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One simple and stable coating of mixed-charge copolymers on poly(vinyl chloride) films to improve antifouling efficiency

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ABSTRACT: An antifouling surface is highly desirable for many biomedical applications. In this study, poly(vinyl chloride) (PVC) films were endowed with the improved properties of resisting nonspecific protein adsorption and platelet adhesion simply through being coated with a kind of mixed-charge zwitterionic polymer, poly(3-sulfopropyl methacrylate–methacrylatoethyl trimethyl ammonium chloride–glycidyl methacrylate) (PSTG), with random moieties of negatively charged 3-sulfopropyl methacrylate potassium, positively charged [2-(methacryloyloxy)-ethyl] trimethylammonium chloride, and glycidyl methacrylate. The PSTG-grafted PVC films were formed by the simple immersion of an amino-functionalized PVC film into a PSTG solution. A grafting density of 220.84 μg/cm^2 of PSTG4-grafted PVC film was successfully obtained. The PSTG4-grafted PVC film showed a lower contact angle (37.5°) than the ungrafted PVC film (98.3°). The *in vitro* protein adsorption results show that the bovine serum albumin adsorption amount decreased 6.72 μg/cm^2 in the case of the PSTG4-grafted PVC film, whereas that on the ungrafted PVC film was 28.54 μg/cm^2. So, PSTG-grafted PVC films could be promising materials for medical devices. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 44632.

KEYWORDS: coatings; films; membranes; poly(vinyl chloride)

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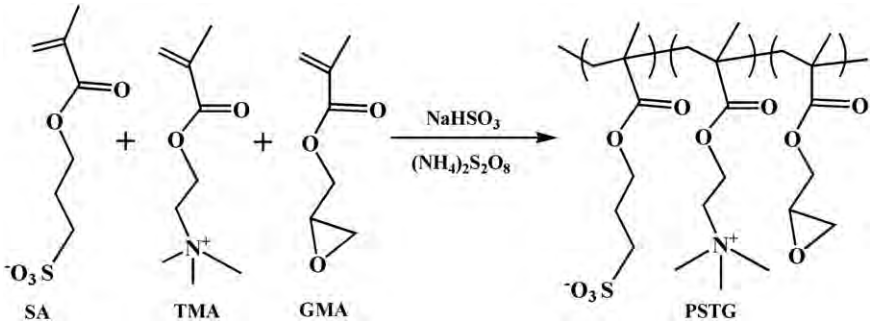
INTRODUCTION

Nonfouling materials are highly desirable in numerous applications; these range from biosensors to medical devices, drug-delivery carriers, and even marine coatings. Because its ability to have a robust mechanical strength, low cost, and other excellent physical and chemical properties, poly(vinyl chloride) (PVC) has been used extensively in blood-contacting materials, such as in bags for blood storage or tubing for extracorporeal circulation.[1] However, the hydrophobic surface of medical PVC facilitates adhesion of blood components, such as proteins and platelet, and leads to variation in the blood components and even coagulation, so the surface properties of medical PVC needs to be improved for wider applications.[2] Various methods for improving PVC's surface antifouling properties and hemocompatibility have been reported in the literature.[3–5] Heparin, a well-known anticoagulant, has been widely applied to the surfaces of medical devices as it can prolong the blood-clotting time.[6] However, it is difficult for heparin maintain its bioactivity when it is immobilized on the surface because its natural conformation is usually changed after it links with the surface.[7]

A novel method for generating a biocompatible interface that has emerged recently is the coating of the surface with a hydrophilic polymer that can function as a barrier to interactions between the interface and physiological components. One kind of hydrophilic material is poly(ethylene glycol) (PEG) based material, which has been long recognized as a most excellent nonfouling material because of the formation of surface hydration via hydrogen bonds.[8] However, PEG is readily subject to oxidation in the presence of oxygen and transition-metal ions; this results in the loss of its resistance in most biochemically relevant solutions.[9] Zwitterionic polymers can overcome these disadvantages of PEG. They can bind water molecules much more strongly than PEG chains via electrostatically induced hydration. So, the use of zwitterionic polymers [such as poly(2-methacryloyloxyethyl phosphorylcholine), poly(sulfobetaine methacrylate), and poly(carboxybetaine methacrylate)] is increasing.[10–14]

Additional Supporting Information may be found in the online version of this article.



Scheme 1. Illustration of the synthesis of the PSTGs.

Mixed-charge zwitterionic copolymers can immobilize proteins. When mixed cation–anion supports are designed to have a null net charge on the surface, they still have the ability to adsorb many proteins when some of them are unable to adsorb on similar fully cationic or fully anionic supports under similar conditions.[15]

It has been demonstrated that excellent resistance to protein adsorption can be readily obtained from mixed-charge copolymers, which can mimic the antifouling properties of zwitterionic polymers.[16] Mixed-charge copolymers, called *pseudo-zwitterionic copolymers*, are composed of positively and negatively charged moieties on different monomer units, which can form a protective hydration layer, provided that a homogeneous mixture of balanced charge groups has been achieved.[17] Shih *et al.*[18] reported a systematic study of the effect of charge-bias variations in poly(3-sulfopropyl methacrylate-*co*-methacrylatoethyl trimethyl ammonium chloride) copolymers on the hemocompatibility in human blood plasma and concluded that pseudo-zwitterionic copolymers of poly(3-sulfopropyl methacrylate-*co*-methacrylatoethyl trimethyl ammonium chloride) with an overall charge neutrality had the best antifouling, anticoagulant, and antihemolytic activities. Jhong *et al.*[19] further developed a wound dressing by growing pseudo-zwitterionic brushes onto polytetrafluoroethylene membranes and investigated the role of the membrane surface charge on the essential wound dressing properties for wound healing. They proved that the pseudo-zwitterionic membranes with a perfect balance of positive and negative moieties exhibited the best antifouling properties and wound-healing abilities. Therefore, it is reasonable to design a medical device based on a mixed-charge copolymer that will constitute a new generation of antibiofouling materials.

