Characterization and Application of Polypropylene Films Modified with Stimuli-Sensitive Copolymers with an Ar-Plasma Postpolymerization Technique

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ABSTRACT: Surface-modified polypropylene (PP) films with thermally and photochemically sensitive copolymers consisting of *N*-(2-hydroxypropyl)methacrylamide (HPMA) and 4-(4-methoxyphenylazo)phenyl methacrylate (MPAP), poly(HPMA-*co*-MPAP)-*g*-PP (abbreviated *g*-PP) film, were prepared by graft copolymerization with an Ar-plasma postpolymerization technique. The surfaces of the *g*-PP films were characterized by means of X-ray photoelectron spectroscopy; the percentage grafting of poly(HPMA-*co*-MPAP) with a number-average molecular weight of 3.28×10^4 was 7.12%, and the molar ratio of HPMA–MPAH in the copolymer was 0.75:0.25. The stimuli-sensitive adsorption of albumin and polystyrene microspheres on the *g*-PP film was also measured. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 143–148, 2003

Key words: poly(propylene) (PP); films; graft copolymers; stimuli-sensitive polymers; *N*-(2-hydroxypropyl)methacrylamide

INTRODUCTION

There has been increasing attention focused on thermosensitive polymers, such as poly(*N*-isopropylacrylamide),[1] poly[*N*-(3-ethoxypropyl)acrylamide],[1,2] and poly(*N*-vinylisobutylamide),[3] that show a reversible phase separation in aqueous solution in response to change in temperature. These polymers have been widely applied to drug-delivery system and other applications.[4–7] A tissue culture system with a thermally reversible poly(*N*-isopropylacrylamide) developed by Okano and coworkers was particularly interesting, for it overcame some problems, such as damage on the cell membrane during the recovery of cells from culture substrates by a hydrolyzing process with trypsin or protease.[8–11] However, light-induced change in the configuration of the azobenzene group in the polymer has also been suggested as a tool for the photocontrol of membrane permeability,[12] antibody–antigen recognition,[13–15] and other properties.[16–18] In a previous article,[19] we reported the characterization of a thermoresponsibly and photoresponsibly amphiphilic copolymer, poly[*N*-(2-hydroxypropyl)methacrylamide-*co*-4-(4-methoxyphenylazo)phenyl methacrylate] [poly(HPMA-*co*-MPAP)], prepared by the radical polymerization of *N*-(2-hydroxypropyl)methacrylamide (HPMA) with 4-(4-methoxyphenylazo)phenyl methacrylate (MPAP). From the absorption spectrum of aqueous poly(HPMA-*co*-MPAP) solution, we found that the azobenzene-containing amphiphile self-organized to form a stable bilayer membrane based on the dimeric chromophore type of aggregate. The aqueous solution of poly(HPMA-*co*-MPAP) exhibited a lower critical solution temperature (LCST) in the dark state than that measured in the photostationary state. The adsorption of poly(HPMA-*co*-MPAP) onto polystyrene microspheres was also found to be photoregulated. In the course of our study on the functionalized HPMA copolymers,[19–21] this article deals with the graft copolymerization of HPMA and MPAP onto the polypropylene (PP) surface by means of an Ar-plasma postpolymerization technique. The surface of the poly(HPMA-*co*-MPAP)-*g*-PP (abbreviated *g*-PP) film modified with poly(HPMA-*co*-MPAP) segment was hydrophobic in nature at temperatures higher than the LCST in the dark state but became hydrophilic as the temperature fell in the photostationary state. The purpose of this study was to obtain basic information on the multistimuli-sensitive polymer film as a scaffold in tissue engineering construction, which would provide us the means of transplanting several kinds of cell to restore lost function.[8,22] The *g*-PP film as a scaffold was evaluated by the effect of thermal and photochemical stimuli on the adsorption of serum proteins

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and polystyrene microspheres as a cell model onto the *g*-PP film.

