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Antibacterial and Hemocompatibility Switchable Polypropylene Nonwoven Fabric Membrane Surface

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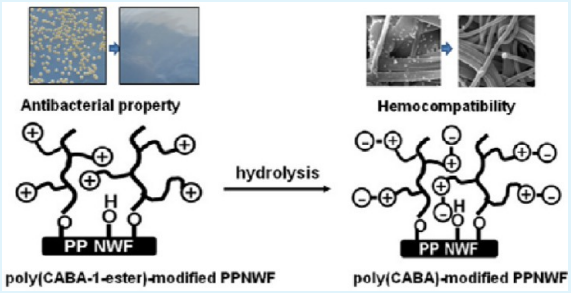
Supporting Information

ABSTRACT: In this article, a facile approach to fabricate a biofunctional polypropylene nonwoven fabric membrane (PP NWF) with a switchable surface from antibacterial property to hemocompatibility is presented. In the first step, a cationic carboxybetaine ester monomer, [(2-(methacryboxy) ethyl)]-*N*,*N*-dimethylamino-ethylammonium bromide, methyl ester (CABA-1-ester) was synthesized. Subsequently, this monomer was introduced on the PP NWF surface via plasma pretreatment and a UV-induced graft polymerization technique. Finally, a switchable surface from antibacterial property to hemocompatibility was easily realized by hydrolysis of poly(CABA-1-ester) moieties on the PP NWF surface under mild conditions. Surface hydrolysis behaviors under different pH conditions were investigated. These PP NWFs grafted with poly(CABA-1-ester) segments can cause significant suppression of *S. aureus* proliferation; after hydrolysis, these surfaces covered by poly[(2-(methacryloxy) ethyl)] carboxybetaine (poly(CABA)) chains exhibited obvious reduction in protein adsorption and platelet adhesion, and remarkably enhanced antithrombotic properties. This strategy demonstrated that a switchable PP NWF surface from antibacterial property to hemocompatibility was easily developed by plasma pretreatment and UV-induced surface graft polymerization and that this surface may become an attractive platform for a range of biomedical applications.



KEYWORDS: *antibacterial, UV-induced graft polymerization, polypropylene nonwoven fabric membrane, hemocompatibility, zwitterionic polymer*

1. INTRODUCTION

Currently, polymers are widely employed in biomedical applications from long-term implants to short-term store and filtration equipment, including vascular, orthopedic and ophthalmologic implants, catheters, hemodialyzers, and blood bags.[1−3] However, the deposition of proteins and platelets and the infection of bacteria in physiological environments cause a biolayer to be formed on these biomedical device surfaces, often leading to the failure of the medical devices.[4] Consequently, two important factors must be taken into account for the safe application of biomedical polymers. The primary one is the hemocompatible relative issue. In general, when blood comes in contact with a foreign surface, the adsorption of plasma proteins occurs in the initial step, followed by platelet adhesion, and activation of the coagulation pathways, leading to the final thrombus formation.[5,6] Thus, there have been considerable research interests in developing a surface that can effectively prevent protein adsorption and acquire excellent hemocompatibility. Tethering hydrophilic materials on a hydrophobic substrate is popularly regarded as an effective way to render a surface of hemocompatibility.[7,8] Up to now, numerous hydrophilic polymers have been grafted onto the substrate surface to prevent nonspecific protein adsorption. For example, the sugar-containing monomer (D-gluconamidoethyl methacrylate (GAMA)) was grafted on a polypropylene microporous membrane with an UV-induced polymerization, and this surface showed excellent hemocompatibility.[9] One kind of thermal cross-linking poly(*N*-vinylpyrrolidone) (PNVP) film was prepared by Neto et al., and the following tests showed that the cross-linking film had an obvious enhancement in its physical and mechanical properties while it still possessed excellent protein-repellent properties.[10]

Among these, poly(ethylene glycol) (PEG)/oligo (ethylene glycol) (OEG) containing copolymers are commonly used protein-resistant materials due to their strong hydrophilicity as well as high configurational mobility.[11−17] However, the susceptibility of PEG/OEG chains to oxidative degradation in aqueous systems, especially in the presence of transition metal ions has limited its long-term application.[18,19] Zwitterionic groups such as phosphorylcholine (PC),[20−22] sulfobetaine (SB),[23,24] and carboxybetaine (CB)[25−27] represent attractive alternatives to conventional PEG/OEG materials because they can bind water molecules even stronger than PEG chains via electrostatically induced hydration. Therefore, CB-based moieties have been widely accepted as hemocompatible materials due to their high resistance to nonspecific protein

Received: March 26, 2013
Accepted: May 13, 2013
Published: May 13, 2013

adsorption and favorable biocompatibility. Zhu et al. reported that CB based monomers were immobilized onto the silicone rubber surface by ozone-induced free-radical polymerization, and the platelet adhesion on the surface of the modified sample was dramatically reduced when compared with that on its control surface.[28] In addition, the protein adsorption behaviors on poly(CB) modified films with different spacer groups between charged dipoles were also investigated.[29]

Sterilization for medical devices is still a challenge, especially for the devices with the need of long-term storage. For these medical devices, it is hard to avoid a second bacterial invasion during long-term storage. Attachment of bacteria to a device's surface can lead to serious problems such as bacterial colonization and biofilm formation, which can easily cause infections of implants and wounds, lead to rejections of transplants, recurrent operations, and sometimes even death.[30] Although several works in the literature have confirmed that the initial attachment and colonization of microbes on surfaces can be reduced or delayed by antifouling coatings,[31,32] there is still a possibility of introducing pathogenic microbes into the physiological environments during blood purification and other biomedical processes. The incorporation of antimicrobial groups onto polymer surface is considered as an active approach to avoiding microbial invasions. An antimicrobial surface containing covalently linked quaternary ammonium compounds (QAC) is of great concern because of its efficient bacterial killing capability and lack of leaking of biocidal moieties into the environment.[33−36] However, these two properties (hemocompatibility and antibacterial activity) are usually incompatible within one surface due to the toxicity and tendency of binding proteins possessed by the QAC group. Therefore, designing a surface that can exhibit antibacterial activity before an application or in the early stage of application to finally transform to a hemocompatible surface will be an efficient way to resolve these problems. Recently, Jiang et al. designed a surface with self-sterilizing and nonfouling capabilities, which was based on a switchable polymer surface by converting cationic polymers to zwitterionic polycarboxybetaines on a gold-coated surface using surface-initiated atom transfer radical polymerization (SI-ATRP).[37] Although these results are encouraging, a limitation is the general feasibility of SI-ATRP for real-world materials due to the restricted kind of initiators and the reaction conditions. In addition, the investigation of hemocompatibility on a film surface, such as platelet adhesion and antithrombotic property, was not covered.

