ACS APPLIED MATERIALS & INTERFACES

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Photoreactive Polymers Bearing a Zwitterionic Phosphorylcholine Group for Surface Modification of Biomaterials

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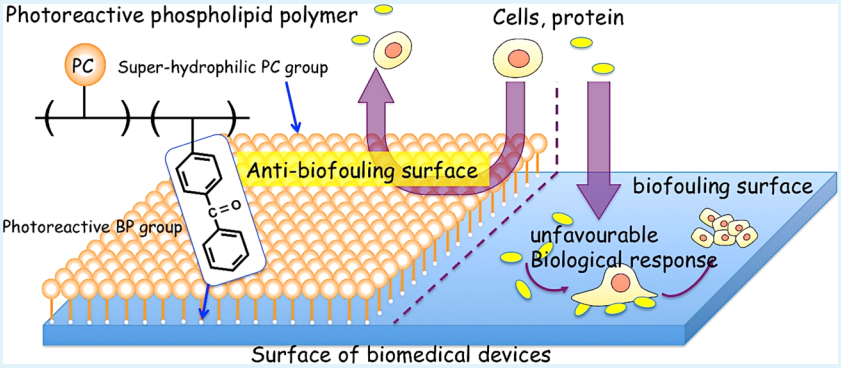
Supporting Information



ABSTRACT: Photoreactive polymers bearing zwitterionic phosphorylcholine and benzophenone groups on the side chain were synthesized and used as surface modification reagents for biomaterials. A photoreactive methacrylate containing the benzophenone group, 3-methacryloyloxy-2-hydroxypropyl-4-oxybenzophenone (MHPBP), was synthesized via a ring-opening and addition reaction between glycidyl methacrylate and 4-hydroxybenzophenone. Then, water-soluble, amphiphilic polymers poly(2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-MHPBP) (PMH) and poly(MPC-*co*-*n*-butyl methacrylate-*co*-MHPBP), with different monomer unit compositions, were synthesized through radical polymerization. Ultraviolet−visible (UV/vis) absorption spectra of these polymer solutions showed that these polymers have maximum absorption peaks at 254 and 289 nm that can be attributed to the benzophenone unit. The intensity of UV adsorption at 289 nm was decreased with increased UV irradiation time, and it was saturated within a few minutes, indicating that the polymers are highly sensitive to UV irradiation. A commercial material (i.e., cyclic polyolefin) was simply modified by a UV irradiation for 1.0 min. Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy analysis results indicated that the stability of the polymer on the surface was dramatically enhanced because of the photochemical reaction of the benzophenone moiety. The air contact angles of PMH surfaces measured in water were up to 160°. Thus, highly hydrophilic surfaces were obtained. The critical surface tension of the PMH-modified surface was 45.7 mN/m. By evaluating the biological reactivity of the treated surface, protein adsorption and cell adhesion were completely inhibited on the surface, which was prepared using a photopatterning procedure using PMH. In conclusion, photoreactive MPC polymers with a benzophenone moiety could be used as a novel and effective surface modifier.

KEYWORDS: *zwitterionic phosphorylcholine group, photoreactive polymer, surface modification, benzophenone, cell adhesion*

INTRODUCTION

Many biomedical devices are commonly used for diagnosis and medical treatment. When in use, they inevitably come in contact with biological components such as proteins, cells, and tissues.[1−4] These materials interact with the surrounding environment through their interfaces. Thus, the control and improvement of material surface properties by modifying chemical structure and physical topography is important for avoiding unfavorable biological responses. Surface modification of conventional materials, such as metals, alloys, ceramics, and polymers, is required to access their intrinsic advantages, including suitable mechanical properties, easy and versatile processing ability, and relative stability under biological conditions.[5−7] When a designed material comes into contact with a biological environment, a nonspecific protein-repelling interface is required to reduce the biological response.[8−10]

Polymeric biomaterials with a zwitterionic phosphorylcholine (PC) group containing phosphate anion and trimethylammonium cation with an inner-salt structure have been studied and applied to biomedical devices.[11−15] Recently, other zwitterionic polymers with carboxybetaine (CB)[16−20] and sulfobetaine (SB) groups[21−25] have been synthesized and investigated as potential biomaterials. Among these zwitterionic polymers, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers are advanta-

Received: June 12, 2015
Accepted: July 23, 2015
Published: July 23, 2015

DOI: 10.1021/acsami.5b05193
ACS Appl. Mater. Interfaces 2015, 7, 17489−17498

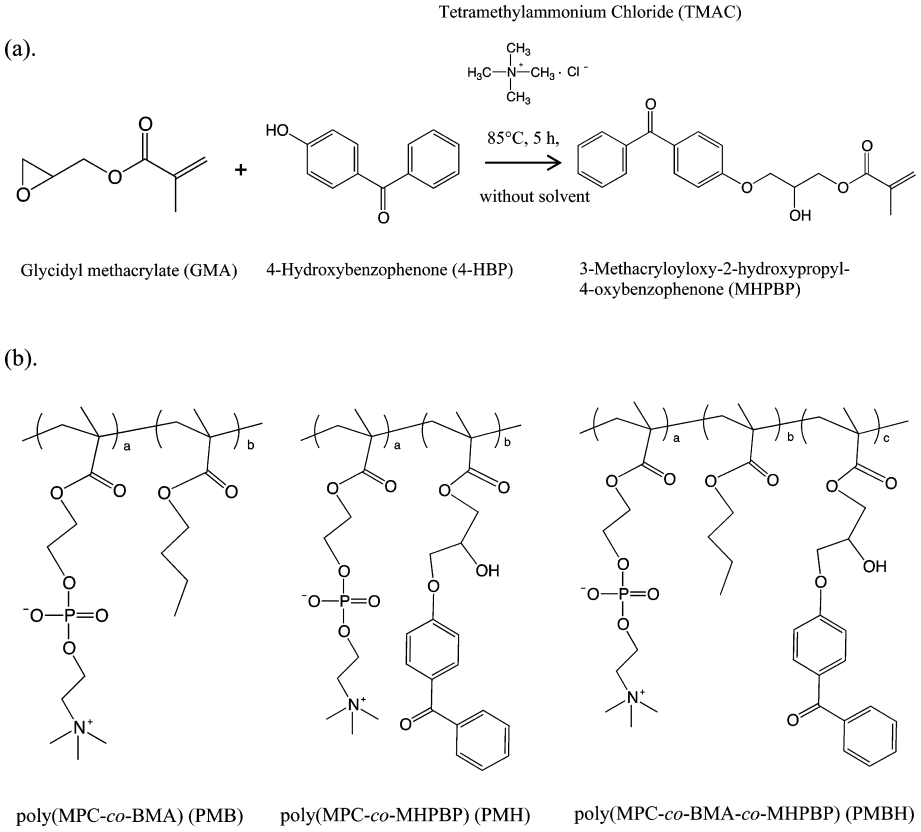


Figure 1. (a) Synthesis of photoreactive monomer 3-methacryloyloxy-2-hydroxypropyl-4-oxybenzophenone (MHPBP) and (b) chemical structures of polymers poly(MPC-*co*-BMA) (PMB), poly(MPC-*co*-MHPBP) (PMH), and poly(MPC-*co*-BMA-*co*-MHPBP) (PMBH).

