Ozone-Induced Grafting of a Sulfoammonium Zwitterionic Polymer onto Low-Density Polyethylene Film for Improving Hemocompatibility

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ABSTRACT: Ozone-induced grafting was developed to improve the hemocompatibility of biomaterials based on low-density polyethylene (LDPE). An LDPE film was activated with ozone and graft-polymerized with *N,N′*-dimethyl-(methacryloylethyl)ammonium propane sulfonate (DMAPS). The existence of sulfobetaine structures on the grafted film was confirmed by X-ray photoelectron spectroscopy and attenuated total reflection/Fourier transform infrared (ATR–FTIR). More DMAPS was grafted onto the LDPE film as the DMAPS concentration increased, as determined by ATR–FTIR. Static contact-angle measurements indicated that the DMAPS-grafted LDPE film had a significant increase in hydrophilicity. The blood compatibility of the grafted film was preliminarily evaluated with a platelet-rich-plasma (PRP) adhesion study. No platelet adhesion was observed on the grafted film incubated with PRP at 37°C for 180 min. This new sulfoammonium zwitterionic-structure-grafted biomaterial might have potential for biomedical applications. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 3697–3703, 2006

Key words: adhesion; polyethylene (PE); film

INTRODUCTION

Polyethylene (PE) is widely used in biomedical fields because of its good biocompatibility and versatility. However, when the surface of PE comes into contact with blood, it still initiates the adsorption of blood proteins, and then platelet adhesion and other components of the blood coagulation system are activated; this leads to thrombus formation.[1,2] A potential solution to the problem of thrombogenic polymers may be realized through the surface modification of polymers.[3–7] Different methods for polymer surface modification have been proposed to obtain more biocompatible polymer materials, including the immobilization of polymer chains onto a polymer surface by coupling reactions[3] and the graft polymerization of monomers via glow discharge,[4] corona discharge,[5] UV radiation,[6] and plasma.[7] In particular, ozone-induced surface grafting[8,9] is being widely applied in biomaterial research because it has the advantage of uniformly introducing peroxides onto the polymer surface even with complicated shapes and is an easy-to-handle, inexpensive technique.[8,9] Peroxides are mainly formed, in addition to carbonyl and carboxyl groups, when a polymer is exposed to ozone gas. The generated peroxides are capable of initiating the radical polymerization of vinyl monomers, which results in surface-grafted polymer chains.

Previous research has indicated that the incorporation of sulfonate groups can significantly reduce platelet deposition.[10,11] It has also been reported that low-density polyethylene (LDPE) films graft-copolymerized with *N,N′*-dimethyl(methacryloylethyl)ammonium propane sulfonate (DMAPS; a zwitterionic monomer) have enhanced wettability and autoadhesion properties.[12] Meanwhile, various studies have demonstrated that zwitterionic interfacial molecular structures have excellent antithrombogenicity.[13–17] In this study, the ozone activated method was used for the first time to graft DMAPS onto the surface of LDPE to improve the hemocompatibility. The surface of the DMAPS-polymer-grafted film was characterized with X-ray photoelectron spectroscopy (XPS), attenuated total reflection/Fourier transform infrared (ATR–FTIR), and static contact-angle measurements. Also, the blood compatibility of the modified film was evaluated with a platelet adhesion study.

EXPERIMENTAL

Materials

An LDPE film about 120 μm thick was commercially obtained. The film was cleaned by Soxhlet extraction