Polymer coatings on the surface of a substrate can be prepared by two main strategies: (1) grafting-to and (2) grafting-from strategies.[20] Grafting-from strategies include the growth of the polymer chain from the surface, whereas grafting-to strategies include the attachment of copolymers to the surface. Even with its many advantages, such as a high grafting density on the surface and a controllable length of polymer chain, the grafting-from method previously needed a tough and complicated process to fix a suitable initiator on the substrate and an inert atmosphere to realize polymerization; this confined this method to large-scale applications. By comparison, the grafting-to method is much more simple and convenient, and one only needs to expose the substrate to the coating solution via dip coating or spin coating. Nevertheless, there is one major challenge in this modification method: most coatings adsorbed on the substrate are driven by physical adsorption, such as hydrophobic interactions, van der Waals forces, and hydrogen bonds. This low affinity for the surface usually cannot ensure complete stability and long-term applications under complex conditions.[21] Hence, the discovery of a simple but robust method to attach pseudo-zwitterionic polymers to PVC surfaces remains a challenge.

In this study, we aimed to develop a simple and effective strategy for PVC-based medical films to enhance their antifouling properties and long-term stability. It is well known that glycidyl methacrylate (GMA) with epoxy pendants is able to react with functional groups, such as amino, hydroxyl, or carboxyl groups, on the surface of a substrate.[22–24] Therefore, poly(3-sulfopropyl methacrylate–methacrylatoethyl trimethyl ammonium chloride–glycidyl methacrylate) (PSTG) copolymers were synthesized by free-radical polymerization. Then the PSTGs were covalently grafted to previously amino-functionalized medical PVC films via a simple immersion method. Except for the reaction of the epoxy groups with $-NH_2$, self-crosslinking between epoxy groups could happen. To the best of our knowledge, no work has been reported on the fabrication of polymer coatings formed by PSTGs. This is also the first study to apply them to medical PVC films for the improvement of the antifouling properties.

EXPERIMENTAL

Materials

PVC (GM1600E) was obtained from Dagu Chemical Factory (Tianjin, China). The PVC granules were extruded into films with a thickness of 1 mm at 165 °C and subsequently cut into squares $2 \times 2\,cm^2$ in size after cooling. [2-(Methacryloyloxy) ethyl] trimethylammonium chloride (TMA; 80 wt % H_2O solution with the monomer), 3-sulfopropyl methacrylate potassium (SA), glycidyl methacrylate (GMA, 97%), ammonium persulfate, sodium bisulfite ($NaHSO_3$) and ethylenediamine were purchased from Aladdin. The deionized (DI) water used in experiments was purified with a Millipore water-purification system with a minimum resistivity of 18.0 MΩ m. Bovine serum albumin (BSA) and a micro bicinchoninic acid protein assay reagent kit were supplied by Sigma-Aldrich and were used without further purification. All of the other chemicals were commercial analytical grade and were used as received.

Preparation of the PSTG Copolymers

The PSTG copolymers were synthesized by conventional free-radical polymerization (Scheme 1). First, N_2 was bubbled into a

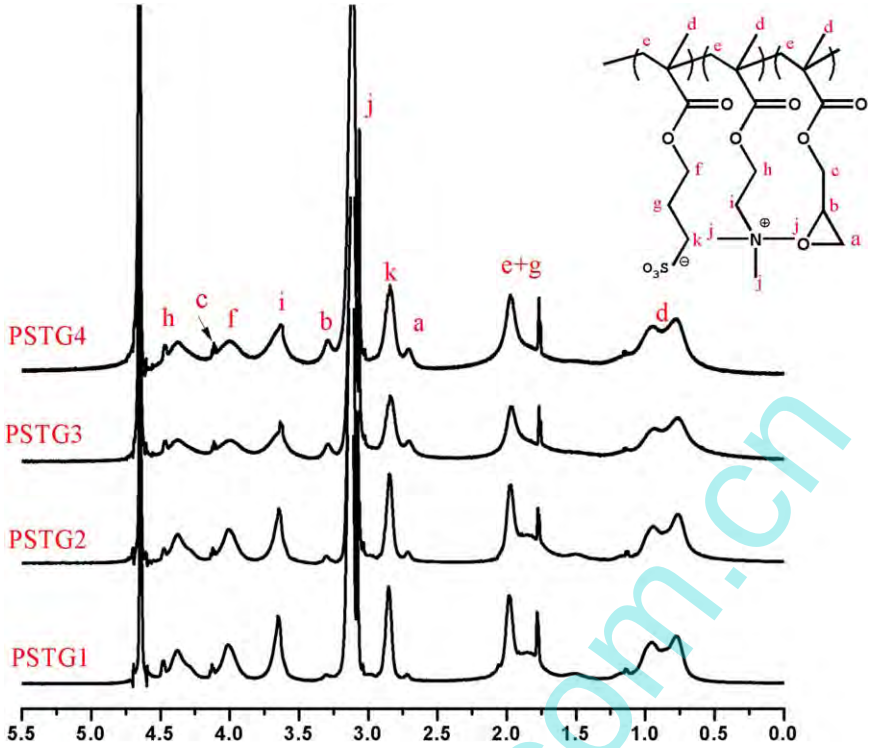


Figure 1. ^{1}H-NMR spectra of the PSTGs in D_2O. [Color figure can be viewed at wileyonlinelibrary.com]

reaction flask containing the solvent deionized water for 30 min to remove residual oxygen. Then, the flask was put into a 45 °C thermostated water bath. The deionized water solution containing SA, TMA, GMA, and the initiator (2 wt % ammonium persulfate and 1 wt % $NaHSO_3$) was dropped into the reaction flask for about 30 min. The polymerization was ended after 5 h. The mixture was added slowly to acetone and redissolved in deionized water repeatedly to obtain the pure copolymer PSTGs. Then, the product was dried *in vacuo* at 30 °C for 24 h. The compositions of the copolymer PSTGs were characterized by ^{1}H-NMR (Varian INOVA 500 MHz) spectral measurements and Fourier transform infrared (FTIR) spectroscopy (Bruker Vertex 70). The number-average molecular weight (M_n) and the polydispersity index [PDI = Weight-average molecular weight (M_w)/M_n] of the copolymer PSTGs were determined by gel permeation chromatography (Agilent 1100). $NaNO_3$ (0.1 *M*) was used as the eluting solvent (1 mL/min), and poly(ethylene oxide) was used as the molecular weight standard. Finally, the ξ potential of the copolymer PSTG solutions [5 mg/mL in phosphate-buffered saline (PBS)] were measured with a Zetasizer Nano ZS90 instrument (Malvern, United Kingdom).

