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Alkylpolyglycoside inducing poly (butylene terephthalate) non-woven graft copolymerization of chitosan

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Abstract: In order to improve the wettability and biocompatibility of the poly (butylene terephthalate) non-woven (PBTNW), the method of surface modification is used to graft copolymerization of chitosan (CS) onto the PBTNW under alkylpolyglycoside (APG) inducing. The product is thoroughly characterized with the Fourier transform infrared spectroscopy (FTIR), the electron spectroscopy for chemical analysis (ESCA), the thermogravimetric (TG) and the scanning electron microscopy (SEM). It is found that chitosan is successfully grafted onto PBTNW. In addition, the water contact angles, hemolysis tests and cytotoxicity evaluation tests show an improvement in wettability and biocompatibility as a result of graft copolymerization of chitosan. So the CS-grafted PBTNW exhibits greater superiority than the original PBTNW. The CS-grafted PBTNW can be a candidate for blood filter materials and other medical applications.

Key words: chitosan; graft; poly (butylene terephthalate) non-woven; alkylpolyglycoside; biocompatibility; wettability
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Various types of polymeric materials have been widely used to reduce pathogenic plasma components or fraction of cells from whole blood in dialysis, filtration, or adsorption devices[1-2]. Especially, the blood filtration is performed to remove leucocytes, inducing a variety of adverse effects on transfusion recipients[3-4]. Filter fabrics have been developed from a wide variety of materials including polyester, polyurethane, and polyamide to provide an effective and flexible method for disposable blood products of leucocytes. Those products require maximum retention of leucocytes on the filter as well as minimum loss of platelet number in the concentrate. However, in lack of prehydration, fabrics trap and activate platelets, it is necessary to synthesize a polymer, which must show platelet compatibility when in contact with blood under dry conditions. Some surface modification methods for obtaining adhesion-resistant properties for platelets have been carried out, but they are still not satisfactory.

Chitosan, a commercially available polysaccharide made of 2-aminoglucose obtained by deacetylation of chitin, has been applied to promote the formation of an extracellular matrix (ECM) in tissue regenerative therapy[5-8]. Recently, it has been widely investigated in the tissue engineering field for its support for the growth of cells such as epithelial cells. However, disadvantages such as low molecular weight and poor solubility have limited the practical application of chitosan. To improve its performance, modified chitosan materials have been manufactured by using graft polymerization, a conventional and useful method for chemical modification[9-13]. To the best of our knowledge, no attempt has been made to graft copolymerization of chitosan onto the poly (butylene terephthalate) non-woven (PBTNW) for blood filter material.

In this paper, chitosan is first introduced on poly (butylene terephthalate) non-woven backbone by free radical grafting polymerization. Alkylpolyglycoside (APG) is nonionic surfactant prepared from renewable raw materials namely glucose and fatty alcohol. Such products are expected to exhibit surface-active properties due to the presence of the hydrophilic sugar moiety and the hydrophobic fatty alcohol residues. Then chitosan is grafted onto the poly (butylene terephthalate) membrane surface by redox reaction using ceric (IV) ammonium nitrate as an initiator and alkylpolyglycoside (APG) as an inducer. The CS-grafted PBTNW is evaluated as blood filter material due to its wettability and biocompatibility.

1 Materials and Methods

1.1 Materials

PBTNW was purchased from Nanjing Jinbond Biomedical Material Co., Ltd. The used alkylpolyglycoside (APG) was from Jingling Petrochemical Plant. The ceric (IV) ammonium nitrate (AR grade) was purchased from Aldrich and used without further purification. Chitosan was purchased from Shanghai Lanji Technology Development Co., Ltd.

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1.2 Preparation of CS-grafted PBTNW

The pre-weighed PBTNW was dipped into 100 mL of APG solution for 30 min. The solid chitosan powder was dissolved in 150 mL of 2% acetic acid aqueous solution by agitation, and then APG was added by agitation. After 30 min stirring under N_2, the Ce (IV) was applied as an initiator. Then the product was washed with water until neutral. The washing process was repeated at least three times to ensure the complete removal of homopolymer and physically absorbed polymer. Finally the CS-grafted PBTNW was dried in vacuum at 60 ℃.

1.3 Characterization

The surface of the film was investigated using an attenuated total reflectance Fourier transfer infrared spectroscope (ATR-FTIR, Nexus-670, Nicolet, Germany).