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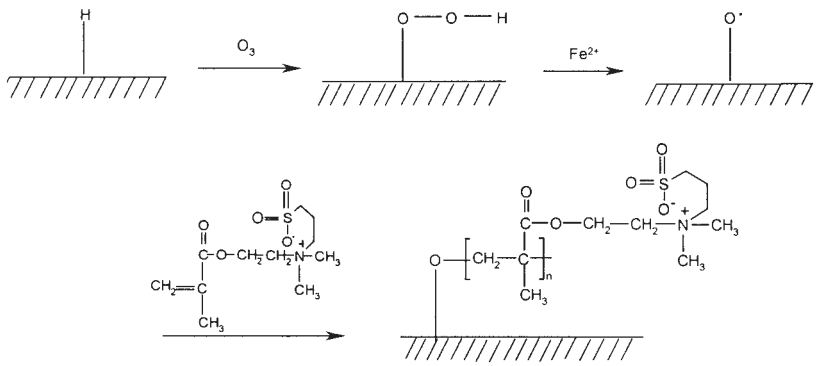


Figure 1 Scheme of surface graft polymerization.

with methanol for 24 h before it was used. The zwitterionic monomer DMAPS (analytical reagent; Raschig, Germany) was used as received. A 25% glutaraldehyde water solution was provided by Shanghai Chemical Reagent Co. (Chinese Medical Group). Freshly prepared platelet-rich plasma (PRP) of human blood was supplied by the Blood Center of the Nanjing Red Cross (Nanjing, China).

Ozonization and surface grafting of DMAPS

Ozone was generated with dried oxygen gas passed through an ozone generator (BX-9, Beijing Environmental Science Institute, Beijing, China). The operating condition was 200 V, and the pure oxygen inlet flow rate was fixed at 200 mL/min to produce an ozone production rate of 0.4 g/h at 30°C. The film was treated with ozonization in a glass vessel. Then, the ozonized film was degassed *in vacuo* below 133 Pa at the ambient temperature for 1 h. The LDPE film was graft-polymerized at 40°C with 1, 3, 5, 8, and 10 wt % aqueous solutions of DMAPS and with an Fe^{2+}-ion concentration of $2.6–3.0 \times 10^{-4}$ mol/L in a sealed tube. The polymerization time was 24 h. To remove the viscous homopolymer from the grafted film, the grafted film was first washed with 40–50°C saline water and then immersed in distilled water under continuous stirring until a constant weight was obtained. The surface graft polymerization is shown schematically in Figure 1.

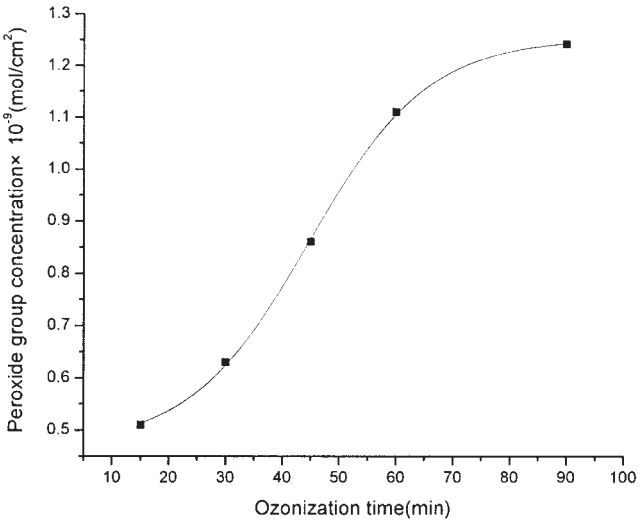


Figure 2 Relationship between the peroxide concentration in the LDPE film and the ozonization time.

