and cytocompatible phospholipid polymer, PMB-PL, was prepared and its surface properties were characterized. The substrate was modified to an extent that it allowed for the study of the photocleaving properties at the surface. Before UV-irradiation, the PMB-PL surface was negatively charged and relatively hydrophobic, which provided protein adsorption and cell adhesion. After irradiation, the surface was neutrally charged and hydrophilic because of the MPC unit. The PMB-PL surface induced cell attachment, and was externally stimulated using UV-light allowing cell detachment from the surface, while maintaining cell viability. Furthermore, the PL monomer unit has a carboxylic group in the side chain, which provides a site for conjugation by desired biomolecules at the PMB-PL surface. The PMB-PL surface is a valuable tool to investigate the bioactivity of conjugated biomolecules, and affords a selective mechanism by which specific cells can be recovered from the surface using UV-light. Selective cell collection and analysis of the cell function at the surface will be reported elsewhere. The development of the PMB-PL surface and the selective detachment of the cells using UV-irradiation has been shown to be a promising and valuable technique for applications in cell analysis and more specifically single-cell analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.colsurfb.2011.08.029.

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Simple surface treatment using amphiphilic phospholipid polymers to obtain wetting and lubricity on polydimethylsiloxane-based substrates

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ABSTRACT

Simple surface treatment of polydimethylsiloxane (PDMS) substrates was performed using an aqueous-ethanolic solution of amphiphilic phospholipid polymers to reduce the hydrophobic and high friction characteristics of PDMS. The phospholipid polymers, poly(2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-2-ethylhexyl methacrylate (EHMA)-*co*-2-(*N*,*N*-dimethylamino)ethyl methacrylate) (PMED) and poly(MPC-*co*-EHMA) (PMEH) were synthesized, and the effects of the electric charge of the polymer chain on the stability of the attachment to the PDMS surface was investigated. The polymers were dissolved in a mixed solvent of ethanol and water, and the PDMS samples were treated by a simple dipping method using the polymer solution. Pure ethanol as the solvent was ineffective for the attachment of the polymers to the PDMS surface. It was considered that the hydrophobic interactions and electrostatic attraction forces between the polymer chains and the PDMS surface were too weak for efficient interaction in this solvent. On the other hand, the surface wettability and lubricity of PDMS could be improved by treatment with an aqueous-ethanolic solution of PMED. The static contact angle was decreased from 90° to 20° by this treatment, and the dynamic friction coefficient against a Co–Cr ball was decreased by nearly 80% compared with that of the untreated PDMS. The hydrophobic interactions and electrostatic attraction forces generated by PMED were both essential for the stable adsorption of the polymer layer on PDMS. Furthermore, the solubilized state of the polymers affected the adsorption of the polymer. We concluded that the surface of PDMS could be stably modified using aqueous-ethanolic solutions of PMED without the need for pretreatments.

1. Introduction

Polydimethylsiloxane (PDMS) is one of the most frequently used materials for medical devices such as catheters, endoscopes, dentures, finger joints, and contact lenses owing to its attractive properties such as high flexibility, processability, good mechanical properties, high gas permeability and optical transparency [1–3]. However, the native hydrophobicity, high friction, and biofouling tendency of PDMS limit its applications in biological environments. A number of attempts have been made to decrease the hydrophobicity and suppress the nonspecific adsorptions of PDMS by means of surface modification [4–10]. Oxygen plasma treatment is one such technique that has been widely used for modification of the surface hydrophobicity. However, the hydrophilicity conferred by this process is temporary and the surface recovers its hydrophobic property within a short time [11–13]. In addition, the oxygen plasma cannot be applied to the inner surfaces of thin tubes such as catheters and endoscopes from the viewpoint of mean free path. Surface modification of PDMS using biocompatible polymers represents an alternative technique for changing the surface properties. The use of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers has proved to be particularly promising for improving the biocompatibility of the surface of biomedical devices [14–17]. MPC polymers contain extremely hydrophilic phosphorylcholine groups in their side chain, and surfaces covered with MPC polymers exhibit good wettability, low friction, and resistance to protein adsorption [18–21]. In fact, MPC polymers have been applied to several medical devices such as artificial hip joints [22,23], implantable blood pumps, cardiovascular stents, and contact lenses. Surface modification of PDMS with MPC has been undertaken in various studies by means of either chemical reaction or physical adsorption. Goda et al. introduced poly(MPC) (PMPC) chains onto the PDMS surface by photoinduced graft polymerization [24,25]. Iwasaki et al. modified the PDMS surface by using well-defined ABA-type triblock copolymers composed of PMPC segments (A) and a central PDMS segment (B) with anchoring vinyl groups. The hydrosilylated PDMS surface reacted with vinyl groups of the B

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