Amination of the PVC Films and Formation of the PSTG Coatings

The method for obtaining the amino-functionalized PVC films was based on a previously reported procedure.[25] Briefly, the PVC films were first cleaned with a mixture of ethanol and water under sonication for 30 min and then dried over a

Table I. Characteristics of the PSTG Copolymers

	Reaction ratios of the comonomers (mol %)[a]			Compositions of the copolymers (mol %)[b]			Characterization of the copolymers		
Sample	SA	TMA	GMA	SA	TMA	GMA	M_w[c]	PDI (M_w/M_n)[c]	ξ potential (mv)[d]
PSTG1	47.5	47.5	5	49.4	45.7	4.9	28,700	2.3	−1.3 ± 0.2
PSTG2	45	45	10	48.1	44.7	7.2	26,400	2.5	−1.3 ± 0.3
PSTG3	42.5	42.5	15	45.0	41.6	13.4	27,600	2.1	−1.5 ± 0.2
PSTG4	40	40	20	43.2	40.1	16.7	28,100	2.3	−1.2 ± 0.1

[a] Feed molar ratios of the GMA, SA, and TMA monomers used in the reaction solution.
[b] The compositions of the PSTG copolymers were calculated on the basis of ^{1}HNMR spectroscopy with the proton signal at 2.85 ppm attributed to $(CH_3)_2SO_3$— of SA, the proton signal at 3.12 ppm attributed to —$N^+(CH_3)_3$ of TMA, and the proton signal at 2.72 ppm attributed to the CH_2 group in the epoxide ring of GMA.
[c] Estimated by gel permeation chromatography.
[d] Estimated with a ξ potentiometer.

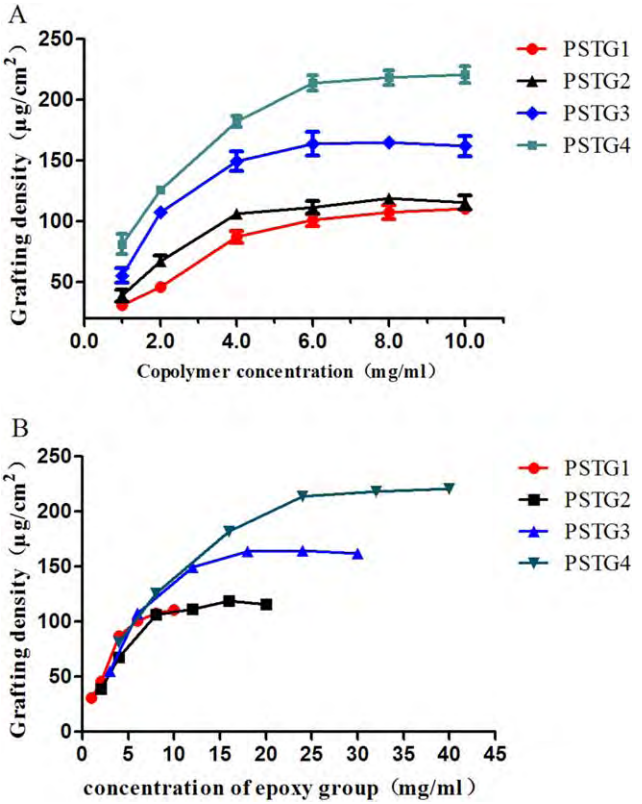


Figure 2. Grafting density of the PSTG copolymers as a function of (A) the copolymer concentration and (B) the concentration of epoxy groups. [Color figure can be viewed at wileyonlinelibrary.com]

nitrogen gas stream. The clean PVC films were immersed into the freshly prepared 80% aqueous solution of ethylenediamine at 70 °C for 1 h. After the reaction, the PVC films were washed with copious amounts of distilled water to remove ethylenediamine and dried in an air oven. Afterward, the amino-functionalized PVC film was dipped in the PSTG solution (PBS solvent, pH 7.4, 10 m*M*, 138 m*M* NaCl, 2.7 m*M* KCl) for 2 h at 60 °C with a concentration range of 1–10 mg/mL. Finally, the PSTG-grafted PVC films were thoroughly washed with deionized water and dried at 25 °C *in vacuo*. The grafting density of the PSTG-grafted PVC films (μg/cm^2) was calculated with the following equation:

$$\text{Grafting density} = (W_1 - W_0)/A_0 \quad (1)$$

where W_0 and W_1 are the masses of the ungrafted PVC films and the PSTG-grafted PVC films (μg), respectively, and A_0 is the area of the film (cm^2).

Surface Morphology Measurement by Atomic Force Microscopy (AFM)

AFM measurements were used to evaluate the surface morphologies and roughnesses of the PSTG-grafted PVC films in air and were obtained by a scanning probe microscope (CSPM5500A, Being Nano-Instruments, Ltd., China) in tapping mode. All of the images were taken of dried films with an area of $5 \times 5\ \mu m^2$ for morphological observations and roughness measurements.

Static Contact Angles

The static water contact angles of the ungrafted and PSTG-grafted PVC films were measured with a Kruss Easy Drop goniometer (Kruss Germany) equipped with a digital photoanalyzer. A 3-μL droplet of water was placed on the surface by a microsyringe, and the contact angle was measured and observed after 5 s. This procedure was carried out at least five times at different positions on each film. The average of these values was determined as the contact angle.

Characterization of the Surface Chemistry

Attenuated total reflectance (ATR)–FTIR spectra of the pristine and PSTG-grafted PVC films were obtained with an FTIR spectrometer (Bruker Vertex 70) coupled with an ATR accessory. The spectra were obtained at a spectral resolution of 4 cm^{-1}, and 16 scans were undertaken. The measurements were conducted at room temperature, and wave numbers ranging from 4000 to 400 cm^{-1} were recorded. The chemical composition of the pristine and PSTG-grafted PVC films was characterized by X-ray photoelectron spectroscopy (XPS; PerkinElmer Phi 1600 ESCA system) with Mg Kα (1254.0 eV) as the radiation source. Data were collected at photoelectron take-off angles of 90° with respect to the sample surface. All spectra were charge-corrected with the C1s peak at 284.5 eV.

