Surface Modification of Polyethylene Membranes Using Phosphorylcholine Derivatives and Their Platelet Compatibility

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ABSTRACT: To fabricate a platelet-compatible polymer, phosphorylcholine (PCe) was introduced onto the surface of polyethylene (PE) using a novel synthetic process. Acrylic acid was graft-copolymerized on the surface of PE by UV irradiation, using both the peroxide preirradiation and the simultaneous methods. Benzophenone and Michler's ketone were used as an initiator and sensitizer in this investigation, respectively. To investigate the spacer effect on the platelet compatibility of the PE membrane surface, PCe with various spacer lengths was introduced onto the surface of PE by a series of chemical reactions. Ethylene glycol, butanediol, poly(propylene glycol), and poly(tetramethyl glycol) were used as spacers. The modifications of the PE membrane surface were analyzed by contact-angle, ATR–FTIR, and ESCA techniques. The platelet compatibility of the PCe-modified polymer was evaluated by the *in vitro* platelet adherent test. It was found that the platelet compatibility of the PE film surface was affected by the existence of various functional groups on the film surface. The length of the lipophilic spacer between PCe groups and the PE surface will affect the biomimetic properties of the membrane surface. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 2947–2954, 1999

Key words: biomimetic; blood compatible; phosphorylcholine; surface modification; polyethylene

INTRODUCTION

When polymer is to be used, surface properties of the material often determine its value as a practical usable material. All solid materials having definite shapes are surrounded by other materials, and, hence, interfacial phenomena should be taken into account. So long as we are using synthetic polymers in the field of biomedical science, most of these materials are nonpolar and hydrophobic. Consequently, the purpose of surface modification has been to provide polarity and hydrophilicity to a polymer surface.

Polymers with phosphorylcholine (PCe) head groups on their surfaces have been shown to reduce thrombus deposition at the blood/polymer interfaces when in contact with blood. The basis of this hypothesis comes from these polymer surfaces having strong affinity with natural phospholipids,[1] having high binding with water,[2] taking part in the coagulation mechanism of blood,[3] and so on. Thus, some investigators are engaged in using copolymerization, graft copolymerization,[4] or adsorbed coating techniques[5] to develop biomimatic biomaterials.

Ishihara et al.[6,7] synthesized a monomer containing a phospholipid polar group, 2-methacryoyloxyethyl phosphorylcholine (MPC), copolymerized with different kinds of hydrophobic monomers. These materials showed excellent blood compatibility and adsorbed natural phospholipids preferentially in the phospholipid/protein adsorption test. Imanishi and coworkers[8] coated some kinds

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of phosphatidyl choline on polyamide, polyethylene (PE), and PVC to mimic the hydrophilic head groups of the outer membrane leaflet of resting platelets and erythrocytes which are naturally blood compatible.

In this study, we synthesized a series of surface-modified PE membranes by introducing PCe head groups using UV irradiation and chemical-modification techniques. To investigate spacer effects on the platelet compatibility of the PE membrane surface, some lipophilic spacers with various chain lengths were investigated. Prelimary *in vitro* platelet adherence measurements were performed to evaluate the blood compatibility of these PCe-modified polymer surfaces. The results of the platelet adhesion test were evaluated using an SEM photograph technique.

EXPERIMENTAL

Materials

PE films with a thickness of about 80 mm were cut into strips of 2 × 4 cm^2 and immersed in 2-propanol at 72°C for 12 h, then treated by ultrasonication for 6 h to remove surface impurities. After being dried under a vacuum for 1 day, all the samples were all stuck on Petri dishes using double-sided adhesive tape. Acrylic acid (AA) was purchased from Ferak and used with no further purification. Triethylamine was distilled over KOH pellets. Nitromethane (Ferak, Berlin, Germany) and organic solvents were all dried over 4 Å molecular sieves after completion of the distillation. Double-distilled water was used for polymer washing and solution preparation.