Herein, we fabricated a switchable polymer surface on a polypropylene nonwoven fabric membrane (PP NWF), which showed many outstanding properties such as controllable pore size distribution, easy fabricating, multiple, good thermal and chemical stabilities, and low cost. First, a cationic monomer, [(2-(methacryboxy) ethyl)]-*N*,*N*-dimethylaminoethylammonium bromide, methyl ester (CABA-1-ester) was synthesized and grafted on the surface of PP NWF to get the resulting product (poly(CABA-1-ester)-modified PP NWF). After hydrolysis, the cationic quaternary ammonium-based PP NWF surface was successfully converted to a zwitterionic polycarboxybetaine-based surface, and the hydrolysis behaviors were also investigated in detail. The biological performances of modified membranes before and after hydrolysis were evaluated, including antimicrobial activity, protein adsorption and platelet adhesion, and antithrombotic properties.

2. EXPERIMENTAL SECTION

2.1. Materials. Polypropylene nonwoven fabric (PP NWF) membrane with an average pore diameter of 0.22 μm was obtained from Beijing JDKR Co., Ltd. (Beijing, China). 2-Dimethylaminoethyl methacrylate (DMAEMA) (>98%), ethyl bromoacetate (>97%), and *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS) (>98%) were purchased from Sigma-Aldrich. Bovine serum albumin (BSA; pI = 4.8), Bovine serum fibrinogen (BFg; pI = 5.6), Lysozyme (Lys; pI = 11.2) and sodium dodecyl sulfate (SDS) were provided by Dingguo Biotechnology (China). Luria−Bertani (LB) broth medium and agar were also provided by Dingguo Biotechnology (China). Micro BCA protein assay reagent kit (AR1110) was purchased from Boster Biological Technology Limited Company (Wuhan, China). Other reagents were of AR grade and used without further purification.

2.2. Monomer Synthesis. 2-Dimethylaminoethyl methacrylate (50 mmol) and methyl bromoacetate (75 mmol) were dissolved with 50 mL of acetonitrile. Then, the mixture was added to a 100 mL round-bottomed flask and stirred at room temperature for 12 h. Subsequently, the precipitate was collected in dried ether and fully washed with 500 mL of anhydrous acetone. Finally, the white product was obtained and dried in a vacuum oven to remove trapped solvents to give the [(2-(methacryboxy) ethyl)]-*N*,*N*-dimethylaminoethylammonium bromide, ethyl ester (CABA-1-ester) monomer in 90% yield. The resulting product was analyzed by both ^{1}H NMR and LC/MS.

^{1}H NMR (300 MHz): 1.87 (s, 3H, CH_3−C═C), 3.32 (s, 6H, $N^+(CH_3)_2$), 3.75 (s, 3H, O−CH_3), 4.01 (t, 2H, CH_2−N^+), 4.40 (s, 2H, CH_2−C═O), 4.60 (m, 2H, CH_2−O−C═O), 5.72 (s, 1H, CH═C−COO-trans), 6.06 (s, 1H, CH═C−COO-cis). LS-MS: *m*/*e* $(M\text{-}Br)^+$ = 230; $(2M\text{-}Br)^+$=539; $(3M\text{-}Br)^+$ = 848. (The ^{13}C NMR as well as other details about ^{1}H NMR and MS spectra are shown in Figures S-1 and S-2 in the Supporting Information.)

2.3. Preparation of Poly(CABA-1-ester)-Modified Membranes. As surface grafting by UV-irradiation has been proved to be an easy and versatile technique to rendering material surface with special property,[38] this method was also adopted in our experiment: First, NWF PP membranes were pretreated with acetone to elute the residual solvent from manufacturing, dried, and weighed. Then, these samples were subjected to oxygen plasma (DT-03 plasma apparatus Suzhou Omega Technology Co., Ltd.) at a pressure of approximately 15 Pa. A certain amount of monomer solution (prepurged with Ar) was deposited onto PP NWF membranes. The membranes were placed between two pieces of quartz plate and irradiated under a high intensity UV lamp for a predetermined time. After grafting, poly(CABA-1-ester)-modified membranes (1.5 × 1.5 cm^2) were washed with deionized water (1000 mL) by shaking 3 times, rinsed with ethanol (500 mL) to remove homopolymer and unreacted monomers on surfaces, and finally dried in a vacuum oven at room temperature for 24 h. The grafting density of poly(CABA-1-ester) (GD, $\mu g \cdot cm^{-2}$) was calculated using the following equation:

$$GD = \frac{W_1 - W_0}{A_0} \quad (1)$$

Here, W_0 and W_1 are the mass of the virgin and modified NWF PP membranes (μg), respectively. A_0 represents the area of the membrane (cm^2).

2.4. Preparation of Poly(CABA)-Modified Membranes by Hydrolysis. Poly(CABA-1-ester)-modified samples were immersed into *N*-cyclohexyl-3-amino-propanesulfonic acid (CAPS) buffer (pH 10.2) at 37 °C. After a certain time interval, the modified PP NWF membranes were washed by deionized water and dried by vacuum. In addition, the poly(CABA-1-ester)-modified membranes were also dipped into aqueous solutions with different NaOH concentrations to investigate the resistance against hydrolysis of the modified membranes. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) was used to verify the surface change of poly(CABA-1-ester)-modified PP NWF membrane and the formation of poly(CABA)-modified membranes by investigating the conversion from ester groups (−COO-CH_3) of poly(CABA-1-ester) to carboxylic acid groups.