geous because of their simple molecular design and synthesis process.[26] In fact, many types of MPC polymers have been synthesized by conventional radical polymerization and living radical polymerization.[27−30] As biomaterials, MPC polymers are used for surface coating through the solvent evaporation method. A strong surface coating layer of MPC polymers can be created through physical interaction by controlling the molecular weight, but it can become detached by mechanical stress due to its swelling properties in biological circumstances.[31,32] To address this issue, the MPC polymers are fixed by chemical covalent bonding between their reactive groups or grafting on the substrate.[33−36] Photoreaction is considered to be an effective and reasonable method for modifying polymer materials on biomedical devices through covalent binding.[37−39] Here, we propose a new photoreactive MPC polymer that can be fixed on the substrate by simple photoirradiation. In addition, the photoinduced reaction can be achieved on a very small scale on the substrate, and patterning with MPC polymer may be attained.

The benzophenone (BP) group can produce a diradical under UV irradiation from 250 to 365 nm that abstracts aliphatic hydrogens, especially those that are α to electron-donating heteroatoms (such as nitrogen, sulfur, and oxygen). Through this process, a covalent binding between the BP group and the biomedical devices made from commercial polymeric materials (e.g., polyethylene (PE), polystyrene, poly(ethylene terephthalate), and cyclic polyolefin (CPO)) can be easily achieved. Hence, benzophenone groups have been widely used as photoinitiators to promote chemical conjugation.[40−43] Methacrylate can be transformed into polymers with different shapes through various radical polymerizations very easily via methods such as conventional polymerization and living radical polymerization.[26,44−46] The successful synthesis of a methacrylate-bearing BP group using this simple method promotes the preparation of photoreactive polymers with various desired architectures for surface modification of biomedical devices.

In this study, we synthesized a photoreactive MPC polymer bearing a BP group for the surface modification of versatile biomedical devices based on the following considerations: (1) a novel photoreactive methacrylate, 3-methacryloyloxy-2-hydroxypropyl-4-oxybenzophenone (MHPBP), was synthesized through nonsolvent epoxide ring-opening and addition reactions, which is believed to be an extremely simple method for obtaining a photoreactive monomer; (2) MHPBP has the inability to react with water, which may eliminate the influence of water molecules and increase the photoinduced binding reaction on the substrate under various conditions;[47] and (3) MHPBP has a relatively high photoirradiation wavelength, which may increase the penetrability of light through host devices and reduce the damage caused by UV light.

The photoreactivity of the synthetic MPC polymer was explored, and the surface properties of the modified materials, including protein adsorption and cell adhesion, were evaluated.

EXPERIMENTAL SECTION

Materials. MPC was purchased from NOF Co., Ltd. (Tokyo, Japan), where it was synthesized according to a previously reported method.[48] *n*-Butyl methacrylate (BMA) was purchased from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). Glycidyl methacrylate (GMA) and tetramethylammonium chloride (TMAC) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 4-Hydroxybenzophenone (4-HBP) and butyltrichlorosilane were obtained from Sigma-Aldrich (St. Louis, MO, USA). 2,2′-Azobis-

DOI: 10.1021/acsami.5b05193
ACS Appl. Mater. Interfaces 2015, 7, 17489−17498

Table 1. Synthetic Results of MPC Polymers[a]

	monomer unit composition (mol %)					molecular weight[c]		solubility[d]	
	in feed MPC/BMA/MHPBP	in copolymer[b] MPC/BMA/MHPBP	initiator (mmol/L)	polymzn time (h)	yield (%)	$M_w \times 10^4$	M_w/M_n	ethanol	water
PMB37	30/70/0	33/67/0	N/A	N/A	N/A	>50	N/A	++	−−
PMBH721	70/20/10	78/11/11	5	3	75	7.9	3.0	++	++
PMH82	80/0/20	82/0/18	5	2	36	8.2	3.1	++	++
PMH91	90/0/10	91/0/9	5	3	42	6.2	2.9	++	++

[a][Monomer] = 0.5 mol/L; initiator for all the polymers was AIBN; polymerization temperature was 60 °C. [b]Determined by ^{1}H NMR spectrum in C_2D_5OD. [c]Molecular weights were determined by GPC in methanol/water = 7/3, [LiBr] = 10 mmol/L, poly(ethylene oxide) standards. M_w and M_n represent weight-average molecular weight and number-average molecular weight, respectively. [d]Solubility was determined with 10 mg/mL each polymer sample and described as soluble (+) or insoluble (−) at 25 °C.

(isobutyronitrile) (AIBN) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Cyclic polyolefin (CPO) was obtained from Sumitomo Bakelite Co., Ltd. (Tokyo, Japan). Poly(MPC-*co*-BMA) (PMB37) with 0.30 mole fraction MPC unit in the polymer was obtained from NOF Co., Ltd. Silicon wafers with ∼10-nm-thick SiO_2 layers on the surface were purchased from Furuuchi Chemical Corp. (Tokyo, Japan). Polyethylene (PE) substrates for tissue culture (Φ = 13 mm) were purchased from Sarstedt, Inc. (Newton, NC, USA). Alexa Fluor 488 conjugated fibrinogen from human plasma (Ex/Em, 495/519 nm) was purchased from Life Technologies, Inc. (Frederick, MD, USA). Fibrinogen from bovine plasma was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture medium and its supplements (Dulbecco's modified Eagle's medium (DMEM) with phenol red, fetal bovine serum (FBS), and trypsin (TrypLE express)) and ultrapure distilled water were purchased from Invitrogen (Grand Island, NY, USA). Other organic reagents and solvents were commercially available as extrapure grade reagents and were used without further purification.

Synthesis of MHPBP. MHPBP was synthesized by a reaction between the epoxide group in GMA and the hydroxyl group in 4-HBP. In brief, GMA (0.10 mol, 14.2 g), 4-BHP (0.11 mol, 21.8 g), and TMAC (1.0 wt %, 0.36 g) were mixed in a 500 mL round-bottomed flask equipped with a magnetic stirrer. Consequently, the flask was kept in an oil bath at 85 °C to dissolve the 4-BHP in GMA and ensure the epoxide ring-opening reaction. After reaction for 5.0 h, a reaction mixture with a light-yellow color was obtained and was dissolved in 100 mL of dichloromethane. The purification of MHPBP was performed using a liquid phase extraction with the addition of another 50 mL of aqueous solution containing 0.10 mol/L NaOH. Finally, the organic phase was collected and dichloromethane was removed under reduced pressure. MHPBP was obtained as a white viscous liquid, and its chemical structure was confirmed by ^{1}H NMR (400 MHz NMR spectrometer; JEOL Ltd., Tokyo, Japan) (Figure S-1a). The procedure for the synthesis of MHPBP is shown in Figure 1a.