Graft Copolymerization

Peroxide Generation Method

PE film was cut into strips of 2 × 4 cm^2 and immersed in an aqueous solution containing a photoinitiator, sensitizer, and various organic solvents. The mixture was placed in a Pyrex glass ampule with a cap and exposed to UV light. Strip films were washed with acetone for 10 min after completion of the irradiation. AA of various concentrations was added and then reacted at room temperature under UV irradiation for a certain period. Peroxide density was determined immediately after peroxide generation.

The density of the peroxides generated was determined by the conventional iodide assay.[9,10] The method is based on the oxidation of sodium iodide by peroxides in the presence of ferric chloride. Isopropyl alcohol (6 mL), 1 mL of benzene, 0.4 mL of 0.123 m*M* $FeCl_3$ in acetic acid, and 0.2 mL of 1.33*M* NaI in isopropyl alcohol were added to the treated polymer samples. After degassing with nitrogen bubbling for 2 min and sealing, the ampules were heated at 60°C for 10 min and then 1 mL of water was added to stop the redox reaction. The peroxide density was calculated from the optical density of the solution at 360 nm.

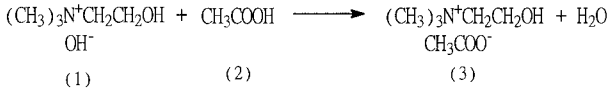
Simultaneous UV-irradiation Method

PE film was cut into strips of 2 × 4 cm^2, which were fixed in a Pyrex glass ampule with a cap. The strip films were treated with 0.1 wt % riboflavin under nitrogen for 40 min, and AA of various concentrations was then added. The mixture was exposed to UV light from a 100-W high-pressure mercury lamp (UVPB-100Y, Jasco Co., 2537–5800). Following UV irradiation at a distance of 10 cm from the mercury lamp for a predetermined time, the film strips were taken out from the ampule and washed with distilled water at 7°C for 24 h to remove the homopolymer. The amount of AA polymers grafted onto the PE film was determined by the gravimetry method.

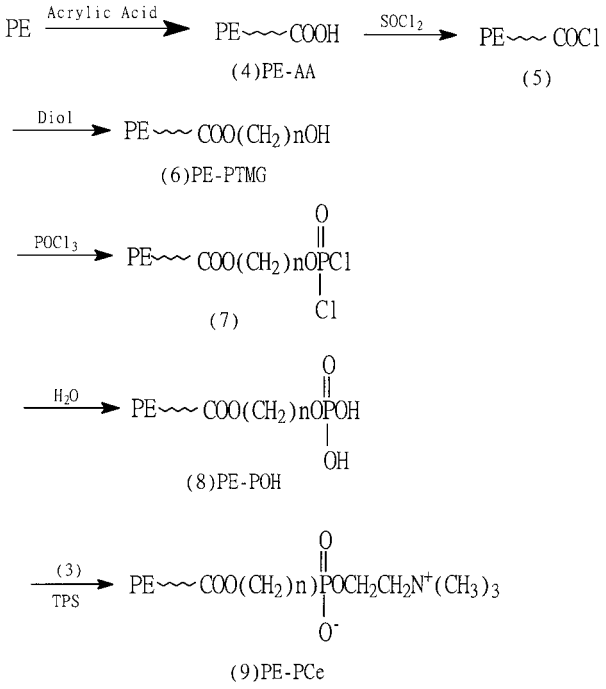
Introducing of PCe

AA-copolymerized PE films were immersed in thionyl chloride containing 0.1 wt % DMF and shaken at 28°C for 2 h. The thionyl chloride solution was decanted and various diols were added. The mixture was shaken at room temperature for a further 2 h and the films were taken out and washed with ethanol and rinsed with acetone. The treated films were then immersed in phosphoryl oxychloride and shaken at room temperature for 2 h. The aqueous solution was decanted and a large amount of water was added. The film was washed with water and then rinsed with acetone and dried in a vacuum.

Choline acetate was synthesized by the following steps (Scheme 1): Choline hydroxide (26.93 g, 45 wt %) in a reaction bottle was neutralized by acetic acid, then evaporated around 70°C to remove excess acetic acid and water.[11] The choline acetate was dried and kept in a vacuum before use.



