Journal of Membrane Science 350 (2010) 387–394

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Surface modification of cellulose membranes with zwitterionic polymers for resistance to protein adsorption and platelet adhesion

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ARTICLE INFO

Article history:
Received 22 October 2009
Received in revised form 4 December 2009
Accepted 8 January 2010
Available online 15 January 2010

Keywords:
Cellulose membrane
Zwitterionic polymers
ATRP
Protein adsorption
Platelet adhesion

ABSTRACT

Three zwitterionic polymers, including poly(*N*,*N*-dimethyl-*N*-(*p*-vinylbenyl)-*N*-(3-sulfopropyl) ammonium) (PDMVSA), poly(2-(methacryloyloxyethyl) ethyl-dimethyl-(3-sulfopropyl)-ammonium) (PDMMSA), and poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) were grafted from cellulose membranes (CMs) via surface-initiated atom transfer radical polymerization (ATRP). The CMs were characterized by attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR), X-ray photoelectron spectroscopy (XPS), static water contact angle (WCA) measurement, and thermogravimetric analysis (TGA). Results showed that zwitterionic polymers were successfully grafted from CM surfaces. The zwitterionic polymer modified surfaces are more hydrophilic than the original CM surface. Total protein adsorption and platelet adhesion on these surfaces were measured *in vitro*. It was found that all the zwitterionic surfaces have improved resistance to nonspecific protein adsorption and possess excellent resistance to platelet adhesion. Moreover, the PDMVSA and the PDMMSA modified surfaces were as effective as the PMPC modified surface at preventing protein adsorption and platelet adhesion.
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1. Introduction

Various polymers, including polyurethane, polyamide, silicone rubber, and polysaccharides, have been widely used in biomedical fields as implant materials, tissue engineering scaffolds, blood-contacting devices, and disposable clinical apparatus during the past half a century [1–4]. Cellulose membrane, one of these polymers, has been frequently used in blood purification therapies. However, the application of cellulose membrane is restricted to some extent by its inadequate blood compatibility.

It is generally believed that when a biomaterial contacts with blood, protein adsorption on the surface is the first step of many undesired bio-reactions and bio-responses [5,6], followed by platelet adhesion and activation of coagulation pathways, leading to thrombus formation [7]. Therefore, the primary target for preparing blood compatible materials is to construct super-low fouling or non-fouling surfaces. Poly(ethylene glycol) (PEG) has always been identified as one of the synthetic no fouling and non-thrombogenic materials [8,9], and PEG-modified surfaces have been extensively studied during the past two decades [8–13]. It is prevalently considered that PEG chains could bind water through hydrogen bond, leading to a "barrier" in addition to the steric repulsion around the PEG chains [14], therefore, the PEG-modified surfaces could resist the close approach of biomacromolecules [15].

In the past decade or so, zwitterionic polymers including polyphosphobetaine, polysulfobetaine and polycarboxybetaine have attracted much attention and have been shown to be among the most effective polymers in this regard [16–24]. It is believed that zwitterionic surfaces could form a hydration layer via electrostatic interaction in addition to hydrogen bond, therefore, it could bind a significant amount of water [25], leading to a strong repulsive force to protein at specific separation distances or making the protein contact with the surface in a reverse manner without a significant conformation change [26]. In other words, zwitterionic surface could maintain the "normal conformation" of biomacromolecules [27,28].

Numerous significant advances have been made in preparing of zwitterionic surfaces, including surface coating or bonding of zwitterionic monomers as functionalized monolayer [20,29–31], in situ random graft polymerization of zwitterions [18,22], blending of interpenetrating polymer networks (IPN) [6,32–34], and controlled grafting of zwitterionic brushes [24,35–37]. Among these methods, surface-initiated ATRP has many advantages including versatility of monomers, mild reaction conditions, and tolerance of impurities [38]. Additionally, uniform chains with tailorable length and

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doi:10.1016/j.memsci.2010.01.015

relative high grafting density are achievable by this approach [39]. In recent years, taking advantage of ATRP, zwitterions have been directly grafted from metallic and inorganic substrates (gold chips [36,40], glass slides [41], and silicon wafers [42]), synthesized as block coating materials for biomedical application [30]. However, there is very little literature reporting on the grafting of zwitterions from polymeric membranes through ATRP.

During the past two decades, several attempts have been performed on the modification of cellulose membrane with zwitterionic polymers for biocompatibility improvement. Ishihara et al. not only prepared polyphosphobetaine modified cellulose dialysis membrane through random radical polymerization using a cerium ion (Ce^{4+}) method [16], but also prepared composed polymers of cellulose acetate and phospholipid as antifouling blood purification membrane [43]. Shen and Lin fabricated sulfobetaine and carboxybetaine monolayer on the membrane by surface chemical conjugation [20], while grafted polysulfobetaine on the surface via the Ce^{4+} method [44].