The electron spectroscopy for chemical analysis (ESCA) spectra was obtained using a V. G. ESCALAB MK Ⅱ spectrometer. Mg Kα radiation (1 253.6 eV) was used as the X-ray source and the experiment was operated at 12 kV, 20 mA.

The thermogravimetric measurements were performed on a Perkin-Elmer TG instrument using a heating rate of 20 ℃/min up to 800 ℃ in N_2.

The static contact-angle of the solution was used to evaluate the hydrophilicity of films by a contact-angle meter (CAM-PLUS, Unicyro Co., Germany).

The scanning electron microscopy (SEM, JEOL JSM-5610LV) was used to examine the surface of the film. The film was coated with gold according to the standard procedures prior to the scan analysis.

1.4 Hemolysis assay

The hemolysis assay was performed to evaluate the blood compatibility of the PBTNW before and after the modification[14]. Both samples were cut into pieces of 1 cm × 1 cm before being transferred into polystyrene tubes. The amount of 10 mL of 0.9% normal saline was poured into each of the tubes and kept at 37 ℃ in a shaking water bath.

After 60 min incubation, 200 μL of diluted ACD blood (8 mL of ACD blood was diluted with 10 mL of normal saline) was dropped into each of the tubes. All of the tubes were further incubated at 37 ℃ for 60 min. Similarly, positive and negative controls were produced in separate tubes by adding 200 μL diluted blood to 10 mL distilled water and normal saline, respectively. The fluid was then transferred into a fresh polystyrene tube and centrifuged at 2 500 r/min for 5 min. The absorbance of supernatants was measured at 545 nm using a UV-visiblespectrophotometer (Shimadzu, UV-2550, Japan). The hemolysis ratio R_H was calculated according to the following formula:

$$R_H = \frac{A_s - A_{nc}}{A_{pc} - A_{nc}} \times 100\%$$

where A_s, A_{pc} and A_{nc} stand for the absorbance of sample supernatant, positive and negative control, respectively.

1.5 Cytotoxicity evaluation

Human umbilical vein endothelial cell (HUVECs) were used to assess the cytotoxicity of the CS-grafted PBTNW fiber film. HUVECs were seeded into 96-well plates with fixed CS-grafted PBTNW fiber film at a density of 5×10^4 cell/mL. After an exposure time of 48 h, the surviving cells number was determined by MTT dye reduction. MTT salt (20 μL of a 5 mg/mL MTT solution, filter-sterilized, Sigma) was added to each well and incubated for 4 h at 37 ℃. Then the MTT reaction medium was removed and formazan crystals were solubilized with the addition of 150 μL per well of dimethylsulfoxide (DMSO). The optical densities were measured in a microplate reader (ELISA, Dynex Technologies, USA) at 570 nm. Cells in wells without fixed CS-grafted PBTNW fiber films were used as the control.

2 Results and Discussion

2.1 Surface characterization

The main purpose of this study is to graft chitosan with PBTNW to form PBTNW-chitosan copolymer by using ceric (IV) ammonium nitrate as an initiator (see Fig. 1).

The Fourier transform infrared spectroscopy is used to analyze the surface modification of the CS-grafted PBTNW copolymer. The spectra are shown in Fig. 2. The IR spectrum of the CS-grafted PBTNW shows an absorbance peak at 1 089 cm^{-1}, which is more obvious than the corresponding peak of the PBTNW. The peak of 1 089 cm^{-1} characterizes the Glucopyranoside bond in chitosan. From the IR spectra, it is confirmed that the CS-grafted PBTNW copolymer is successfully synthesized.

The changes in the chemical structure of the PBTNW surface after modification were investigated by the ESCA. The compositions of the PBTNW surfaces are shown in Tab. 1. Carbon and oxygen can only be detected on the original PBTNW surface. However, the new content of nitrogen can be observed on the CS-grafted PBTNW surface. The nitrogen content of the PBTNW surface dramatically increases after modification, and the carbon and oxygen contents of the PBTNW surface also change. Details of the C1s spectra for the modified PBTNW surface are shown in Fig. 3. Furthermore, in contrast with the ESCA spectra of the original PBTNW surface, the new peak (N1s) appears at that of the CS-grafted PBTNW as shown in Fig. 4. And in Fig. 5, the N1s chart reflects the nitrogenous carbon peak (NH—C) at 399.84 eV[15]. The

ESCA results also indicate that CS has been successfully grafted onto the PBTNW surface.

