

Synthesis of Photoreactive Phospholipid Polymers for Use in Versatile Surface Modification of Various Materials to Obtain Extreme Wettability

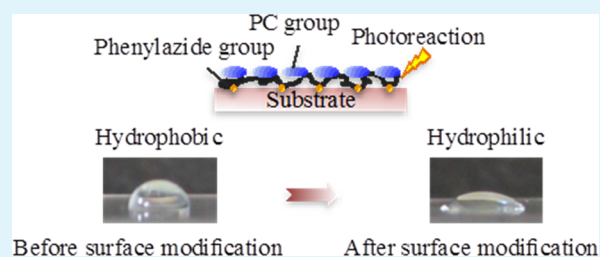
Kyoko Fukazawa[†] and Kazuhiko Ishihara^{*,†,‡}

[†]Department of Materials Engineering, [‡]Department of Bioengineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

S Supporting Information

ABSTRACT: We synthesized photoreactive phospholipid polymers for use in the versatile surface modification of various materials. The photoreactive methacrylate derivative 2-methacryloyloxyethyl-4-azidobenzoate (MPAz) was synthesized. MPAz was copolymerized with 2-methacryloyloxyethyl phosphorylcholine (MPC) to obtain poly(MPC-co-MPAz) (PMPAz) and with both MPC and *n*-butyl methacrylate (BMA) to obtain poly(MPC-co-BMA-co-MPAz) (PMBPAz). PMPAz and PMBPAz were bound to the surfaces of various materials, including polymers, glass, and metals, by exposure to ultraviolet irradiation. The azide groups in the MPAz units played a role in the surface anchoring; the polymer was bound covalently to the substrate. After the photoreaction, the surfaces were converted from hydrophobic to superhydrophilic, and their cell adhesion was effectively suppressed.

KEYWORDS: biomaterials, modification, photochemistry, phospholipid polymer, wettability



Before surface modification After surface modification

1. INTRODUCTION

The surface properties of materials are important factors in determining interfacial phenomena such as adhesion, adsorption, and reactions occurring on the surface. In particular, materials used in biomedical applications require well-controlled interfacial phenomena under biological conditions to avoid unfavorable bioresponses.^{1–3} The hydrophilicity of a material is known to influence biological responses such as the adsorption of proteins from the surrounding fluid.^{4–6} In the development of medical devices and implants, materials such as polymers, metals, and ceramics are chosen based on their mechanical properties, processing ability, and stability under biological conditions. On the basis of this information, a versatile surface modification process that is applicable to various materials should be widely useful.

One candidate for surface modification purposes is 2-methacryloyloxyethyl phosphorylcholine (MPC), which possesses extremely hydrophilic phosphorylcholine groups. MPC polymer surfaces exhibit superhydrophilicity, possess low friction, and are nonbiofouling by virtue of their resistance to protein adsorption and cell adhesion. MPC-based polymers have already been applied to the surfaces of some medical devices, including artificial hip joints, implantable blood pumps, cardiovascular stents, and contact lenses.⁷

The challenge in using MPC polymers for surface modification is to develop a process to bind MPC polymers on various base material surfaces in a simple and stable manner. The selling points of photoreactions are their short reaction time and efficient reaction conversion; this type of chemistry

can also be applied during the fabrication of medical devices. Also, photoirradiation does not significantly damage the bulk properties of the prospective materials when using selected wavelengths.^{8,9} We designed a new photoreactive monomer bearing a phenylazide group (MPAz), which polymerizes with other monomers by a conventional radical polymerization procedure, resulting in a polymer with phenylazide side chains. The phenylazide groups are decomposed by ultraviolet (UV) irradiation and form nitrene groups—highly reactive radical groups that can covalently bind to alkyl groups.^{10–12} Thus, MPC-based polymers containing MPAz units can be bound to the surfaces of different materials by applying UV irradiation. The photoreaction of the polymer and the wettability of the various materials after the surface modification were investigated.