In a previous work [45], we reported the direct grafting of poly-*p*-vinyl benzyl sulfobetaine brushes on cellulose membranes via surface-initiated ATRP. In the present study, three different zwitterionic polymers (one polyphosphobetaine and two polysulfobetaines) were grafted from cellulose membranes under the same conditions. The surface composition, wettability, and thermal stability of the CM substrates were characterized by ATR-FTIR, XPS, WCA, and TGA, respectively. The resistance to nonspecific protein adsorption and platelet adhesion of the modified and unmodified surfaces were measured *in vitro*. In addition, the antifouling properties of the three zwitterionic surfaces that prepared under the same conditions were compared.

2. Experimental methods

2.1. Materials

Cellulose membrane (CM) was purchased from Sigma–Aldrich and cut into circular pieces. 2-Bromoisobutyryl bromide (BIBB, 97%), and 2-dimethylaminopryridine (DMAP, 97%) were purchased from Alfa Aesar. copper (I) bromide (CuBr, 98.5%), 2,2′-bipyridine (BPY, 99.5%), triethylamine (TEA, 99%), chloroform ($CHCl_3$, AR) and tetrahydrofuran (THF, AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. [2-(Methacryloyloxyethyl) ethyl]-dimethyl-(3-sulfopropyl)-ammonium (DMMSA, 97%) was purchased from Sigma–Aldrich. 2-Methacryloyloxyethyl phosphorylcholine (MPC, 98%) was purchased from Joy-Nature Technology Institute, Nanjing, China. Fresh whole blood and fresh platelet rich plasma (PRP) were provided by Blood Center of Jiangsu Red Cross. Phosphate buffered saline (PBS, 0.02 M phosphate, 0.15 M sodium chloride, pH 7.4) were purchased from Boster Bio-technology Co., Ltd. An enhanced BCA protein assay reagent kit and sodium dodecyl sulfate (SDS, 10 wt% in PBS) were purchased from Beyotime Institute of Biotechnology, China.

2.2. Preparation of DMVSA monomer

DMVSA monomer was prepared as reported in our previous work [18]. Typically, 7.27 g (40 mmol) *N*-(4-vinylbenzyl)-*N*,*N*-dimethyl amine (dissolved in 80 mL chloroform) was added into a 250 mL flask equipped with magnetic stirring, after which 6.07 g (48 mmol) 1,3-propanesulfone (dissolved in 80 mL chloroform) was added droplet into the flask. The reaction was reacted more than 10 h at 30 °C. A white powdered crude product was obtained after filtration of the suspension. Finally, the typical yield of the product was about 60% after recrystallized in ethanol twice. ^{1}H NMR was recorded on a Bruker spectrometer (300 MHz). For DMVSA (in D_2O): δ 7.50 (q, 4H, aromatic), 6.75 (q, 1H, =CH), 5.85 (d, 1H, cis, $=CH_2$), 5.30 (d, 1H, trans, $=CH_2$), 4.42 (s, 2H,–CH_2), 3.36 (m, 2H, CH_2–N), 2.97 (s, 6H, –CH_3), 2.88 (t, 2H, –CH_2), 2.23 (m, 2H, –CH_2–).

2.3. Surface grafting of zwitterionic polymers from CM using ATRP

The surface-initiated ATRP was performed by a two-step process as shown in Scheme 1. The first step is the esterification of hydroxyl groups with BIBB. Typically, CM substrates were immersed in 25 mL THF solution containing 2.22 g TEA (22 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred under ice bath and 4.6 g BIBB (20 mmol) was added into the mixture dropwise. Then, the reaction proceeded at ambient temperature for 24 h. After been thoroughly washed with dichloromethane and ethanol ultrasonically, the initiator functionalized CMs (refer to CM-Br) were obtained.

The second step, the surface-initiated ATRP from CM, was performed similar to that reported in our previous work [45]. Typically, six CM-Br sheets and CuBr (71 mg, 0.5 mmol) were placed in a dry flask, after the flask was evacuated and back-filled with nitrogen five times. Then a 20 mL of degassed solution (methanol and distilled water in 1:1 volume ratio) containing single zwitterionic monomer (4.0 mmol) and BPY (156 mg, 1.0 mmol) was transferred into the flask under nitrogen protection and polymerized for 1 h. After the polymerization, the substrates were thoroughly washed with PBS and distilled water ultrasonically, and dried under vacuum. The obtained cellulose membranes here are referring to CMG-X, where X is the corresponding zwitterionic monomer.