Synthesis of Photoreactive Polymer. The water-soluble amphiphilic polymers, poly(MPC-*co*-MHPBP) (PMH) and poly(MPC-*co*-BMA-*co*-MHPBP) (PMBH), were synthesized by a conventional radical polymerization method using AIBN as an initiator. The desired amounts of MPC, BMA, MHPBP (total monomer concentration 0.50 mol/L), and AIBN (5.0 mmol/L) were dissolved in 30 mL of ethanol at room temperature. Polymerization was performed in a sealed flask at 60 °C under an atmosphere of argon gas. After that, the reaction mixture was poured into a mixed solvent of ether/chloroform (90/10, v/v) to precipitate the polymer. The polymer was filtered off and collected as a white powder after vacuum desiccation, followed by dialysis against distilled water through a standard regenerative cellulose membrane (molecular weight cutoff, 3.5 kDa; Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) for 7 days. Finally, the polymers were obtained as a white powder after lyophilization using an EYELA FDU-1100 freeze drier (Tokyo Rikakikai Co., Ltd., Japan) at −47 °C for 48 h. The chemical structures of the polymers were confirmed using ^{1}H NMR (Figure S-1b−e), a UV/vis spectrophotometer (V-560; JASCO, Tokyo, Japan) (Figure S-2), and a Fourier transform infrared spectrometer (FT/IR-6300; JASCO) (Figure S-3). The average molecular weights of the polymers were measured using a gel permeation chromatography (GPC) system (JASCO system) in a water/methanol mixture (30/70, v/v) containing 10 mmol/L lithium bromide (LiBr). Poly(ethylene oxide) (Tosoh Co., Tokyo, Japan) was used as the standard for the calibration curve. The chemical structures of PMH and PMBH are shown in Figure 1b, and the molecular properties of the polymer are summarized in Table 1.

Surface Modification Using Photoreactive Polymers. CPO and silicon substrates (1.0 cm × 1.0 cm) were ultrasonically washed in ethanol for 10 min. CPO substrates were used directly for modification of MPC polymers, and silicon substrates were treated with butyltrichlorosilane (2.5 mM, toluene) for 1.0 h before surface modification with polymers. The MPC polymers (PMB37, PMBH721, PMH82, and PMH91) were dissolved in ethanol with a 0.20 wt % concentration. Each substrate was immersed in a polymer solution for 10 s, followed by solvent evaporation at room temperature under atmospheric pressure in an ethanol vapor-protective environment. The surface of the coated substrate was subsequently irradiated with a UV lamp (intensity 250−400 nm, 10 mW/cm^2) for a specific time (1.0, 3.0, and 5.0 min), followed by rinsing with ethanol for 1.0 h at room temperature to remove the unreacted or physically absorbed unstable MPC polymers and dried at 25 °C.

In order to check the photosensitivity of the BP group in the photoreactive MPC polymers, a CPO substrate (0.90 cm × 4.0 cm) was coated with 0.20 mL of PMH82 solution (0.20 wt %, ethanol). Subsequently, the coated substrate was dried at room temperature and put into a rectangular cell of 1.0 cm optical path length (3.0 mL capacity) cell (FP-1004; JASCO), followed by measurement of the absorbance spectrum at different UV irradiation times (intensity 250−400 nm, 10 mW/cm^2).

Surface Characterization. Photoreaction between MPC polymers and the substrate (CPO) was evaluated through the measurement of surface functional groups and elements by Fourier transform infrared (FT-IR) spectroscopy with attenuated total reflection (ATR) equipment (IMV-4000; JASCO) and X-ray photoelectron spectroscopy (XPS). XPS analysis was performed using a Shimadzu/Kratos AXIS-His 165 spectrometer (Kyoto, Japan) equipped with a 15 kV Mg Kα radiation source at the anode. The takeoff angle of the photoelectron was maintained at 90°, and the binding energy (BE) was corrected using the C_{1s} peak at 285 eV as a reference. The presence of characteristic absorption peaks for carbonyl (1720 cm^{-1}) and phosphate (1240, 1080, and 970 cm^{-1}) groups indicates the successful modification of MPC polymer on CPO substrates. XPS analysis was used to confirm that the elements remained on the dip-coated substrate surface after photoirradiation for 1.0 min (intensity 250−400 nm, 10 mW/cm^2) and rinsing with ethanol for 1.0 h. Four elements—carbon (C_{1s}, BE = 285 eV), oxygen (O_{1s}, BE = 533 eV), nitrogen (N_{1s}, BE = 403 eV), and phosphorus (P_{2p}, BE = 133 eV)—were measured. The differences in the surface functional groups and elements were compared using the following five samples: original CPO substrate, PMB37-modified CPO, PMBH721-modified CPO, PMH82-modified CPO, and PMH91-modified CPO substrates.

The surface wettability of each substrate was characterized by measuring the static water and air contact angle with a static contact angle goniometer (CA-W; Kyowa Interface Science Co., Tokyo,

DOI: 10.1021/acsami.5b05193
ACS Appl. Mater. Interfaces 2015, 7, 17489−17498

Japan). Distilled water droplets were deposited onto substrate surfaces, and the water contact angles were measured within 10 s through the analysis of photographic images. For the measurement of the air contact angle, all the samples were immersed into distilled water for 3.0 h before measurement. A custom holder and two magnets were used to attach and fix the sample, which was subsequently immersed into distilled water in a glass vessel. Air bubbles were introduced underneath each sample in contact with the measurement surface through a U-shaped needle. For each sample, five different points were measured.

Critical surface tension values of unmodified CPO substrate and photoreactive MPC polymer modified CPO substrate were obtained using the Zisman-plot method,[49] where test liquids including water (surface tension 72.0 mN/m), glycerol (surface tension 63.7 mN/m), formamide (surface tension 57.8 mN/m), and ethylene glycol (surface tension 47.3 mN/m) droplets were deposited onto substrate surfaces, followed by the evaluation of contact angles within 10 s through the analysis of photographic images with a static contact angle goniometer. For each sample, five different points were measured. A surface tension value against the cosine of the contact angle (θ) of the test liquid was obtained, and the surface energy of the substrate was defined by extrapolating the plot curve to a zero contact angle where the corresponding surface tension value was equivalent to the surface energy.