2.5. Surface Characterization. ATR-FTIR spectra of the virgin and modified NWF PP membranes were obtained from a Fourier transform infrared spectrometer (FTIR, BRUKER Vertex 70) with a resolution of 4 cm^{-1} in absorbance mode.

Surface elemental composition of the samples was determined via X-ray photoelectron spectroscopy (XPS, VG Scientific ESCA MK II Thermo Avantage V 3.20 analyzer) with an Al/K ($h\nu$ = 1486.6 eV) anode mono-X-ray source. All of the samples were completely vacuum-dried prior to use. The releasing angle of the photoelectron for each atom was fixed at 90°. Surface spectra were collected over a range of 0−1200 eV, and high-resolution spectra of C1s, O1s, and N1s regions were collected. The atomic concentrations of the elements were calculated by their corresponding peak areas.

The static contact angles of water on the surfaces of the virgin and modified NWF PP membranes were carried out at room temperature using a contact angle goniometer (DSA KRÜSS GMBH, Hamburg 100) by injecting 3 μL of distilled water on the membrane surfaces. The value of water contact angle was recorded after 2S. Five measurements were made on each sample to obtain the average value of contact angles.

2.6. Antibacterial Property. In this test, *S. aureus* was used as a test organism since it represents one of the most common nosocomial (originating in a hospital) pathogens. *S. aureus* cells (Shanghai Paitong Biotechnology Co., Ltd.) from a 15% glycerol stock were cultured in Luria−Bertani (LB) broth with the pH value of 7.4 at 37 °C for 24 h. The concentration of *S. aureus* cells in the broth was determined by a cell counter and diluted to the desired concentration with deionized water. The actual number of cells used for a given experiment was determined by standard serial dilution.

The shaking flask method[34,39] as a concise yet effective approach was adopted here to investigate the antimicrobial activity of the modified samples (Virgin PP NWFs, poly(CABA-1-ester), and poly(CABA) modified PP NWF with different grafting densities). These samples (1.5 × 1.5 cm^2) were shaken with 5 mL of suspension of *S. aureus* containing approximately 4.8 × 10^5 cell at 37 °C for 2 h. After that, 10 min of ultrasonic treatment was applied to these suspensions, removing these adsorbed *S. aureus* on sample surfaces. Then, the suspensions from each flask were taken out and diluted appropriately. Finally, 50 μL volume of each suspension was taken out and plated on gelatinous LB agar plates. The agar plates were incubated at 37 °C for 24 h, and the number of viable bacteria on the plates was counted as colony forming units (CFU) at the end of the incubation period. The antibacterial activity was assessed by the number of viable cells because each surviving cell developed into a distinct colony after incubation. Therefore, the surface antibacterial activity of samples was represented by a kill percentage, which was calculated by the following equation:

$$\text{killing percentage } (\%) = \frac{N_{\text{virgin}} - N_{\text{sample}}}{N_{\text{virgin}}} \times 100$$

Here, N_{virgin} and N_{sample} are the number of CFU on the LB agar plates of the virgin PP NWF and modified samples, respectively.

2.7. Protein Adsorption. Virgin, poly(CABA-1-ester), and poly(CABA)-modified PP NWFs (1.5 × 1.5 cm^2) were placed in individual wells of a 24 well tissue culture plate. After equilibration by PBS at 37 °C for 12 h, we immersed the membranes into single protein solutions of BSA, BFg, and Lys (1 mg/mL in PBS) at 37 °C for 2 h, respectively. After rinsing several times with fresh PBS, we transferred the samples to clean wells, and the samples were washed in an aqueous solution of 1.0 wt % SDS, subjected to shaking for 60 min, and sonicated for 20 min at room temperature to remove the protein adsorbed on the surfaces. On the basis of the bicinchoninic acid (BCA) protein assay kit method, the protein concentration in the SDS solution was determined by using a microplate reader (TECAN SUNRISE, Switzerland), and the amount of proteins adsorbed on the surfaces was calculated. The reported data were the mean values of triplicate specimens for each membrane.

2.8. Platelet Adhesion. The virgin and modified PP NWF samples (1.5 × 1.5 cm^2) were placed into a 6-well polystyrene plate, sterilized by ethanol for 2 h, dried under vacuum at room temperature for 12 h, and then equilibrated by PBS at 37 °C for 2 h. Twenty microliters of fresh PRP (the platelet-rich plasma obtained from the fresh rabbit blood by centrifugation at 800 rpm for 10 min) was dropped onto the center of each sample and followed by incubation for 30 and 90 min at 37 °C. After being rinsed with PBS moderately, the platelet adhering on the membrane was fixed by 2.5 wt % glutaraldehyde at 4 °C for 10 h. Finally, the membranes were washed with PBS several times and dehydrated with a series of ethanol−water mixtures (10, 30, 50, 70, 90, and 100 vol % ethanol) for 30 min in each step. The samples were observed with a field emitted scanning electron microscopy (FESEM, XL 30 ESEM FEG, FEI Company).

2.9. Antithrombotic Properties. The antithrombotic properties of these virgin and modified membranes (GD = 327.5 $\mu g \cdot cm^{-2}$) were usually evaluated using the free hemoglobin method.[40−42] Briefly, the whole blood with sodium citrate as anticoagulant (10.0 mL) was drawn from healthy adult rabbits and activated with the addition of 1 mL of $CaCl_2$ (0.1 M). After that, a 100 μL volume of the activated blood was carefully added to virgin and modified specimens, which were placed in the wells of a 12-well plate at 37 °C. After predetermined time intervals of 5, 10, 20, and 30 min, the clotting procedure was terminated by adding a definite amount of deionized water to the plate. The red blood cells that had not been trapped in a thrombus were hemolyzed with the addition of deionized water, and the free hemoglobin was dispersed in liquid. The concentration of free hemoglobin in the solution was determined colorimetrically by monitoring the absorbance at 540 nm using a 96 well plate reader. The mixture of blood, $CaCl_2$, and deionized water was used as a reference. Three trials were performed, and the average results are presented.