2.4. Characterization

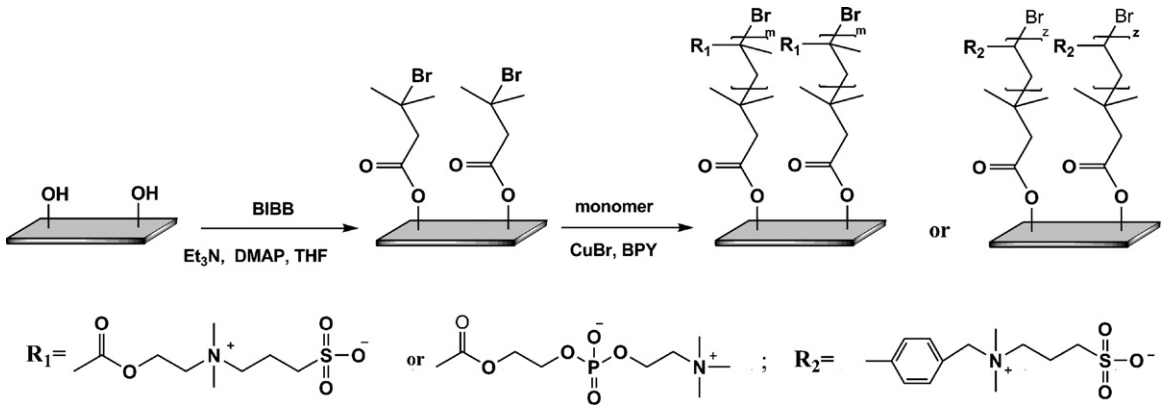
ATR-FTIR measurements of the CM substrates were performed on a Nicolet 170 sx FTIR equipped with an Omni sampler over 32 scans. The spectra were recorded at a 45° angle using a Ge crystal and with a resolution of 4 cm^{-1}. The X-ray photoelectron spectroscopy measurements were obtained on an ESCALAB 250 (Thermo Scientific, USA), using an Al Kα radiation source ($h\nu$ = 1486.6 eV) and operating at 150 W. The take-off angle of the photoelectron and the X-ray beam spot were kept at 90° and 500 μm, respectively. All the binding energies were referenced to the C_{1s} hydrocarbon peak at 284.6 eV. Water contact angles were measured and calculated in static mode on a DataPhysics Instrument (OCA-30, Germany) at ambient temperature. One drop of water (3 μL) was put on the surface of the film with an automatic piston syringe and photographed. Three spots were performed for each sample. TGA results were obtained in nitrogen atmosphere with a PerkinElemer thermogravimetric analyzer (Pyris 1, USA). Samples were heated at a constant rate of 20 °C/min from ambient temperature to 750 °C.

2.5. Protein adsorption [6]

After being equilibrated with PBS overnight, the CM substrates were immersed in 2 mL of platelet-poor plasma (PPP) made from fresh blood [46] at 37 °C for 90 min and then rinsed with PBS three times. The adsorbed protein was detached in 1% SDS for 60 min and the concentration of the adsorbed protein was determined by a BCA method at 562 nm. Independent measurements were performed in triplicate samples and the amount of the adsorbed protein was calculated from the concentration of the standard protein solution.

2.6. Platelet adhesion [47]

The CM substrates were placed in individual wells of 24-well tissue culture plate and equilibrated with PBS overnight. A 500 μL of PRP was added into each well and incubated at 37 °C for 120 min



Scheme 1. Schematic illustration of surface-initiated ATRP on the CM.

under static condition. After being rinsed with PBS, the substrates were immersed into 2.5% glutaraldehyde in PBS for 30 min, subjected to a series of graded alcohol–water solutions (25%, 50%, 75%, 95% and 100%) for 20 min in each step and dried under vacuum. Finally, the substrates were examined by a scanning electron microscope (SEM, Shimadzu, SSX-550) after coating with gold. Three different spots were observed on each sample.

3. Results and discussion

ATRP process can create polymer brushes on surface with control over surface density and chain length of polymers [38]. In the preset work, all the zwitterionic monomers were polymerized from the initiator immobilized CM surfaces for 1 h in a mixture solvent of water and methanol using CuBr and bipyridine as catalysts. The time of reaction is sufficient to create a high density polymer according to our previous work [45].

3.1. Surface characterization

Surface properties of the original cellulose membranes and the zwitterionic polymer modified membranes were investigated by ATR-FTIR and XPS.

3.1.1. ATR-FTIR analysis

Three different zwitterionic polymer modified surfaces (CMG-DMVSA, CMG-DMVSA, and CMG-MPC), as well as CM-Br and the original CM were firstly characterized by ATR-FTIR. The transmittance spectra and their corresponding absorbance spectra are shown in Fig. 1.

For the original CM, ATR-FTIR spectra in Fig. 1a and a′ show the characteristic peaks of cellulosic structures (stretching vibration of –OH at 3340 cm^{-1}, broad stretching vibration of C–H in $–CH_3$ and $–CH_2$ centered at 2894 cm^{-1}, while their bending vibrations at 1426, 1369 and 1315 cm^{-1}; asymmetric stretching of C–O–C at 1159 cm^{-1} and skeletal vibration involving the C–O stretching at 1058 cm^{-1} and 1024 cm^{-1}) [20,48]. The characteristic peaks in spectra of CM-Br (Fig. 1b and b′) were almost unchanged as compared with those in the spectra of pristine CM. This is because there was just a very thin layer of initiator immobilized on surfaces. The thicknesses or the contents of this layer may be below the detection limit of ATR-FTIR.

