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In vitro studies of platelet adhesion on UV radiation-treated nylon surface

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Abstract

The aim of the study was to prepare a novel blood compatible of nylon film by grafting *O*-butyrylchitosan (OBCS). The immobilization was accomplished by irradiating with ultraviolet light, OBCS being coated on the film surface to photolyze azide groups, thus crosslinking OBCS and nylon together. Surface properties of nylon were investigated by attenuated total reflection Fourier transform infrared spectroscopy, electron spectroscopy for chemical analysis and water contact angle measurements. The blood compatibility of the OBCS-grafted nylon films was evaluated by platelet rich plasma contacting experiments and the results were observed by scanning electron microscopy. The blood compatibility of OBCS grafted nylon films seems better than that of blank nylon films. The modifications could be carried out to tailor nylon biomaterial to meet the specific needs of different biomedical applications. These results suggest that the blood compatible of nylon/OBCS films make them suitable biomaterials for some applications.

Keywords: Surface modification; Radiation grafting; *O*-Butyrylchitosan; Nylon; Blood compatibility; Platelet adhesion

1. Introduction

Various polymers have been widely applied in clinical fields as biomedical materials (Kyröläinen, Reddy, & Vadgama, 1997; Nho, Kwon, & Jie, 2000; Risbud, Saheb, Jog, & Bhonde, 2001; Zhang et al., 2002). With the progress of prosthetic surgery, blood-compatible polymers have been increasingly in demand, and a large number of investigations have been carried out. Nylon is an engineering plastic with excellent mechanical properties and is mainly used in applications such as automobile, appliances and engineering components. It has also been used in biomedical applications (García Páez et al., 2001; Shinde, Phadke, Nair, Mungantiwar, Dikshit, & Saraf, 1999; Uff, Scott, Pockley, & Phillips, 1995). However, when in direct contact with blood, it is still prone to initiate the formation of clots, as platelets and other components of the blood coagulation system are activated.

It is well known that the formation of a thrombus is dependent upon either or both the behaviors of platelets at or near the surface and on the protein-based coagulation cascade (Rhodes, Kumary, & Williams, 1996). A potential solution to the problem of thrombogenic polymers may now be realized by modifying the surfaces of polymers (Amiji, 1997; Dadsetan, Mirzadeh, Sharifi-Sanjani, & Salehian, 2001; Han, Jeong, & Kim, 1989; Kim, Kang, Huh, & Yoon, 2000; Park, Okano, Nojiri, & Kim, 1988; Yang, Chen, & He, 1997).

In contrast to previous techniques for pre-coating and entrapment to modify the nylon surface, we employed a novel surface modification technique via covalent immobilization of *O*-butyrylchitosan (OBCS) with a photosensitive hetero-bifunctional crosslinking regent, 4-azidobenzoic acid.

Chitosan, a (1→4)-linked 2-amino-2-deoxy-β-D-glucan, is prepared by N-deacetylation of chitin, which is the main structural component of crab and shrimp shells. Chitosan has both reactive amino and hydroxyl groups, which can be used to chemically alter its properties under mild reaction conditions. Therefore, there are many interesting chitosan derivatives, especially for biomedical applications (Hirano,

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Zhang, Nakagawa, & Miyata, 2000; Jie, Nho, Kwon, & Hoffman, 1999; Zhu, Zhang, Wu, & Shen, 2002). *N*-acyl chitosans have been reported to be blood compatible materials (Lee, Ha, & Park, 1995). In the present study, by irradiating with ultraviolet light, OBCS, a water-soluble derivative of chitosan, was covalently immobilized onto the nylon film surface using the photosensitive hetero-bifunctional crosslinking reagent, 4-azidobenzoic acid, which was previously bound to OBCS by a reaction between an acid group of the crosslinking reagent and the free amino group of OBCS.

Surface properties of the grafted-OBCS nylon 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 grafted-OBCS nylon 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. Experimental

2.1. Materials

Nylon (polycaproamide) films with a thickness of 0.2 mm were used as a substrate for the graft reaction. The nylon film was cut into 3.0×5.0 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 6.7×10^5 g mol^{-1}, while the degree of deacetylation was 90%.

PRP of human blood was supplied by Blood Center of Nanjing Red Cross.

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 ml/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%) (Hayashi et al., 1992).

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. 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 (Grant, Blair, & Mckay, 1988).

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 unbound azide completely, vacuum dried and dissolved in water to prepare 0.1% 4-azidobenzoic bonded *O*-butyrylchitosan (Az-OBCS) solution.

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

The Az-OBCS solution was cast on a nylon 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 with water completely and then dried (Ono et al., 2000).

