# Surface Modification of Polyethylene by Plasma Pretreatment and UV-induced Graft Polymerization for Improvement of Antithrombogenicity

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**ABSTRACT:** The purpose of this study was to enhance blood compatibility of polyethylene (PE) films. Glycidyl methacrylate (GMA) was grafted onto the surface of PE by Ar plasma pretreatment and UV-induced graft polymerization without photo-initiator, then heparin was immobilized onto the poly (glycidyl methacrylate) segments. The surface compositions and microstructure of GMA graft polymerized PE films were studied by X-ray photoelectron spectroscopy (XPS) and Attenuated Total Reflectance Fourier Transfer Infrared (ATR-FTIR) spectroscopy. It was confirmed that heparin was successfully immobilized onto the surface of PE films by XPS analysis. The antithrombogenicity of the samples was determined by the activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), and plasma recalcification time (PRT) tests and platelet adhesion experiment. Results indicated that the antithrombogenicity of modified PE was improved remarkably. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 93: 2014–2018, 2004

**Key words:** polyethylene; plasma; UV-induced graft polymerization; surface modification; antithrombogenicity

## INTRODUCTION

Polyethylene (PE) was widely used in medical devices because of its excellent mechanical and chemical resistance and good thermal property. Its various applications in medical and biomedical fields had emerged such as medical catheters, artificial blood vessels, and some blood-contacting materials in extracorporeal circulation equipment during operations. However, like other synthetic polymers, a major problem with PE is that its working surface is foreign to blood and tends to initiate red-cell destruction and coagulation, and as a result, to form clots. Much work has been done to find ways to impart nonthrombogenicity on the surface of synthetic materials.[1–4] Surface heparinization is one of the most important ways to realize it. The surface of a polymer is inert and needs to be modified before heparinization. Various methods for surface modification have been used such as corona discharge treatment, electron beam treatment, X-ray irradiation, plasma-induced graft polymerization, and photoinitiated graft polymerization.[5–9] In addition to these methods, recently surface modification of plasma pretreated polymers via UV-induced graft polymerization with specific functional monomers has been shown to be an effective approach to adhesion enhancement with polymers.[10–13]

In this work, surface modification of Ar plasma pretreated PE films is carried out via UV-induced graft polymerization with a bifunctional monomer (glycidyl methacrylate, GMA), and then heparin is immobilized in poly (glycidyl methacrylate) branches.

## EXPERIMENTAL

### Materials

PE films having a thickness of about 0.1 mm were used in this study and were obtained from Hongda Ltd., Jiangxi, China. The surfaces of PE films were cleaned with analytical reagent grade acetone (A.R.) in an ultrasonic water bath for 45 min before use. GMA and the solvent 1,4-dioxane were graded analytical reagents from Aldrich Chemical Co., Milwaukee, WI, USA. Heparin sodium was supplied by Sanpu Chemical Co., Shanghai, China.

### GMA graft polymerization and heparin immobilization

The PE films (20 mm × 30 mm) were placed into a plasma treatment system shown in Figure 1. Samples were mounted on the sample holder in the glass chamber (1500 $cm^3$) equipped with two external electrodes, then the chamber was fixed to a vacuum system. The

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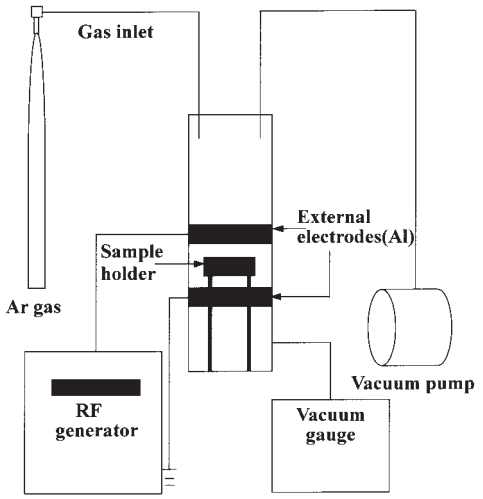



**Figure 1** Schematic diagram of plasma treatment system.

plasma treatment was conducted using a 13.56 MHz radio-frequency (rf) generator (SY-300, Institute of Microelectronics Chinese Academy of Science, Beijing, China.) under the following conditions: rf power = 80 W, Ar pressure = 20 Pa, treatment time = 5 min. The plasma pretreated PE films were then exposed to the atmosphere for 10 min to facilitate the formation of surface peroxides and hydroperoxides for the subsequent UV-induced graft polymerization experiments.[14] The pretreated PE films were immersed in 30 mL of 1,4-dioxane (for GMA) monomer solution, which was sealed in a glass tube. The concentration of the monomer solution was varied from 1 to 10 vol %. The graft polymerization was carried out by irradiating the PE films with 1000 W high-pressure mercury lamp (wavelength: 365 nm; Philips Co., Holland) for 60 min. The apparatus is schematically shown in Figure 2. After the graft polymerization reaction, the films were washed in pure 1,4-dioxane for more than 12 h to remove homopolymers and unreacted monomers.