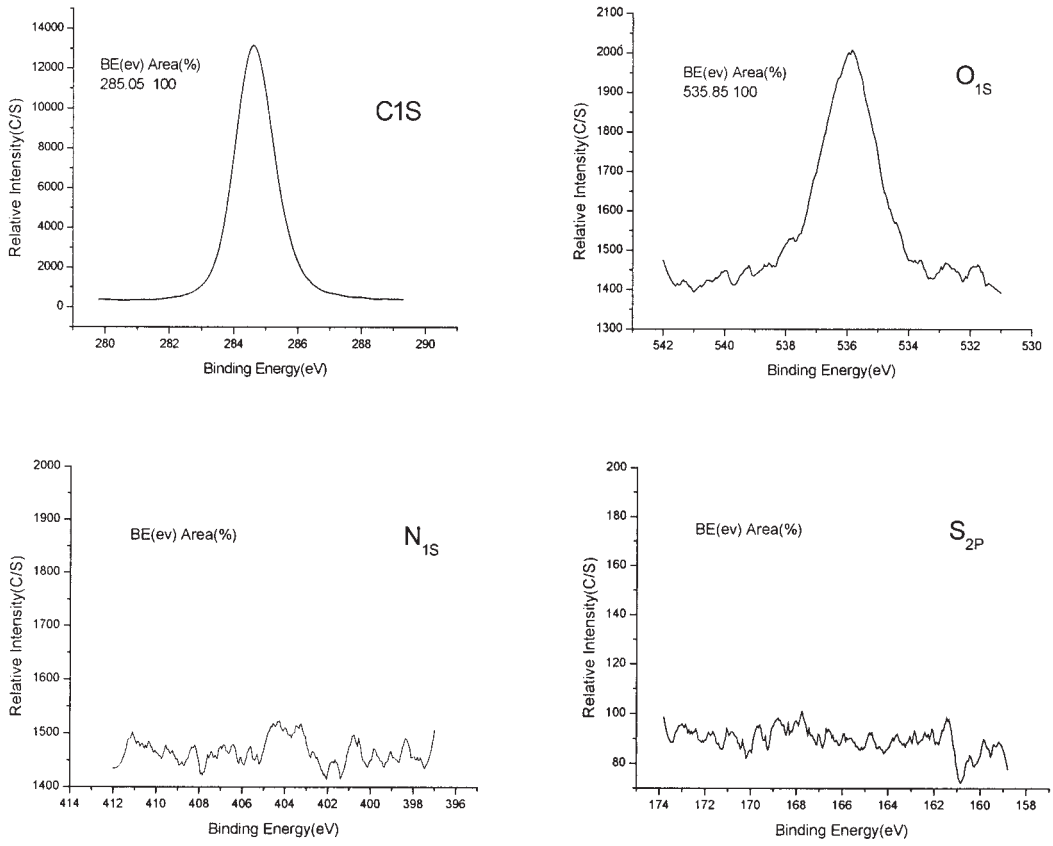
Surface analysis

The amount of the peroxide generated on the film was determined according to ref. 18. In brief, 25 mL of isopropanol was added to the sample, and this was followed by 1 mL of saturated potassium iodide and 1 mL of glacial acetic acid. The mixture was heated almost to boiling, kept at incipient boiling for 2–5 min with occasional swirling and without cooling, and titrated with standard sodium thiosulfate until the yellow color disappeared. It was possible to titrate solutions with as little as $10^{-4}N$ peroxygen to within 0.1 mL with 0.005*N* thiosulfate. The amounts reported are the mean values for three specimens.

Electron spectroscopy for chemical analysis (ESCA; XPS) spectra were obtained on an Escalab MK II spectrometer (V.G. Scientific Co., Ltd., UK) with Al Kα radiation. The releasing angle of the photoelectron for each atom was fixed at 45°. The IR spectrum of the surface of the LDPE film was measured with an ATR–FTIR spectrophotometer (Nicolet, United States). Static contact-angle measurements were performed at 25°C with the sessile drop method (model 100-0, Rame-Hart, Inc., United States).

TABLE I
XPS Elemental Surface Composition (%) of LDPE and LDPE-*g*-PDMAPS Films

Polymer film	C_{1s}	O_{1s}	N_{1s}	S_{2p}
LDPE	97.48	2.22	0.30	0
LDPE-*g*-PDMAPS	64.54	27.78	3.81	3.87



(a)

Figure 3 XPS spectra of the film surfaces of (a) LDPE and (b) LDPE-*g*-PDMAPS.