EXPERIMENTAL

Materials

HPMA was prepared according to the method of Strohalm and Kopecek:[23] melting temperature (mp) 67–68°C (ref. 23 mp 67–68°C):

ANAL.Calcd for $C_7H_{13}NO_2$ = 143.186: C, 58.72%; H, 9.15%; N, 9.78%. Found: C, 58.37%; H, 9.03%; N, 9.58%.

MPAP was prepared by the reaction of methacryloyl chloride (Tokyo Kasei Co., Tokyo, Japan) with 4-(4-methoxyphenylazo)phenol according to the method used in the preparation of 1-{[(*E*)-3-(alkoxycarbonyl)acryloyl]oxy}-4-(4-methoxy-phenylazo)benzene[24] (mp = 116.2–117.5°C):

^{1}H-NMR ($CDCl_3$, δ, ppm): 2.08 (CH_3, 3H), 3.89 (OCH_3, 3H), 5.79, 6.38 (CH_2═, 2H), 7.00–.02 (ArH, 4H), 7.92–7.94 (ArH, 4H). Calcd. for $C_{17}H_{16}N_2O_3$ = 296.326: C, 68.91%; H, 5.44%; N, 9.45%. Found: C, 68.63%; H, 5.59%; N, 9.20%.

PP film (1.0 mm thick) was obtained from Sanplatec Corp. (Osaka, Japan). Styrene was purified according to the method described in a previous article.[19] Argon (99.99 mol %) was purchased from Taiyo-Toyo-Sanso Co. (Osaka, Japan). 2,2-Diphenyl-1-picrylhydrazil (DPPH) was obtained from Wako Pure Chemicals (Japan) and was used without further purification. Potassium peroxodisulfate was recrystallized from water. Bovine serum albumin tetramethylrhodamine conjugate (Alb; Molecular Probes, Oregon) was used without further purification. Distilled deionized water was used throughout the experiments.

Preparation of the *g*-PP film

A radio-frequency (13.56 MHz) plasma reactor consisting of a glass chamber (7 cm in height and 21 cm in diameter) and copper plate electrodes was used as described in previous articles.[25,26] The conditions determined as optimum for the Ar-plasma treatment were as follows: initial pressure = 1.3 Pa, radio frequency (RF) power = 10 W, Ar flow rate = 10 sccm, and irradiation time = 120 s.

A strip of the PP film (6 × 0.6 cm, ca. 1.0 mm thick) was washed with methanol, dried in a vacuum oven at 50°C, weighed, and then fixed in the plasma reactor.[26] After being exposed to Ar plasma, the PP film was exposed to air for 1 min and then placed into a glass ampule containing 2.0 g (14.0 mmol) of HPMA and 0.22 g (0.75 mmol) of MPAP in 10 mL of absolute ethanol. The glass ampule was degassed by a freeze–thaw technique with a liquid nitrogen bath and sealed *in vacuo*. After postpolymerization at 60 ± 0.2°C for 15 h, the grafted the *g*-PP film was washed thoroughly with methanol and soaked for 24 h in methanol to eliminate residual monomers and copolymer adhered to the surface. Two samples were prepared for the *g*-PP film. The amount of poly(HPMA-*co*-MPAP) grafted was 0.56 mgcm^{-2}. The molar ratio of azo monomer in the copolymer grafting on the PP film was determined by X-ray photoelectron spectroscopy (XPS).