Scheme 1



Scheme 2

The phosphoryl oxychloride/water-treated PE films were placed in a reaction bottle, and 1 g of choline acetate, 0.42 g of triisopropylbenzenesulfonyl chloride (TPS), and 15 mL of pyridine/nitromethane (1:1) were added. The mixture was reacted at 50°C for 4 h. The films were taken out and washed with water completely. The reaction procedures are shown in Scheme 2.

In Vitro Platelet Adherence Test

Grafted and PCe-modified films were cut into 6 $\times$ 6 mm^2 and fixed in a Petri dish. The films were immersed in Milli-Q water overnight, then soaked in a phosphate-buffered solution (PBS, pH 7.4; ionic strength, 0.15*M*) for 12 h to equilibrate the film surfaces. After that, the PBS solution was decanted and 0.03 mL of platelet-rich plasma (PRP) was poured onto each film surface and kept in an incubator at 37°C with 5% CO_2 flow for 2 h. The samples were rinsed twice with PBS with mild shaking; 2 vol % glutaraldehyde in PBS was then poured into the Petri dish to fix the cells on the film surface at room temperature for 1 h. Again, the sample was rinsed twice with PBS and then the PBS replaced gradually with Milli-Q water. The membranes were freeze-dried by liquid nitrogen, and the surfaces were analyzed by SEM following sputter-coating of a gold layer.

Characterization of Modified Films

ATR–FTIR analyses were performed using a Jasco Volar III infrared spectrometer equipped with an ATR attachment. The spectra were recorded at an incident angle of 45° through a KRS-5 crystal; the penetrating depth is up to about 3 mm. The choline acetate compound was analyzed by the Ge crystal of an FTIR liquid cell.

ESCA (XPS) spectra of UV-irradiated films and PCe-modified films were recorded with a spectrometer using MgKα (1253.6 eV) excitation. The element analyses were compared with each normalized area of the element.

Water contact angles of the films were evaluated using a face contact anglemeter (Kyowa Kaimen Kagaku CA-DP A type). Seven different spots were measured for each sample by reading the data within 20 s to ensure that the hydrophilic groups would not diffuse away from the surface.

The grafted membranes were analyzed by scanning electronic microscopy (SEM) measurement to estimate their morphology changes and energy-dispersive X-ray spectroscopy (EDX) was used to estimate the P content with sputter-coated carbon layers on them. The morphology and composition distribution of the membrane surface were also checked through focusing on different micron spots.

RESULTS AND DISCUSSION

Modification of PE film

Schemes 1 and 2 show the synthesis of choline acetate and PE film modification, respectively. The IR spectrum of choline acetate shows two strong bands at 1575 and 1390 cm^{-1}, corresponding to the COO^- group's absorption. Moreover, the fingerprint section is very similar to that of choline hydroxide. The structure of choline acetate was also confirmed using ^{1}H-NMR. Its chemical shifts lie at 1.78 ppm (3H, CH_3COO^-), 3.03 ppm [9H, $—N^+(CH_3)_3$], 3.35–3.50 ppm (2H, $—N^+CH_2$), and 3.85–3.90 ppm(2H, $—OCH_2$).

AA was graft-copolymerized onto the surface of PE by UV irradiation, using both the peroxide preirradiation and the simultaneous methods. Benzophenone and Michler's ketone were used as a photoinitiator and sensitizer in the peroxide generation method, respectively. The peroxide density was calculated from the optical density of the solution at 360 nm. The method is based on the oxidation of sodium iodide by peroxides in the