3. RESULTS AND DISCUSSION

3.1. Preparation of Poly(CABA)-Modified PP NWF. As illustrated in Figure 1, the poly(CABA)-modified PP NWF was

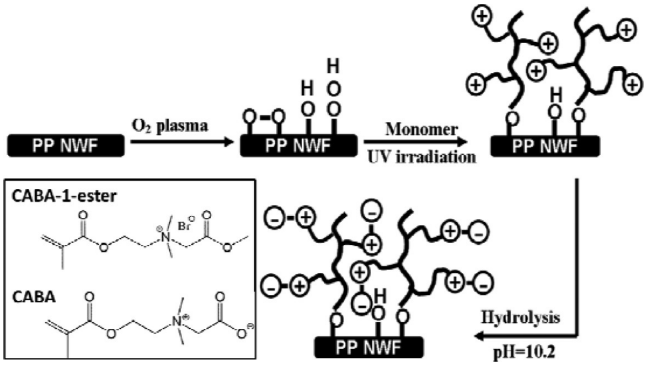


Figure 1. Schematic illustration of the procedure for the preparation of the poly(CABA)-modified NWF membrane.

prepared in a three-step process. In the first step, the surface was activated by low-temperature oxygen plasma treatment, and the polar and peroxide functional groups were produced on the substrate surface. Then, poly(CABA-1-ester)-based polymers were formed by UV-induced graft polymerization using a sandwiched setup.[43] In this experiment, the grafting density was effectively influenced by experiment parameters such as the plasma treatment time, UV irradiation time, and monomer concentration. Finally, poly(CABA)-modified surfaces were formed by the hydrolysis of poly(CABA-1-ester)-modified PP NWF in CAPS buffer (pH 10.2) at 37 °C for 8 h. This approach has several advantages for real-world applications: first, plasma pretreatment produced a peroxide group, and UV irradiation triggered the formation of a radical group; therefore, the grafting polymerization can be initiated without prior

modification of a surface by conventional or living radical initiators. Second, UV-induced grafting is relatively simple and well suited for commodity polymers with no active groups, such as polypropylene, polyethylene, and polystyrene, etc. Third, this procedure is more energy-efficient and cost-effective and more useful for applications.

3.2. Chemical Changes of the PP NWF Membrane Surface. Incorporation of poly(CABA-1-ester) and poly-(CABA) segments to PP NWF was first tested by ATR-FTIR (Figure 2). The grafting polymerization of cationic monomer,

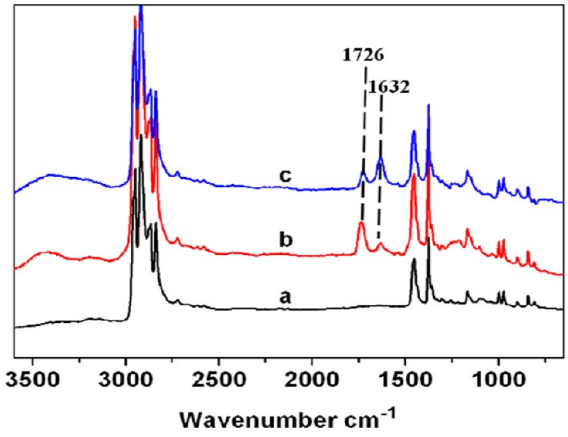


Figure 2. FTIR-ATR spectra for the (a) virgin PP NWF membrane; (b) poly(CABA-1-ester)-modified and (c) poly(CABA)-modified PP NWF (GD = 327.5 $\mu g \cdot cm^{-2}$).

CABA-1-ester, onto the PP NWF surface was successfully demonstrated since a new peak appeared at about 1726 cm^{-1} coming from the ester stretching band of the carboxyl group (C═O). In addition, a weak adsorption peak at 1632 cm^{-1} corresponding to the stretching vibration of the carboxylic acid groups (COO^-) from a small amount of hydrolysis of poly(CABA-1-ester) was also observed (Figure 2b). As for the poly(CABA)-modified membrane, these two peaks mentioned above also appeared on the spectrum (Figure 2c). However, clear differences were observed between these two spectra. The intensity of the peak at 1726 cm^{-1} on poly(CABA-1-ester)-modified PP NWF decreased obviously, while the peak at 1632 cm^{-1} increased significantly as compared with the poly(CABA)-modified PP NWF, which confirmed the surface transformation from cationic polymers to zwitterionic polymers.

XPS is a highly surface-specific analytical technique, with typical sampling depths for organic films of around 5−10 nm. This measurement can precisely reveal the elemental compositions of the outermost layer of the membrane surface. Herein, the surface composition of samples was further examined by XPS (in Figure 3). For the virgin PP NWF surface (Figure 3a), an obvious peak at 285 eV and a small peak at 525 eV attributed to C_{1s} and O_{1s} were observed (oxidation or contamination of the virgin PP NWF membrane). Meanwhile, an enhanced peak of O_{1s} was observed on the plasma-treated PP NWF membrane (Figure 3b). In the case of modified membranes before and after hydrolysis (Figure 3c and d), the appearance of a new peak at 403 eV (N_{1s}) was a clear indication of the successful graft polymerization on membranes.

In addition, the C_{1s} core-level spectra of these samples are also shown in Figure S-3 (see Supporting Information). For the virgin NWF PP membrane, there is only one peak component

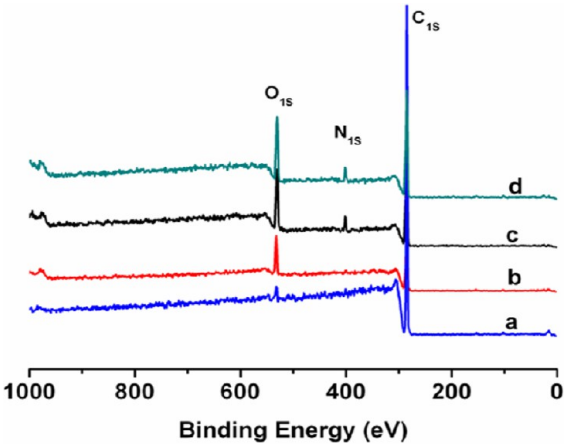


Figure 3. XPS survey spectra of the (a) virgin NWF membrane, (b) plasma-treated NWF membrane, (c) poly(CABA-1-ester)-modified NWF membrane, and (d) poly(CABA)-modified PP NWF (GD = 327.5 $\mu g \cdot cm^{-2}$).