Detection of peroxides

A strip of the PP film (6 cm×0.6 cm, ca. 1.0 mm) pretreated with an Ar plasma at 10 W for 120 s was exposed to air for 1 min, followed by placement in a glass ampule containing a 0.1 mmol L^{-1} DPPH in 10 mL of benzene. The ampule was cooled and sealed *in vacuo* in a similar manner as mentioned previously. After the ampule was shaken in a thermostat maintained at 60°C for a given time, the benzene solution was submitted to UV measurement with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The amount of peroxides generated on the PP film was determined spectrophotometrically by measurement of the disappearance of the adsorption of DPPH at 520 nm (absorption coefficient ε = 8870 L mol^{-1} cm^{-1}).[26]

Preparation of polystyrene microspheres

Polystyrene microspheres were prepared by the emulsifier-free emulsion polymerization of styrene initiated with potassium peroxodisulfate in water at 70°C for 24 h, according to a method in a previous article.[27] Polystyrene microspheres were 840 ± 100 nm and 990 ± 10 nm in diameter, as obtained from scanning electron microscopy (SEM) measurements of dried sample and the size distribution of particles measured with a Particle Sizing Systems NICOMP370 (Florida) in water, respectively.

XPS measurements

The surface of the *g*-PP film was analyzed by means of XPS with a 90° incident angle of the X-ray with a Shimadzu ESCA 750. The samples used were the freeze-dried *g*-PP films at −78°C after immersion in water at 25°C for 24 h. Measurements were made with MgKα radiation and at a pressure less than 6.6 × 10^{-5} Pa. The atom ratios of nitrogen and oxygen to carbon were obtained based on the factors, 1.77 and 2.85 for N_{1s}/C_{1s} and O_{1s}/C_{1s}, respectively. The spectra of N_{1s} and O_{1s} were taken with 25 and 3 scans, respectively.

Measurements of contact angle (θ)

Static θ's between the *g*-PP film and pure water were measured at ambient temperature (ca 22°C) by the

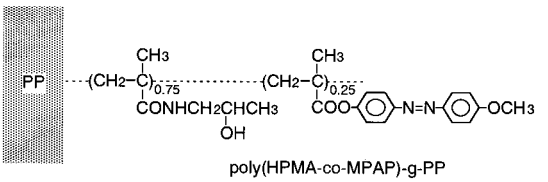

Figure 1 Structure of the *g*-PP film.

sessile drop method with an Erma θ meter (Tokyo, Japan; model G-I). The values given are the average of 10 measurements of each sample taken at 70 s of contact with the 15-μL water droplet on the air-exposed side.

Protein adsorption

The adsorptions of Alb on the *g*-PP film in the dark state and in the photostationary state were evaluated by means of microscopic Fourier transform spectrometry. The *g*-PP film was well rinsed with water, immersed, and equilibrated in the Alb solution (initial concentration of Alb = 0.05 wt %) at different temperatures from 25 to 45°C for 4 h at pH 5.6. The *g*-PP film was then immersed in a large amount of water for a few seconds and rinsed carefully to remove unadsorbed Alb; it was then by freeze-dried at −78°C. After that, the *g*-PP film was measured immediately with a Hitachi model U-6000 microscopic Fourier transform spectrometer (Tokyo, Japan). The amount of Alb was calculated from the fluorescence intensity with a calibration ($y = 0.014x$, where x and y represent the fluorescence and the amount of Alb, respectively). The adsorption experiments were also carried out with the *g*-PP film after UV irradiation for 2 h.

Adsorption of polystyrene microspheres on the *g*-PP film

The adsorption of polystyrene microspheres on the *g*-PP film was evaluated by means of SEM. The *g*-PP film was well rinsed with water, immersed, and equilibrated in the polystyrene emulsion (0.54 g L^{-1}) at 25 and 46°C for 4 h in the dark state. The *g*-PP film was then immersed in a large amount of water for a few seconds and rinsed carefully to remove unadsorbed polystyrene microspheres; it was then freeze-dried at −78°C. The adsorption experiments were also carried out with the *g*-PP film after UV irradiation for 2 h. The amount of polystyrene microspheres adsorbed on the *g*-PP film was evaluated immediately by means of SEM at 25 kV with a Hitachi S-2150.