The thickness of the dip-coated polymer layer on the silicon substrate was measured under dry conditions with a spectroscopic ellipsometer (α-SE; J. A. Woolam Co., Inc., Tokyo, Japan) at an incident angle of 70° in the visible region. The thickness of the modified polymer layer was measured and calculated using a Cauchy layer model with an assumed refractive index of 1.49 at 632.8 nm.[50−52]

Protein Adsorption Analysis. Protein adsorption on the surface of a material is very important for evaluating the implanted or blood contact application. A mixture of Alexa Fluor 488 conjugated fibrinogen from human plasma (Ex/Em, 495/519 nm) (0.1 mg/mL) and fibrinogen from bovine plasma (0.9 mg/mL) in PBS (pH 7.4, 1×) was used to evaluate the protein adsorption behavior on the surface of PE substrate modified by PMH82. For the surface modification, PMH82 was dissolved in distilled water with 0.20 wt % concentration, and the polymer solution was spotted on the PE substrate with one drop for every 250 μm interval (0.75 cm × 0.75 cm) using a piezoelectronic inkjet-printing instrument (DeskViewer, Cluster Technology, Osaka, Japan) with a 60-μm-sized nozzle (voltage 14.0 V, frequency 1000 Hz, number 1, wave B, and normal mode). After being irradiated with a UV lamp for 1.0 min (intensity 250−400 nm, 10 mW/cm^2), the substrate was sufficiently rinsed with ethanol (1.0 h, 25 °C). Subsequently, the protein solution (2.0 mL, 1.0 mg/mL) was added to a 24 well plate (Iwaki, Tokyo, Japan), where the modified PE substrate was preplaced on the bottom and pretreated with 2.0 mL of distilled water for 30 min. The plate was further covered with aluminum foil and put into an oven (3 h, 37 °C). Eventually, the PE substrate was rinsed with PBS (pH 7.4, 1×) and observed using the fluorescence mode of a phase-contrast microscope (Model IX 71; Olympus, Tokyo, Japan).

Cell Adhesion Experiment. A cell adhesion experiment was performed using human cervical cancer (HeLa) cells stably expressing fluorescent ubiquitination-based cell cycle indicator (Fucci) (HeLa-Fucci; RCB2812, RIKEN Bio-Resource Center) on the PE substrate. The PE substrate modified with PMH82 using the same protocol described above was rinsed with ethanol again and dried on a clean bench before sterilization under UV. HeLa-Fucci cells were seeded in a polystyrene tissue culture dish (Φ = 10 cm, 5.0 × 10^4 cells/mL) in DMEM supplemented with 10% FBS at 37 °C in a humidified atmosphere containing 5.0% CO_2. Subconfluent cell cultures were passaged using 0.25% trypsin/EDTA. The cells were transferred to a 24 well plate (Iwaki, Tokyo, Japan; 5.0 × 10^4 cells/cm), where the modified PE substrate was preplaced on the bottom and pretreated with 2.0 mL of distilled water for 30 min, with DMEM supplemented with 10% FBS and incubated at 37 °C in 5.0% CO_2. After 48 h, the PE substrate was rinsed with PBS (pH 7.4, 1×) and transferred into a polystyrene tissue culture dish (Φ = 10 cm). The morphology of the cells on the surface of substrate was observed using a phase-contrast microscope (Model IX 71; Olympus, Tokyo, Japan).

Statistical Analysis of the Data. All graphs and bar charts are expressed as the mean ± standard deviation (SD) of five repeated experiments as described above. Student's t-test was carried out to determine whether the observed differences were statistically significant ($p < 0.001$).

RESULTS AND DISCUSSION

Characterization of Photoreactive Polymer. Herein, an extremely simple method was used to synthesize photoreactive methacrylate (MHPBP). The monomer was produced through a simple mixture of two compounds without solvent. The ring-opening reactions occurred between the epoxide group of GMA and the hydroxyl group of 4-HBP at 85 °C and produced a 90% yield after 5 h. This nonsolvent reaction process is much more simple and effective than ring-opening reactions using solvents with properties that strongly affect the reaction.[53] The chemical structure of MHPBP was confirmed by ^{1}H NMR (Figure S-1a). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 1.95 (s, 3H, α-CH_3); 3.50−4.70 (m, 6H, $-OCH_2-CH(OH)-CH_2O-$); 5.64, 6.14 (s, s, 1H, 1H, α-$CH_3-C{=}CH_2$); 7.09, 7.78 (d, d, 2H, 2H, $-CH_2O-$aromatic$(-H)-C(=O)-$); 7.50, 7.60, 7.70 (m, m, d, 2H, 1H, 2H, $-C(=O)-$aromatic$(-H)$).

The MPC polymers, PMBH721, PMH82, and PMH91, were synthesized through a conventional radical polymerization method, and details about these polymers are shown in Table 1. The chemical structures of these MPC polymers are shown in Figure 1b and were confirmed by ^{1}H NMR (Figure S-1b−e). From the calculation of the integral values of characteristic peaks, the monomer unit composition was confirmed based on the following analysis: 3.25 ppm ($-N^+(CH_3)_3$, 9H) for the MPC unit, 1.45−1.63 ppm ($-CH_2-$, 4H) for the BMA unit, and 6.80−7.85 ppm (benzophenone-H, 9H) for the MHPBP unit. These monomer units in the polymer chain were randomly distributed, with a total composition approximately equal to that of the monomer feed solutions (Table 1). Water insoluble PMB37 has been widely used for surface modification of biomedical devices through only physical interaction. Actually, 30% of MPC unit in the PMB polymer is a boundary condition to determine the solubility of PMB polymers in aqueous solution. PMB polymers will be easy to dissolve in aqueous solution when the amount of MPC unit is more than 30%, which will decrease the stability of physical immobilized polymer. Thus, PMB37 was prepared with a higher polymerization degree resulting in a larger molecular weight (M_w > 5.0 × 10^5), so it does not dissolve in aqueous solution. Thus, it has been widely used for coating materials because of the strong physical interaction between alkyl methacrylate (BMA unit) and the host materials.[31,54,55] Herein, PMB37 was just chosen as a control polymer to compare with photoreactive polymer, further to prove the importance of photoreactive group on the surface modification of biomaterials. The yields of all the synthetic photoreactive polymers, PMBH721, PMH82, and PMH91, were 75, 36, and 42%, respectively. The yields of PMH polymers can be increased by prolonging the polymerization time. The weight-average molecular weights of all the synthetic photoreactive polymers were less than 1.0 × 10^5, and they could dissolve in both ethanol and water. The UV spectrum measurements demonstrated that similar maximum absorption peaks appeared at 254 and 289 nm in the polymer/ethanol solutions (Figure S-2).