After the polymerization, the intensity of the peak at 1024 cm^{-1} in both Fig. 1c′ and d′ was enhanced, this may be because the overlapping of the skeletal vibration of C–O originated from CM substrate and the vibration of $–SO_3^-$ originated from the side chains of grafted PDMVSA and PDMMSA. On the other hand, in Fig. 1e′, an obvious characteristic peak attributing to the bending vibration of C–H in $–POCH_2–$ which originated from the side chains of PMPC appeared at 1060 cm^{-1} [49]. Moreover, both in Fig. 1d and e, a distinct peak attributing to the stretching vibration of C=O in ester groups which originated from the side chains of PDMMSA and PMPC (as shown in Scheme 1) appeared around 1730 cm^{-1}. These results preliminarily indicated that zwitterionic polymers were successfully fabricated on the CM surfaces.

3.1.2. XPS analysis

XPS has been utilized as the method for tracking the surface composition variations of the pristine CM, CM-Br and three zwitterionic polymer modified surfaces. Table 1 lists the detailed data from XPS scans on different surfaces.

According to the data, after the reaction of the first step occurred for 24 h, the content of carbon (C_{1s}) was slightly increased while the content of oxygen (O_{1s}) was slightly decreased, and a small amount of bromine (0.82%, Br_{3d}) appeared with a binding energy of about 69 eV (Fig. 2b), indicating the existence of initiator on the surface. As respect to the narrow scan of C_{1s} (Fig. 2a′ and b′), the C_{1s} spectrum of CM can be mainly curved into three components with binding energies of about 284.6, 286.2, and 287.6 eV attributing to the C–H (containing C–C), C–O, and C–C^*=O species, while that of CM-Br can be curved into four main peak components with binding energies of about 284.6, 286.2, 287.6, and 288.6 eV attributing to the C–H (containing C–C), C–O/C–Br, C–C^*=O, and O–C=O species, respectively [50]. The appearance of O–C=O species confirmed that the existed initiator was indeed covalently bonded on the surface through esterification.

After the ATRP reactions occurred for 1 h, the contents of carbon (C_{1s}) were sharply increased, while the contents of oxygen (O_{1s}) were remarkably decreased. For the survey scan, the characteristic signal of bromine disappeared while characteristic signals of sulfobetaines (PDMVSA and PDMMSA, S_{2p} at 164.0 eV and N_{1s} at 402.5 eV in Fig. 3a and b) and phosphobetaine (PMPC, P_{2p} at 129.0 eV and N_{1s} at 402.5 eV in Fig. 3c) were obviously observed as compared with the survey scan spectrum of CM-Br in Fig. 2b, indicating the successful in situ grafting of zwitterionic polymers from the surfaces. Some researchers suggested that the disappearance of bromine signal may be because some of the living chains have underwent termination, these chains are “dead” and there are less living chains with bromine-end left [25]. On the other hand, some researchers believed that the XPS method only measures a depth of ~10 nm, the chains on the surface are entangled and the living chain-ends may therefore not located directly in the outermost layer [51].

As respect to the narrow scan spectra of C_{1s} (Fig. 3a′–c′), remarkable variations in the intensity of characteristic peaks were observed. In comparison with the narrow scan spectrum of CM-Br

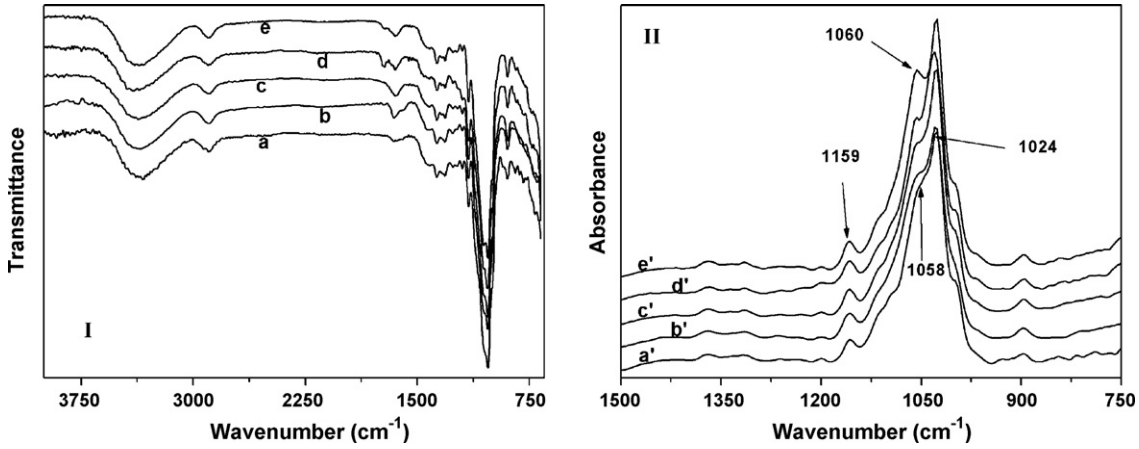


Fig. 1. Transmittance (I) and absorbance (II) spectra of original CM (a and a′), CM-Br (b and b′), CMG-DMVSA (c and c′), CMG-DMMSA (d and d′), and CMG-MPC (e and e′).