Heparin was immobilized onto the surface of GMA-grafted PE film by placing the grafted materials in a flask with 50 mL $H_2O$ and 2 g heparin, then slowly vibrating for 12 h at 40°C. After reaction the films were immersed with distilled water for 24 h and dried at 50°C.

## Surface analytical methods

Attenuated total reflectance (ATR) FTIR spectra were performed with a NEXUS-670 FTIR spectrometer with an ATR unit (ZnSe crystal, 45°, Nicolet Instrument Co., Madison, WI). Each spectrum was obtained by cumulating 32 scans at a resolution of 4 $cm^{-1}$.

XPS measurements were recorded on a Perkin–Elmer PHI-5400 (Wellesley, MA) with a $MgK_\alpha$ X-ray source (1253.6 eV) of 15 kV, 250 W. The vacuum degree is 1–4 $\times$ $10^{-8}$ Torr and the photoelectron take-off angle is 45°. The degree of grafting GMA was calculated by comparing the found oxygen content with the theoretical value of the pure GMA using the formula[15]:

$$\text{Degree of grafting GMA} = \frac{10O/C}{3 + 3O/C} \times 100$$

The contact angles to water of the GMA-grafted PE films were measured on a JY-82 contact goniometer (Chengde Test Instrument Co., Hebei, China) at ambient humidity and temperature. Six data for three water drops for one sample were obtained, with the deviation range within ± 2–3°, and its averaged value was adopted as the contact angle for sample.

## Antithrombogenicity test in vitro

Activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) measurements[16]: The tested films were incubated with healthy human blood plasma in a transparent plastic tube, and the reagents for each coagulation time test were added to the tube immediately. The clotting times were measured by a photo-optical clot detection instrument CA-6000 (Sysmex Co., Koube, Japan). The data were mean values of those obtained from three repeated experiments.

Plasma recalcification time (PRT) measurement: Platelet poor plasma (PPP, 3 $\times$ $10^{-2}$ mL) was incubated on the samples. 0.025 M $CaCl_2$ aqueous solution was added to the PPP. The plasma solution was monitored for clotting by manually dipping a stainless steel wire hook coated with silicone into the solution for detec-

