



# Introduction of anticoagulation group to polypropylene film by radiation grafting and its blood compatibility

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Received 31 October 2003; received in revised form 16 December 2003; accepted 16 December 2003

## Abstract

Based on in vitro tests for an improvement of the blood compatibility of polypropylene (PP) films by grafting *O*-butyrylchitosan (OBCS), we prepared a novel biocompatible film. The immobilization was accomplished by irradiating with ultraviolet light, OBCS being coated on the film surface to photolyze azide groups, thus cross-linking OBCS and PP together. The grafted sample films were verified by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), electron spectroscopy for chemical analysis (ESCA) and the water contact angle measurements. The blood compatibility of the OBCS-grafted PP films was evaluated by platelet rich plasma (PRP) contacting experiments and protein adsorption experiments using blank PP film as the control. It demonstrated that blood compatibility of the OBCS-grafted surfaces is better than that of the blank PP. The suitable modifications could be carried out to tailor PP biomaterial to meet the specific needs of different biomedical applications. These results suggest that the photocrosslinkable chitosan developed here has the potential of serving as a new biomaterial in medical use.

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PACS: 42.62.Be; 42.72.Bj; 61.41.+e

Keywords: Surface modification; Radiation grafting; *O*-butyrylchitosan; Polypropylene; Blood compatibility

## 1. Introduction

A substantial part of current research on biomaterials is focused on the design and preparation of polymers with perfect or near-perfect blood compatibility [1,2]. The ultimate goal of that work is to find materials featuring complete absence of surface-induced thrombus formation, a phenomenon that

poses serious problems in the functioning of implants and other medical devices. A potential solution to the problem of thrombogenic polymers may now be realized by modifying the surfaces of polymers [3–6]. Different methods for polymer surface modification have been attempted in order to obtain more blood compatibility polymer materials [7–9].

Water-soluble monomers have been grafted onto the polymeric materials to improve blood compatibility [10]. On this purpose, radiation grafting with  $\gamma$ -ray and electron beam, photografting and other methods have frequently been used to bond

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hydrophilic monomers to the surface of hydrophobic polymers.

Chitosan is a derivative of chitin, that is the second most plentiful natural biopolymers. This naturally occurring polymer has a repeating structural unit of 2-acetamido-2-deoxy- $\beta$ -D-glucose, which yields chitosan when partially or fully deacetylated. It has both reactive amino and hydroxyl groups, which can be used to chemically alter its properties under mild reaction conditions. Chitosan exhibit properties that makes them desirable candidates for biocompatible and blood-compatible biomaterials [11–13]. *N*-Acyl chitosans are already reported as blood-compatible materials [14]. In the present study, by irradiating with ultraviolet light, *O*-butyrylchitosan (OBCS) [15] was covalently immobilized onto polymer film surface using the photosensitive hetero-bifunctional cross-linking reagent, 4-azidobenzoic acid, which was previously bonded to OBCS by reaction between an acid group of the cross-linking reagent and a free amino group of OBCS.

Various polymers have been widely applied in clinical fields as biomedical materials. With the progress of prosthetic surgery, blood-compatible polymers have been increasingly in demand, and a large number of investigations have been carried out [1,9,11,16,17]. We are focusing on surface modification of polypropylene (PP).

Surface properties of the OBCS-grafted PP films were investigated by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), electron spectroscopy for chemical analysis (ESCA) and water contact angle measurements. The blood compatibility of OBCS-grafted PP films was evaluated by platelet rich plasma (PRP) contacting experiments and the results were observed by scanning electron microscopy (SEM). The state of platelet adhesion was described.

## 2. Experiments

### 2.1. Materials

PP films with a thickness of 0.3 mm were used as a substrate for the graft reaction. The PP film was cut into 3.0 cm  $\times$  5.0 cm pieces and ultrasonically cleaned twice in methanol for 5 min each, and dried. It was stored in a desiccator before irradiation.