Table 1
Elemental surface composition of CM and surface-modified CM substrates determined from XPS[a,b].

Sample	Element (atom %)					
	C	O	S	N	P	Br
CM	52.15	47.85				
CM-Br	52.85	46.33				0.82
CMG-DMVSA	70.45	21.54	4.19	3.82		
PDMVSA	(73.69)	(15.79)	(5.26)	(5.26)		
CMG-DMMSA	60.80	30.06	4.10	5.04		
PDMMSA	(61.10)	(27.78)	(5.56)	(5.56)		
CMG-MPC	57.23	35.96		3.44	3.37	
PMPC	(57.89)	(31.59)		(5.26)	(5.26)	

[a] The values in the parentheses are the theoretical atomic percentages of related bulk polymers.
[b] Data precision is ~±5%.

in Fig. 2b′, the peak around 284.6 eV, instead of the peak around 286.2 eV, became the dominant peak on the CMG-DMVSA surface (Fig. 3a′), and the peak at 288.6 eV corresponding to the O–C=O species disappeared. The intensity of the peaks around 286.2 eV on both CMG-DMMSA surface (Fig. 3b′) and CMG-MPC surface (Fig. 3c′) was decreased by different extents, and the intensity of the peak at 288.6 eV corresponding to O–C=O species was largely increased. These variations are associated with the different characteristic structure of zwitterionic polymers as shown in Scheme 1: PMPC has more C–O species than PDMMSA and possesses equal amount of O–C=O species as compared to PDMMSA, while PDMVSA has none of these two species in its structure.

3.2. WCA analysis

Water affinity of the five surfaces was also investigated with a static mode. Table 2 lists the detailed water contact data from the measurements on these surfaces. Due to pristine CM contains massive hydrophilic groups, a low water contact angle of 47.64° was observed on its surface. After the esterification, the water contact

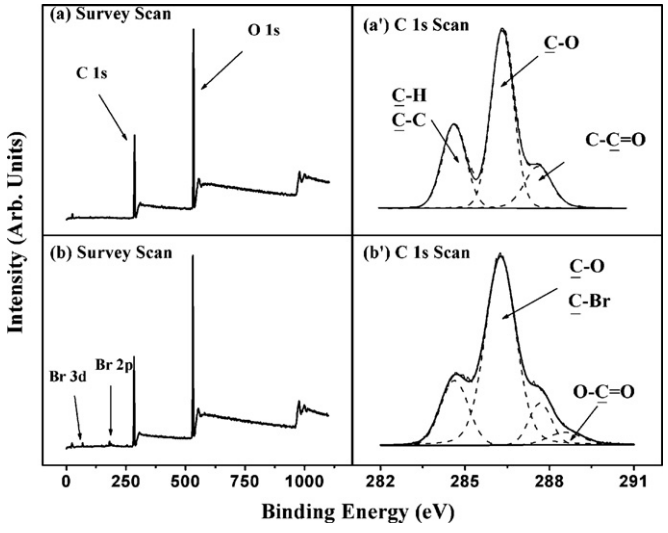


Fig. 2. XPS spectra of CM (a and a′) and CM-Br (b and b′).

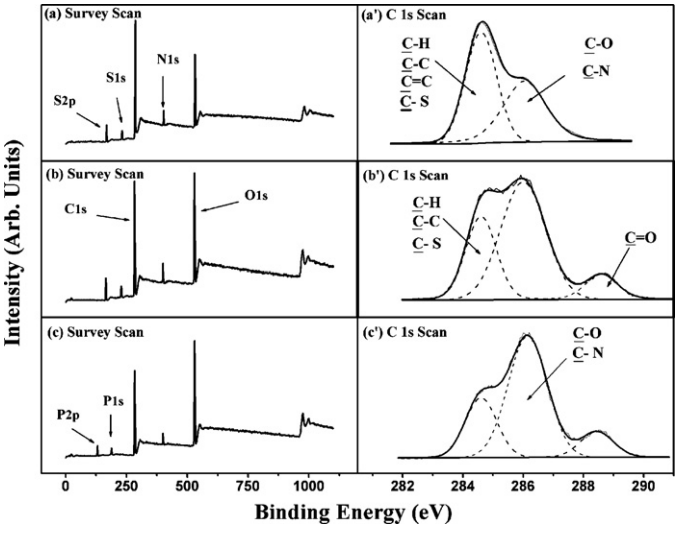


Fig. 3. XPS spectra of CMG-DMVSA (a and a′), CMG-DMMSA (b and b′), and CMG-MPC (c and c′).

Fig. 4. Water contact angle images of CM, CM-Br, and zwitterionic polymers modified surfaces.

Table 2
Water contact angles of CM, CM-Br and zwitterionic surfaces.