DOI: 10.1021/acsami.5b05193
ACS Appl. Mater. Interfaces 2015, 7, 17489−17498

Photosensitivity of Photoreactive Polymers. The CPO substrate (0.90 cm × 4.0 cm) coated with 0.20 mL of PMH82 solution (0.20 wt %, ethanol) was dried at room temperature and put into a rectangular cell of 1.0 cm optical path length (3.0 mL capacity) cell. The UV degradation curves as a function of irradiation time of PMH82 on the surface of CPO substrate were measured with a UV/vis spectrophotometer. As shown in Figure 2, the absorbance peaks of the BP group at 254 and 289 nm decreased dramatically from 0 to 180 s even within an interval time of 10 s, compared to those measured after photoirradiation (intensity 250−400 nm, 10 mW/cm^2) for 180 s. This result demonstrates that the BP group in the photoreactive MPC polymer is very sensitive to external photoirradiation, and a 300 s irradiation could ensure the complete bonding of modified photoreactive MPC polymer to the CPO substrate.

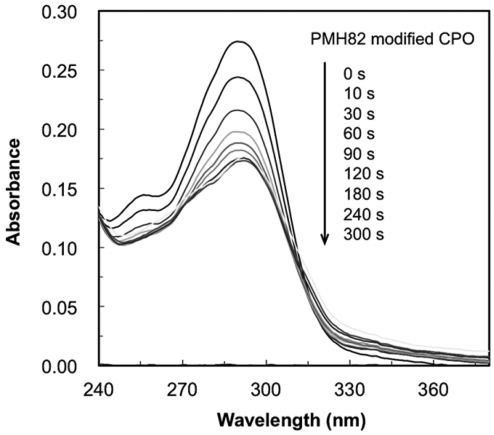


Figure 2. UV/vis absorption spectrum of 0.2 mL of PMH82 solution (0.2 wt %, ethanol) drop-coated CPO substrate (0.9 cm × 4.0 cm) as a function of UV irradiation time (intensity 250−400 nm, 10 mW/cm^2).

Surface Characterization of the Modified Substrate. The functional groups of polymers coated on the CPO substrate were confirmed by FT-IR spectroscopy with attenuated total reflection equipment, where the presence of the characteristic absorption peak of the carbonyl (1720 cm^{-1}) and PC groups (1240, 1080, and 970 cm^{-1}) represents the successful coating of MPC polymer on the CPO substrates. A peak around 1454 cm^{-1} from the CH bending was used to calculate the amount of modified MPC polymer by comparing to the peak of the carbonyl group from carboxylic ester (1720 cm^{-1}/1454 cm^{-1}). The CPO substrates coated with PMB37, PMBH721, PMH82, and PMH91 were exposed to photoirradiation (intensity 250−400 nm, 10 mW/cm^2) for 0, 1.0, 3.0, or 5.0 min. The FT-IR absorption spectra of each modified substrate before and after rinsing with ethanol were obtained to confirm the stability of the MPC polymer modified layer (Figure S-3). The characteristic peak ratio values (1720 cm^{-1}/1454 cm^{-1}) are shown in Figure 3a. Substrates modified with all MPC polymers showed characteristic peaks of both carbonyl and PC groups before rinsing with ethanol, unlike the unmodified original CPO substrate. The characteristic peak ratio value (1720 cm^{-1}/1454 cm^{-1}) of the PMB37-modified CPO substrate calculated from the spectrum decreased to the same value as that obtained on the original CPO substrate (Figure 3a). The peak integration of the carbonyl group (1720 cm^{-1}) decreased and disappeared after rinsing with ethanol, and this was not related to the irradiation time (Figure S-3a). This result demonstrates that physical adsorption cannot ensure the stability of the modified amphiphilic MPC polymer on the hydrophobic surface when it is exposed to strong solvents. In contrast, the characteristic peak ratio value (1720 cm^{-1}/1454 cm^{-1}) of substrates modified with PMBH721, PMH82, and PMH91 through photoinduced covalent binding after rinsing decreased but was still much larger than that obtained on the original CPO substrate (Figure 3a). Both absorption peaks of the carbonyl and PC groups could be observed on the substrates modified with PMBH721 (Figure S-3b), PMH82 (Figure S-3c), and PMH91 (Figure S-3d) both before and after rinsing with ethanol, even when irradiation was performed for only 1.0 min. Without irradiation, the peak ratio decreased close to the level of the original CPO because there were no chemical interactions between the photoreactive polymer and CPO substrate. When irradiation was performed, the peak ratio also decreased but still remained much higher than that of the original CPO. The MHPBP unit can enhance the stability of the coated MPC polymer through covalent bonding, compared to PMB37, which attaches to the surface by physical adsorption force. A comparison of the XPS profiles of unmodified CPO substrates with those of MPC polymer modified substrates also confirmed the successful modification of photoreactive MPC polymers on the CPO substrate; i.e., the characteristic signals of the MPC unit attributed to nitrogen (N_{1s}) and phosphorus (P_{2p}) were observed at 403 eV (triethylammonium cation) and 133 eV (phosphate anion), respectively (Figure 3b). The peaks from nitrogen and phosphorus with relatively smaller integrations were also observed on the PMB37-modified CPO substrate after rinsing with ethanol, indicating that a small amount of PMB37 still remained on the CPO substrate. Although the FT-IR spectrum did not show the characteristic peaks of the carbonyl and PC groups after rinsing with ethanol, the more sensitive XPS spectrum showed the existence of PMB37 on the CPO substrate. Contact angle measurement and cell adhesion tests were performed to further evaluate the properties of the PMB37-modified CPO substrate after rinsing with ethanol.

Figure 4a shows the water contact angles of the CPO substrates after surface modification with different MPC polymers and treatment with various photoirradiation times. The water contact angles of the CPO substrate coated with MPC polymers containing the photoreactive groups, PMBH721, PMH82, and PMH91, were dramatically decreased with increasing photoirradiation, compared with those obtained without photoirradiation. Moreover, the CPO substrate modified with the PMH containing a higher composition of MPC had a lower water contact angle in the range 30−60°. The photochemical reaction between the alkyl group in the CPO substrate and the BP group in the polymer under photoirradiation promoted the stabile fixation of the MPC polymer layer, and the hydrophilic PC group in the MPC unit could increase the surface free energy. This result further demonstrates that 9% of the MHPBP unit in the polymer chain (PMH91) was enough to ensure the fixation of the MPC polymer layer to the substrate. The same conclusion could be acquired from the results of the air contact angle measurement (Figure 4b). The air bubble in water showed a lower affinity for the hydrophilic surface, which resulted in a larger air contact angle value. The PMB37 surface showed a similar air contact angle value, which was much higher than that obtained on the original CPO substrate because 67% of the hydrophobic BMA unit can strongly induce the physical absorption of polymer on