Chitosan powder was obtained from Lianyungang Biologicals Inc. Its viscosity average molecular weight was  $6.7 \times 10^5$  g/mol, while the degree of deacetylation was 90%.

PRP of human blood was supplied by Blood Center of Nanjing Red Cross. Bovine fibrinogen (BFG, Sigma, F-8630) was obtained as lyophilized powders.

Other chemicals were reagent grades and used without further treatment.

### 2.2. Preparation of 4-azidobenzoic acid

4-amino benzoic acid (0.050 mol) in HCl aqueous solution (concentrated HCl/water = 20/200 ml) was reacted with aqueous sodium nitrite (0.055 mol) at  $-10$  to  $0$  °C for 15 min, followed by aqueous sodium azide (0.060 mol) at room temperature for 1 h to yield 4-azidobenzoic acid (yield 80%) [18].

### 2.3. Preparation of *O*-butyrylchitosan (OBCS)

Chitosan (2.1 g) were added to methanesulphonic acid (11 ml) and the mixture was stirred at  $0$  °C for 15 min until homogeneous [19].

Butyric anhydride (20 ml) was added dropwise and the total mixture stirred at between  $0$  and  $5$  °C for 2 h. The resulting gel was stored at  $-15$  °C overnight. Pouring into acetone precipitated the thawed product, after which the acylated chitosan was dried in vacuo.

### 2.4. Preparation of the solution of 4-azidobenzoic bonded *O* butyrylchitosan (Az-OBCS)

OBCS (1 g, powder), (1-ethyl-3-(3-dimethylamino-propyl)) carbodiimide (EDC) (0.35 g) and 4-azidobenzoic acid (0.2 g) were added to 100 ml methanol. The mixture was stirred at room temperature for 8 h and then concentrated. The thawed product was precipitated by pouring into acetone (100 ml), filtered and extracted for 18 h with methylene chloride to remove the unbonded azide completely, vacuum dried and dissolved in water to prepare 0.1% Az-OBCS solution.

### 2.5. Immobilization of Az-OBCS onto PP film surface to prepare OBCS modified PP film

The Az-OBCS solution was cast on a PP film surface and dried in a brown colored desiccator.

The film was irradiated by a mercury lamp (8 W, 254 nm UV-tube light, China) for 3 min, washed 20 times with deionized water by mild shaking (fresh deionized water each time) to remove the unbonded Az-OBCS completely and then dried.

## 2.6. Characterization

ATR-FTIR and transmission infrared (IR) spectra were obtained using a Nicolet 170sx Fourier transform infrared spectrometer. Ultraviolet spectroscopy analysis was completed on an UV 3100 spectrophotometer. The ESCA spectra was obtained using a V.G. ESCALAB MK II spectrometer. The X-ray source was Mg K $\alpha$  radiation (1253.6 eV) operated at 12 kV, 20 mA. The take-off angle was fixed at 45° relative to the sample surface. The measurements were made in a vacuum better than  $2 \times 10^{-8}$  mbar at room temperature. The water contact angle was determined at 25 °C by a contact angle meter (Rame-Hart-100) employing drops of pure deionized water. The readings were stabilized and taken in 60 s after dropping. Each sample was measured at five different locations and the readings were averaged.

The grafting was verified by FTIR-ATR, ESCA and water contact angle measurements.

## 2.7. Platelet adhesion

The result of blood platelet adhesion in vitro was observed through SEM (JSM model 6300, JOEL, Japan). The OBCS-grafted films were rinsed with PBS first and contacted at 37 °C for 1 h with freshly prepared PRP of human blood. Samples were rinsed with PBS and then treated with 2.5% glutaraldehyde for 30 min at room temperature. The samples were washed with PBS again and dehydrated by systemic immersion in a series of ethanol–water solutions (50, 60, 70, 80, 90, 95, 100% (v/v)) for 30 min each and allowed to evaporate at room temperature. The platelet-attached surfaces were coated with gold prior to being observed by SEM. The blank PP film was used as a reference.