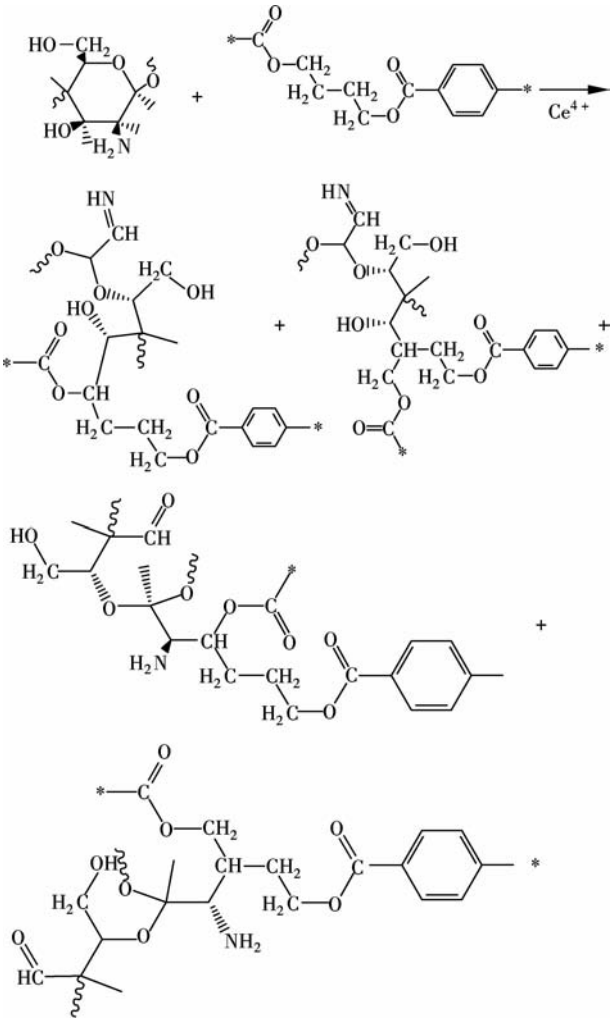

Fig. 1 The synthesis procedure of CS-grafted PBTNW

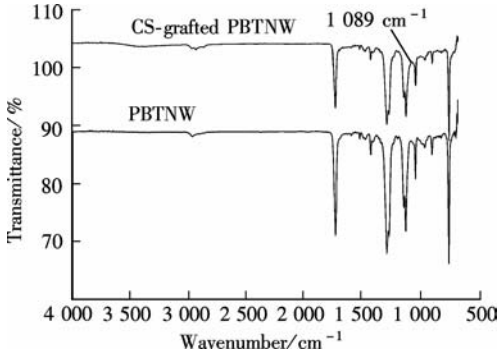

Fig. 2 ATR-FTIR spectra

Tab. 1 Elemental composition of original PBTNW and CS-grafted PBTNW fiber film calculated from XPS %