DOI: 10.1021/acsami.5b05193
ACS Appl. Mater. Interfaces 2015, 7, 17489−17498

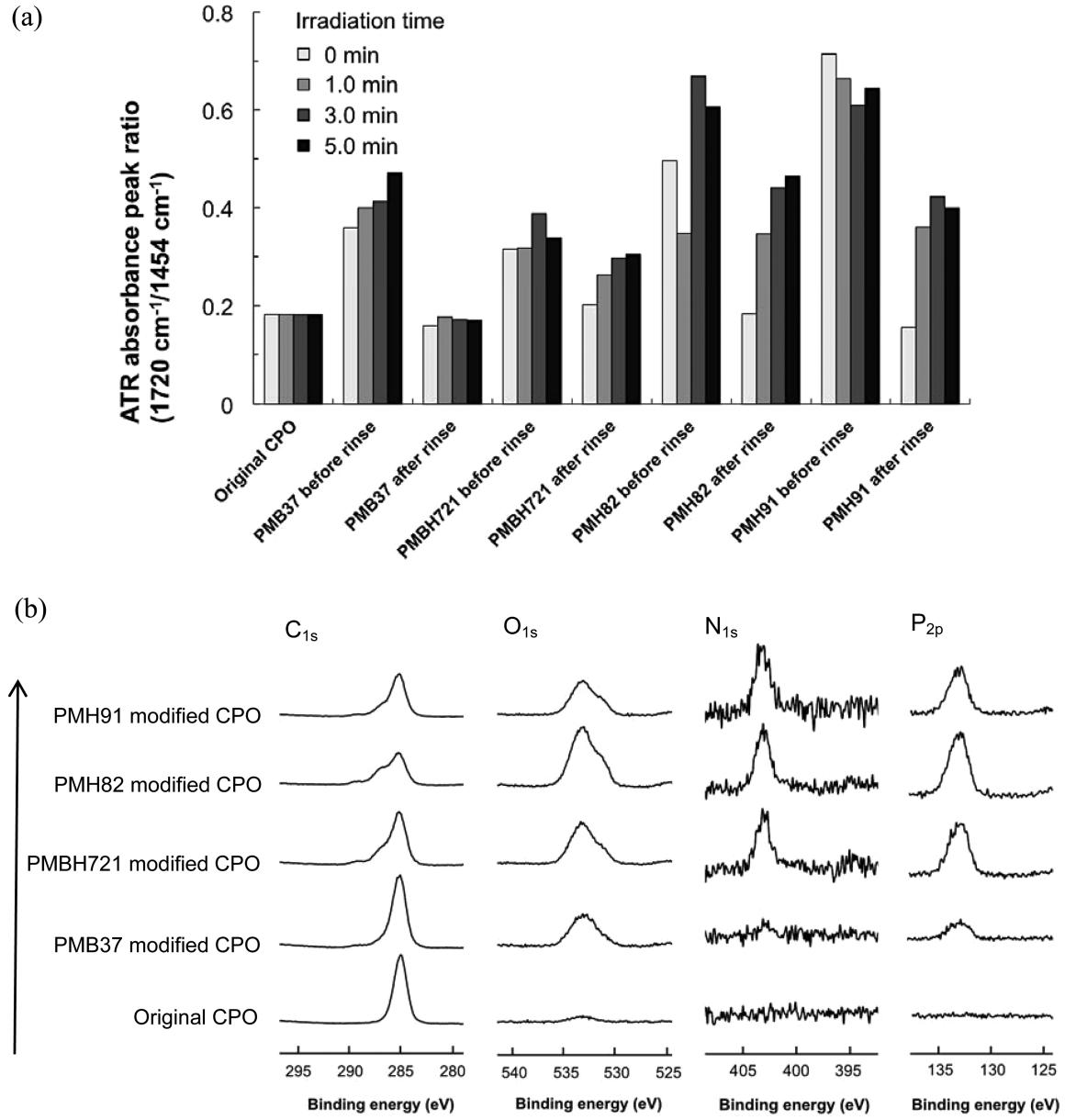


Figure 3. (a) Characteristic peak ratio (1720 cm^{-1}/1454 cm^{-1}) of FT-IR absorbance spectrum of CPO substrates modified with different MPC polymers before and after rinsing with ethanol. (b) Representative XPS charts of original CPO substrate and CPO substrate modified with MPC polymers after rinsing with ethanol for 1.0 h.

the hydrophobic CPO surface. A small amount of PMB37 still remained on the surface of the substrate even after rinsing with ethanol, as confirmed by XPS measurement (Figure 3b), and the remaining hydrophilic PC group in the PMB37 polymer could gradually orient to the interface with distilled water during pretreatment for 3.0 h immersion in water. A surface equilibrium is required for a dried amphiphilic MPC polymer modified surface to allow for PC group arrangement.[56] In the case of photoreactive polymers, air contact angle values were much higher after UV irradiation. This indicates the successful modification of the photoreactive polymer on the CPO surface by covalent binding after only 1.0 min of irradiation. The hydrophilic PC group in the photoreactive polymer on the CPO substrate can gradually orient with distilled water at the interface, which causes the air contact angle values to be in the range 150−180°. Notably, after rinsing with ethanol, a small amount of physically absorbed MPC polymers remained, which could significantly increase the air contact angle in water. This phenomenon can be observed in the PMB37-modified CPO and the photoreactive polymer modified CPO without exposure to irradiation. It is also important to emphasize that the differences between air contact angles of photoreactive polymer (PMBH721, PMH82, and PMH91) modified CPO substrates and the PMB37-modified CPO substrate are mainly caused by the chemical reaction of the BP group rather than the physical properties of the photoreactive polymers themselves. Although the photoreactive MPC polymers also contained hydrophobic MHPBP and/or hydrophobic BMA units, the surface contact angles were different as a result of exposure to UV irradiation.

The contact angle of liquid droplets on the substrate can also be explained as a balance between the attractive forces of internal molecules (cohesive force) and the attraction of the liquid molecules to the surface (adhesive force).[57] The surface