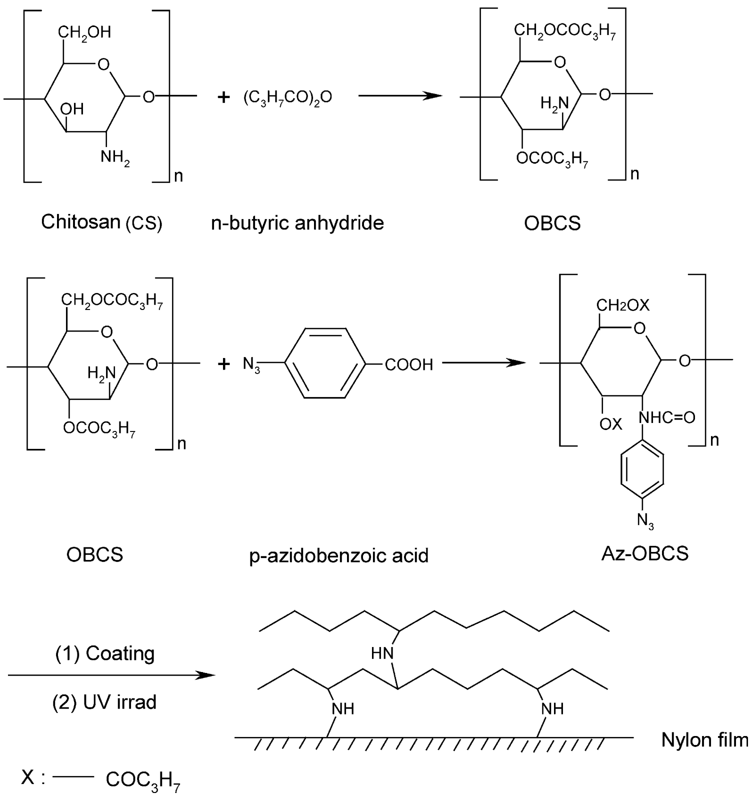
2.6. Characterization

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 (V.G. Scientific Co. Ltd. UK). The X-ray source was Mg Kα 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 2×10^{-8} mbar at room temperature.

The OBCS grafted nylon films were characterized by water contact angle measurements using an optical contact angle goniometer (Rame-Hart-100). The water contact angle for each OBCS grafted nylon sample was measured five times by a sessile drop method at room temperature. Drops of purified water (3 μl) were deposited onto the modified nylon film, and the readings were stabilized and taken in 60 s after dropping. Water sessile drops were deposited from a glass syringe onto the sample surfaces, and the water contact angle was measured after a defined period of time, in order to allow the establishment of equilibrium.

2.7. Platelet adhesion

In order to determine the potential blood compatibility of the materials, platelet adhesion studies were conducted since platelet adhesion is one of the most important steps during blood coagulation on artificial surfaces. Blood platelet attachment in vitro was evaluated by SEM (JSM Model 6300 scanning electronic microscopy, JOEL, Japan). The



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

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 treated with 2.5% glutaraldehyde for 30 min at room temperature. The samples were rinsed with PBS 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 gold deposited in vacuum and examined by SEM. The blank nylon film was used as a reference.

3. Results and discussion

3.1. Preparation Az-OBCS

OBCS was immobilized covalently on the nylon film surface by using the photosensitive hetero-bifunctional crosslinking 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

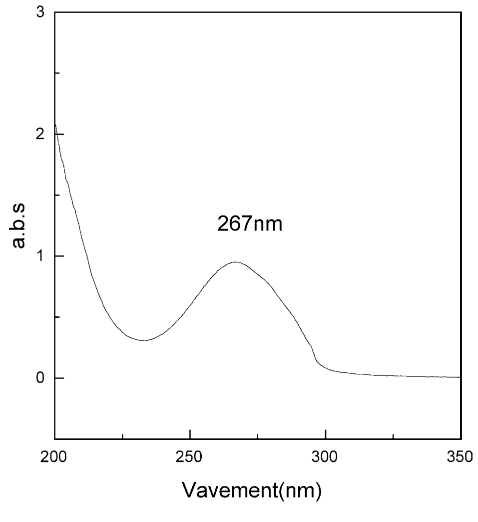


Fig. 1. UV spectrum of Az-OBCS.