Stability of the PSTG-Grafted PVC Films

The tests for the long-term stability of the PSTG-grafted PVC films were carried out by contact angle measurements. All of the polymer-grafted PVC films were soaked in PBS at 37 °C. For defined time intervals, the films were removed from the PBS, washed with deionized water, and dried under a stream of nitrogen. The contact angle was measured at least five times to calculate the average contact angle. The pristine and amino-functionalized PVC films were treated with the same method as the control.

Protein Adsorption

BSA was used as a model protein to evaluate the protein resistance of the PVC films in PBS (pH 7.4). Pristine, amino-functionalized and PSTG-grafted PVC films (2×2 cm^2) were placed in individual wells of a 24-well tissue culture plate. After equilibration by PBS at 37 °C for 12 h, all of the samples were immersed into single protein solution of BSA at concentrations of 1.0 and 2.0 mg/mL at 37 °C for 2 h, respectively. After three rinsing cycles with fresh PBS, the samples were transferred to clean wells and washed with an aqueous solution of 1.0 wt % SDS; this was followed by shaking for 60 min and sonication for 20 min at room temperature to detach the adsorbed protein on the surface. A bicinchoninic acid protein assay kit was used to test the protein concentration in the sodium dodecyl sulfate (SDS) solution; it was measured with an ultraviolet–visible spectrophotometer at 562 nm. The reported data are the mean values of triplicate specimens for each film.

Platelet Adhesion

Fresh blood collected from a healthy rabbit was mixed immediately with a 3.8 wt % solution of sodium citrate at a dilution ration of 9:1. The blood was centrifuged at 1200 rpm for 15 min to obtain platelet-rich plasma. Clean PVC samples were placed in individual wells of a 24-well tissue culture plate and

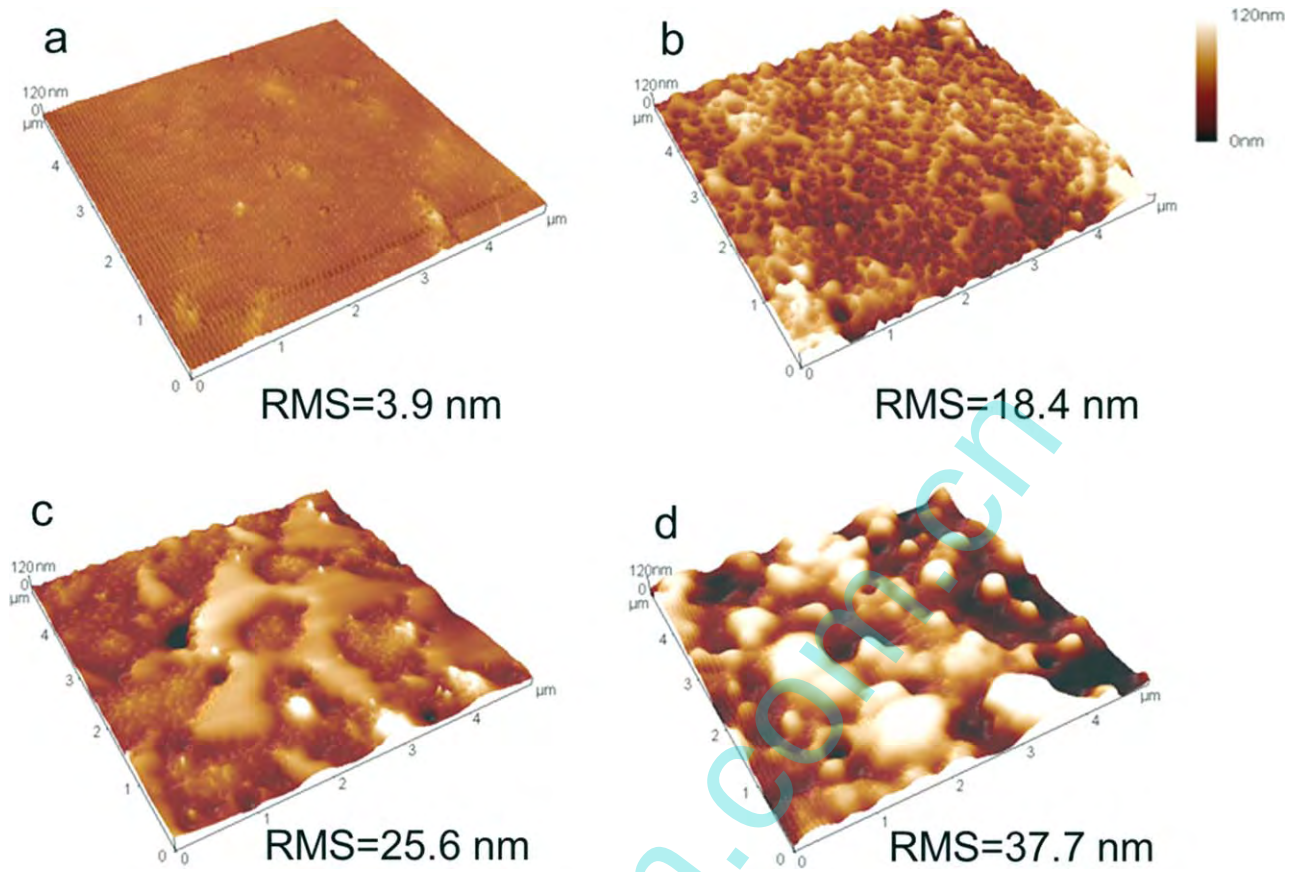


Figure 3. Tapping-mode AFM images of the PVC films: (a) PVC film before grafting, (b) PSTG1-grafted PVC film, (c) PSTG3-grafted PVC film, and (d) PSTG4-grafted PVC film. The scan size was $5 \times 5\,\mu m^2$. [Color figure can be viewed at wileyonlinelibrary.com]

equilibrated with PBS for 2 h at room temperature (25 °C). A volume of 50 μL of the platelet-rich plasma prepared previously was added to the wells after the PBS solution was removed with an aspirator, and we left them static for 30 min. After 2 h of incubation, the PVC films were carefully washed with PBS solution (pH 7.4) five times to remove nonfirmly adhered platelets. Then, the PVC samples were immersed into 2.5 wt % glutaraldehyde in PBS and kept at room temperature for 1 h to immobilize the platelets; they were then rinsed thoroughly with deionized water. The platelets adhering to the films were dehydrated with a serials of concentration-graded aqueous ethanol solutions (50, 60, 70, 80, 90, and 100%) and air-dried at room temperature. Finally, the film surfaces were observed by scanning electron microscopy (SEM; Hitachi SU1510, Japan) after a gold-sputtering treatment.