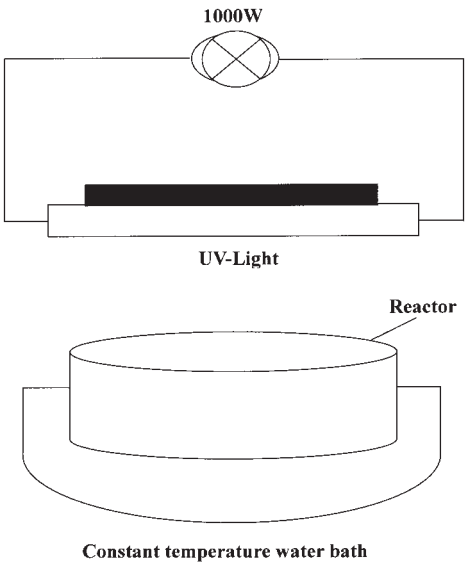



**Figure 2** Schematic diagram of UV-light graft equipment.

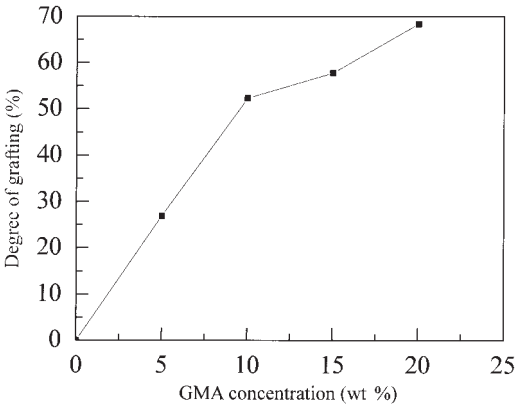



**Figure 3** Degree of grafting as a function of GMA concentration.

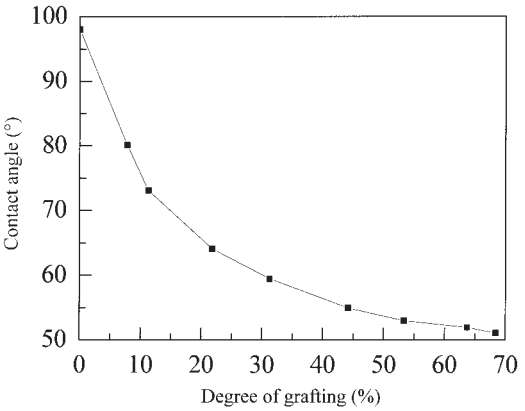



**Figure 4** Contact angle as a function of degree of grafting.

tion of fibrin threads. Clotting times were recorded at the first signs of fibrin formation on the hook.

Platelet adhesion experiment: Samples were brought to contact with human fresh platelet-rich plasma and incubated at 37°C for 60 min. Then, samples were carefully and slowly rinsed by phosphate buffered saline (PBS) three times to remove unadhered platelet. Adhered platelets on the samples were fixed by using 2.5% glutaraldehyde for 30 min, followed by a dehydration procedure using a series of ethanol-water mixture (50, 60, 70, 80, 90 and 100 vol % of ethanol) 20 min each. After critical point drying with $CO_2$, samples were gold coated and examined by scanning electron microscopy (SEM) (GSM-5800, JEOL Co., Tokyo, Japan).

## RESULTS AND DISCUSSION

### Ar pretreatment and UV-induced graft polymerization

It is well known that the epoxy group can react with a lot of different functional groups. When GMA was grafted onto the surface, a reactive polymer surface containing epoxy groups was formed. To this surface, some substances can be attached through reaction with the epoxy group. Thus, its surface property can be further modified.

We study the effect of GMA concentration on grafting reaction in a 1,4-dioxane solution (see Fig. 3). The degree of grafting increases with increases in the monomer concentration. The higher monomer concentration gives the higher degree of grafting. The maximal concentration used for the grafting solution (20 wt %) is far from a possible gel effect that would lead to a decrease of graft amount because of the insolubility of poly (glycidyl methacrylate) in its own monomer.

Figure 4 shows contact angle as a function of degree of grafting. Contact angle decreases with the degree of grafting increasing. After grafting, the contact angle against water decreases from 98 to 51°. As epoxy groups of GMA are hydrophilic, it probably contributes to the decrease in the contact angle.

Figure 5 shows the ATR-FTIR spectra of pristine PE and GMA-grafted PE. As can be seen, a new peak appears at 1733 $cm^{-1}$ in the spectrum of GMA-grafted PE due to carbonyl stretching. New absorption peaks of the epoxy group and ester group of GMA are observed at 848 $cm^{-1}$ and 1149 $cm^{-1}$, respectively. These results indicate that GMA is grafted onto the surface of PE film.

Figure 6 shows wide scan spectra of pristine PE and GMA-grafted PE. For the pristine PE, the spectrum indicates the presence of C1s peak only. In the spectrum of GMA-grafted PE O1s peak appears, which is attributed to the grafted GMA. The results can also be seen in Table I. Table I gives the data of binding energy and concentration of elements on the surface of pristine PE, GMA-grafted PE, and heparinized PE. Clearly, the surface of GMA-grafted PE contains oxy-

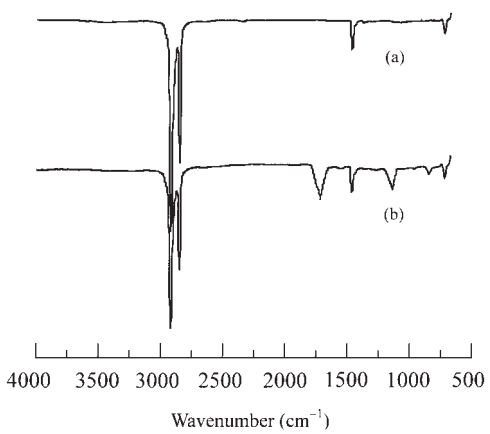



**Figure 5** ATR-FTIR spectra of (a) pristine PE and (b) GMA-grafted PE (rf power, 80 W; treatment time, 5 min; UV irradiation time, 60 min; GMA, 20 wt %).