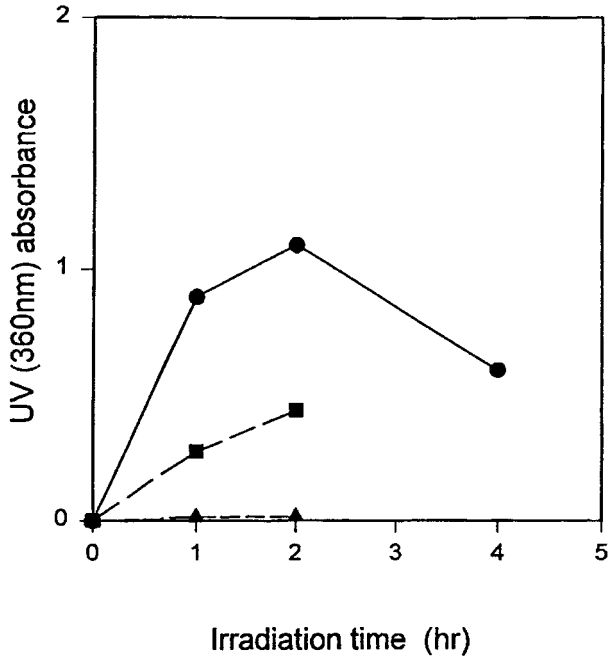


Figure 1 Dependence of absorbance of 360 nm on the UV-irradiation time. Sensitizer: (●) benzophenone + Michler's ketone; (■) benzophenone; (▲) none.

presence of ferric chloride.[9,10] Figure 1 shows the result of the peroxide generation under various conditions. As shown in Figure 1, the photochemical reaction was enhanced effectively by the addition of Michler's ketone as a sensitizer. AA of various concentrations was added and then reacted at room temperature under UV irradiation for a certain period. It was found that the amount of AA introduced onto the PE film by this method is too small to progress to the following PCe modification.

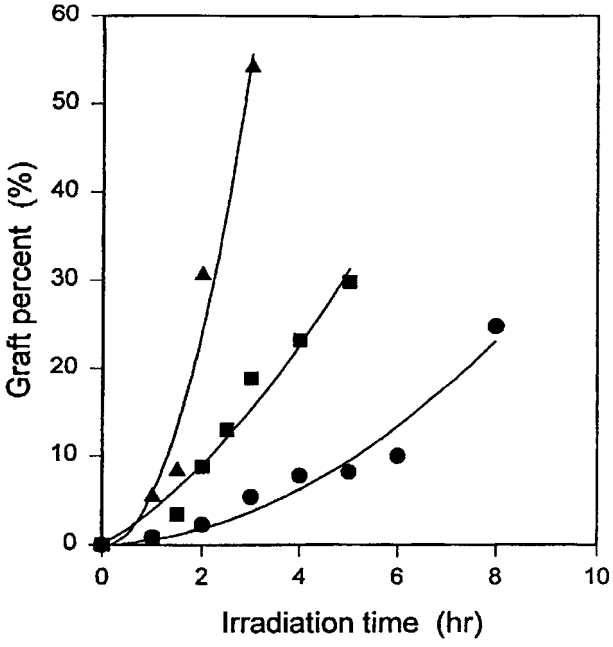


Figure 2 Dependence of graft percent of AA on the irradiation time. AA concentration: (●) 30 wt %; (■) 50 wt %; (▲) 70 wt %.

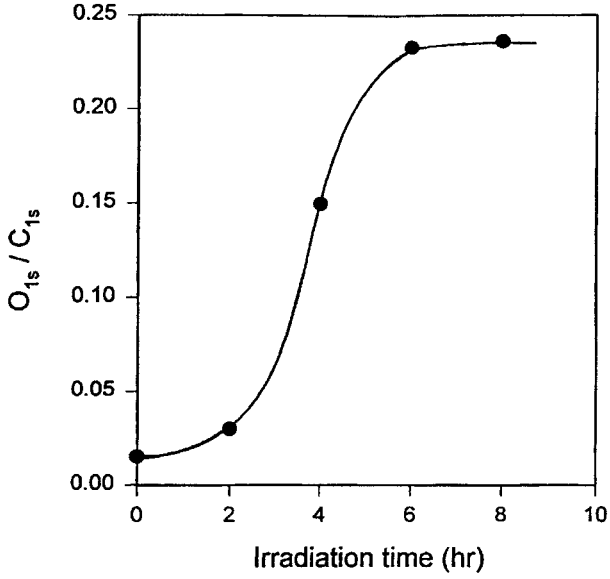


Figure 3 Dependence of O_{1S}/C_{1S} value on the UV-irradiation time of PE film in 30 wt % AA solution.