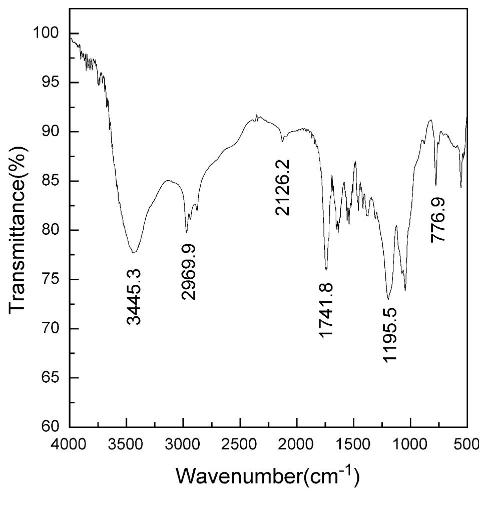


Fig. 2. FTIR spectrum of Az-OBCS.

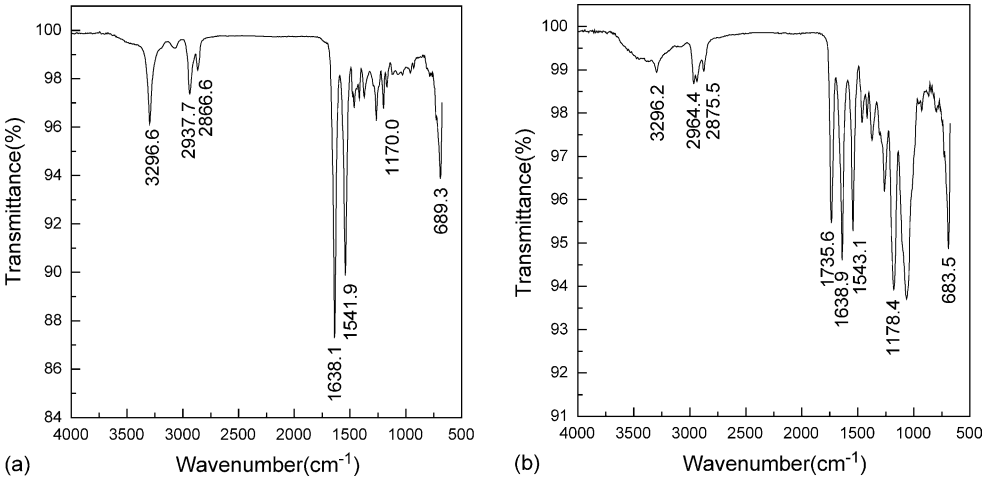


Fig. 3. FTIR spectra of film surface (a) blank nylon film and (b) OBCS grafted nylon film.

is assignable to the azidophenyl group, 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 cm^{-1}, which is the characteristic of –N_3. This result confirmed the above conclusion that the azide group had been bound to on the OBCS molecules.

3.2. Immobilization of Az-OBCS on nylon surface

The method of immobilizing OBCS was simple as described in Section 2. Unbound OBCS molecules were washed out with water. Azide 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

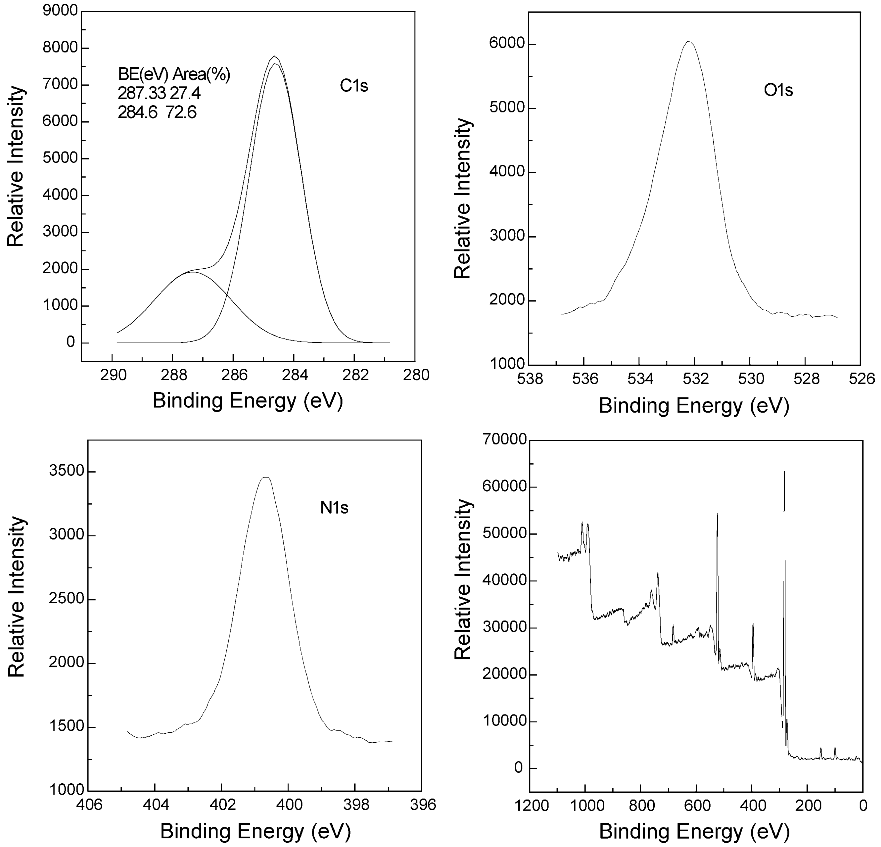


Fig. 4. ESCA spectra of blank nylon film surface.