at the binding energy of 284.6 eV attributed to (CH_2) species (Figure S-3a, Supporting Information). The plasma-treated membrane showed obvious changes compared to the virgin NWF PP, and the new peak components at binding energies of about 286.3 and 288.4 eV were assigned to the C−O and COO species, respectively (Figure S-3b, Supporting Information). In the case of modified membranes before and after hydrolysis (Figure S-3c and -d, Supporting Information), the appearance of a new peak at 403 eV (N_{1s}) was a clear indication of the polymer immobilization on the surface of PP NWF. The C_{1s} core-level spectra of poly(CABA-1-ester)-modified NWF PP membranes could be curve-fitted with four peak components. These peak components at the binding energies of 284.6, 286.1, 286.6, and 288.7 eV were assigned to the (CH_2), C−N^+, C−O, and COO species, respectively. The C_{1s} core-level spectra of the poly(CABA)-modified membrane could also be curve-fitted with four peak components as shown above, but a minor decrease in the intensity of C−O was observed due to the hydrolysis of the methyl ester. Furthermore, the elemental percentages and atomic ratio of N/O were also calculated by the XPS results (see Supporting Information, Table S-1).

In order to investigate hydrophilic behaviors of these modified surfaces, Water contact angles were tested (see Supporting Information Figure S-4). Among these, the virgin NWF PP surface showed an initial water contact angle value of 126° due to its inherent hydrophobic property. While poly(CABA-1-ester)-modified PP NWFs exhibited a remarkable decrease in contact angle of 58°, even a relatively low grafting density (GD = 121.3 $\mu g \cdot cm^{-2}$), and the values further reduced from 38° to a minimum value of about 22° by increasing GD from with 201.4 to 414.2 $\mu g \cdot cm^{-2}$ (Figure S-4a, Supporting Information); under the same conditions, after being subjected to hydrolysis in CAPS buffer (pH 10.2) at 37 °C for 8 h, a slightly decreased tendency showed up on the water contact angle value of poly(CABA)-modified samples when compared with poly(CABA-ester)-modified samples. These values reduced from 58° on poly(CABA-1-ester)-modified PP NWF to 45° after hydrolysis at a GD of 121.3 $\mu g \cdot cm^{-2}$. The contact angle further decreased from 29° to 19° with increasing GD from 201.4 to 414.2 $\mu g \cdot cm^{-2}$ (Figure S-4b, Supporting Information). All in all, a slight but consistent decrease in contact angle value was observed on poly(CABA)-

modified samples compared to poly(CABA-ester)-modified membranes, indicating the change of chemical groups occurred on these surfaces after hydrolysis.

3.3. Hydrolysis of Carboxybetaine Ester Polymers on the PP NWF Surface. In this test, the hydrolysis of poly(CABA-1-ester)-modified PP NWFs was conducted and the hydrolytic stability of the ester(I) (−COO-CH_2CH_2−) and the ester(II) (−COO-CH_3) of poly(CABA-1-ester) was studied. The poly(CABA-1-ester)-modified PP NWFs with the same grafting density were immersed in CASP buffer (pH 10.2) and different concentrations of NaOH (0.2, 0.5, and 1.0 M), and their hydrolysis behaviors were investigated. After a certain time of hydrolysis in CAPS buffer, ester hydrolysis was analyzed by measuring the absorption peaks of remaining ester groups and the generated carboxylic acid on the polymers by ATR-FTIR. The results showed that obvious changes occurred on both peaks at 1737 cm^{-1} and 1632 cm^{-1}. Compared with the membrane before hydrolysis, a significant decrease of 1737 cm^{-1} and a peak shift (from 1737 to 1726 cm^{-1}) were displayed on the spectra after hydrolysis, indicating the successful transformation from carboxybetaine ester to carboxybetaine surface; prolonging hydrolysis time from 8 to 24 h did not show any substantial change on the ATR-FTIR spectra, and it can be deduced that a completely hydrolyzed surface was obtained within 8 h in CAPS. After exposure to NaOH solution with different concentrations for 24 h, the poly(CABA-1-ester)-modified membranes were analyzed by ATR-FTIR to investigate the degradations of ester(I) group (−COO-CH_2CH_2−) and ester(II) group (−COO-CH_3). The results showed that a small decrease in peak intensities of 1632 cm^{-1} and 1726 cm^{-1} occurred, implying that only a small amount of hydrolysis occurred on ester(I) (−COO-CH_2CH_2−). Thus, it can be deduced that ester(I) groups were substantially more stable to hydrolytic degradation than ester(II) groups even with a high concentration of NaOH solution (1.0 M) (Figure 4). In addition, in order to get further information about hydrolysis behaviors of poly(CABA-1-ester) segments on the PP NWF surface, a gravimetric method was applied to detect the weight variation of these membranes before and after hydrolysis.

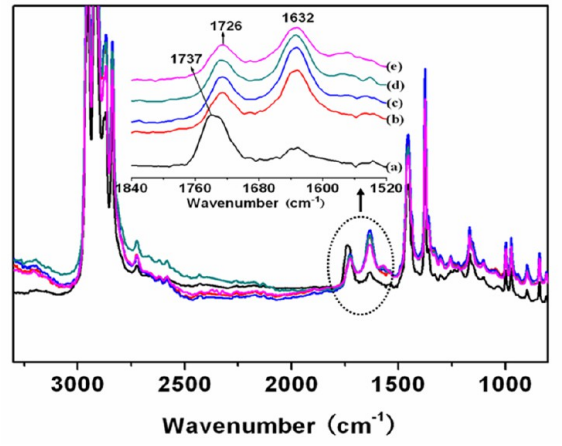


Figure 4. FTIR-ATR spectra for the hydrolysis of poly(CABA-1-ester)-modified PP NWF: (a) modified PPNWF before hydrolysis, (b) modified PPNWF after hydrolysis with CASP buffer, and with different concentrations of NaOH: (c) 0.2M, (d) 0.5 M, and (e) 1.0 M. The upper inset is a magnified view of the FTIR-ATR spectra from 1520 to 1840 cm^{-1}.