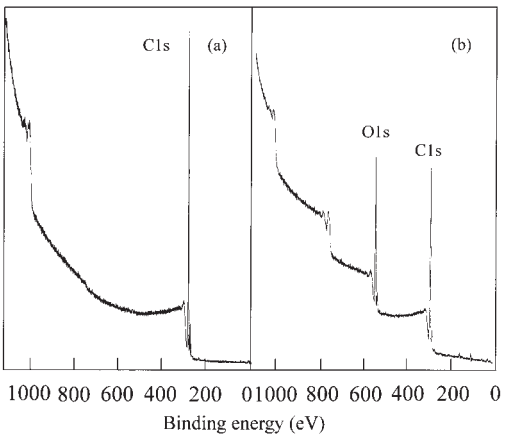



**Figure 6** Wide-scan spectra of (a) pristine PE and (b) GMA-grafted PE (rf power, 80 W; treatment time, 5 min; UV irradiation time, 60 min; GMA, 20 wt %).

gen at much higher level than pristine PE. It can be concluded that GMA is indeed grafted onto the PE surface on the basis of the analysis results of ATR-FTIR and XPS.

### Immobilization of heparin

Heparinized polymeric materials, which were first described by Gott,[17] are relatively thromboresistant. Heparin is a water-soluble acidic mucosaccharide; therefore, it is difficult to immobilize heparin on the surface of materials. Heparin can be immobilized onto polymeric materials by plasma.[18] This is a kind of covalent end-point immobilization. The covalent end-point immobilization of heparin can preserve anticoagulant activity. By being anchored to the surface with a single chemically stable linkage at one terminus, the molecule extends from the surface into the fluid phase and is free to interact with coagulation factors and inhibitors in the blood. To increase the flexibility of heparin, it was attached to surface of polymer through a hydrophilic GMA spacer. Heparin contains acid, amine, and alcohol groups, which can react with epoxy groups of GMA. Figure 7 shows the respective Na1s, N1s, and S2p core-level spectrum of GMA-grafted PE and heparinized PE. The results indicate that after heparinization reaction, traces of nitrogen, sulfur, and sodium that belong to the heparin are detected. Data in Table I also indicate traces of nitrogen, sulfur, and sodium. Therefore, the analysis result of XPS suggests that heparin is successfully immobilized onto the surface of GMA-grafted PE.

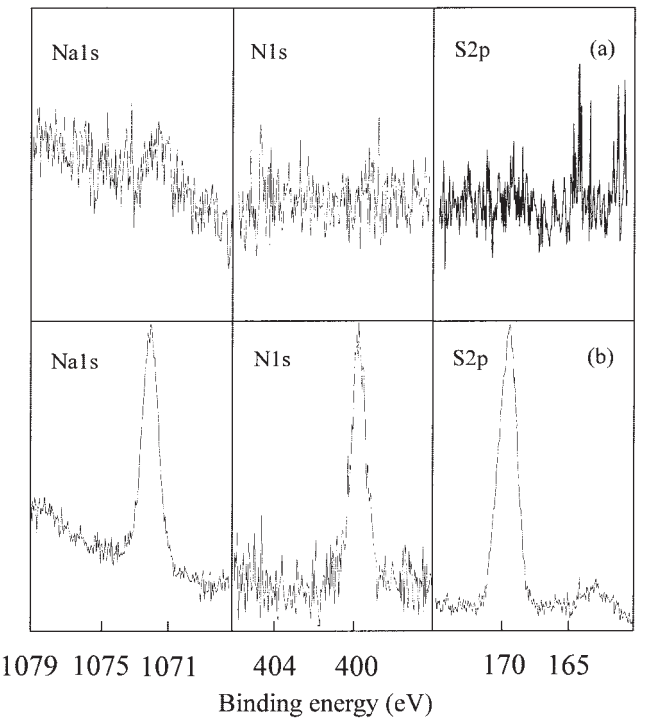



**Figure 7** Na1s, N1s, and S2p core-level spectra of (a) GMA-grafted PE and (b) heparinized PE (rf power, 80 W; treatment time, 5 min; UV irradiation time, 60 min; GMA, 20 wt %; heparinization, 2 g heparin, 50 mL $H_2O$, 12 h at 40°C).