Platelet adhesion[1]

To determine the potential blood compatibility of the materials, we conducted platelet adhesion studies because platelet adhesion is one of the most important steps during blood coagulation on artificial surfaces.[19] Blood platelet attachment *in vitro* was evaluated with scanning electron microscopy (SEM; model 15300VP, Leo, Germany). The grafted film was rinsed with phosphate-buffered saline (PBS) first and was placed into contact, at 37°C for 180 min, with freshly prepared PRP of human blood supplied by the Blood Center of the Nanjing Red Cross. Each sample was rinsed with PBS and treated with 2.5% glutaraldehyde for 30 min at room temperature. It was rinsed with PBS and dehydrated by systemic immersion in a series of ethanol–water solutions [50, 60, 70, 80, 90, 95, and 100% (v/v)] for 30 min each, which were allowed to evaporate at room temperature. The platelet-attached surfaces were gold-deposited *in vacuo* and examined with SEM. The LDPE film was used as a reference.

RESULTS AND DISCUSSION

Effect of ozonization on the LDPE surface

Figure 2 shows the concentration of the peroxides that evolved on the LDPE surface treated by ozone. The concentration increased quickly with increasing ozonization time during the first 60 min. However, the peroxide concentration increased slowly after 60 min of exposure to the ozone gas. Perhaps the concentration neared saturation on the LDPE surface. Therefore, 60 min was set as the ozonization time.

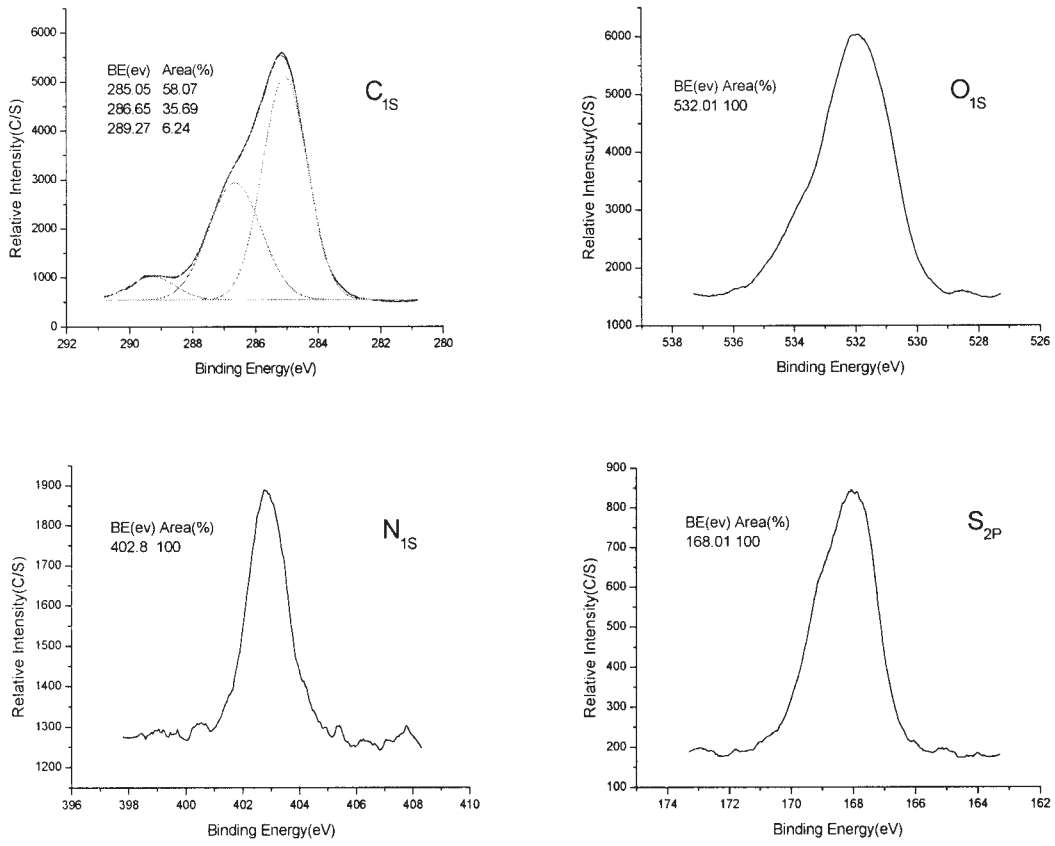
DMAPS polymer grafted onto the LDPE surface