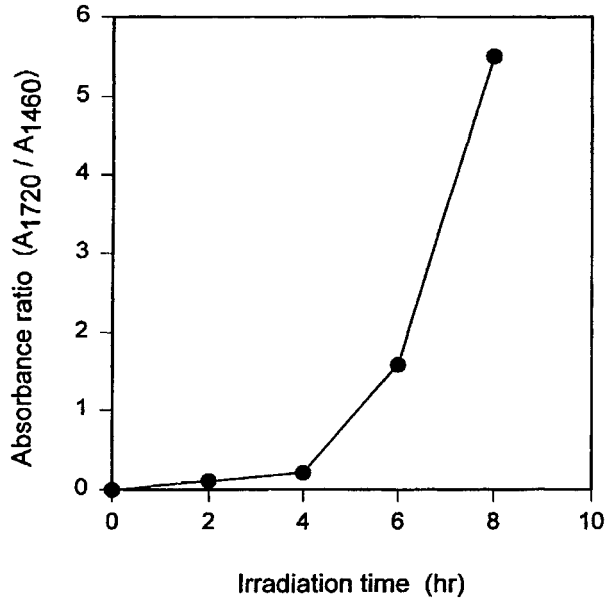


Figure 4 Effect of UV-irradiation time on the absorbance ratio (A1720/A1460) PE film surface in 30 wt % AA solution.

Table I Results of ESCA Analyses of Membrane Surface

Membrane	C_{1S}	O_{1S}	N_{1S}	P_{2P}	O_{1S}/C_{1S}	P_{2P}/C_{1S}
PE	98.5	1.5	—	—	0.015	—
PE–AA[a]	81.1	18.9	—	—	0.233	—
PE–PTMG[b]	86.2	13.8	—	—	0.160	—
PE–POH[c]	82.5	17.1	—	0.4	0.207	0.00485
PE–PCe[d]	79.8	18.0	2.0	0.3	0.226	0.00307

[a] Acrylic acid-grafted polyethylene.
[b] PTMG-grafted polyethylene.
[c] Phosphoryl oxychloride/water-treated PE film.
[d] Phosphorylcholine-grafted polyethylene.

To increase the graft-copolymerized AA amount on the PE membrane surface, a simultaneous UV-irradiation method was carried out. The amount of AA polymers grafted onto the PE film was determined by the gravimetry method. The results of graft-copolymerization of AA onto the PE film are summarized in Figure 2. As shown in Figure 2, the introduced AA percentage increased with increasing UV-irradiation time and was affected significantly by the concentration of AA. Figure 3 shows the dependence of the O_{1S}/C_{1S} value (estimated from the ESCA analyses) on the UV irradiation of the PE film, which was modified in a 30 wt % AA aqueous solution. As can be seen in Figure 3, the O_{1S}/C_{1S} values increased with increasing irradiation time. The results suggest that the amount of grafted AA groups onto the PE film surface increased with the progress of the photochemical reaction. The dependence of the IR absorbance ratio of 1720 cm^{-1} (—COOH of AA) to 1460 cm^{-1} (—CH_2— of PE) on UV-irradiation time is summarized in Figure 4. The results in Figure 4 show that the amount of introduced AA increased with increasing irradiation time. The results are consistent with those observed in Table I.

The contact angle measurement is a sensitive method for obtaining outermost surface information. The membrane samples were dipped into water for more than 24 h to equilibrate the surface energy; then, the water contact angle was detected within 20 s after absorbing water on the sample by using a soft tissue. The contact angle decreases rapidly and appears to level off for more than 6 h of UV irradiation, as shown in Figure 5.