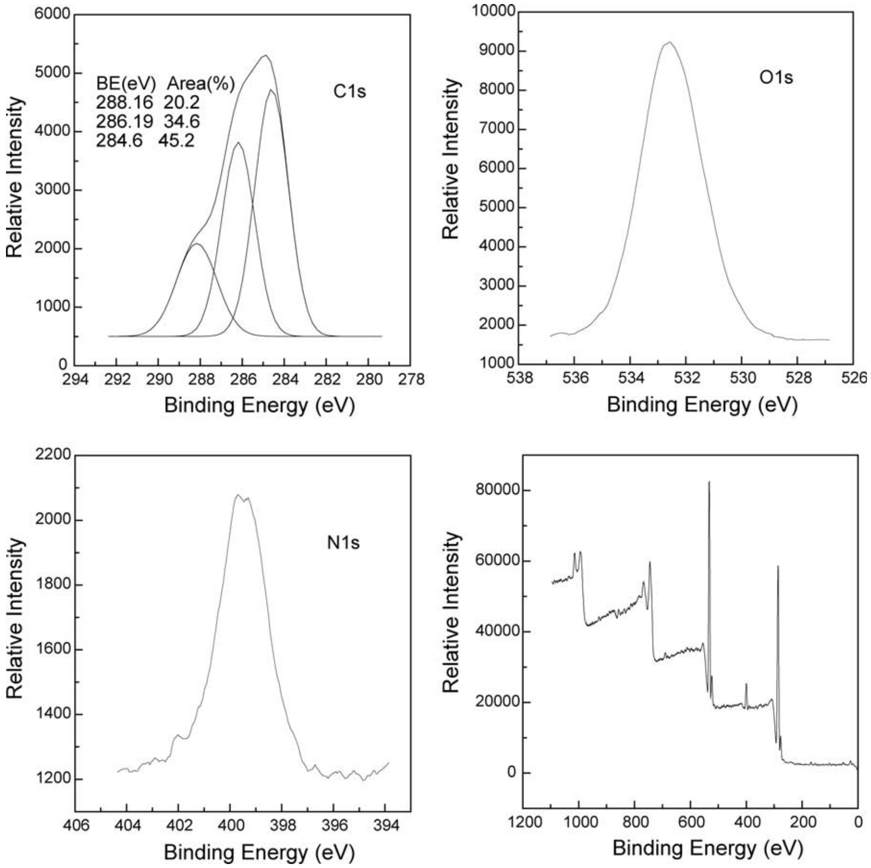


Fig. 5. ESCA spectra of OBCS grafted nylon film surface.

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 nylon film surface.

In Fig. 3, ATR-FTIR spectra of blank nylon film and OBCS grafted nylon film are shown. After reaction with OBCS a sharp band at 1735.6 cm^{-1}, characteristic of –C=O stretching vibration frequency of the butyryl group, appeared in the OBCS grafted nylon film spectrum. This result indicated that OBCS had been introduced to the nylon film.

The C_{1s} peak for the blank nylon film could be resolved into two component peaks: a hydrocarbon (C–C–C) peak at 284.6 eV and an amide (NH–CO) peak at 287.33 eV. Its relative composition ratio based on area was been indicated in Fig. 4. The C_{1s} peak for OBCS surface modified nylon could be resolved into three component peaks: a hydrocarbon (C–C–C) peak at 284.6 eV, an ether (C–C–C) peak at 286.19 eV, an amide (NH–CO) peak at 288.16 eV. Its relative composition ratio based on area was also been indicated in Fig. 5. The different in surface elemental composition from ESCA between blank nylon film and OBCS grafted nylon film was indicated in Table 1. All the above results indicated that OBCS was bound to the nylon film surface.

The water contact angle measurement results of nylon film and OBCS grafted nylon film were listed in Table 2. The water contact angle of OBCS grafted films (85°) decreased greatly in comparison with blank nylon films (43°). In other words, the OBCS grafted films were more hydrophilic than blank films.