## 2.8. Protein adsorption

BFG, Sigma, F-8630 was obtained as lyophilized powders. The buffer solution used in the protein

adsorption experiments was PBS, pH 7.4. Quantification of adsorbed protein on the polymer surfaces was performed using  $^{125}\text{I}$ -labeled protein.  $^{125}\text{I}$ -labeled protein was added to unlabelled protein solution in order to obtain a final activity of about  $10^7$  cpm/mg. The samples were immersed into 1 ml buffer solution at 37 °C, and then 1 ml fibrinogen solution (0.2 mg/ml) was added and mixed. Adsorption tests were carried out at 37 °C during 1 h. After protein adsorption, samples were rinsed three times with 2 ml of buffer solution. The gamma activities were counted with the samples placed in radio-immunoassay tubes by a Gamma Counter (FH-408, Beijing Nucleus Instrument Factory). Four replicates were used. The counts from each sample were averaged and the surface concentration was calculated by the equation:

$$\text{BFG } (\mu\text{g}/\text{cm}^2) = \frac{\text{counts (cpm)} \times C_{\text{solution}} (\mu\text{g}/\text{ml})}{A_{\text{solution}} (\text{cpm}/\text{ml}) \times S_{\text{sample}} (\text{cm}^2)}$$

where the count measures the radioactivity of the samples, the  $S$  sample measure the surface area of the samples, the  $C_{\text{solution}}$  and  $A_{\text{solution}}$  are the concentration and the specific activity of the protein solution, respectively.

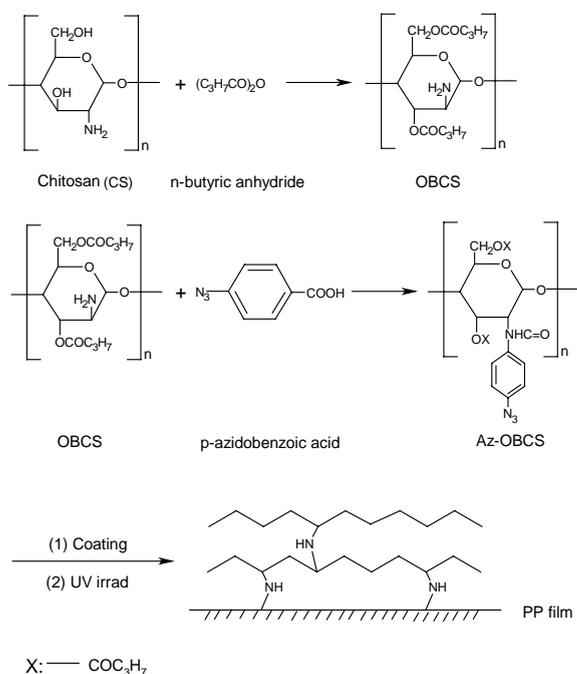
## 3. Results and discussion

### 3.1. Preparation Az-OBCS

OBCS was immobilized covalently on the substrate by using the photosensitive hetero-bifunctional cross-linking reagent, 4-azidobenzoic acid. The reaction scheme for the immobilization was shown in Scheme 1.

Fig. 1 showed the UV spectra of Az-OBCS (water was used as solvent). An absorption at 267 nm, which is assignable to the azidophenyl group [18], was observed. This result indicated that the azide group had been introduced to the OBCS molecules.

Fig. 2 showed FTIR spectrum of Az-OBCS, from which it could be seen that there was obvious absorption peak at  $2126.2 \text{ cm}^{-1}$ , which is the characteristic of  $-\text{N}_3$  [20]. This result confirmed the above conclusion that the azide group had been bonded on the OBCS molecules.



Scheme 1. Reaction scheme of Az-OBCS and immobilization scheme of OBCS on PP film surface.