	Samples				
	CM	CM-Br	CMG-DMVSA	CMG-DMMSA	CMG-MPC
Contact angle (°)	47.64 ± 1.40	73.09 ± 0.45	34.07 ± 2.20	21.7 ± 1.86	20.3 ± 1.33

angle of the surface was sharply increased from 47.64° to 73.09°. The water affinity of CM mainly attributes to the hydration effect between its hydrophilic groups and water. The increase in water contact angle is attributed to the transition from hydrophilic to hydrophobic caused by the immobilization of hydrophobic initiator.

However, after the ATRP reactions occurred, the contact angles of these grafted surfaces were significantly decreased to the levels even lower than that of pristine CM, and this tendency is clearly shown by the images in Fig. 4. This is because zwitterionic groups could form a hydration layer via electrostatic interaction in addition to the hydrogen bond [25], therefore, the zwitterionic surfaces are more hydrophilic than the original CM surface.

3.3. TG analysis

Thermal stability of the zwitterionic polymer modified membranes, as well as the pristine CM, has also been investigated. All samples were dried under vacuum for 24 h and kept in vacuum before use in order to reduce the experiential error. The TG curves and detailed data are shown in Fig. 5 and Table 3, respectively.

As can be seen from Fig. 5, all the samples underwent weight loss in three stages. The first stage is the loss of the moisture while others are attributing to the pyrolysis. In the first stage, the pristine CM has the lowest weight loss of 7.7%, while CMG-DMVSA, CMG-DMMSA and CMG-MPC have higher weight losses of 8.7%, 12.1% and 10.2%, respectively (Table 3), suggesting that the zwitterionic surfaces indeed could bind more water than the pristine CM under the same conditions due to the additional electrostatic interaction. This result is consistent with the result of water contact angle measurement.

Table 3
Thermal analytical data of original CM and zwitterions grafted substrates.

Samples	Weight loss[a] (%)	T_i[b] (°C)	Char (%)
CM	7.7	161.8	16.8
CMG-DMVSA	8.7	206.9	18.4
CMG-DMMSA	12.1	202.2	18.7
CMG-MPC	10.2	183.2	16.9

[a] Values for the first stage of weight loss.
[b] Temperature of the initial decomposition (the second stage of weight loss).

In addition, from the data listed in Table 3, we can clearly see that the initial pyrolysis temperatures and the percent chars at 750 °C of the grafted substrates were increased compared with those of the pristine CM, indicating that the zwitterionic polymer covered substrates are more thermal stable than the pristine substrate.

3.4. Protein adsorption

Due to the protein adsorption on biomaterials' surfaces is always considered as the first step of many undesired bio-reactions and bio-responses [5,6], the primary target for preparing biomaterials is to construct super-low fouling or non-fouling surfaces. Protein adsorption is affected by numerous factors including protein size, charge, shape, hydrophobicity, pH, surface charge, surface

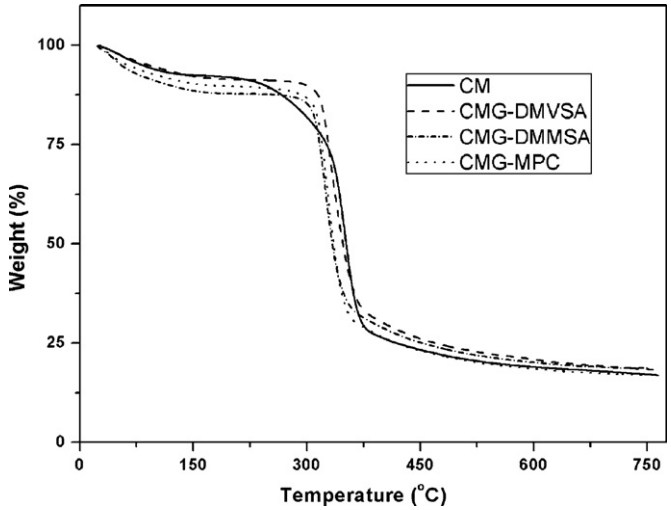


Fig. 5. TGA curves of original CM and zwitterions modified substrates.

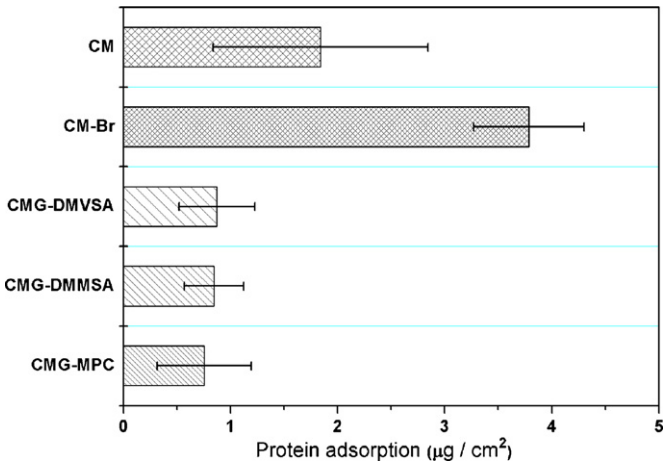


Fig. 6. Amount of protein adsorbed on the CM substrates. Data from three separated experiments are shown as mean ± SD.