Peroxides generated by ozonization were reduced into radicals to initiate the grafting of DMAPS onto the LDPE film surfaces. However, it was inevitable that POOH decompose by heat to produce PO · and OH ·. PO · induced copolymerization, whereas OH · induced homopolymerization. In the reaction, Fe^{2+} ion acted as follows:[20]

$$POOH + Fe^{2+} \xrightarrow{k_1} PO\cdot + OH^- + Fe^{3+}$$

$$PO\cdot + Fe^{2+} \xrightarrow{k_2} PO^- + Fe^{3+}$$

Here POOH represents the polymeric peroxide. PO · was also reduced by Fe^{2+} ($k_2 \ll k_1$). As a result, the homopolymerization was restrained.



(b)

Figure 3 (*Continued from the previous page*)

Table I lists the ESCA surface elemental compositions of the LDPE and low-density polyethylene-*g*-poly[*N*,*N*′-dimethyl(methacryloylethyl)ammonium propane sulfonate] (LDPE-*g*-PDMAPS) film. There was no content of N_{1s} and S_{2p} on the surface of LDPE, but the content of S on the surface of the LDPE-*g*-PDMAPS film was as high as 3.87%. Also, the approximately equal content of N, 3.81%, strongly confirmed the presence of sulfoammonium zwitterions grafted onto the LDPE film.

Figure 3(a,b) shows detailed ESCA spectra for the LDPE and LDPE-*g*-PDMAPS film, obtained at a 45° takeoff angle. The C_{1s} core-level spectrum of the pristine LDPE film consisted predominately of a single peak component with the binding energy at 285.05 eV, whereas the C_{1s} peak for LDPE-*g*-PDMAPS skewed toward the high binding-energy side and could be resolved into three component peaks: a hydrocarbon (*C*—C—C) peak at 285.05 eV, an ammonium (—N^+—*C*) peak at 286.65 eV, and an ester (—*C*OO—) peak at 289.27 eV.

A Gaussian peak at 532.05 eV was used to fit the LDPE-*g*-PDMAPS O_{1s} peak. The high-energy and low-energy tailings on the O_{1s} peak reflected a combination of contributions from ester oxygen and sulfonate oxygen (SO_3^-). The N_{1s} core-level spectrum consisted of predominantly a peak component at 402.85 eV, which was characteristic of a positively charged nitro-

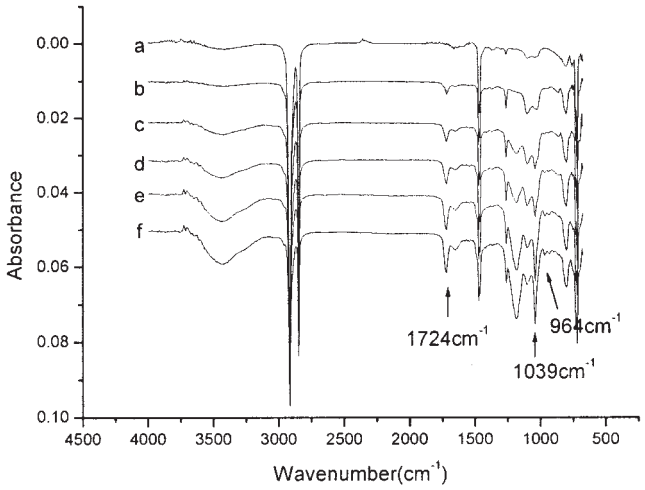


Figure 4 ATR–FTIR spectra of (a) LDPE and (b–f) LDPE-*g*-PDMAPS films. The LDPE-*g*-PDMAPS films were grafted with concentrations of (b) 1, (c) 3, (d) 5, (e) 8, and (f) 10 wt % DMAPS.