2. EXPERIMENTAL SECTION

2.1. Materials. MPC was synthesized by a previously reported method.¹³ Thionyl chloride and 4-azidobenzoic acid were reagent grade (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). Triethylamine (TEA), 2-hydroxyethyl methacrylate (HEMA), ligroin, and *n*-butyl methacrylate (BMA) were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). *n*-Butyltrichlorosilane was purchased from Sigma-Aldrich (St. Louis, MO, USA). HEMA and TEA were purified by

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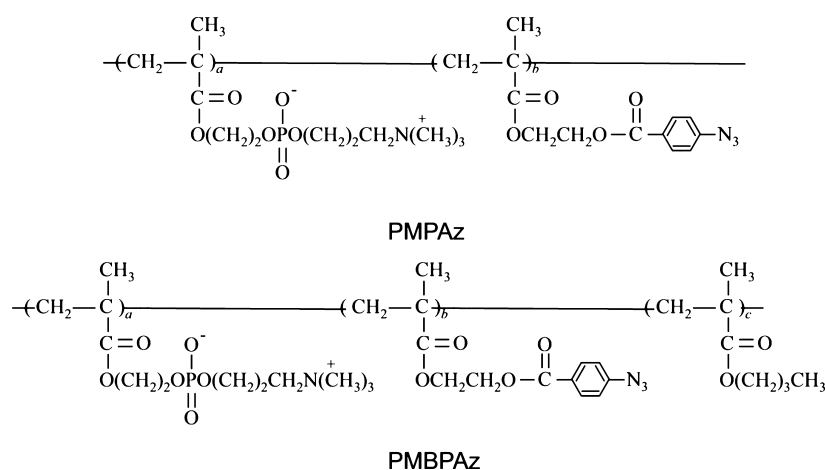


Figure 1. Chemical structures of photoreactive phospholipid polymers.

Table 1. Synthetic Data of Photoreactive Phospholipid Polymers^a

abbrev.	composition (mol %)						yield (%)	M_w^c ($\times 10^4$)	M_w/M_n
	In feed			In copolymer ^b					
	MPC	BMA	MPAz	MPC	BMA	MPAz			
PMPAz73	70	0	30	75	0	25	67	6.1	3.1
PMPAz82	80	0	20	85	0	15	66	7.8	3.3
PMPAz91	90	0	10	94	0	6	65	7.4	3.1
PMBPaz631	60	30	10	70	21	9	58	7.4	3.0
PMBPaz721	70	20	10	79	12	9	61	7.9	3.2
PMBPaz811	80	10	10	87	5	8	67	8.1	3.2

^a[Monomer] = 0.5 M, [AIBN] = 2.5 mM. Reaction temperature 60 °C. ^bDetermined by ¹H NMR. ^cWeight-averaged molecular (M_w) and number-averaged molecular weight (M_n) were determined by GPC measurement in water/methanol = 3:7 with PEG standard.

distillation, and the fractions at the boiling points (74 °C/0.6 kPa and 87 °C, respectively) were collected and used. Other reagents and solvents were commercially available. Cyclic polyolefin (CPO) was obtained from Sumitomo Bakelite Co., Ltd. (Tokyo, Japan). Polyethylene (PE), polystyrene (PS), poly(methyl methacrylate) (PMMA), and polypropylene (PP) were commercially available products. Surface alkyl groups were introduced on glass and titanium using *n*-butyltrichlorosilane. After cleaning with PR500, an oxygen plasma reactor (Yamato Science, Tokyo, Japan), for 1 min under an oxygen flow of 100 mL min⁻¹, the glass and titanium substrates were immersed in a toluene solution containing *n*-butyltrichlorosilane (2.5 mM) for 1 h at room temperature. Next, these substrates were sonicated in toluene and methanol to obtain alkyl-group-treated glass (AG) and alkyl-group-treated titanium (AT).