RESULTS AND DISCUSSION

Preparation and characterization of the *g*-PP films

HPMA is known as a biocompatible monomer[28–30] and gives not only a thermoresponsible homopolymer but also a copolymer[20,21] with methacrylates as comonomers. The random copolymer poly(HPMA-*co*-MPAP), containing the azobenzene moiety was a thermosensitive and photosensitive amphiphilic copolymer. The poly(HPMA-*co*-MPAP) segments grafted with the PP film (i.e., *g*-PP) was prepared by means of an Ar-plasma postpolymerization technique (Fig. 1).

The low-temperature plasma treatment postpolymerization technique was applied to modify the surface properties of the PP film with water-soluble HPMA and a small amount of MPAP as an azo monomer. In plasma treatment postpolymerization, the graft copolymerization is initiated with peroxides generated by oxidation when the PP film is brought into contact with air after exposure to Ar plasma.[31] In the plasma treatment, several kinds of peroxide are formed because hydroperoxides are generated during irradiation but may be transformed to more highly oxidized species, such as peroxyester and peroxycarbonate. Here, we could not identify the kind of species involved in this reaction. The generated peroxides on the surface of the PP film were detected by the DPPH method described in the literature.[32] Figure 2 shows the concentration of free radicals on the PP film calculated from the DPPH consumption as a function of heating time at 60°C. The concentration of free radicals increased monotonously with heating time.

The weight-average molecular weight of poly(HPMA-*co*-MPAP) grafted onto the PP surface was calculated to be 3.28×10^4 on the basis of the amount of grafted copolymer (0.56 mg cm^{-2}) and the amount of peroxides generated (1.59×10^{-5} mmol cm^{-2}). The surface of the *g*-PP films was analyzed by XPS. Figure 3 illustrates the signals of C_{1s}, N_{1s}, and O_{1s} of the *g*-PP film, in which the peak intensity was normalized in percentage. The Ar-plasma-treated polypropylene film (PP*) showed an atom ratio for O/C of 0.39 due to

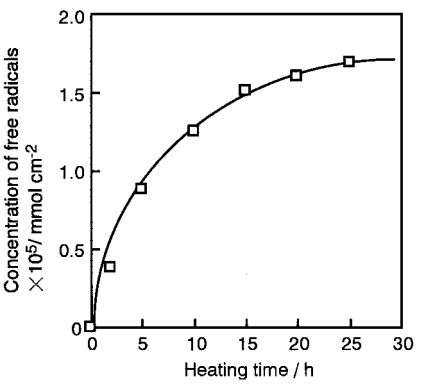

Figure 2 Concentration of peroxide produced on the PP surface as measured by the DPPH method as a function of reaction time. Ar-plasma preirradiation conditions: RF power = 15 W, initial pressure = 9.8 mTorr, and time = 10 min. DPPH reaction conditions: [DPPH] = 1.0×10^{-4} mol dm^{-3}, temperature = 60°C, and times varied.

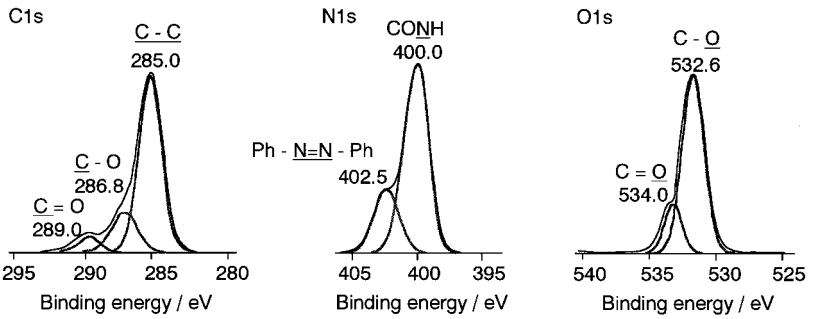

Figure 3 XPS spectra of *g*-PP.