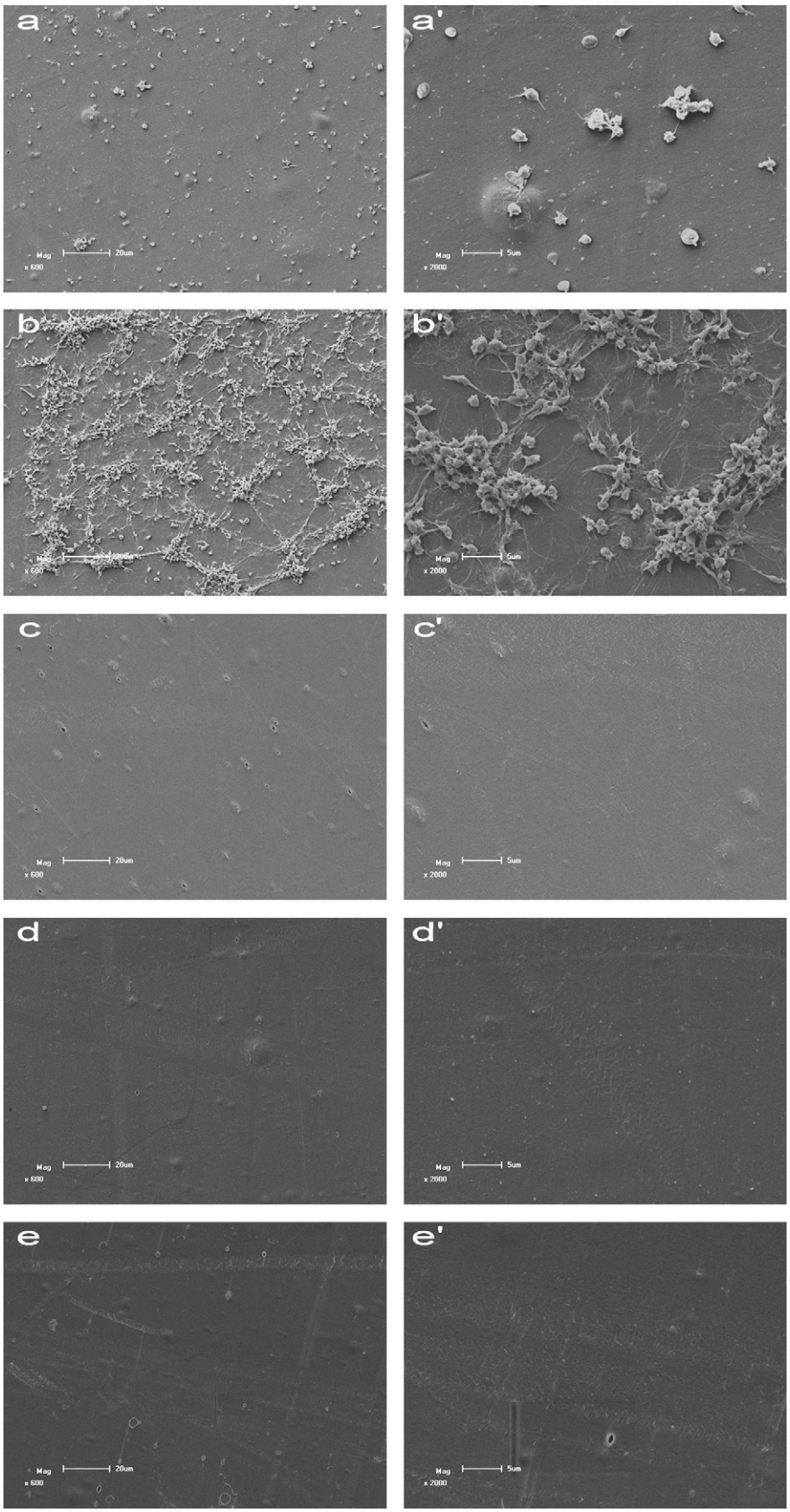


Fig. 7. SEM photographs of (a and a′) CM, (b and b′) CM-Br, (c and c′) CMG-DMVSA, (d and d′) CMG-DMMSA, and (e and e′) CMG-MPC after 120 min incubation in the human PRP (left: magnification are 600, bars are 20 μm; right: magnification are 2000, bars are 5 μm).

topology, coadsorption of low-molecular-weight ions, intermolecular forces between adsorbed molecules, strength of functional groups, composition of the protein solution, and chemistry of the surface [2,52].

Fig. 6 shows the total protein adsorption from fresh human PPP on the five surfaces. As shown in the figure, the adsorbed amount of total protein on the pristine CM is about 1.8 $\mu g/cm^2$, while the adsorbed amount of total protein on the initiator immobilized surface (CM-Br) is about double the amount of that on the pristine CM surface. This may be attributed to the immobilization of initiator on the surface resulting in the transition from a hydrophilic surface to a hydrophobic surface. As investigated, hydrophobic surfaces have greater interaction from the outer layer and possibly from the inner core of the protein, and protein adsorption is even more energetically favorable on the hydrophobic surfaces [53,54]. Moreover, the bromine group on the initiator may have a strong interaction with proteins, so the adsorbed amount of proteins on the CM-Br is more than that on the pristine CM.