TABLE II
A_1/A_2 and Contact-Angle Values of LDPE-*g*-PDMAPS Films with Different DMAPS Concentrations

	DMAPS concentration (wt %)					
	0	1.0	3.0	5.0	8.0	10.0
A_1/A_2	0	0.086	0.181	0.215	0.339	0.397
Contact angle (±3°)	91.5	61.9	45.7	38.7	34.5	32.0

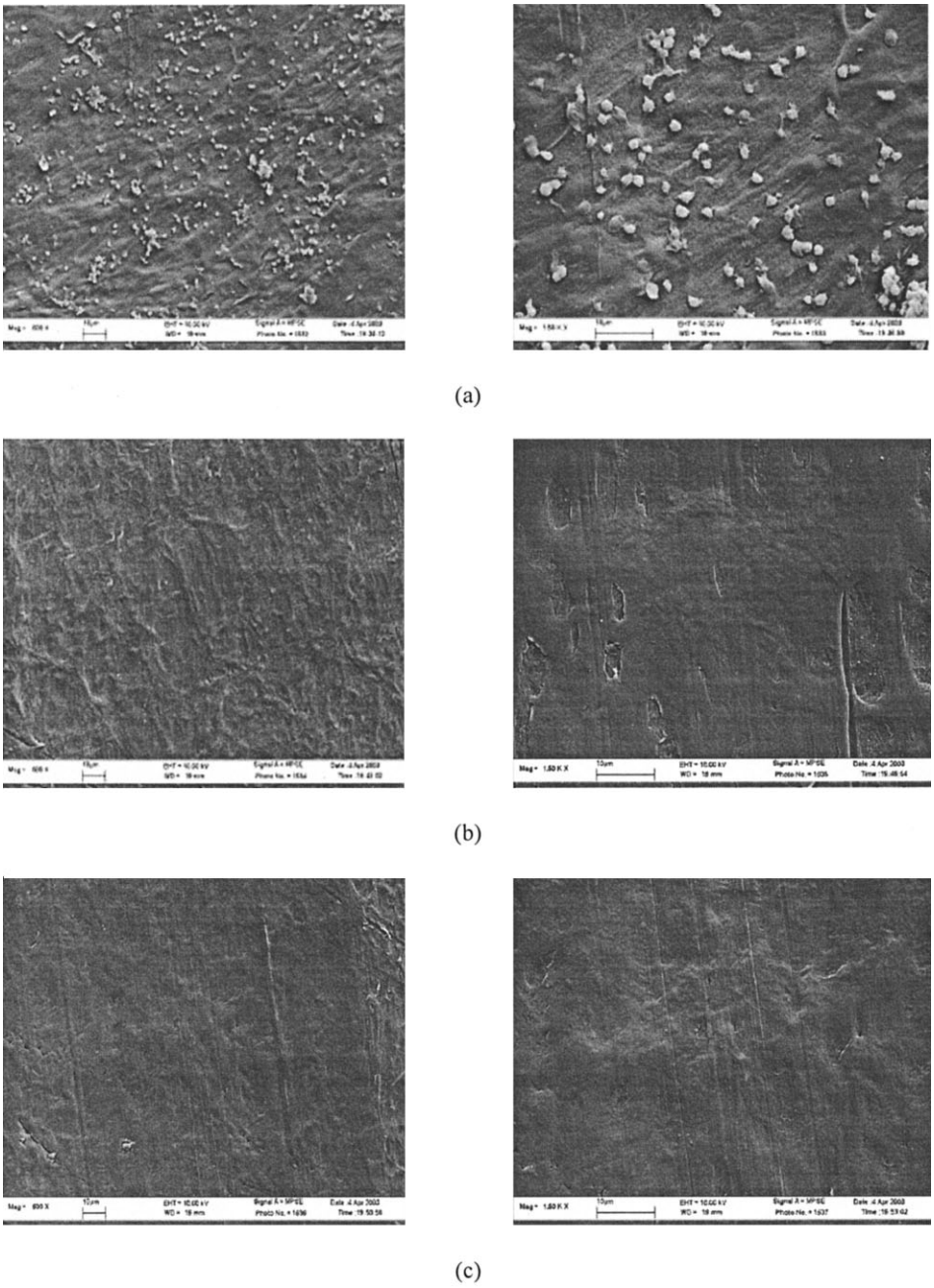
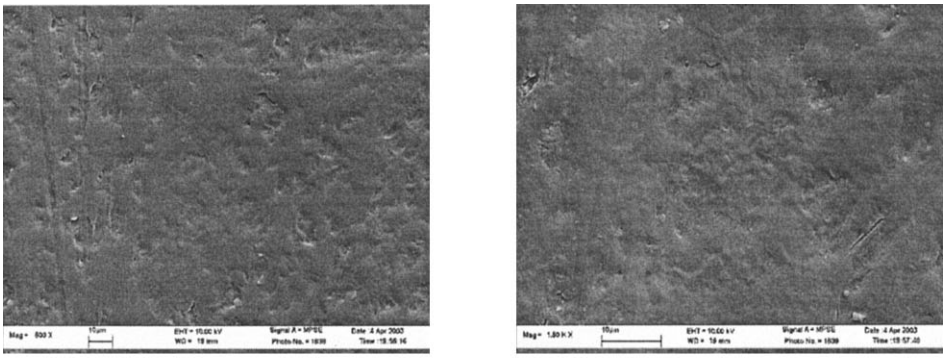