### 3.2. Immobilization of Az-OBCS on PP surface

The method of immobilizing OBCS was simple as described in the experimental section. Unbonded OBCS molecules were washed out with water. Azide

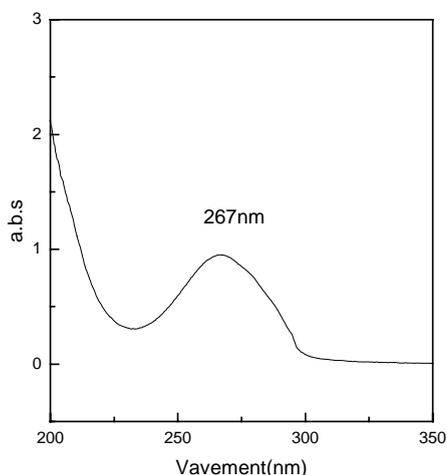


Fig. 1. UV spectra of Az-OBCS.

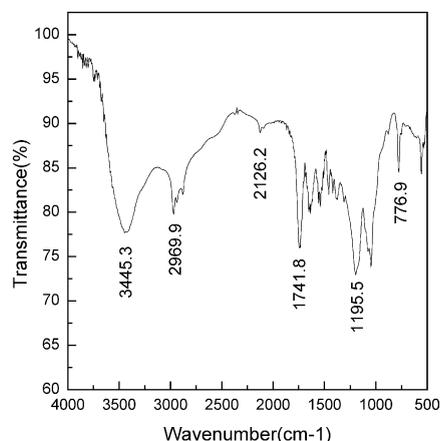


Fig. 2. FTIR spectra of Az-OBCS.

groups ( $-N_3$ ) are known to release  $N_2$  under UV irradiation and to be converted into highly reactive nitrene groups. Nitrene groups were supposed to undergo insertion reaction with the underlying substrate molecules and made OBCS chains cross-linked as well (Scheme 1).

FTIR, ESCA and water contact angle measurements were carried out to check if OBCS had been immobilized on the PP film surface.

In Fig. 3, ATR-FTIR spectra of blank PP film and OBCS-grafted PP film are shown. After reaction with OBCS a sharp band at  $1737.8\text{ cm}^{-1}$ , characteristic of  $-C=O$  stretching vibration frequency of the butyryl group [19], appeared in the OBCS-grafted PP film spectrum. This result indicated that OBCS had been introduced to the PP film.

The C 1s peak for the blank PP film is the peak: a hydrocarbon ( $C-C-C$ ) peak at 284.6 eV was indicated in Fig. 4. The C 1s peak for OBCS surface modified PP could be resolved into three component peaks: a hydrocarbon ( $C-C-C$ ) peak at 284.6 eV, an amide ( $NH-CO$ ) peak at 286.21 eV, and an ether ( $C-O-C$ ) peak at 287.8 eV [21]. Its relatively composition ratio based on area was also been indicated in Fig. 5. Furthermore, the new peak at 399.26 eV was the characteristic peaks of nitrogen. The different of surface elemental composition from ESCA between blank PP film and OBCS-grafted PP film was indicated in Table 1. All the above results indicated that OBCS did bond on the PP film surface.

The water contact angle measurement results of blank PP film and OBCS-grafted PP film were listed