After completion of the AA modification, PCe with various spacer lengths was then introduced onto the surface of PE by a series of chemical reactions. Ethylene glycol (EG), butanediol (BDO), polypropylene glycol (PPG), and poly(tetramethyl glycol) (PTMG) were used to investigate the spacer effect on the platelet compatibility of the PE membrane surface.

The surfaces of functional group-modified PE films were analyzed by ESCA analyses. The results are summarized in Table I. It was found that the O_{1S}/C_{1S} value increased while AA was introduced onto the PE film surface. As can be seen in Table I, the further introduction of the long carbon chain of diol onto the film surface leads to decrease of the O_{1S}/C_{1S} value. The introduced PCe groups on the PE film were also confirmed by ESCA spectra of the element absorption of P and N at 134 and 400 eV, respectively. The distribution of the element P on the PE film surface can also be found in the EDX spectra of SEM.

Figure 6 shows the SEM photographs of platelet adhesion tests, in which PRP contacted with

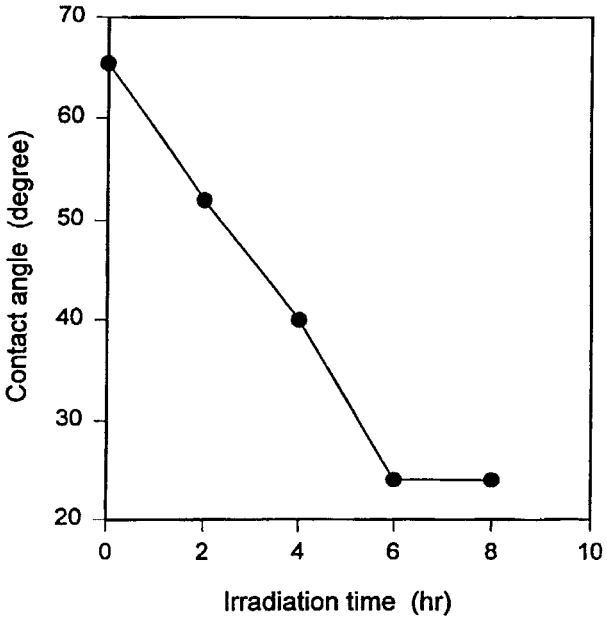


Figure 5 Dependence of contact angle of PE film surface on UV-irradiation time in 30 wt % AA solution.