RESULTS AND DISCUSSION

Synthesis of the PSTG Copolymers

In this study, PSTG copolymers were synthesized by SA, TMA, and GMA monomers via free-radical polymerization with an oxidation–reduction initiator. Compared with living radical polymerization, such as atom transfer radical polymerization or reversible addition–fragmentation chain transfer, free-radical polymerization is less demanding in terms of the reaction conditions and easier to realize in industrial production. Here, we mainly researched the influence of the content of GMA moieties on the grafting density of the PSTGs because GMA behaved as a bridge between the coatings and PVC films. To determine the optimal amount of GMA, four different kinds of PSTG copolymers (PSTG1, PSTG2, PSTG3, and PSTG4) with different molar ratios of SA, TMA, and GMA monomer were synthesized. The molar percentages of the GMA monomer in the PSTG1, PSTG2, PSTG3, and PSTG4 were 5, 10, 15, and 20%, respectively. A summary of the compositions and characteristics of PSTG copolymers is given in Table I. As shown, the molar fraction of each segment in the PSTG copolymers was, to some extent, in good agreement with the feeding composition of the PSTG copolymers. Figure 1 displays the ^{1}H-NMR results of four kinds of PSTG copolymers. The relative peak at 2.85 was attributed to the $-(CH_3)_2SO_3^-$ proton resonance of the SA side groups, and the peak at 3.12 ppm corresponded to signals of $-N^+(CH_3)_3$ proton resonance of the TMA side groups. Peaks at 3.30 and 2.72 ppm were assigned to the CH and CH_2 groups, respectively, in the epoxide ring. The FTIR spectra of these PSTG samples given in the Supporting Information (Figure S1) further confirmed the results of ^{1}H-NMR for the successful synthesis of the PSTG copolymers. Gel permeation chromatography data showed that the average molecular weights of the PSTG copolymers were similar at about 27.7 ± 0.2 kDa. However, the PDIs (2.1–2.5) of the PSTG copolymers showed a broad molecular weight distribution; this was probably due to the nature of radical polymerization mechanism.[26] The ξ potentials of the PSTG copolymers were

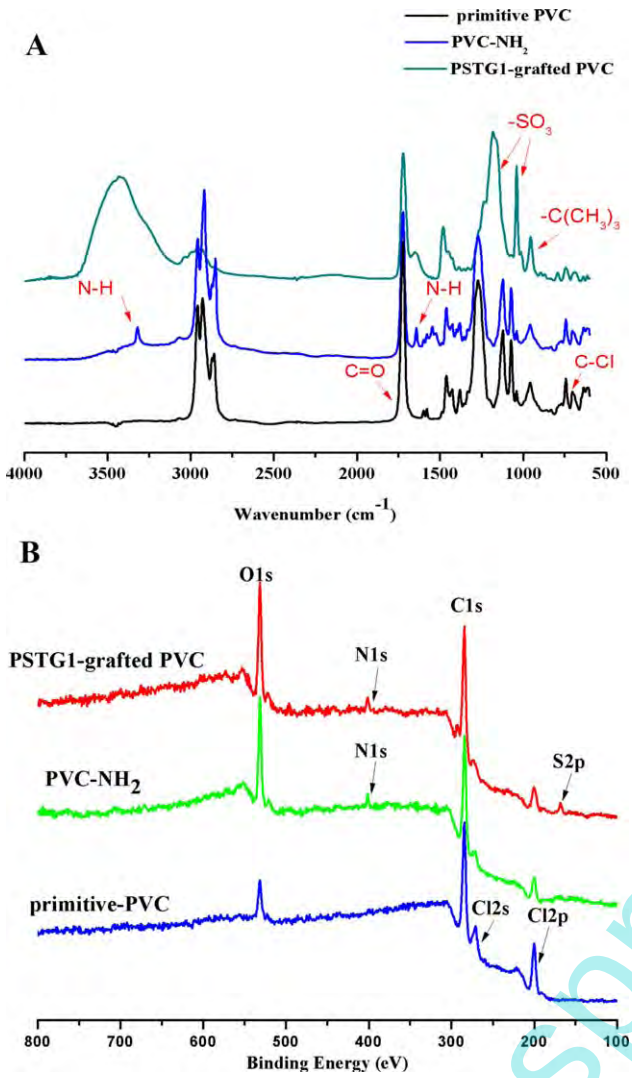


Figure 4. (A) ATR–FTIR spectra of the primitive PVC film, amino-functionalized PVC film, and PSTG1-grafted PVC film. (B) XPS spectra of the primitive PVC film, amino-functionalized PVC film, and PSTG1-grafted PVC film. [Color figure can be viewed at wileyonlinelibrary.com]

estimated with a ξ potentiometer at a polymer solution concentration of 5 mg/mL in PBS solution. The results (Table I) showed that all of the ξ potentials were close to zero; this suggested that almost no net electric charge was carried in the PSTG copolymers. This may have been due to the similar chemical reactivities