the highly oxidized species mentioned previously. The atom ratio for N/C (0.007) found in PP* arose from nitrogen as an impurity in argon gas. The signals of C_{1s} depicted in Figure 3 at binding energies (BEs) of 285.0, 286.8, and 289.0 eV appearing in all of the *g*-PP films were assigned to the methylene group and C—O and C═O carbons, respectively. The peak for nitrogen at BEs of 400.0 and 402.5 eV were assigned to the amide group in the HPMA and the azo group in the MPAP moiety in addition to the O_{1s} signals at 532.6 and 534.0 eV assigned to the C—O and C═O oxygens. Considering the N/C ratio in the *g*-PP film, the percentage substitution of the PP surface was calculated to be 7.12% for the *g*-PP film. From the ratio of areas of N_{1s} signal CONH (400 eV) based on the HPMA moiety to the azo group (402.5 eV) based on the MPAP moiety, the monomer ratio in poly(HPMA-*co*-MPAP) grafted on the PP film was calculated to be HPMA–MPAP = 0.75:0.25. These data indicates not the bulk copolymer composition but a composition of the thin surface with several tens of nanometers of *g*-PP.

The *g*-PP films were still yellowish and transparent like the plasma untreated one. The original PP film with a θ of 101.6° shed water well, whereas the *g*-PP film showed an improved wettability due to the grafting of the hydrophilic polymer. θ's of the *g*-PP film were measured to evaluate the wettability in the dark state and in the photostationary state at ambient temperature. The *g*-PP film showed a lower static θ of 50.8° in the dark state and a θ of 40.3° in the photostationary state. This difference in the θ is explained by the difference in polarity change based on the trans–cis isomerization of the methoxyazobenzene group in the MPAP moiety. From the photochemical study of poly(HPMA-*co*-MPAP), we found that the trans-to-cis isomerization of the methoxyazobenzene group in the copolymer occurring on UV irradiation reached the photostationary state within 2 h and that the rate of cis–trans relaxation was very slow. The half-life period ($t_{1/2}$) was 12 h.[19] The $t_{1/2}$ was enough to enable the adsorption experiments that carried out the condition in either the trans or cis configuration of the methoxyazobenzene moiety. The *g*-PP film modified with the azobenzene-containing amphiphiles was, therefore, expected to be a photoresponsive material with biocompatibility due to the formation of a stable biomembrane-like surface with a hydrogel structure.[19,26,33] Although the effect of temperature on θ of the *g*-PP film was not measured, we could estimate that the *g*-PP exhibited hydrophobic and hydrophilic behavior at low and high temperatures, respectively. Transparency was found below 34°C, poly(HPMA-*co*-MPAP) exhibited a remarkable phase change in response to the change in temperature, and the molar ratio of HPMA–MPAP influenced the LCST. In the case of HPMA–MPAP = 0.78:0.22, the copolymer solution showed transparency below 34°C, and it sharply turned opaque when the temperature was raised to 50°C, whereas the LCST exhibited the lower temperature by 10°C when the ratio was 0.71:0.29. [19] The poly(HPMA-*co*-MPAP) with HPMA–MPAP = 0.75:0.25 grafted on the PP film was expected to show the LCST in the temperature range 40–50°C.; it formed a hydrated and extended conformation at low temperatures and formed extensively dehydrated and compact chain conformation at high temperatures.[19] Because the θ's of the *g*-PP films decreased with the temperature change from high to low or with UV irradiation, *g*-PP films were expected to have a detachable cell culture substrate with a biocompatibility based on cell adhesion and propagation at a high-temperature and in the dark state and cell detachment at a low temperature and in the photostationary state.