2.2. Synthesis of 2-Methacryloyloxyethyl-4-azidobenzoate (MPAz). MPAz was synthesized according to a previously reported method.¹⁴ Briefly, 4-azidobenzoyl chloride (9.0 g, 0.050 mol) was dissolved in diethyl ether (80 mL) and cooled to -2 °C. A solution of diethyl ether (50 mL) containing both HEMA (50 mmol) and TEA (50 mmol) was added to the stirred solution over a period of 1 h. After the mixture was reacted for 20 h, the resulting triethylamine hydrochloride was filtered, and the unreacted portion was extracted using aqueous HCl (10 mM). The diethyl ether layer was dehydrated using anhydrous magnesium sulfate. After removing the magnesium sulfate by filtration, the solvent was evaporated under reduced pressure. MPAz was obtained as a light yellow oil. Upon storing at -20 °C, MPAz formed a light yellow solid. The structure of MPAz was analyzed using ¹H

nuclear magnetic resonance (NMR) spectroscopy in CDCl₃ and Fourier-transform infrared spectroscopy (FT-IR; FT-IR-6300, JASCO, Tokyo, Japan). The NMR results are as follows (ppm): 1.95 (s, 3H, α -CH₃), 4.47–4.53 (q, 2H, -CH₂CH₂), 4.55–4.58 (q, 2H, -CH₂CH₂), 5.59 (s, 1H, =CH₂), 6.14 (s, 1H, =CH₂), 7.06–7.09 (d, 2H, benzyl), 8.02–8.05 (d, 2H, benzyl). The IR results are as follows (cm⁻¹): 3022 (*p*-Ar), 2121 (C–N₃), 1718 (C=O), 1604 (C=C).

2.3. Synthesis of Photoreactive Phospholipid Polymer. Water-soluble photoreactive phospholipid polymers, poly(MPC-*co*-MPAz) (PMPAz) and poly(MPC-*co*-BMA-*co*-MPAz) (PMBPaz), were synthesized using a conventional radical polymerization method in ethanol, using 2,2-azobisisobutyronitrile (AIBN) as an initiator at 60 °C. The reaction mixture was poured into an excess of diethyl ether/chloroform (80:20 v/v) to precipitate the polymer. The precipitated polymer was collected by filtration and redissolved in water to purify by dialysis against water in the dark, using a dialysis membrane (molecular weight cutoff of 3000). Next, the aqueous polymer solution was freeze-dried. The chemical structures of PMPAz and PMBPaz were confirmed using ¹H NMR in CD₃CD₂OD. The molecular weight of the polymers was evaluated using gel permeation chromatography in a water/methanol mixture (30:70 v/v), and the retention times were compared with that of poly(ethylene glycol) standard samples (Tosoh Co., Tokyo, Japan). The chemical structure and the results from the synthesis of PMPAz and PMBPaz are shown in Figure 1 and Table 1, respectively.

2.4. Surface Modification Process. PMPAz and PMBPaz were dissolved in ethanol to make a 0.20 wt % solution. The

various material substrates were washed in ethanol before the surface modification. Each substrate was immersed in a polymer solution for 10 s, followed by solvent evaporation at room temperature and atmospheric pressure. The surfaces were subsequently irradiated with a UV lamp for 1 min (254 nm, 500 mJ cm⁻²). The photoreaction of the phenyl azide group of the MPAz unit was confirmed by FT-IR spectroscopy with attenuated total reflection equipment (IMV-4000; JASCO) (see Figure S1 in the Supporting Information).

2.5. Surface Characterization. The hydrophilicity of each surface after the photoreaction was evaluated by water and air contact angle measurements with a static contact angle goniometer (CA-W; Kyowa Interface Science Co., Tokyo, Japan). All substrates were immersed in water for 1 h after the photoreaction to remove unreacted polymer and then dried in air before the contact angle measurement. For the measurement of water contact angles under dry conditions, water droplets were brought into contact with the modified surfaces and the contact angles were measured within 10 s. Photographic images were used to determine the contact angles. Air contact angles were measured in water by attaching the samples to a custom holder, which was transferred to a glass vessel filled with distilled water. After 5 min, air bubbles were introduced underneath each sample through U-shaped needles. The contact angles were measured using photographic images from 30 positions for each sample.