Poly(CABA-1-ester) modified samples (1.5 × 1.5 cm^2) with GD of 327.5 $\mu g \cdot cm^{-2}$ were accurately weighed and subsequently immersed in CAPS buffer and sodium hydroxide solution (1.0 M). After a certain time of hydrolysis (from 4 to 20 h), these membranes was taken out, rinsed by water, dried completely in an oven, and weighed again. Thus, the weight change with different hydrolysis time was obtained, and its corresponding apparent grafting density was also calculated. Therefore, the hydrolysis degree can be evaluated with respect to the theoretical grafting density of poly(CABA). As shown in Figure S-5a (Supporting Information), the apparent GD of poly(CABA-ester) modified PP NWF decreased sharply in the first 8 h when treated with CAPS buffer. This value changed from 327.5 $\mu g \cdot cm^{-2}$ to 220.9 $\mu g \cdot cm^{-2}$; however, the apparent GD reached a plateau and became constant at 217.5 $\mu g \cdot cm^{-2}$ after prolonging the hydrolysis time from 8 to 20 h, which is very close to the theoretical grafting density of poly(CABA) modified membrane (GD = 227.1 $\mu g \cdot cm^{-2}$). We ascribed this change to a complete hydrolysis of the ester(II) group (−COO-CH_3), with almost no effect on the ester(I) group (−COO-CH_2CH_2−). After NaOH solution treatment (Figure S-5b, Supporting Information), two main differences were observed on the apparent GD of modified sample as compared with the sample in CAPS buffer: (1) The apparent GD reached its plateau within 4 h of hydrolysis treatment; (2) the final value after 20 h of treatment was slightly lower (212.3 $\mu g \cdot cm^{-2}$) than the one in CAPS buffer. These results suggested that degradation of the ester(II) group was more easy in NaOH solution (1.0 M) than ester(I), which were in a good agreement with results of the hydrolysis investigation by FTIR-ATR.

3.4. Antibacterial Property of Poly(CABA-1-ester)-Modified PP NWF Surface. Figure 5 shows the digital photographs of agar plates incubated with the diluted *S. aureus* suspension, which had been in contact with virgin, poly(CABA-1-ester)-modified, and poly(CABA)-modified PP NWFs for 2 h of incubation. Compared to the control (Figure 5a), poly-

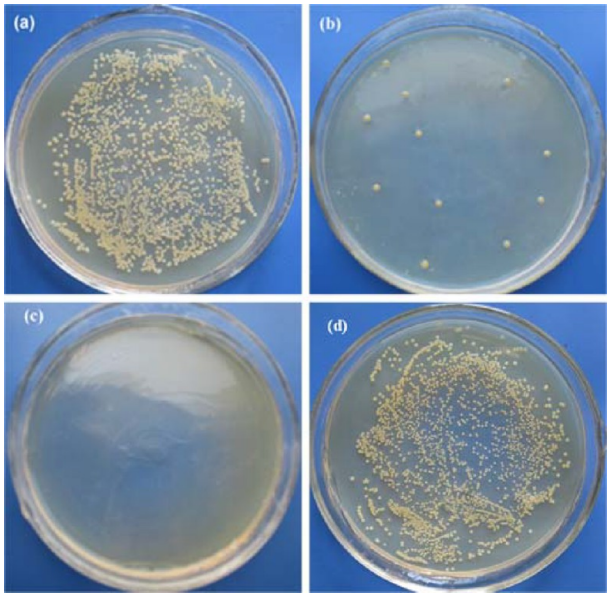


Figure 5. Photographs of agar plates onto which the *S. aureus* suspension of distilled water treated with (a) virgin PP NWF, (b and c) poly(CABA-1-ester)-modified PP NWF (GD = 279.5 $\mu g \cdot cm^{-2}$ and GD = 327.5 $\mu g \cdot cm^{-2}$), and (d) poly(CABA)-modified PP NWF (GD = 327.5 $\mu g \cdot cm^{-2}$) were deposited and incubated for 24 h at 37 °C.

dx.doi.org/10.1021/am401098u | ACS Appl. Mater. Interfaces 2013, 5, 5260−5268

(CABA-1-ester)-modified PP NWFs can efficiently inhibit the growth of *S. aureus*, and their antibacterial abilities become stronger with increasing grafting density (Figure 5b and c). Among these, more than 90% *S. aureus* was killed by poly(CABA-1-ester)-modified PP NWF with a GD of 279.5 $\mu g \cdot cm^{-2}$ with respect to virgin PP NWF. Furthermore, nearly no bacterial colony appeared on the plate when the *S. aureus* suspension was disposed with the modified membrane at a higher grafting density (GD = 327.5 $\mu g \cdot cm^{-2}$) (Figure 5c). All of these results demonstrated that the antibacterial property of poly(CABA-1-ester)-modified PP NWF is rooted in the covalent immobilization of quaternary ammonium compounds on surface. The antibacterial property of the quaternary ammonium groups was attributed to the interaction between the positively charged quaternary ammonium groups and the negatively charged bacterial cell biomembrane.[35] The biocidal activity was further enhanced with increasing grafting density of poly(CABA-1-ester). These results were in good agreement with other reports on the antibacterial activity of quaternary ammonium compound-modified materials.[44] Under the same condition, the antibacterial capability of the poly(CABA)-modified sample was also investigated, and the result showed no obvious inhibition on bacterial growth as compared to that of virgin PP NWF(Figure 5d). Recently, some interesting works focused on the antibacterial activity of the zwitterionic sulfopropylbetaine surface were carried out by Chen et al.[45] They attributed this antibacterial mechanism to a way different from that of traditional QAC killing properties, which also aroused our interest in further works about the investigation of zwitterionic carboxybetaine antibacterial activity.