Adsorption of protein on the *g*-PP film

The plasma protein adsorption, which is the initial event in cell–material interaction, influences subsequent cell adhesion and propagation. Therefore, the plasma protein adsorption plays a major role in cell adhesion at the cell–material interface, and the control of protein adsorption is an important factor when the design of scaffolds are considered. From this perspective, the interactions between the *g*-PP film and Alb as a typical serum proteins were studied in the dark and photostationary states to obtain basic information for

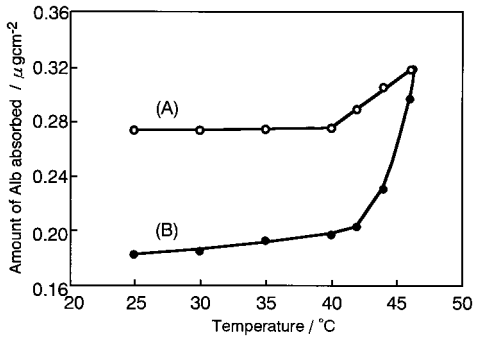

Figure 4 Effect of the temperature and UV irradiation on the amount of Alb adsorbed on the *g*-PP film: (A) *g*-PP film before UV irradiation and (B) *g*-PP film after UV irradiation for 2 h.

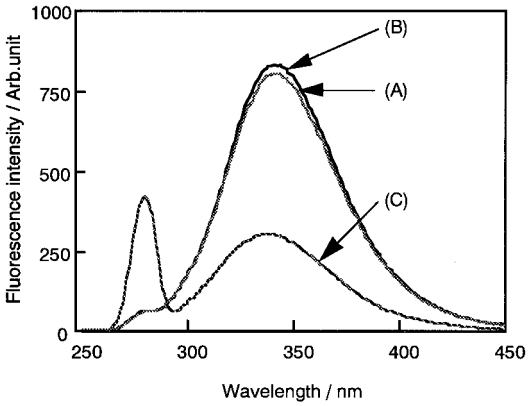

Figure 5 Fluorescence spectra of Alb in the pH 5.6 phosphate buffer solution at various temperatures ([Alb] = 0.05 wt % and excitation wavelength, λ_{EX} = 280 nm): (A) 25, (B) 46, and (C) 60°C.

detachable cell culture substrate. The adsorption of Alb on the *g*-PP film was examined after equilibration at different temperatures from 25 to 46°C for 4 h in aqueous solution (initial concentration of Alb = 0.05 wt %) at an ionic strength of 0.01 adjusted with aqueous NaCl solution at pH 5.6, which was so close to the respective isoelectric points in which Alb took the most compact form and in which the maximum amount of adsorption was expected. Figure 4 shows the effect of temperature on the Alb adsorption on the *g*-PP film in the dark and photostationary states. The amount of adsorption increased at around 40°C. This result was compatible with the phase transition of poly(HPMA-*co*-MPAP) from a hydrated and extended conformation to an extensively dehydrated and compact chain one. The phase transition of grafting poly-(HPMA-*co*-MPAP) on the *g*-PP film from a hydrophilic to a hydrophobic condition at around 40°C resulted in an increase in the adsorption of Alb. In the photostationary state, the high polarity of the cis isomer of the methoxy azobenzene group due to high dipole moment seemed to be the reason for the increase in LCST; that is, the higher the polarity stabilized, the more hydrogen bonding there was.[19] The difference in the amount of the adsorbed protein is thus explained by the differences in both polarity change and hydrogel structure based on the trans–cis isomerization of the methoxyazobenzene group.

From the results obtained, we expected that cultured cells on the *g*-PP film could detach on temperature change from 42 to 20°C and on condition changes from the dark to the photostationary state. As it is known that the fluorescence spectra of protein in various media provide a sensitive means of characterizing proteins and their conformation, the spectra indicate the polarity of the environment of the tryptophan and tyrosine residues as well as their specific interaction.[34] Figure 5 shows the fluorescence spectra of Alb solution after it was kept at various temperatures for 6 h in a pH 5.6 phosphate buffer solution. There was no denaturation in the structure of Alb at 46°C because no reduction in the intensity of the fluorescence occurred.