However, after the in situ ATRP of single zwitterionic monomer on the CM-Br, respectively, the total protein adsorption on these zwitterionic polymer modified surfaces were sharply decreased to the levels even lower than that of pristine CM. This tendency is very similar to the tendency found in the water contact angle measurement. As expected, many researches associated the decrease in protein adsorption with the increase in water affinity of the surfaces. It is believed that zwitterions form a hydration layer via electrostatic interaction in addition to hydrogen bond. Therefore, it could bind a significant amount of water on the surface [25], leading to a strong repulsive force to protein at specific separation distances or making the protein contact with the surface in a reverse manner without a significant conformation change [26]. On the other hand, it is considered that the improved resistance to protein adsorption could be attributed to the zwitterionic structure groups [18]. In aqueous (blood) medium, the zwitterionic structure molecules cannot diffuse into the interior of protein, which is maintained by hydrophobic bonds and hydrogen bonds, to effect the synergetic interaction between main chains and side portions. Moreover, it could minimize the effect on exterior surface ions of proteins, and thus could maintain the "normal conformation" of biomacromolecules [27,28].

3.5. Platelet adhesion

Fig. 7 shows the scanning electron microscope images of the five surfaces after incubating in fresh human PRP for 120 min. It can be seen from Fig. 7a (with a magnification of 600) that several platelets were adhered on the pristine CM surface. Some of these adhered platelets were deformed with extended pseudopodia (Fig. 7a′). After the initiator being immobilized, there were massive platelets adhering and aggregating on the surface with a network structure (Fig. 7b and b′), and some of these platelets were fully spread on the surface (Fig. 7b′), presenting the highly activated state [55]. This is in agreement with the trend of protein adsorption. And this may be explained by the higher protein adsorption on the hydrophobic CM-Br surface because platelet adhesion is thought to be the step next to the protein adsorption with a link by link relationship in thrombosis process [7].

After the in situ ATRP of zwitterions, there was almost no platelet adhering on the zwitterionic polymer modified surfaces (Fig. 7c–e), showing all the three zwitterionic surfaces have excellent resistance to platelet adhesion. However, the result of the platelet seems incomplete in agreement with the result of protein adsorption because there was a small quantity of proteins adsorbed on these surfaces. Two probable explanations could be addressed to this "inconsistent": The protein source in this study is fresh human PPP, which is a mixture of hundreds of proteins [56]. However, fibrinogen, the key player of conveying the surface chemical feature to platelets in material–platelet interactions [57,58], is just one of them. On the other hand, according to the studies of Barbucci et al. [57] and Ranter et al. [58], the conformation of the adsorbed fibrinogen, more than its amount, takes the primary responsibility for platelet adhesion and activation. Therefore, though there was a small quantity of proteins adsorbed on the zwitterionic surfaces, the zwitterionic structures could maintain the normal or the nature conformation of these adsorbed proteins, and consequently have less interaction with platelet [18,28].

In addition, in the present study, three zwitterions were polymerized from CM under the same conditions, such as CM and CM-Br substrates from the same batch, equal concentrations of monomer and catalyst, the same reaction temperature and time. On the basis of the results above, it can be clearly seen that the antifouling property of sulfobetaines is as effective as that of phosphobetaine, which has been identified as one of the synthetic non-fouling and non-thrombogenic materials [17]. This is not in agreement with Lloyd's findings. According to Lloyd's research, sulfobetaine-based polymers were thought to be less fouling-resistant than phosphobetaine-based polymers [59].

This may be because different methods have been performed in preparing the zwitterionic polymers. The method used in Lloyd's research is random free radical polymerization while that in our present study is ATRP, by which well-defined chains with tailorable length and relative high grafting density are achievable. Accordingly, when the zwitterionic surfaces are well-defined, super-low fouling could be achieved on these surfaces [25,60].

4. Conclusions

Three zwitterions were polymerized from CM via in situ ATRP under the same conditions. The FTIR result and the XPS element analysis confirmed that the initiator was indeed covalently bonding on the CM surface, and zwitterionic polymers were successfully grafted from the CM. The WCA measurement and the TGA test revealed that the zwitterionic surfaces not only possess a stronger hydrophilicity than the original CM surface, but also could bind more water compared with the original CM. In addition, all the zwitterionic surfaces have improved resistance to nonspecific protein adsorption and possess excellent resistance to platelet adhesion. More importantly, it is demonstrated that the sulfobetaine modified surfaces were as effective as the phosphobetaine modified surface at resisting protein adsorption and platelet adhesion. This work also provides a facile method for modifying cellulose membranes or other polymers to achieve low fouling property.

Acknowledgments

We would like to thank the National High Technology Research and Development Program of China (2006AA03Z445) and the Changzhou Science and Technology Research Project.

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