3.3. Platelet adhesion

Platelet adhesion results are shown in Fig. 6. The blank nylon film showed the highest platelet adhesion, most of the adhered platelets were distorted with pseudopodia. Surprisingly, the surfaces of OBCS grafted films have nearly no adhered platelets. The platelet adhesion test revealed that

Table 1
Surface elemental composition from ESCA

Polymer film	C_{1S}	O_{1S}	N_{1S}
Blank nylon	73.9	16.6	9.5
OBCS grafted nylon	67.4	28.0	4.6

Table 2
Water contact angle of blank nylon film and OBCS grafted nylon film

Polymer film	Water contact angle (°)
Blank nylon	85 ± 3
OBCS grafted nylon	43 ± 3

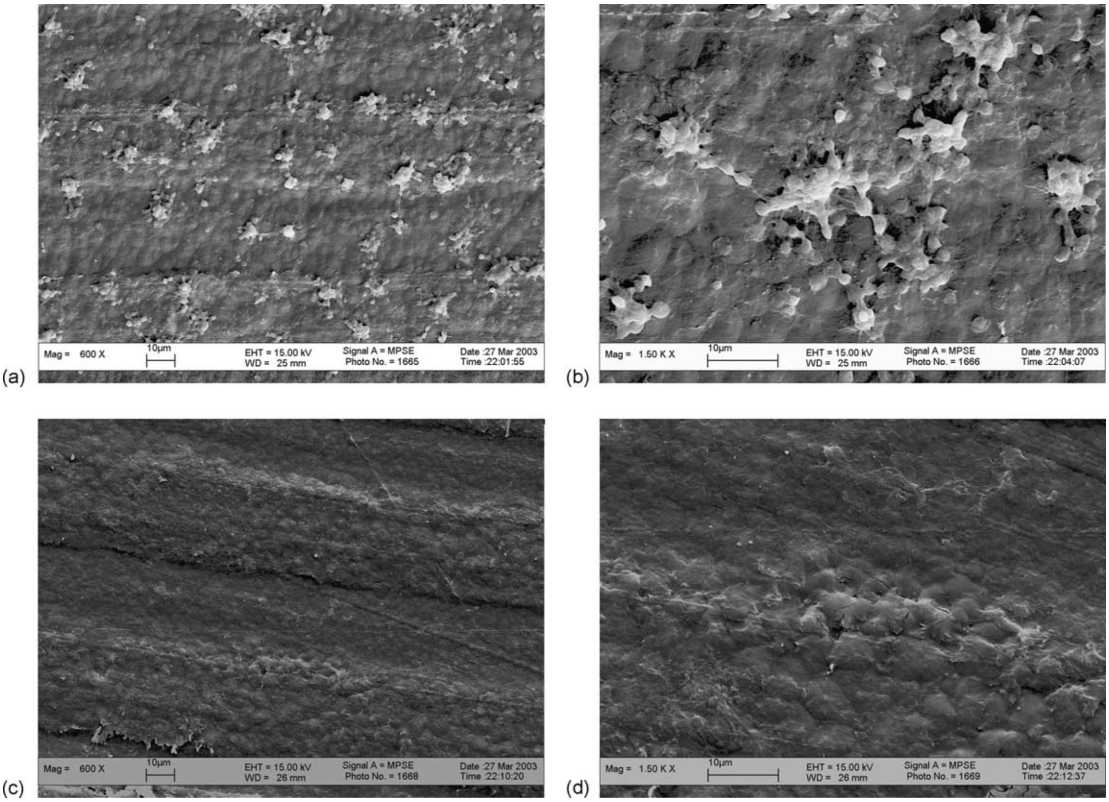


Fig. 6. SEM photographs for the morphologies of PRP contacted surfaces. (a) Blank nylon-1 h (×600), (b) blank nylon-1 h (×1500), (c) OBCS grafted nylon-1 h (×600) and (d) OBCS grafted nylon-1 h (×1500).

films grafted OBCS show excellent anti-platelet adhesion. It is considered that the improved antithrombogenicity can be attributed to OBCS. The antithrombogenic function of OBCS was similar to those of *O*-diacetylchitosan and *N*-hexanoylchitosan (Hirano et al., 2000). The results may offer the possibility of using this for biomaterial devices, which are directly in contact with blood.

4. Conclusions

In conclusion, we have shown that aryl azide with OBCS can be used for the photochemical surface modification of nylon. The hemocompatibility of the modified surfaces was shown to be improved by this procedure. This suitable modification could be carried out to tailor nylon biomaterial to meet the specific needs of different biomedical application. OBCS exhibit properties that make it desirable candidates for biocompatible and blood-compatible biomaterials.

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