Adsorption of polystyrene microspheres onto the *g*-PP film

The adsorption of polystyrene microspheres with a ζ potential of -99 mV[35] as the cell model onto the stimuli responsive *g*-PP was examined by the batch

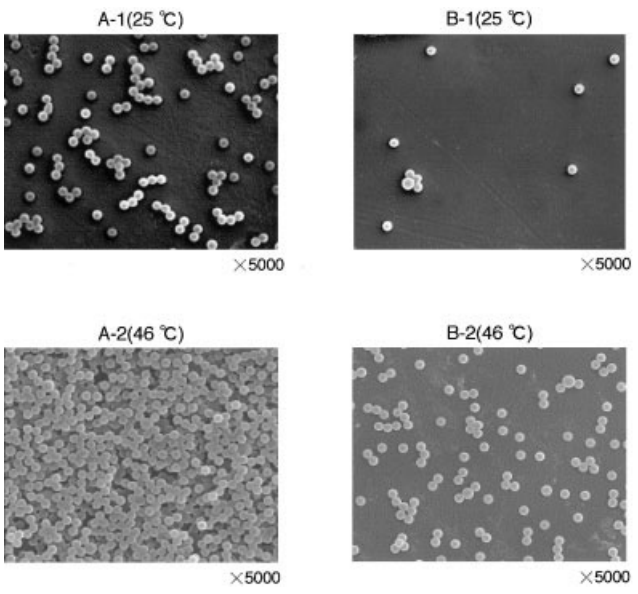

Figure 6 SEM images of the *g*-PP film after immersion in the polystyrene emulsion (0.54 g dm^{-3}) at 25 and 46°C for 4 h (plasma irradiation time = 120 s and postpolymerization time = 25 h). (A-1) *g*-PP film before UV irradiation measured at 25°C. The amount adsorbed was 6.41×10^8 cm^{-2}. (A-2) *g*-PP film before UV irradiation measured at 46°C. The amount adsorbed was 69.1×10^8 cm^{-2}. (B-1) *g*-PP film after UV irradiation measured at 25°C. The amount adsorbed was 2.57×10^8 cm^{-2}. (B-2) *g*-PP film after UV irradiation measured at 46°C. The amount adsorbed was 8.46×10^8 cm^{-2}.

method. Figure 6 shows the SEM micrographs of the *g*-PP film after immersion in the polystyrene microspheres at 25 and 4°C with the *g*-PP film before and after UV irradiation for 2 h. The polystyrene microspheres adsorbed on the *g*-PP film with a hydrophobic surface at 46°C in the dark state, whereas the *g*-PP film covered with hydrophilic poly(HPMA-*co*-MPAP) with a fully hydrated and extended conformation at 25°C in the photostationary state drastically reduced adsorption. The amount of polystyrene microspheres adsorbed on the *g*-PP film through hydrophobic interaction was larger than that of the hydrophobicity of the poly(HPMA-*co*-MPAP) segments grafted. We believe that polystyrene microspheres adsorbed onto the *g*-PP film through hydrophobic interactions at 25°C in the dark state were desorbed as a result of increased hydrophilicity at 46°C in the photostationary state.

CONCLUSIONS

The surface of the *g*-PP film modified with an amphiphilically biocompatible copolymer was hydrophobic in nature with a static θ of 50.8° in the photostationary state, and it became hydrophilic in nature with a θ of 40.3° in the dark. The adsorption of Alb took place more easily in the photostationary state than in the dark, and the amount of Alb adsorbed increased with increasing temperature from 25 to 45°C. Polystyrene microspheres as a cell model adsorbed on the *g*-PP film at 46°C in the dark state, whereas the amount of adsorption was drastically reduced at 25°C in the photostationary state. Therefore, we conclude that the *g*-PP film could thermally and photochemically control the adsorption of Alb and polystyrene microspheres as basic information. Because cells adhere and grow more easily on a hydrophobic surface than on a hydrophilic one, we expected that cultured cells would adhere and develop on the *g*-PP film at around 42°C in the dark state and that cultured cells would detach easily at 20°C in the photostationary state.

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