The thickness of the polymer layer on the various substrates was determined under dry conditions with a spectroscopic ellipsometer (J.A. Woolam Co. Inc., Tokyo, Japan). The PS, PE, PP, and AT substrates, and the polymer layer on each, were measured at an incident angle of 70° in the visible region. The thickness of the modified polymer layer was determined using the Cauchy layer model with an assumed refractive index of 1.49 at 632.8 nm. Data were collected at three positions for each sample.

2.6. Cell Adhesion. A cell adhesion test was performed using HeLa cells on the PMPAz73-modified PE substrate. PMPAz73 as an aqueous solution (0.2 wt %) was spotted on the PE at five drops over 500 μm intervals using a piezoelectronic inkjet-printing instrument, (DeskViewerTM, Cluster technology, Osaka, Japan) with a 60-μm-sized nozzle, and then irradiated with a UV lamp. The substrate was sterilized with ethanol and then dried and placed in a 24-well tissue culture plate. The HeLa cells (4.0 × 10⁴ cells cm⁻²) were routinely cultured on the substrate in the culture medium (Dulbecco's modified Eagle's medium, Gibco, Grand Island, USA) containing 10% fetal bovine serum at 37 °C for 2 d in a humidified atmosphere of air containing 5% CO₂. After the medium was removed, the substrates were rinsed twice with phosphate buffered saline (PBS) and then transferred to new wells. The cells on the surface were observed using an Olympus phase-contrast microscope (model 1 × 71, Tokyo, Japan).

3. RESULTS AND DISCUSSION

3.1. Surface Characterization of Modified Substrates.

The hydrophilicity of the substrate is a commonly used parameter to determine its antifouling properties. Figure 2 shows the water contact angle values of various substrates before and after surface modification with the (a) PMPAz series and (b) PMBPaz series. For all the substrates, the water contact angles decreased after surface modification with PMPAz73, PMBPaz631, and PMBPaz721. In the PMBPaz series, the water contact angles were approximately 20° higher