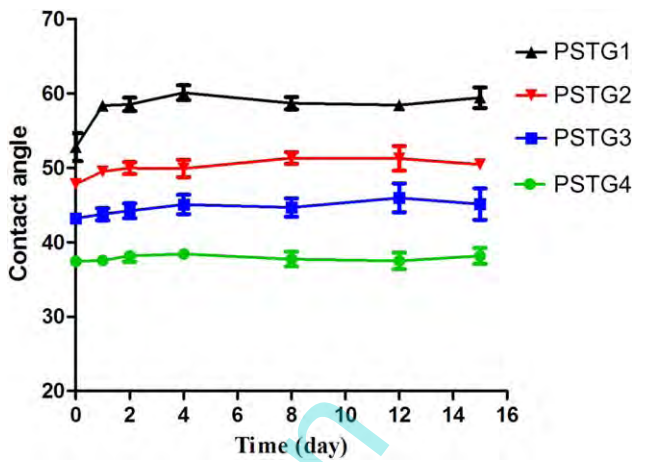


Figure 5. Contact angle measurements as a function of time for the PSTG-grafted PVC films. [Color figure can be viewed at wileyonlinelibrary.com]

of the TMA monomers and SA monomers in aqueous solution, as confirmed previously.[19] Therefore, the charge-bias variation of the PSTG copolymers could be controlled at a low level via the regulation of the feed ratio of the SA to TMA monomer at 1:1.

Amination of the PVC Films and Formation of the Polymer Coatings

The amination of PVC films has been reported in many literatures. One of the most widely used amino-functionalized PVC techniques is the exposure of the surface to ammonia plasma.[27] Plasma treatment can alter the surface performances of a substrate while maintaining the bulk properties. However, the method of surface modification can last only for a relatively short period of time because of its ease of surface reconstruction and hydrophobicity recovery.[28] In addition, this method requires vacuum equipment; this leads to a significant increase in technical complexity and manufacturing costs. A recent amino-functionalized PVC technique is based on the dechlorination of PVC and is mostly fulfilled through wet chemical modification, in which a nucleophile is used to substitute chlorine atoms in PVC through the S_N2 reaction.[29] Zou *et al.*[30] reported the introduction of amino groups to the surface of a PVC sheet by wet chemical modification with 4-mercaptobenzyl as the nucleophilic agent, which was dissolved in a mixed solution of dimethylformamide and water at 50 °C. Jiang *et al.*[31] prepared amino-functionalized PVC films via *in situ* amination

Table II. Surface Elemental Atomic Percentages and N/S Molar Ratios for Different Samples on the Basis of an XPS Examination

	Elements (mol %)					
Sample	C1s	Cl2p	N1s	O1s	S2p	N/S ratio
Primitive PVC	75.6	19.2	—	5.2	—	—
Aminated PVC	77.8	10.2	1.2	10.8	—	—
PSTG1-grafted PVC	71.2	4.0	1.5	21.6	1.7	0.88
PSTG2-grafted PVC	64.7	4.5	2.1	26.4	2.3	0.91
PSTG3-grafted PVC	67.0	3.6	2.8	23.9	2.7	1.04
PSTG4-grafted PVC	62.3	2.9	3.4	28.3	3.1	1.09

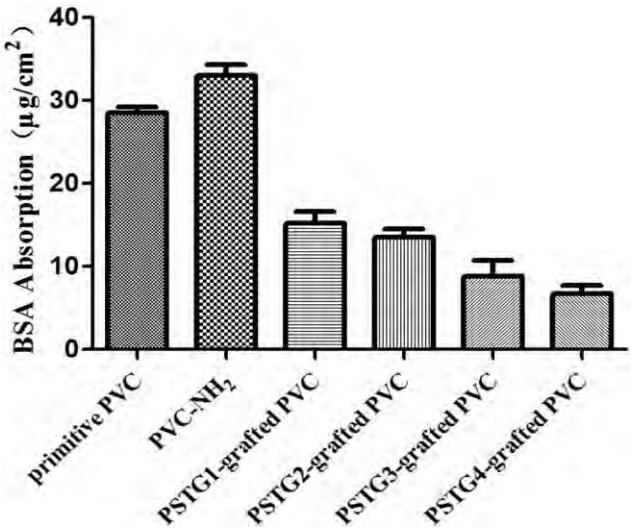


Figure 6. Amounts of protein adsorption on the PVC substrates. Data from six separate experiments are shown as means and standard deviations.

with triethylenetetramine in DMAc casting solution. In this study, the amination process of the PVC films followed a process reported by Balakrishnan *et al.*,[25] which was an effective and convenient method for providing active chemical sites on the film surface. Ethylenediamine substituted the liable Cl atoms in the PVC chains to introduce free primary amino groups on the surface, with the aim of generating active groups on the surface for further chemical modification.

We selected GMA as the comonomer to tether the PSTG copolymers onto the surface of the PVC films. The epoxy groups of GMA possess high reactivities toward amino groups.[32–34] In this study, the obtained PSTG polymers had both epoxy groups distributed randomly along the backbone and were grafted onto the amino-functionalized PVC surface simply through the soaking of the films in PSTG solutions at 60 °C for 2 h. The grafting density of the PSTG copolymers (micrograms per square centimeter) as a function of the copolymer concentration was assessed through measurement of the weight increase of the films per unit surface area. The results in Figure 2(A) shows that the grafting density of the PSTG copolymers on the surface of the PVC films increased with increasing copolymer concentration. Also, the increase in the content of GMA moieties facilitated the grafting of PSTG onto the PVC films. These could have been due to the fact that the amino groups on the surface had more chances to contact the anchorage sites of epoxy groups in the higher concentration solution; this led to a higher grafting density.[35] In addition, we observed that the grafting densities tended toward their respective platforms for all of the PSTG copolymers. Figure 2(B) shows the plots of the grafting densities versus the concentration of the epoxy groups; this more clearly shows the dependence of the grafting density on both the concentration of the epoxy groups and the content of epoxy groups on the PSTG copolymer chains. In the case of the same concentration of epoxy groups, the highest grafting density was obtained with PSTG4, whose molecular chains had the highest content of epoxy groups. Importantly, as a function of the concentration of epoxy groups, the equilibrium points of the grafting density also increased with the content of GMA moieties on the PSTG copolymers; this indicated that in addition to the reaction between the epoxy groups and the amino groups on the PVC surface, interchain crosslinking due to the ring-open reaction of the epoxy groups might have contributed highly to the grafting density and formed a PSTG self-crosslinking hydrogel coating. It was reported that a crosslinked hydrogel could benefit the antifouling properties;[36] this was confirmed by the BSA adsorption results discussed later. Therefore, a thicker coating on the PVC surfaces was produced at higher concentrations of PSTG copolymer with higher GMA moieties through a combination of the grafting reaction of the epoxy groups with the amino groups on the PVC surface and the ring-open reaction of epoxy groups.