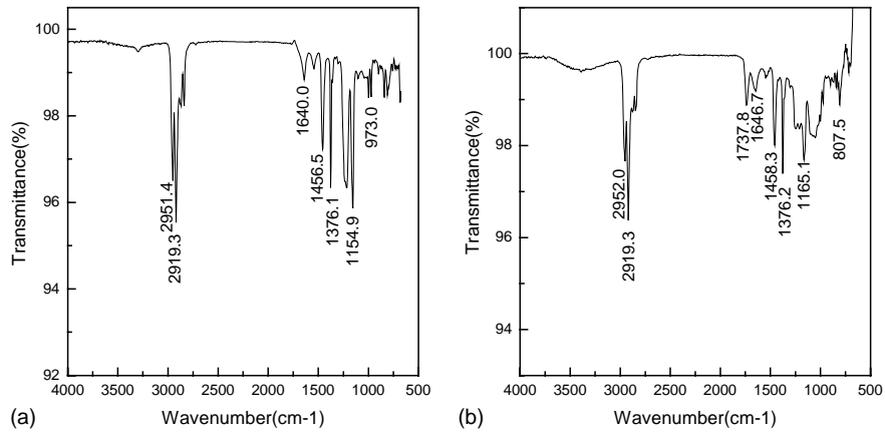


Fig. 3. FTIR spectra of film surface: (a) blank PP film; (b) OBCS-grafted PP film.

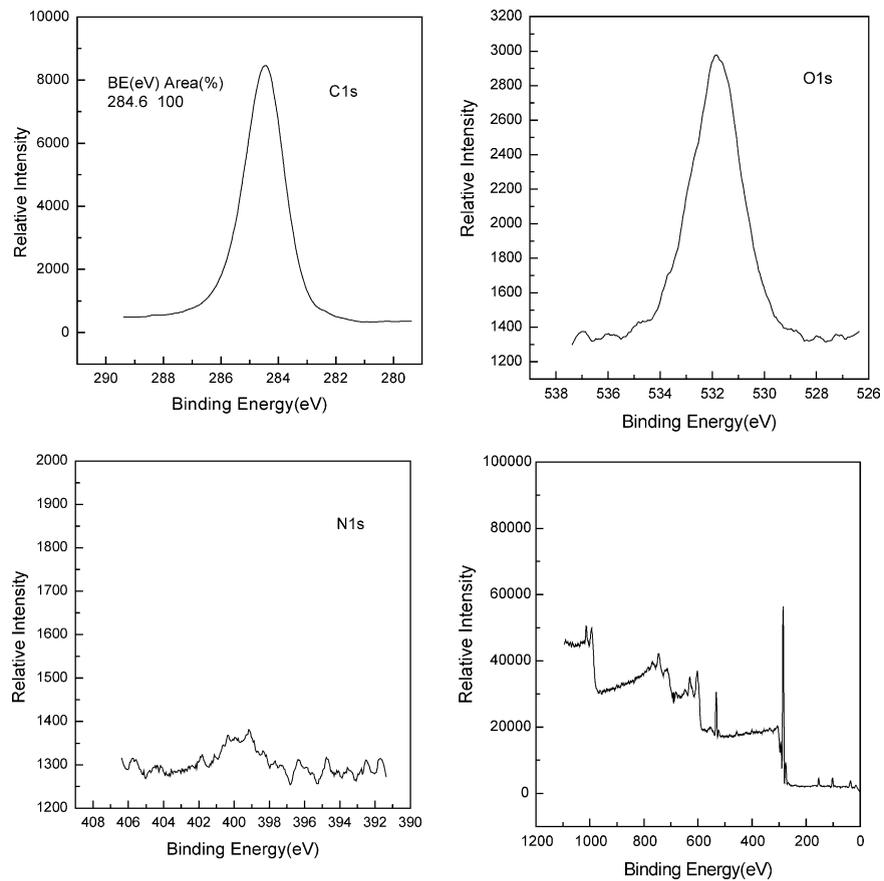


Fig. 4. ESCA spectra of blank PP film surface.