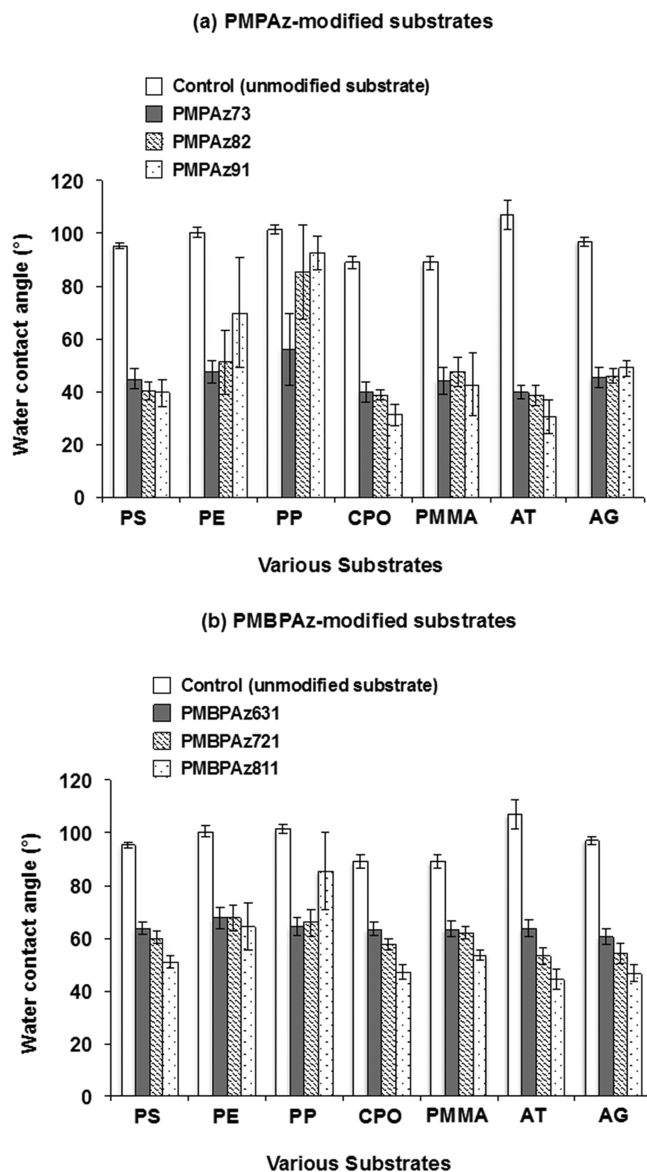


Figure 2. Water contact angles of various substrates before and after surface modification with (a) PMPAz series and (b) PMBPaz series.

than those seen in the PMPAz series, because of the presence of the BMA unit. Normally, poly(MPC-co-alkyl methacrylate) is used as a coating material, and the amount of MPC unit is controlled at 30 mol % to avoid the detachment of the polymer after the coating process. The conventional coating process results in surfaces with larger water contact angles under dry conditions, almost similar to the contact angles of the untreated surfaces.

The hydrophobic alkyl methacrylate group is enriched at the air interface, and it decreases the surface free energy. Thus, a surface equilibrium with an aqueous solution is needed to arrange the phosphorylcholine group.¹⁵ However, using surface modification through chemical bonding, an MPC polymer with a higher percentage of MPC unit can be used. One advantage of this method is that PMPAz- and PMBPaz-modified surfaces showed the expected hydrophilicity without requiring a surface equilibrium step. In the PMPAz series, the water contact angles of PP and PE increased with decreasing content of MPaz unit. The results indicate that at least 30 mol % MPaz unit is needed for stable surface modification. In the PMBPaz series, although

the MPAz unit is only present at 10 mol %, PMBPaz631 and PMBPaz721 were bound onto the substrates evenly. We hypothesize that the azide group of the MPAz unit is compatible with the substrate because of the hydrophobic BMA group, and thus can react with the substrate easily. However, the PMBPaz811-modified PP surface showed high contact angles. When the MPC unit content increases, the hydrophilic MPC unit prefers to exist near the substrate after the solvent evaporates, because air is hydrophobic. Thus, the azide group of the MPAz unit cannot react with the substrate evenly. Figure 3 shows the air contact angles of the various