Surface Morphology

To investigate the morphologies of the PVC surfaces before and after coating with the PSTG copolymers, we performed an AFM study; this is a direct and accurate method for detecting the thicknesses of coatings. The primitive PVC surface was relatively smooth and uniform [Figure 3(a)], with a surface root mean square (RMS) roughness of 3.9 nm. The RMS roughness of the PSTG1-grafted PVC film increased to 18.4 nm [Figure 3(b)]; this did not show a distinct difference from the PSTG2-grafted PVC film (data not shown). Because denser and thicker polymer coatings were formed on the PVC films because of interchain crosslinking, the RMS roughness of the PSTG3-grafted and PSTG4-grafted PVC films were 25.6 and 37.7 nm, respectively [Figure 3(c,d)]. The results imply that the PSTG copolymers grafted onto the PVC films; the results of the grafting density are given in Figure 4.

Static Contact Angles

To investigate the wettability of the primitive and PSTG-grafted PVC films, the static water contact angle of each sample was measured. The results are shown in the Supporting Information (Figure S2). The surface of the primitive PVC film was mostly rich in C—C, C—H and C—Cl moieties and was, thus, hydrophobic; it exhibited an average contact angle of 98.3 °. After amination of the PVC film, the contact angle decreased to 85.5 °; this indicated that the amino groups were incorporated into the surface, and this may have contributed to the hydrophilicity of the surface. For the PSTG-grafted PVC films, all of the films showed further decreases in the water contact angles compared to the primitive and amino-functionalized PVC films; this indicated enhanced hydrophilicity with the introduction of the PSTG copolymer. This superhydrophilic characteristic was attributed to its strong hydration capacity. The lowest water contact angle was achieved by the PSTG4-grafted PVC film (37.5 °) because more PSTG copolymers were grafted onto the PVC film.

Surface Chemical Structures

ATR–FTIR spectroscopy was first used to determine the surface chemical changes of medical PVC films after the surface modification. The ATR–FTIR spectra of the primitive, amino-functionalized, and PSTG-grafted PVC films are shown in Figure 4(A). For the primitive PVC film, the absorption bands at

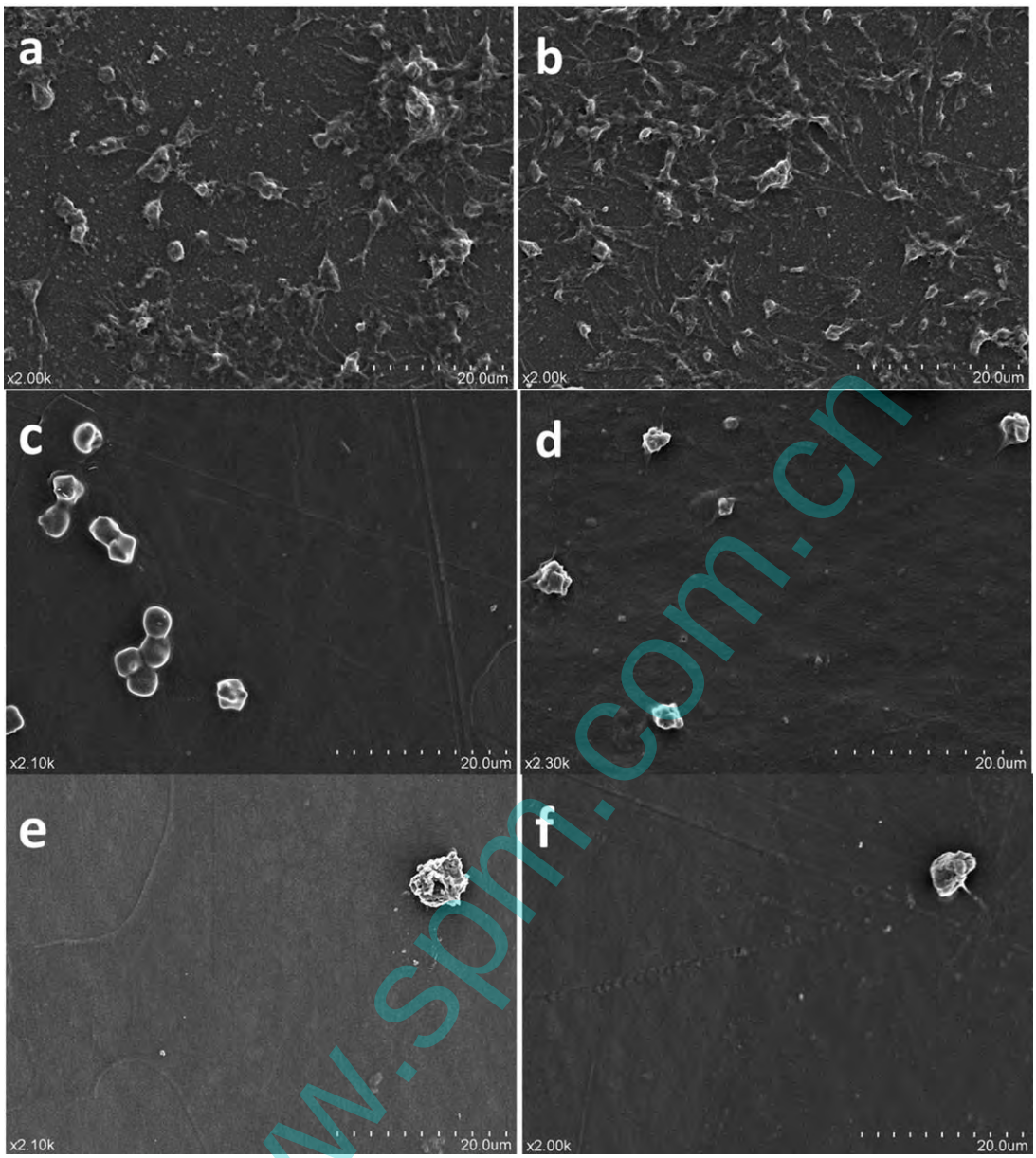


Figure 7. SEM images of platelets adhering to the surface of the ungrafted and grafted PVC films: (a) virgin PVC film; (b) aminated PVC film; and (c–f) polymer-grafted PVC films (PSTG1, PSTG2, PSTG3, and PSTG4, respectively).