Sample	C	O	N
Original PBTNW	73.72	26.24	0
CS-grafted PBTNW	73.33	24.36	2.31

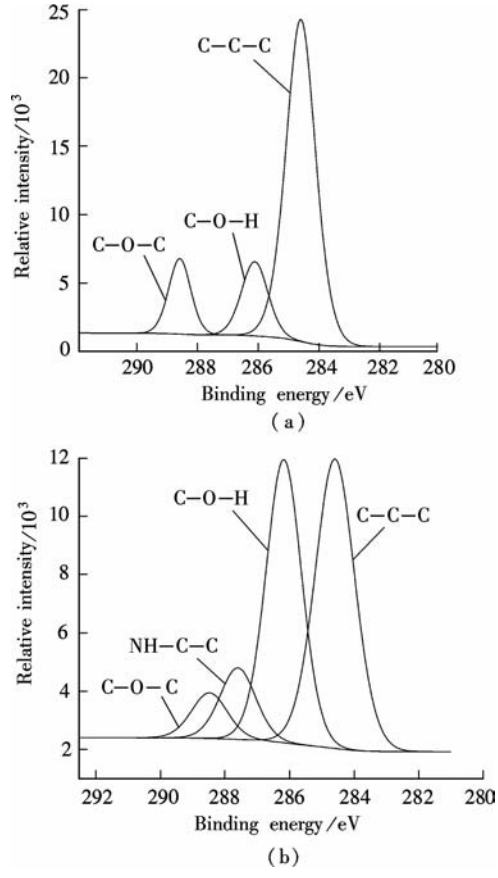

Fig. 3 XPS spectra of C1s. (a) Original PBTNW; (b) CS-grafted PBTNW

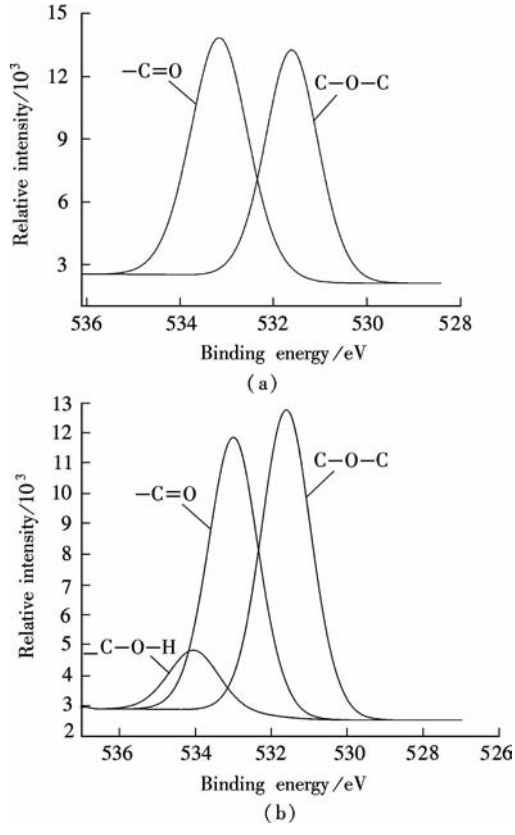

Fig. 4 XPS spectra of O1s. (a) Original PBTNW; (b) CS-grafted PBTNW

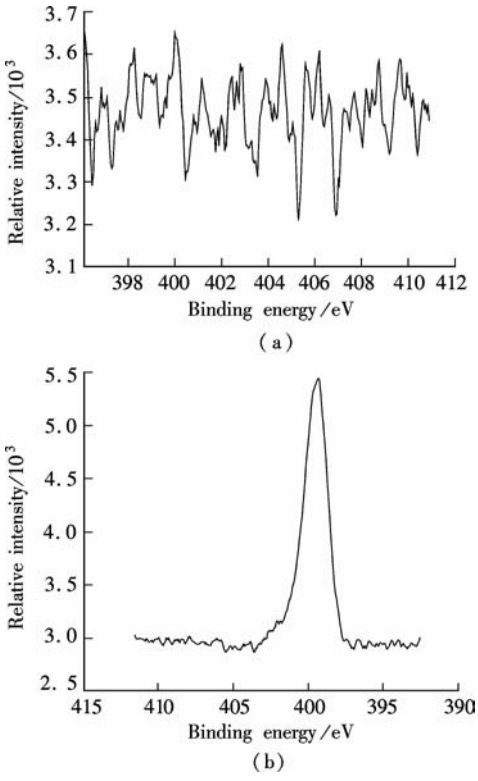

Fig. 5 XPS spectra of N1s. (a) original PBTNW; (b) CS-grafted PBTNW

2.2 Thermal stability of CS-grafted PBTNW

The degradation process and thermal stability of the PBTNW, chitosan and CS-grafted PBTNW copolymer are evaluated through thermo-gravimetric analysis (TGA) experiments, and the results are shown in Fig. 6. For the TGA curve of the PBTNW, in the first stage (from 30 to 379 ℃), the mass loss of absorbed water is 1.0%; in the second stage (from 380 to about 400 ℃), the relative mass of the PBTNW is only 10%, which is due to the scission of the PBTNW backbone; and in the last stage (from 400 to about 700 ℃), the relative mass of the PBTNW is 5.0%, which is due to the thermal decomposition of the PBTNW.