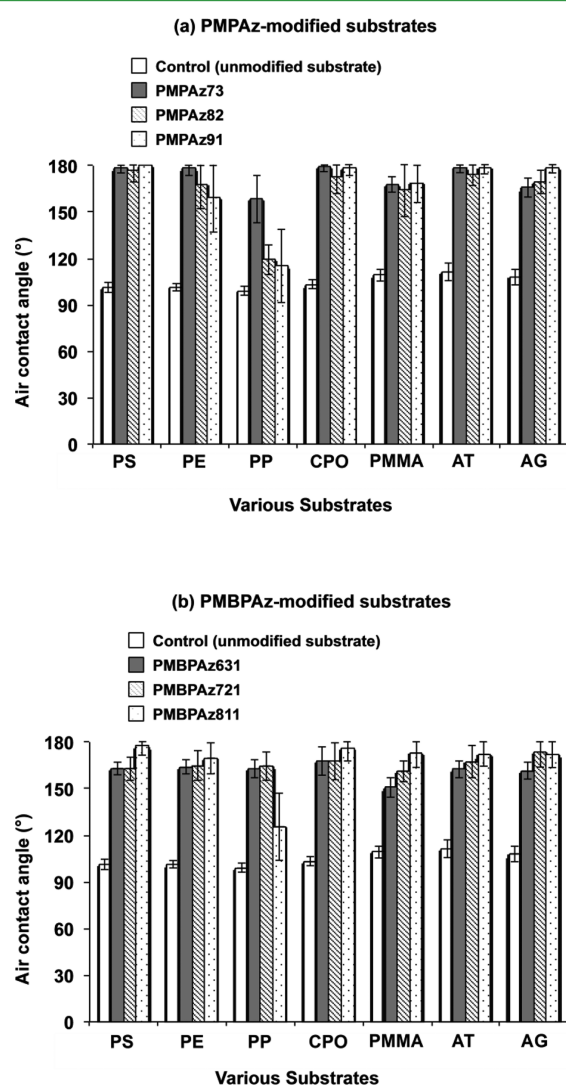


Figure 3. Air contact angles, as measured in water, of various substrates before and after surface modification with (a) PMPAz series and (b) PMBPaz series.

substrates, which were measured in water, before and after surface modification with the (a) PMPAz series and (b) PMBPaz series. In all substrates, the air contact angles increased after the surface modification with PMPAz73, PMBPaz631, and PMBPaz721. For both the water and air contact angle measurements, there was a variability in the contact angle values of PP after modification with PMPAz73 (see Figures S2 and S3 in the Supporting Information). On the other hand, in the case of PMBPaz631 and PMBPaz721, all the surfaces had air contact angle values in the range 150–180°,

measured at more than 20 positions for each sample (see Figure S3 in the Supporting Information). All the treated substrates exhibited superhydrophilicity in water as a result of exposure of the hydrophilic MPC units, which reduced the interfacial free energy.

The thickness of the PMPAz73 layer was approximately 20 nm. The thickness of the PMBPaz721 layer was larger, between 10 and 100 nm. The thickness was unchanged even after immersion in water for 5 days, i.e., the polymer layer was stably fixed on the substrate. This result indicates that the polymers were covalently bound to the substrate, as PMPAz73 and PMBPaz721 are soluble in water.

3.2. Cell Adhesion Behavior. Figure 4 shows a microscopic image of the adhesion of HeLa cells on the PMPAz73-

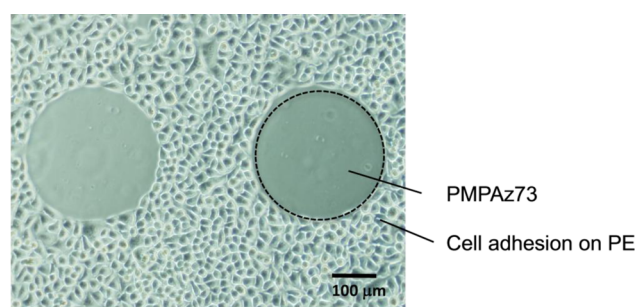


Figure 4. Cell adhesion on pattern-modified PE with PMPAz73.

modified PE substrate. It is clearly observed that the cells did not adhere to the PMPAz-modified areas, even when the cell adhesion reached confluency. PMPAz73 suppressed the adhesion of cell-adhesive proteins from the cell culture medium and did not induce cell adhesion. The characteristics of the MPC polymers prevent protein adsorption and cell adhesion.¹⁶

4. CONCLUSIONS

Photoreactive MPC polymers possessing both extremely hydrophilic phosphorylcholine groups and photoreactive groups were successfully synthesized. The surfaces of various materials were functionalized to become superhydrophilic by a convenient and simple technique using the MPC polymers, which bind covalently to the surface. Furthermore, cell adhesion was suppressed completely at the modified areas. This surface modification procedure is useful for the development of biomedical devices such as implantable artificial organs.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and information (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: 81-3-5841-7124. Fax: 81-3-5841-8647. E-mail: ishihara@mpc.t.u-tokyo.ac.jp

Notes

The authors declare no competing financial interest.

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