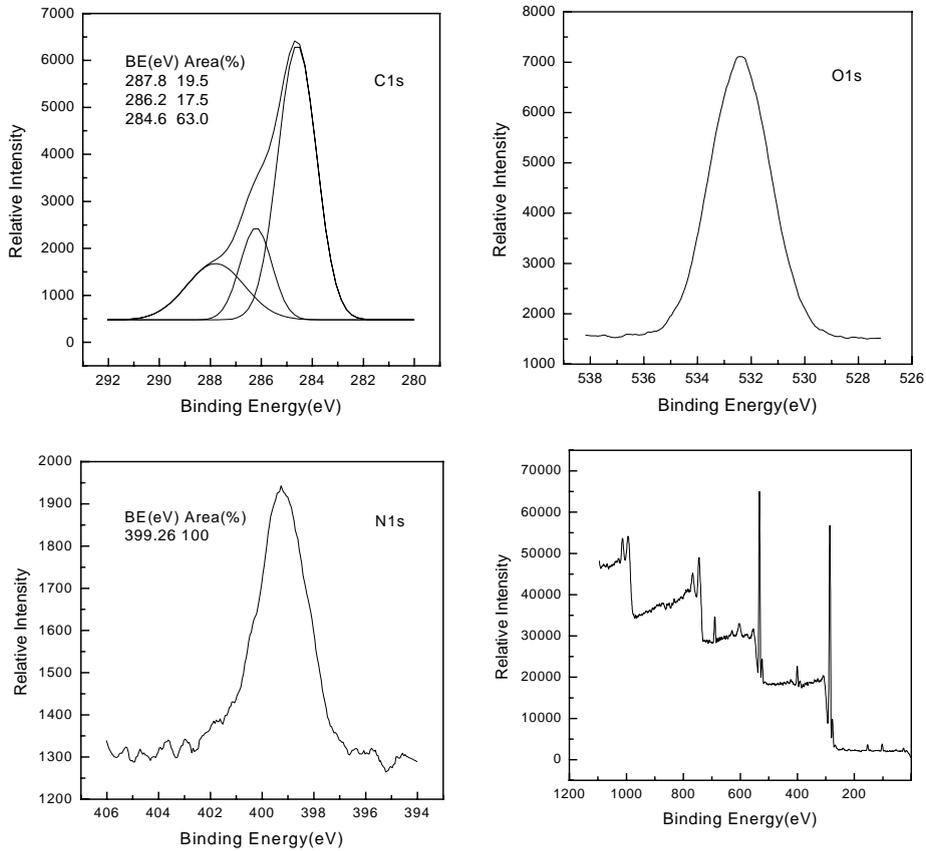


Fig. 5. ESCA spectra of OBSCS-grafted PP film surface.

in Table 2. The water contact angle of OBSCS-grafted films ( $65^\circ$ ) decreased greatly in comparison with blank PP films ( $116^\circ$ ). In other words, the OBSCS-grafted films were more hydrophilic than blank films.

### 3.3. Platelet adhesion

Platelet adhesion results are shown in Fig. 6. The blank PP film showed highest platelet adhesion, most of the adhered platelets were distorted with pseudo-

podia. But to the surprise, the surfaces of OBSCS-grafted films have nearly no platelet adhered. The platelet adhesion test revealed that OBSCS-grafted films show excellent anti-platelet adhesion. It is considered that the improved anti-thrombogenicity can be attributed to OBSCS. The anti-thrombogenic function of OBSCS was similar to those of *O*-diacetylchitosan and *N*-hexanoylchitosan [22]. The results may offer the possibility of the usage for biomaterial devices, which are directly in contact with blood.

Table 1  
Surface elemental composition from ESCA

Polymer film	C 1s	O 1s	N 1s
Blank PP	90.6	9.4	0
OBSCS-grafted PP	72.5	23.9	3.6

Table 2  
Water contact angle of blank PP film and OBSCS-grafted PP film

Polymer film	Water contact angle ( $^\circ$ )
Blank PP	$116 \pm 3$
OBSCS-grafted PP	$65 \pm 2$