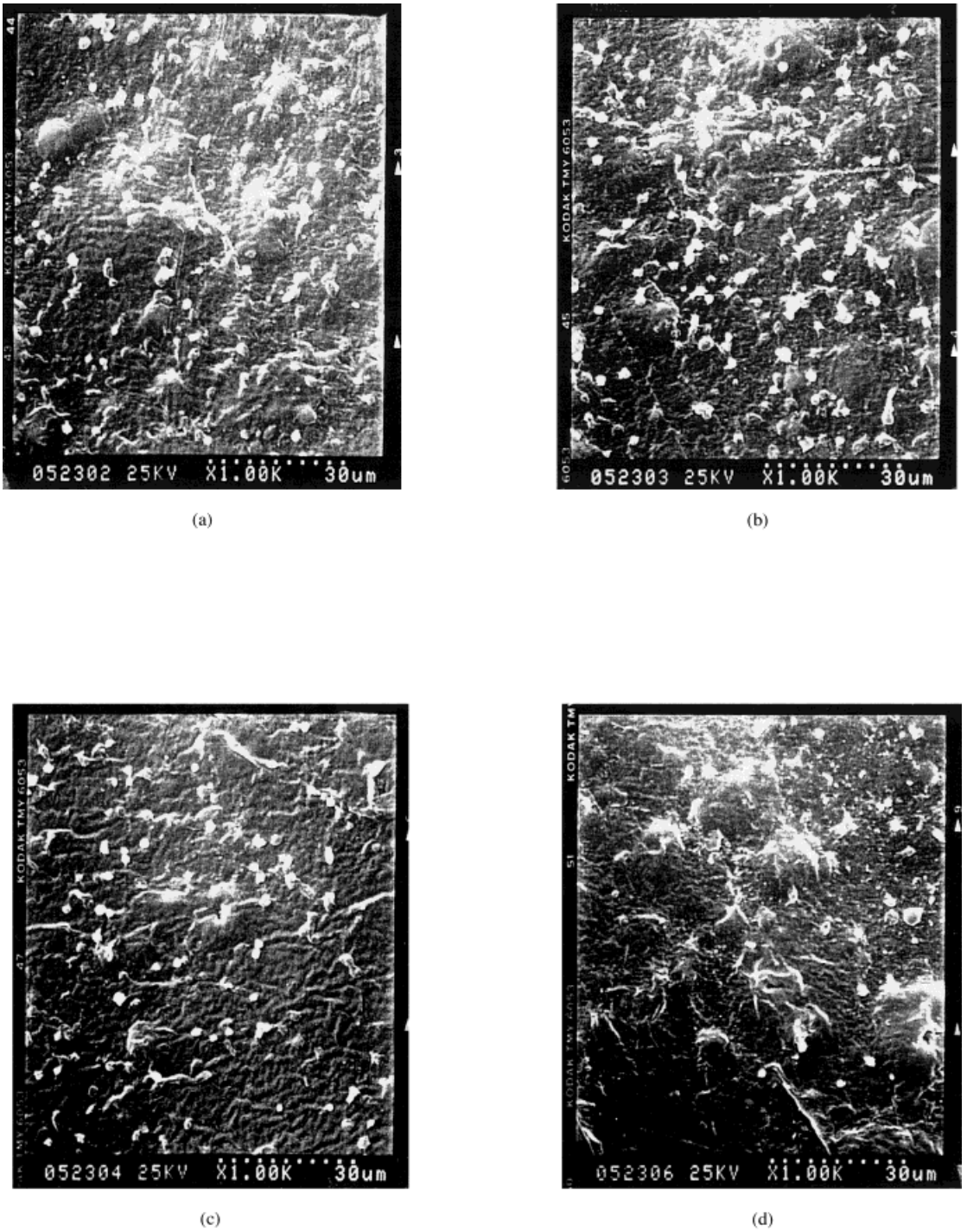


Figure 6 Comparison of SEM pictures of platelets adhered onto functional group-grafted PE film surface: (a) PE–AA; (b) PE–PTMG; (c) PE–POH; (d) PE–PCe.

the surfaces of functional group-grafted PE and PCe-modified PE for 2 h of incubation, respectively. It was found that the amount of adhered platelets decreased in the order PE–POH > PE–PTMG > PE–AA > PE–PCe. It seems that surface functional groups and charge character, as well as wettability, play important roles for platelet adhesion. The results suggest that the existence of PCe on the polymer surface could reduce platelet adhesion and deformation.

To investigate the spacer effect on the platelet adhesion, PCe's with various lipophilic lengths onto