3.5. Protein Adsorption. Protein adsorption onto a surface plays an important role in the following platelet adhesion and thrombosis formation. In this test, two kinds of negatively charged proteins, bovine serum albumin (BSA) and bovine fibrinogen (BFg), and one kind of positively charged protein, lysozme (Lys), were chosen as model proteins to evaluate protein-resistant behaviors of the sample. Among these, albumin is the most abundant plasma protein (60%), and fibrinogen plays a vital role in the formation of thrombosis as it is converted by thrombin to insoluble fibrin.[46] Bicinchoninic acid (BCA) assay,[47] based on the formation of the Cu^{2+}–protein complex under alkaline conditions, was performed followed by the reduction of Cu^{2+} to Cu^{+}. The amount of reduction is proportional to the protein present, which is quantified spectrophotometrically at 560 nm. This assay is widely accepted as a protein quantified method due to its sensitivity, stability, and application in a broad range of protein concentrations. In this work, the bicinchoninic acid (BCA) protein assay kit method was used to quantify adsorbed proteins on these PP NWFs.

Protein adsorptions on virgin, poly(CABA-1-ester)-modified and poly(CABA)-modified PP NWFs were studied (Figure 6). Poly(CABA-1-ester)-modified PP NWFs (GD = 327.5 $\mu g \cdot cm^{-2}$) showed higher adsorption of BFg and BSA and a lower adsorption of Lys in comparison to virgin PP NWF (Figure 6a and b). The possible interpretation for this finding could be that protein adsorption can easily be triggered by these surfaces modified with QAC groups, while Lys adsorption can be affected by the electrostatic force between the cationic surface and positively charged proteins. In contrast, the poly(CABA)-modified PP NWF displayed considerable low adsorption values of all three proteins; these were about 70%, 88%, and 85% adsorption reductions of BSA, BFg, and Lyz,

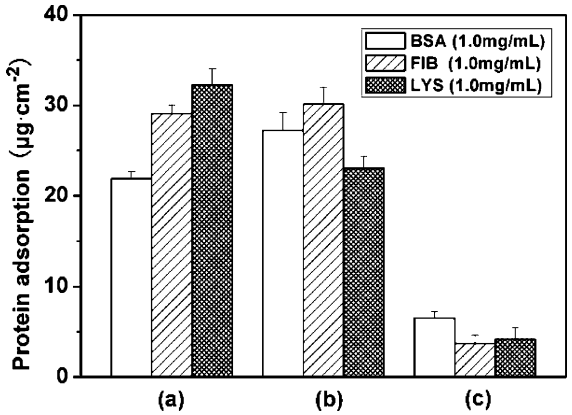


Figure 6. Protein adsorptions on (a) virgin PP NWF, (b) poly(CABA-1-ester)-modified PP NWF (GD = 327.5 $\mu g \cdot cm^{-2}$), and (c) poly(CABA)-modified PP NWF (GD = 327.5 $\mu g \cdot cm^{-2}$).

respectively, as compared to the control (Figure 6c). These observations can be explained by the fact that the hydration layers formed on the poly(CABA)-modified modified surface is regarded as the key to enhance the resistance to protein adsorption, which shows great contribution to low and reversible protein adsorption on the surface without a significant conformation change in protein molecules.[48−52]

Furthermore, it was also reported that the surface covered with albumin can show thromboresistance, while that with fibrinogen promotes platelet adhesion and activation.[53−55] Hence, the relationship of albumin and fibrinogen adsorptions on the sample was tested to evaluate its antiplatelet adhesion capability. Herein, the BSA/BFg adsorbed ratios on different samples were measured according to protein adsorption results. Compared with the value of virgin PP NWF (0.74), it apparently increased on poly(CABA)-modified PP NWF (1.74), while only a slightly increase appeared on poly-(CABA-1-ester)-modified PP NWF (0.90). As mentioned before, a higher adsorption ratio (BSA/BFg) means a lower platelet adhesion behavior. On the basis of these results, we might speculate that poly(CABA-1-ester)-modified PP NWF can transform from a strong platelet affinity surface to a low platelet affinity surface after hydrolysis.

3.6. Platelet Adhesion. The platelet adhesion test has already become a proven technique to estimate surface hemocompatibility. This test was also applied to evaluate the hemocompatibility of these modified samples in vitro by analyzing the status of adhered platelets on a surface during a certain period of incubation. Figure 7 showed the scanning electron micrographs of platelets fixed on the surface of virgin and modified PP NWF (GD = 327.5 $\mu g \cdot cm^{-2}$) before and after hydrolysis. After 30 min of incubation with PRP, a large number of disc-shaped and pseudopodia platelets adhered on the virgin membrane surface; normally, the disc-shaped platelet is considered the nonactivated status, and the pseudopodia shape means a typical stage of early activation (Figure 7a). As for poly(CABA-1-ester)-modified PP NWF, platelet adhesion behavior on its surface is different. A great amount of adhered platelets were highly spread, forming aggregates and focal clumps (Figure 7b), indicating that the surface modified with QAC groups can trigger serious platelet activation. A few platelets in rounded shape (nonactivated state) were observed on the poly(CABA)-modified PP NWF surface (Figure 7c). After 60 min of PRP incubation, new changes were observed on

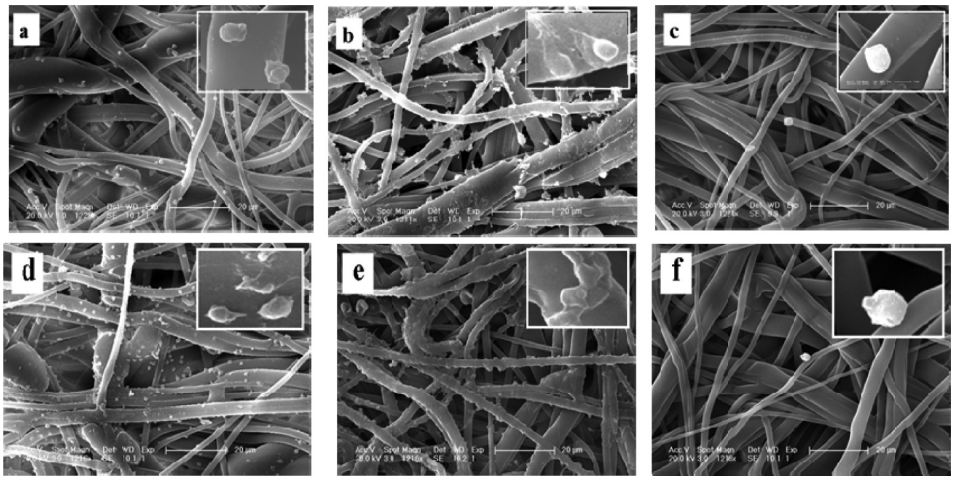


Figure 7. SEM pictures of adhered platelets on (a) virgin, (b) poly(CABA-1-ester)-modified, and (c) poly(CABA)-modified PP NWF after 30 min of incubation and (d) virgin, (e) poly(CABA-1-ester)-modified, and (f) poly(CABA)-modified PP NWF after 60 min of incubation. The insets in all pictures are magnified views of adhered platelets with different activation levels.