DOI: 10.1021/acsami.5b05193
ACS Appl. Mater. Interfaces 2015, 7, 17489−17498

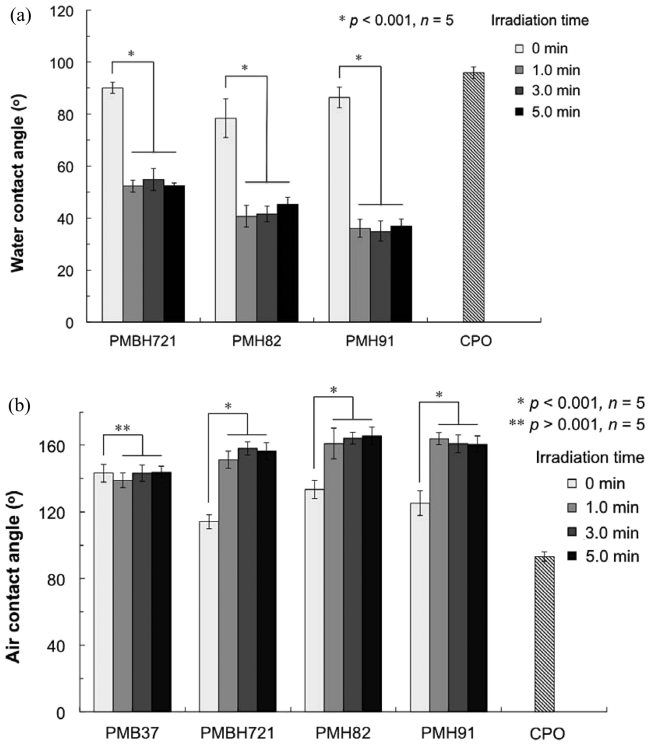


Figure 4. (a) Water contact angle and (b) air contact angle (measured in distilled water) of CPO substrates modified with different MPC polymers after rinsing with ethanol for 1.0 h. Single asterisk (∗) indicates statistically significant difference ($p < 0.001$, $n = 5$), and double asterisk (∗∗) indicates no statistically significant difference ($p > 0.001$, $n = 5$).

energy (γ_{sv}), which strongly relates to the surface wettability of the substrate, can be quantitatively evaluated by a force balance between the liquid−vapor surface tension of the drop liquid (γ_{lv}) and the interfacial tension between a solid substrate and liquid (γ_{sl}), and is further manifested through the liquid contact angle of drop liquid (θ) shown as $\gamma_{sv} = \gamma_{lv} \cos(\theta) + \gamma_{sl}$. By extrapolating the Zisman plots to a zero contact angle, the corresponding surface tension (critical surface tension, γ_{cr}) is approximately equivalent to the surface energy (γ_{sv}). Thus, we obtained a critical surface tension for evaluating the hydrophilic/hydrophobic nature of the substrate.

As shown in Figure 5, the contact angles of different liquids (water, glycerol, formamide, and ethylene glycol) on the surface of unmodified CPO and modified CPO substrates were different. The calculated $\cos(\theta)$ values of the same liquid on the modified surface were much higher than those on the original CPO surface, and the critical surface tensions (γ_{sv}) of PMH82-modified CPO and the original CPO were calculated as 45.7 and 17.4 mN/m, respectively. This indicates that the original CPO substrate was hydrophobic in nature, and the surface became hydrophilic by modification with PMH82.

The thicknesses of the modified MPC polymer layers on the silicon substrates were evaluated (Figure S-4). The thickness range of the photoreactive polymers, PMBH721, PMH82, and PMH91, on the silicon substrates was 15−64 nm and did not significantly change after the substrates were rinsed with ethanol. In contrast, the thickness of PMB37 was significantly reduced after rinsing with ethanol. Hence, the physical adsorption was not stable compared to the photoinduced chemical binding.

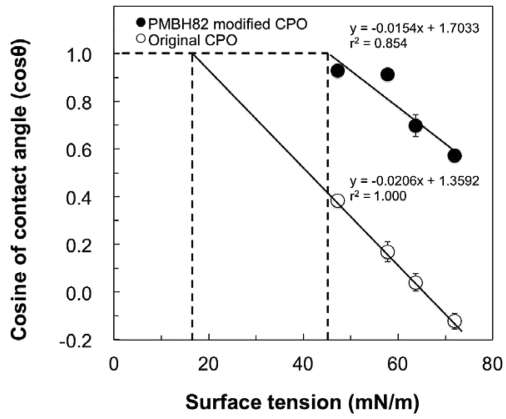


Figure 5. Zisman plot of original CPO substrate (○) and CPO substrate modified with photoreactive PMH82 polymer (●). The polymer-modified substrate was rinsed with ethanol for 1.0 h at room temperature to remove the unreacted or physically absorbed unstable MPC polymers and dried at room temperature.

Protein Adsorption and Cell Adhesion. MPC polymers have been widely used for the surface modification of various materials to avoid the nonspecific adsorption of biomolecules, especially proteins, which can subsequently interfere with cell adhesion.[9,10] It has been demonstrated that a surface covered with a bioinspired PC group can strongly reduce molecular interactions with biomolecules and further inhibit subsequent biological responses (e.g., protein adsorption, cell adhesion, and blood coagulation).[7,9,11,15,58−61] As described above, a photoreactive polymer (PMH82) was dissolved in distilled water with a 0.20 wt % concentration, and the polymer solution was spotted on a PE substrate with one drop for every 250 μm interval (0.75 cm × 0.75 cm). After being dried, the substrate was irradiated by a UV lamp (intensity 250−400 nm, 10 mW/cm^2) and was rinsed with ethanol to remove the unreacted polymer and dried into a clean bench for UV sterilization. The morphology of prepared PE substrate with spotted PMH82 was observed with phase-contrast microscopy, where polymer coated area showed a circular dotlike structure with a 100 μm diameter (Figure S-5). The pretreatment of the substrate with distilled water was performed to ensure the correct orientation of the PC group on the polymer surface, which can inhibit the unexpected hydrophobic interaction between cells/proteins and the substrate.

Protein adsorption characterization is very important to evaluate a designed biomaterial for implanted or blood-contact application. A thrombogenic interaction caused in the blood circulation usually starts from protein adsorption, platelet and leukocyte activation/adhesion, to complement activation and coagulation. As a plasma protein, fibrinogen can stimulate the platelet adsorption on the material's surface. Thus, the adsorption behavior of fibrinogen protein can be used to evaluate the biocompatibility of implanted or blood-contact biomaterials.[62,63] Figure 6a shows the fluorescent image of PMH82 modified PE after contacting with Alexa Fluor 488 conjugated fibrinogen protein. The green fluorescence from fibrinogen can be observed in the unmodified PE substrate, while PMH82 modified area did not show fluorescence. This is because PMH82 modified area can form a repelling surface and dramatically reduce adsorption of protein due to the PC group.