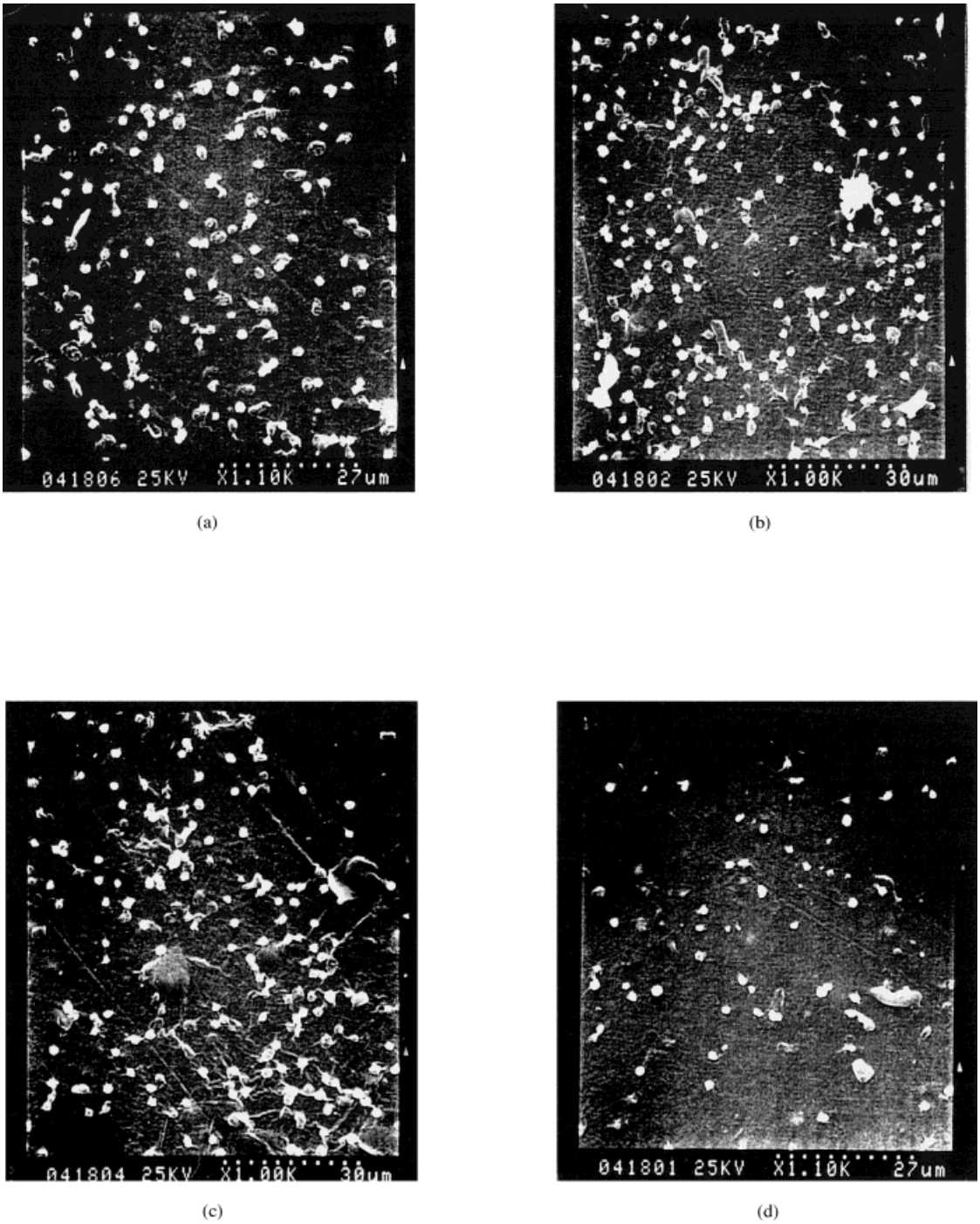


Figure 7 SEM pictures of platelets adhered onto PCe-grafted PE film surface with various spacer lengths. Spacer: (a) EG; (b) BDO; (c) PPG; (d) PTMG.

the PE film surface were synthesized. EG, BDO, PPG, and PTMG were used in this investigation. The results of the platelet adhesion tests are shown in Figure 7. As can be seen in the figure, platelets that adhered to the modified membrane decreased with increasing spacer length. The results suggest that a sufficient lipophilic length existed between the PCe groups and that the PE membrane may stabilize the PCe layer.

CONCLUSIONS

PCe groups with various lipophilic spacer lengths can be introduced onto the PE film surface by a

series of photochemical and chemical processes. The platelet compatibility of the PE film surface will be affected by the existence of various functional groups on the film surface. It was found that the amount of adhered platelets decreased in the order PE–POH > PE–PTMG > PE–AA > PE–PCe. The length of the lipophilic spacer between PC groups and the PE surface will affect the stability of the film surface. A sufficient lipophilic length exists between the PCe groups and the PE membrane may stabilize the PCe layer, leading to an increasing of platelet compatibility of the film surface.

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