these samples except for poly(CABA)-modified PP NWF. More platelets aggregated and adhered on the virgin PP NWF surface than before, and most of them displayed an activated state (Figure 7d). More serious conditions appeared on poly(CABA-1-ester)-modified PP NWF than that for 30 min of incubation; this result originated in the mutual interactions between platelets and the cationic surface, revealing that prolonging incubation time can lead to serious platelet adhesion and activation (Figure 7e). As for poly(CABA)-modified PP NWF, the platelet morphology is similar to that of the 30 min-incubation, and no further platelet adhesion was observed on this surface (Figure 7f). The whole platelet adhesion results revealed that poly(CABA-1-ester)-modified PP NWF displayed strongly adhered and activated platelet properties, while poly(CABA)-modified PP NWF showed excellent platelet resistance regardless of prolonging induction time. It is normally considered that the improved hemocompatibility can be attributed to the formation of zwitterionic groups on a membrane surface. Zwitterion-based polymers can highly resist nonspecific protein adsorption, especially for Fg adsorption, and effectively avoid the following platelet adhesion and aggregation.[56] These results were also consistent with protein adsorption results that higher adsorption ratio of BSA/BFg results in stronger resistance to platelet adhesion on surface.

3.7. Antithrombotic Assessment. An in vitro induced blood clotting test was carried out to assess the formation of thrombus on the virgin and modified PP NWFs (GD = 327.5 $\mu g \cdot cm^{-2}$) before and after hydrolysis. In this test, the percentages of free hemoglobin defined as the ratio of the released hemoglobin from sample solution to the mixed blood reference were shown (Figure. 8). The percentage of free hemoglobin varied with incubation time; a higher hemoglobin value normally means better thromboresistance of a material. As for virgin NWF, prolonging the incubation time can sharply reduce the free hemoglobin within 20 min. In the case of poly(CABA-1-ester)-modified PP NWF, the free hemoglobin value decreased quickly in the first 10 min of incubation, then the tendency became slower in the next 10 min when compared with virgin PP NWF. More than 30% of free hemoglobin was detected on the solution treated by poly-(CABA)-modified PP NWF with respect to 5% of the

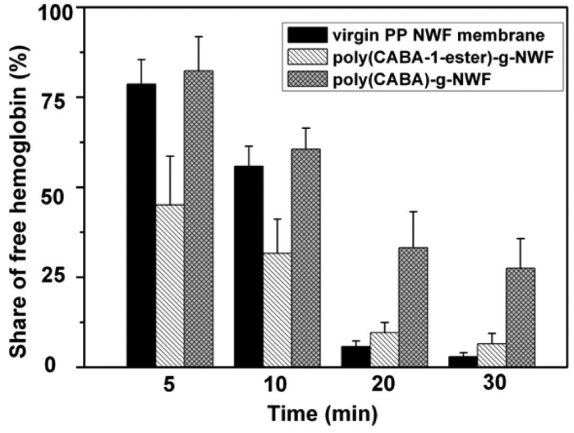


Figure 8. Share of hemoglobin released from the virgin and grafted membranes before and after hydrolysis.

hemoglobin on control. These results confirmed that poly-(CABA)-modified PP NWF possessed good antithrombotic property due to the formation of the carboxybetaine polymer layer surface.

4. CONCLUSIONS

Herein, we present a facile approach for the preparation of switchable polymer surface from antibacterial property to hemocompatibility on PP NWF via plasma pretreatment and UV-induced graft polymerization technique. A range of poly(CABA-1-ester)-modified PP NWFs with different grafting densities were synthesized. Both FTIR-ATR spectra and gravimetric method were adopted to investigate the hydrolysis behaviors of poly(CABA-1-ester) segments on PPNWF, and results showed that ester(I) group ($-COO-CH_2CH_2-$) has a much higher hydrolysis resistance than the ester(II) group ($-COO-CH_3$) and that these products can easily transform from a cationic poly(CABA-1-ester)-modified surface to a zwitterionic poly(CABA)-modified surface under mild hydrolysis conditions. Biological tests showed that poly(CABA-1-ester)-modified PP NWFs exhibited effective antibacterial property against *S. aureus*, while the poly(CABA)-modified sample displayed obvious resistance to protein adsorption, platelet adhesion, and activation; in addition, obvious

prolonged clotting time was observed on poly(CABA)-modified PP NWF. All of these results revealed its potential application as dual-functional biomaterials.

■ ASSOCIATED CONTENT

Supporting Information

Elemental percentages, atomic ratio (N/O), and core-level XPS spectra from XPS, ^{1}H NMR spectrum for CABA-1-ester, and water contact angle values. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the financial support of the National Natural Science Foundation of China (Project No. 21274150).

■ ABBREVIATIONS

CABA-1-ester = [(2-(methacryboxy)ethyl)]-*N*,*N*-dimethylamino-ethylammonium bromide, methyl ester
PP NWF = polypropylene nonwoven fabric membrane
poly(CABA) = poly(carboxybetaine methacrylate)
PC = phosphorylcholine
SB = sulfobetaine
CB = carboxybetaine
CAPS = *N*-cyclohexyl-3-aminopropanesulfonic acid
BSA = bovine serum albumin
BFg = bovine serum fibrinogen
Lys = lysozyme
QACs = quaternary ammonium compounds
LB = Luria−Bertani
W_0 = mass of the virgin NWF membrane (μg)
W_1 = mass of the modified NWF membrane (μg)
A_0 = the membrane area (cm^2)
N_{virgin} = number of bacterial colonies in solution after 2 h of contact with virgin PP NWF
N_{sample} = number of bacterial colonies in solution after 2 h of contact with modified samples

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