A phase-contrast microscopic image of the cell adhesion test results on the PMH82-modified PE substrate is shown in

DOI: 10.1021/acsami.5b05193
ACS Appl. Mater. Interfaces 2015, 7, 17489−17498

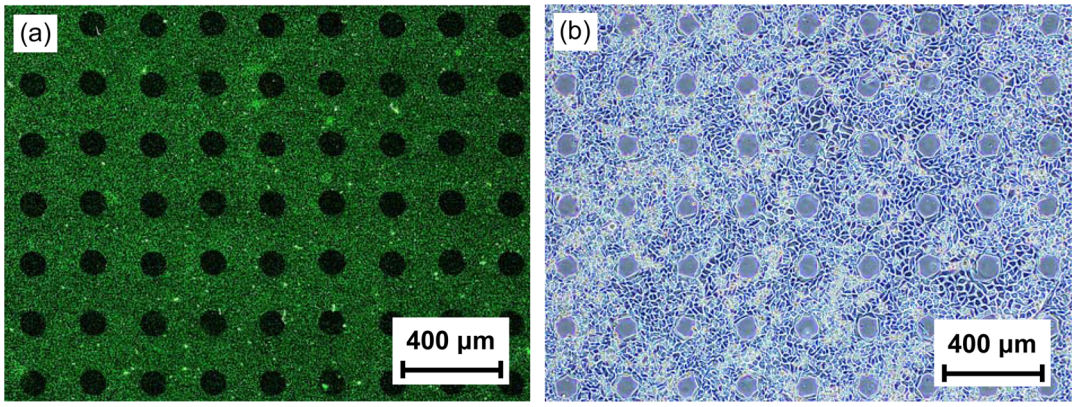


Figure 6. Phase-contrast microscopic images of (a) adsorption of Alexa Fluor 488 conjugated fibrinogen on patterned PMH82 modified polyolefin (PE) substrate, and (b) adhesion of cells on patterned PMH82 modified polyolefin (PE) substrate. The dot area was modified by photoreactive MPC polymer PMH82.

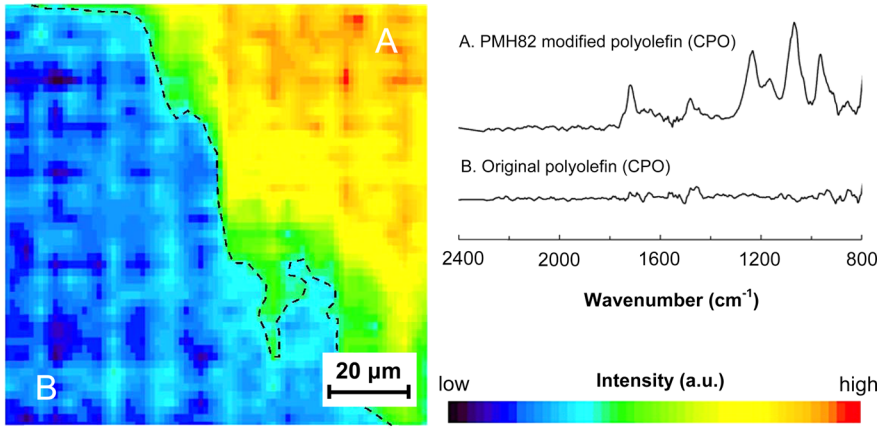


Figure 7. FT-IR ATR mapping results of PMH82 modified polyolefin (CPO) substrate, and spectra of PMH82 modified area (A) and unmodified original CPO area (B). The carbonyl group (1720 cm^{-1}) intensity was utilized for surface analysis to export the mapping image with different colors.

Figure 6b. As shown, HeLa-Fucci cells adhered to the unmodified PE area, remained on the PMH82-modified area as circular dots, and reached confluence. We did not observe any adverse effects on the cells that adhered to the dot area. In order to confirm that the dot area was correctly modified by PMH82, the FT-IR ATR equipment was used for mapping the surface functional group (carbonyl group, 1720 cm^{-1}) contained in PMH82. The intensity of the carbonyl group was utilized for surface analysis to export the mapping image with different colors (Figure 7). The spectra of PMH82-modified area (open triangle) and unmodified original polyolefin (CPO) area (open circle) have totally different absorption spectra. The absorption peaks from carbonyl (1720 cm^{-1}) and PC groups (1240, 1080, and 970 cm^{-1}) can be clearly observed in the PMH82-modified area. Therefore, the PC group on the polymer surface (dot area) repelled the adsorption of cell adhesion protein, inhibiting cell adhesion and demonstrating the success of the photoinduced covalent binding of MPC polymer with a small pattern (Φ = 100 μm). In order to further verify the importance of the photoreactive group in the preparation of a cell adhesion repelling surface, PE substrates were dip-coated with PMB37/distilled water solution (0.2 wt %) using the same methods mentioned above. Cells (5.0×10^4 cells/cm) were seeded onto PMB37-modified PE substrates treated with and without ethanol rinsing. As shown in Figure S-6, cells compactly adhered to the original PE substrate. In the absence of rinsing with ethanol, the PMB37 polymer layer could dramatically inhibit cell adhesion (Figure S-6a), but cell adhesion was observed on the polymer after rinsing with ethanol (Figure S-6b). This indicates that the PMB37 that was physically adsorbed on the CPO surface was not stable, and the remaining PMB37 (detected by XPS) could not effectively repel cell adhesion. This demonstrates the potential use of these photoreactive MPC polymers for surface modification of biomedical devices.

CONCLUSIONS

Photoreactive MPC polymers were designed as effective surface modifiers of conventional substrates. A methacrylate, MHPBP, was synthesized using a new simple process. The photoreactive MPC polymers containing both the zwitterionic and hydrophilic PC groups and the photoreactive BP group were synthesized successfully. These polymers have high sensitivity to photoirradiation, and they can be used to modify the surfaces of conventional materials with versatile aliphatic hydrogen through covalent bonding via photoirradiation for only 1.0 min. The stability of the MPC polymer layer on the surface was enhanced. The MPC polymer modified surface strongly inhibited the nonspecific adsorption of protein and adhesion of living cells. For the nonpolymeric surface, pretreatment of chemicals with alkyl end group on the surface can promote the

DOI: 10.1021/acsami.5b05193
ACS Appl. Mater. Interfaces 2015, 7, 17489−17498

subsequent immobilization of photoreactive MPC polymer. For biomaterials with complicated geometry, the penetrability of light to the desired area has to be considered well to ensure the photoreaction. Thus, we concluded that photoreactive MPC polymers containing BP group could be utilized for surface modification of various biomedical devices via a simple photoirradiation process.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b05193.

^{1}H NMR charts of the MHPBP, PMB37, PMBH721, PMH82, and PMH91; UV/vis absorption spectrum of polymers in ethanol with a concentration of 0.2 mg/mL; FT-IR spectroscopy with attenuated total reflection (ATR) equipment spectra of CPO substrate coated with various polymers before and after rinsing with ethanol under different UV irradiation times; thickness of modified MPC polymer layer on silicon substrate before and after rinsing with ethanol; phase-contrast microscopic image of patterned PMH82 modified polyolefin (PE) substrate; cell adhesion test on bare PE and PMB37-modified PE substrate (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Dr. Y. Inoue, Mr. S. Hachiya, and Mr. S. Sakata, Department of Materials Engineering, and Ms. Y. Liu and Mr. K. Suzuki, Department of Bioengineering, The University of Tokyo, for their valuable and constructive discussions. This research was supported by S-innovation from Japan Agency for Medical Research and Development (AMED), Japan.

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