Figure 5 SEM photographs of the film surfaces of (a) LDPE and (b–f) LDPE-*g*-PDMAPS films after 180 min of exposure to human PRP [original magnification = 600× (left) or 1500× (right)]. The LDPE-*g*-PDMAPS films were grafted with concentrations of (b) 1, (c) 3, (d) 5, (e) 8, and (f) 10 wt % DMAPS.

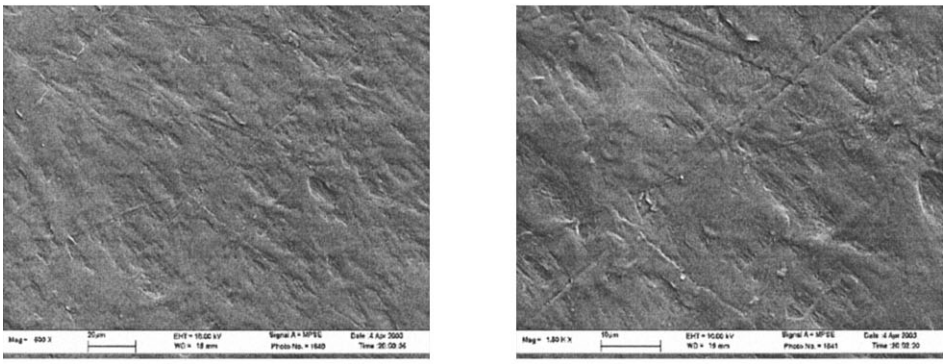
gen of DMAPS.[12] Similarly, a Gaussian peak at 168.05 eV, the characteristic $S_{2P3/2}$ binding energy, was attributed to the covalently bonded sulfonate species (SO_3^-).[12]

Figure 4 shows the ATR–FTIR spectra of the LDPE and LDPE-*g*-PDMAPS film. A comparison of these spectra shows a significant difference due to the grafting of DMAPS onto the LDPE surface. Only CH stretching vibrations at 2916 and 2849 cm^{-1} and CH bending vibrations at 1463 and 1366 cm^{-1} can be observed in the spectrum of the LDPE surface. In the spectrum of the LDPE-*g*-PDMAPS film, the adsorption of SO_3^- (1180 and 1039 cm^{-1}), N^+—$(CH_3)_3$—CH2— (964 cm^{-1}), and —COO— (1723 cm^{-1}) can be observed, and this confirmed the successful grafting of DMAPS.