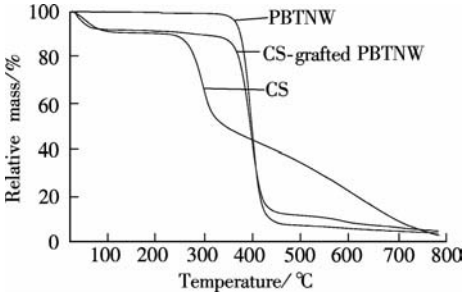

Fig. 6 TGA thermograms

For the TGA curve of the chitosan, in the first stage (from 55 to 191 ℃), the mass loss of absorbed and bounded water is 6.3%, indicating the hygroscopic nature of the chitosan; in the second stage (from 230 to 327 ℃), the mass loss of the chitosan is 45.7%, which is due to the scission of the ether linkage in the chitosan backbone; and in the third stage (from 327 to 703 ℃), the mass loss of the chitosan is 35.0%, which is due to the thermal decomposition of glucosamine residue.

However, the thermal degradation of the CS-grafted PBTNW copolymer is different from that of the chitosan. In the first stage (from 30 to 180 ℃), the mass loss of absorbed water is about 3.0%; in the second stage (from 180 to 320 ℃), the mass loss of the CS-grafted PBTNW copolymer is about 8.6%, which is also due to the scission of the ether linkage in the chitosan backbone. The onset temperatures of both the dehydration and the thermal degradation are lower than those of the chitosan. That is, the thermal stability of the chitosan decreases slightly after graft copolymerization. This is because more hydroxy and amino groups exist in the chitosan structure so that the crystalline regions form easily compared to the CS-grafted PBTNW copolymer. In the third stage (from 320 to 480 ℃), the mass loss of the CS-grafted PBTNW copolymer is 55.5%, which is due to the thermal decomposition of the side chains of the PBTNW.

2.3 Surface properties

The surface properties of the PBTNW are tested by measuring the contact angles of surfaces. As shown in Tab. 2, surface modification leads to a considerable decrease in the water contact angle of the PBTNW film. PBTNW is a kind of hydrophobic polymer, which has a water contact angle of 120°. Due to the increase in the polar group density, the contact angle is decreased to 0° by grafting polymerization of chitosan onto PBTNW film.

Tab. 2 Water contact angle of original and CS-grafted PBTNW

Sample	Contact angle/(°)
Original PBTNW	120 ± 0.3
CS-grafted PBTNW	0

The surface morphologies of PBTNW before and after graft polymerization are observed using the SEM. Fig. 7 shows SEM images of the original and CS-grafted PBTNW. It seems that the graft polymerization takes place on both the outer and inner sides of the PBTNW. Compared with the original PBTNW, the pore size of the CS-grafted PBTNW decreases gradually. Additionally, after the modification, the surface becomes significantly more porous. This change in surface morphology supports the occurrence of graft polymerization.

2.4 Hemolysis ratio

Hemolysis of the blood is an important problem associated with the biocompatibility of materials. Erythrocytes hemolyze upon contact with water. This problem may be aggravated in the presence of an implantable material. Less than 5% hemolysis is regarded as a nontoxic effect

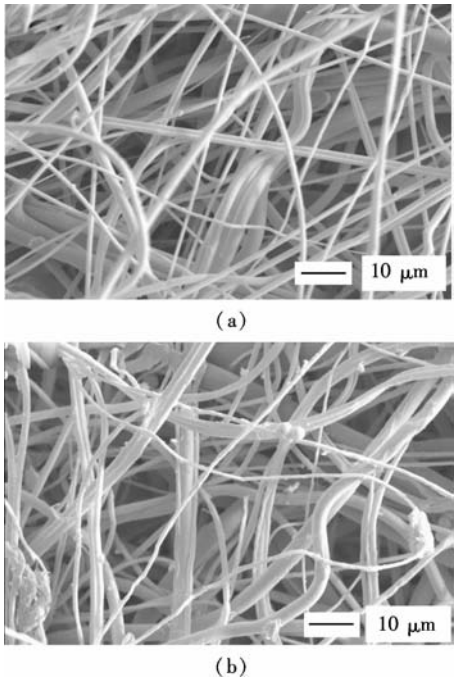

Fig. 7 SEM images. (a) Original PBTNW; (b) CS-grafted PBTNW

level, according to Petrini and Autian et al[16-17].

The hemolysis assay results are summarized in Tab. 3, showing that the R_H of the CS-grafted PBTNW is lower than the permissible limit of R_H for acceptable biomaterials, i.e. 5%.