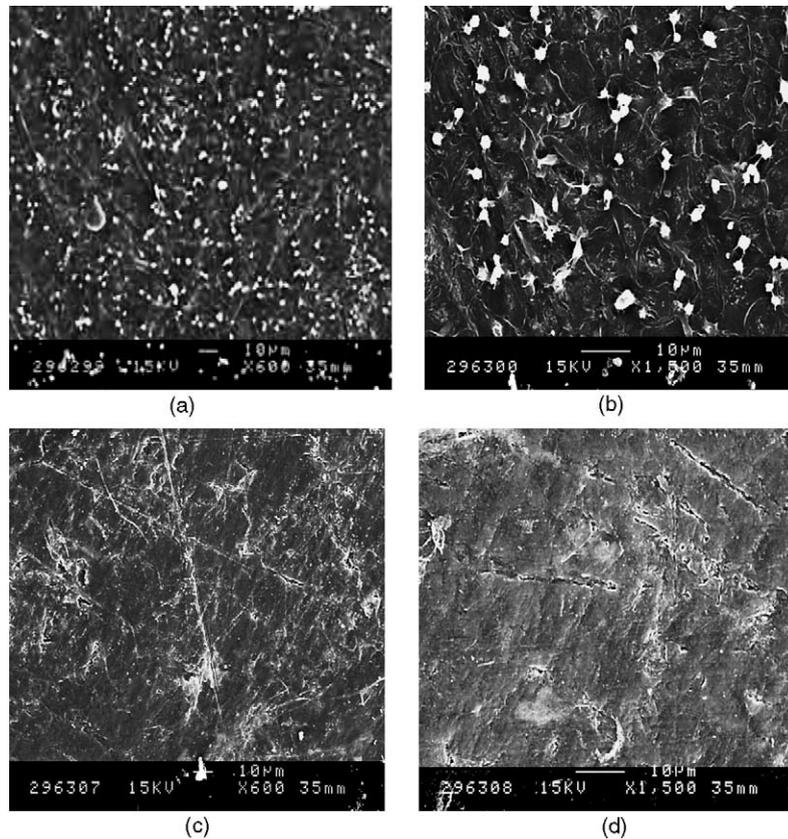


Fig. 6. SEM photographs for the morphologies of PRP contacted surfaces: (a) blank PP-1 h (600 $\times$ ); (b) blank PP-1 h (1500 $\times$ ); (c) OB-CS-grafted PP-1 h (600 $\times$ ); (d) OB-CS-grafted PP-1 h (1500 $\times$ ).

### 3.4. Protein adsorption

Material biocompatibility is generally considered to have relation with protein adsorption process, because adsorbed proteins may trigger the coagulation sequence [23]. In the present work, the blank PP surface and OB-CS modified PP surface were studied in relation to adsorption of BFG in vitro. Table 3 shows the adsorption of BFG onto PP surfaces from the protein solution (0.1 mg/ml). These results demon-

strate that graft of OB-CS onto PP surface decreases fibrinogen adsorption. Its improved anti-thrombogenicity may also be due to OB-CS. So the PP-g-OB-CS film had improved blood compatibility.

## 4. Conclusions

In this study, we have been working on a photochemical method for the surface modification of PP in order to improve blood compatibility. OB-CS was grafted onto PP surface successfully using a UV irradiation grafting technique. The surface composition of films and the hydrophilicity on the PP surface were investigated by ATR-FTIR, ESCA and water contact angle measurements, respectively. It was found that OB-CS-introduced PP film has a good blood-compatible property. OB-CS exhibit properties

Table 3  
Bovine fibrinogen (BFG) adsorption onto blank PP film and OB-CS-grafted PP film

Polymer film	BFG adsorption ( $\mu\text{g}/\text{cm}^2$ )
Blank PP	$2.723 \pm 0.103$
OB-CS-grafted PP	$1.475 \pm 0.077$

that make it desirable candidates for biocompatible and blood-compatible biomaterials.

### Acknowledgements

The authors wish to thank the financial support provided by the Special Funds for Major State Basic Research Projects of PR China (G1999064705) and the High Technology Research Project of Jiangsu Province (2002HXXSB9B222).

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