**TABLE I**
**Data of XPS (rf power, 80 W; treatment time, 5 min; UV irradiation time, 60min; GMA, 20 wt %)**

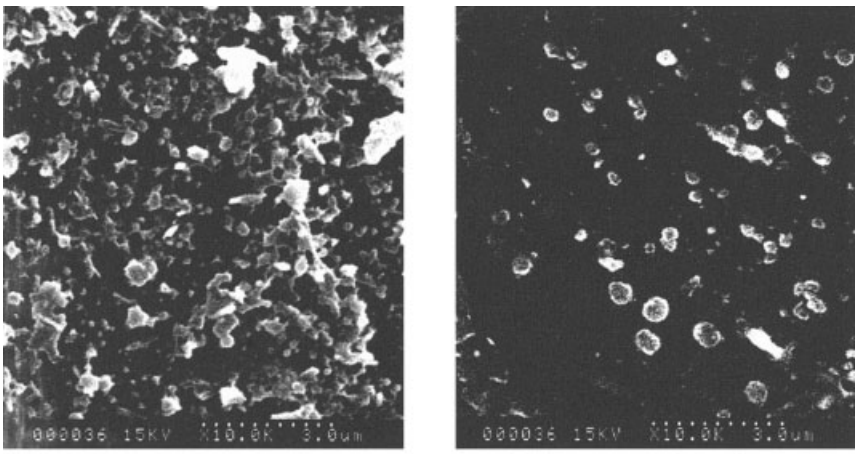
| | Pristine PE | | GMA-grafted PE | | Heparinized PE | |
|---|---|---|---|---|---|---|
| Element | B.E. (eV) | Conc. (%) | B.E. (eV) | Conc. (%) | B.E. (eV) | Conc. (%) |
| C1s | 284.6 | 100 | 285.0 | 78.91 | 285.1 | 56.87 |
| O1s | | | 532.4 | 21.09 | 532.2 | 34.67 |
| N1s | | | | | 400.2 | 2.02 |
| S2p | | | | | 169.0 | 3.22 |
| Na1s | | | | | 1072.2 | 3.23 |

### Antithrombogenicity test in vitro

The PT, APTT, and TT tests were widely used for the clinical detection of the abnormality of blood plasma and for the primary screening of the anticoagulative chemicals.[19] They were recently applied in the evaluation of the antithrombogenicity of biomaterials. The normal ranges of PT, APTT, and TT for a healthy

**TABLE II**
**PT, APTT, and TT of human blood plasma incubated with pristine PE and heparinized PE**

| Samples | PT (s) | APTT (s) | TT (s) |
|---|---|---|---|
| Blood plasma | 10.5 | 32.3 | 14.8 |
| Pristine PE | 10.4 | 33.9 | 15.6 |
| Heparinized PE | 11.3 | >90 | >60 |



(a) (b)

**Figure 8** SEM micrographs of platelets adhered on (a) pristine PE and (b) PE after heparinization (rf power, 80 W; treatment time, 5 min; UV irradiation time, 60 min; GMA, 20 wt %; heparinization, 2 g heparin, 50 mL $H_2O$, 12 h at 40°C).

blood plasma are regarded to be 11 ± 3, 28 ± 10, and 16 ± 5 s, respectively. When the blood plasma is incubated with pristine PE, the corresponding data of PT, APTT, and TT are 10.4, 33.9, and 15.6 s, indicating PE itself has little influence on the thrombogenicity of the blood plasma (Table II). However, when the blood plasma is incubated with heparinized PE, the APTT and TT become undetectable, because they both are over the maximums of 90 and 60 s set to the instrument, respectively (Table II). Therefore, it is thought that heparin immobilized on the surface of PE plays an important role in inhibiting the activities of some clotting factors of blood plasma involved in APTT and TT.

PRT is an easy method to examine antithrombogenicity of materials. PRT of heparinized PE (~ 296 s) is relatively longer than that of pristine PE (~ 170 s). This indicates that blood-clotting factors are difficultly activated on the surface of heparinized PE. Heparinized PE has a compatible surface for blood.

SEM was used to study the morphology of adhered platelets and to compare the platelets' shape change responses on the surfaces of pristine PE and heparinized PE after 60 min incubation (Fig. 8). It is clear that the amount of adhered platelets is significantly decreased for heparinized PE. The adhered platelets on the surface of heparinized PE have few aggregations and pseudopodiums occurred in contrast to pristine PE. This result provides evidence for the improved blood compatibility by heparin immobilized on the surface of PE.

## CONCLUSION

GMA was successfully grafted onto the surface of PE by Ar plasma pretreatment and UV-induced graft polymerization, allowing heparin immobilized onto the surface of GMA-grafted PE. PT, APTT, TT, and PRT tests indicated the property of antithrombogenicity of PE was greatly improved after heparinization. SEM observation of platelets' adhesion showed that platelets were effectively suppressed on heparinized PE, leading to good *in vitro* blood compatibility.

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