Both the original and the CS-grafted PBTNW are incubated with human blood. As a control, a blood sample is incubated in an isotonic solution under the same conditions. The ratio of released hemoglobulin from the red blood cells is accepted as 100% for the blood incubated in distilled water. As shown in Tab. 3, the incorporation of chitosan onto the PBTNW results in a decrease in its hemolytic activity.

Tab. 3 Hemolysis test of original PBTNW and CS-grafted PBTNW fiber film

Sample	Hemolysis/%
Original PBTNW	1.92 ± 0.17
CS-grafted PBTNW	1.54 ± 0.15
Positive control	100
Negative control	0

Hemolysis is the breakage of the cell membrane that occurs when cells swell to a critical bulk. Meanwhile, ADP released from the broken red blood cells intensifies the assembly of blood platelets, which accelerates the formation of clotting and thrombus. In this study, chitosan presents high anticoagulation activity. A significant decrease in the hemolysis rate is observed (see Tab. 3), which fits well with the permissible limit. Moreover, it can be deduced that only the requisite concentration of the CS-grafted PBTNW should be used, avoiding any excess amount that might cause additional hemolysis. These results all show that the CS-grafted PBTNW has good anticoagulation characteristics.

2.5 Cytotoxicity evaluation

The cytotoxicity of the PBTNW fiber films is evaluated by MTT-based colorimetric assay[18]. In Fig. 8, the ratio of HUVECs viability and the control are shown, during 48 h exposure of the CS-grafted PBTNW fiber film. Compared to the control (PBS as control, taken as 100%), the CS-grafted PBTNW fiber film has a slightly higher cytotoxicity, but it is within acceptable limits, which may be related to the surface hydrophilicity of CS.

Thus, we can confirm that the CS-grafted PBTNW fiber films are blood compatible with a low level of cell cytotoxicity, and has significant potential to use in vivo.

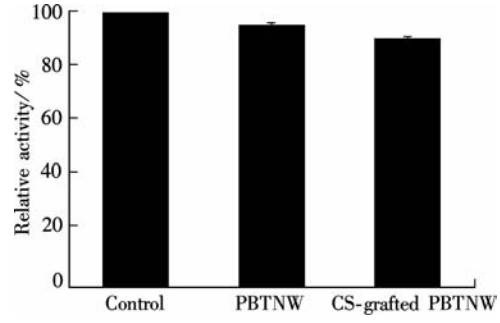

Fig. 8 Results from the MTT assay for HUVECs incubated with the PBTNW samples of 48 h

3 Conclusion

Chitosan is successfully grafted onto the PBTNW backbone in a homogeneous solution. The study of the FT-IR spectra indicates that the graft copolymerization does take place. The TGA result shows that the thermal properties of chitosan are slightly changed by the graft copolymerization. The surface wettability of the CS-grafted PBTNW is significantly better than that of the original PBTNW. The CS-grafted PBTNW exhibits excellent biocompatibility through hemolysis tests. We conclude that the CS-grafted PBTNW can be applied to achieve the desired wettability and biocompatibility and can be used as blood filter materials.

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烷基糖苷引导壳聚糖共聚接枝聚对苯二甲酸丁二醇酯非织造布

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摘要:为了提高聚对苯二甲酸丁二醇酯非织造布(PBTNW)的亲水性和生物相容性,采用表面改性的方法,在烷基糖苷(APG)的引导作用下将壳聚糖(CS)通过共聚接枝修饰到 PBTNW 的表面. 利用傅里叶全反射红外光谱(FTIR)分析仪、化学分析电子光谱学(ESCA)检测仪、热重(TG)分析仪及扫描电镜(SEM)对修饰前后的 PBTNW 材料进行表征,发现壳聚糖被成功接枝到 PBTNW 表面. 此外,对修饰前后的 PBTNW 进行水接触角、溶血率和细胞毒性实验,结果表明壳聚糖接枝后的 PBTNW 具有良好的亲水性和生物相容性. 接枝后的 PBTNW 较接枝前更具优越性,在血液过滤材料及其他的药用领域都可能是一种很好的替补材料.

关键词:壳聚糖;接枝;聚对苯二甲酸丁二醇酯非织造布;烷基糖苷;生物相容性;润湿性
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