The ratio of the peak area of >C═0 (A_1) to that of —CH_2— (A_2) was calculated to indicate the amount of PDMAPS grafted onto the LDPE surface and is summarized in Table II. The A_1 data suggest that the >C═O group, representing the amount of DMAPS, was present on the surface of LDPE. As a result of the



(d)



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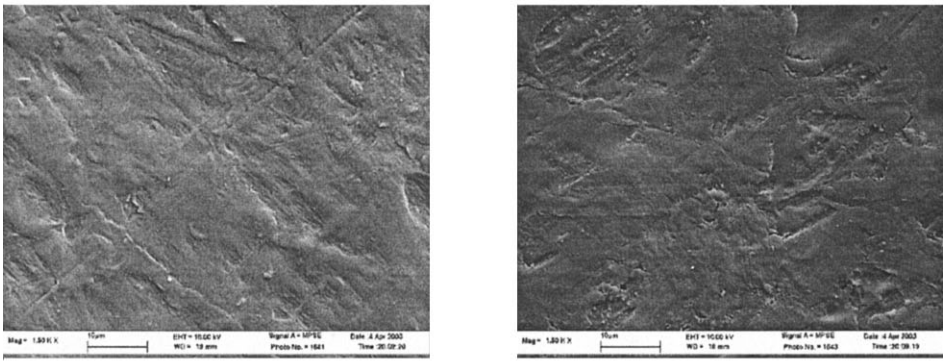

(f)

Figure 5 (*Continued from the previous page*)

DMAPS polymer grafting, >C═O showed a tendency of increasing gradually with increasing DMAPS monomer concentration.

Static water contact-angle measurements were carried out to investigate the hydrophilicity of the surface of the LDPE and LDPE-*g*-PDMAPS film. The results summarized in Table II suggest that the LDPE film was relatively hydrophobic, and the hydrophilicity of the grafted film increased with increasing DMAPS concentration. This also indicated that the graft polymerization took place in higher yields.

Platelet adhesion

The antithrombogenicity of the LDPE-*g*-PDMAPS film was assessed by the degree and nature of the platelet adhesion resulting from incubation in fresh human PRP for 180 min, with LDPE as a reference. Blood platelet attachment *in vitro* was viewed by SEM. Typical SEM photographs of the LDPE-*g*-PDMAPS film and LDPE film are shown in Figure 5. No platelet attachment happened on the surfaces of modified LDPE, even when the film was immersed in PRP 180 min. On the contrary, the LDPE film surface exhibited a large amount of adhered platelets, some of which had some degree of shape variation. Moreover, there were several platelet aggregates on the surface of the LDPE film.

It is thought that the LDPE-*g*-PDMAPS film surfaces were covered with a layer of the DMAPS polymer. The improved antithrombogenicity could be attributed to the zwitterionic structure of DMAPS. In an aqueous (blood) medium, the zwitterionic structure molecules not only cannot diffuse into the interior of the protein's tertiary/quaternary structure, which is mainly maintained by hydrophobic interactions and hydrogen bonds, but also minimize the effect on exterior surface ions (cations/anions) of the protein and are thus favorable to the maintenance of normal conformations of proteins and their assemblies.[12,13]

CONCLUSIONS

DMAPS, a sulfobetaine structure, was grafted onto an LDPE film surface by ozone-induced polymerization, and this was confirmed by XPS and ATR–FTIR. The surface hydrophilicity of the grafted film was greatly improved. The platelet adhesion on the grafted film was efficiently restrained because of the sulfoammonium zwitterionic interfacial structure.

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