about 615 and 688 cm^{-1} were due to the stretching vibrations of C—Cl bonds. The characteristic bands of ester at 1728 cm^{-1} were observed in the primitive PVC; these indicated that several additives existed in the current medical-grade PVC. New peaks at 1700–1500 cm^{-1}, corresponding to the bending vibrations of primary ammine, and 3320 cm^{-1}, attributed to the stretching absorption of the N—H bonds, were observed on the amino-functionalized PVC films; these suggested the introduction of amino groups on the PVC films.[27] For the PSTG-grafted PVC films, typical peaks for $—SO_3$ stretching at 1038 cm^{-1} and $—N^+(CH_3)_3$ at 950 cm^{-1} were found; this indicated that the PSTG copolymers were successfully grafted onto the PVC films.

To further confirm that the copolymers were indeed grafted to the PVC film, we performed XPS measurements; this indicated the change of chemical element within the order of 1–5 nm.[37] Table II and Figure 4(B) show quantitative elemental composition data and typical XPS survey spectra for the PVC films before and after modification, respectively. As the spectra of the PSTG-grafted PVC films were almost the same except for the small difference in the peak intensity, the PSTG1-grafted PVC film is shown as a representative. On the primitive PVC surface, carbon (C1s at 284.5 eV) and chlorine (Cl 2p peak at 200.6 eV and Cl 2s peak at 270.9 eV) were clearly present; this was in agreement with the elemental composition of the PVC film. The signal of O1s at 531.6 eV observed in the spectrum may have been due to the presence of ester additives. As shown in Table II, the N 1s atom signal with an atomic concentration of 1.2%, which was absent for the primitive PVC film, appeared in the

amino-functionalized PVC film. The XPS spectrum of the PSTG1-grafted PVC film exhibited an S2p signal at 168.2 eV; this originated from the C—S bond and corroborated the successful grafting of the PSTG copolymer. In addition, the N/S molar ratio was close to 1:1; this suggested that the surface charge could be well controlled.

According to the result of the S element content on the surface given in Table II and the elemental contents of the PSTG copolymers, we calculated that about 38% of the C element on the surface of the PSTG1-grafted PVC film was provided by PSTG1, and about 93% was provided in the case of PSTG4. Therefore, the XPS results further confirmed that more PSTG copolymers were grafted onto the PVC surface by PSTG4.

Stability of the PSTG-Grafted PVC Films

The stability of the PSTG-grafted PVC films is always a primary concern, especially in complicated and turbulent blood environments. Tests for the long-term stability of PSTG-grafted PVC films were carried out by contact angle measurements. As shown in Figure 5, almost all of the PSTG-grafted PVC films maintained their original contact angle after 15 days of soaking in PBS at 37 °C. However, there was a significant increase in the contact angle (from 50 to 60 °) of PSTG1 up to day 3. Similar trends were also observed for PSTG2, PSTG3, and PSTG4 but to a decreasing extent in sequence. The PSTG4-grafted PVC film was more stable compared with the other three PSTG-grafted PVC films because it had more anchor sites, which could result in a high crosslinking density. Anyway, the almost unchanged contact angle after a long soaking in PBS at 37 °C suggested that the PSTG copolymer was immobilized on the PVC films by strong covalent bonding.

Protein Adsorption

In general, it is well acknowledged that nonspecific protein adsorption is the first step when blood comes into contact with a foreign surface; this is followed by platelet adhesion and thrombus formation.[38] Therefore, protein adsorption on the surface is considered one of the most important factors in the evaluation of the biocompatibility of materials.[39] To investigate the antifouling properties of the PSTG-grafted PVC films, BSA was used as a model protein. Figure 6 displays the quantity of protein adsorbed on the surface of the substrates per unit area. The data were averaged from three independent experiments to ensure reproducibility. We observed that BSA was adsorbed seriously on the primitive PVC films because of the hydrophobic interaction between the PVC films and BSA. The amounts of BSA adsorption was 28.2 and 33.5 $\mu g/cm^2$ for the primitive and amino-functionalized PVC films, respectively. The higher adsorption may have been due to the fact that the amino-functionalized PVC films were positively charged and could adsorb BSA more easily by electrostatic force. In contrast, the PSTG-grafted PVC films displayed a lower protein adsorption compared with the control samples. In addition, the amount of protein adsorption decreased with increasing GMA content in the PSTG copolymers because a higher graft density was achieved. These results clearly indicate that the antifouling properties were effectively improved by the introduction of PSTG copolymers to the hydrophobic PVC films; this was probably due to the formation of a hydrated layer through the binding of a significant amount of water; this weakened the interactions between the protein and PVC films.

Platelet Adhesion

Platelet spreading and aggregation have been considered as major causes of thrombosis.[40] To investigate the blood compatibility of the PSTG-grafted PVC films, we conducted a platelet adhesion test to examine the amount and morphology of the adhered platelets. Figure 7 shows typical SEM images of platelets fixed onto different PVC films. It was clear that many platelets aggregated and adhered to the primitive and amino-functionalized PVC films [Figure 7(a,b)]. The adhered platelets exhibited deformed shapes with extended pseudopodia; this indicated that most attached platelets were highly activated. In contrast, platelets incubated by the PSTG-grafted PVC films showed reduced attachment [Figure 7(c–f)]. Meanwhile, most of the platelets maintained a rounded morphology with less aggregation and pseudopodia; this suggested that the PSTG-grafted PVC films showed improved blood compatibility. The results confirm that the PSTG-grafted PVC films provided excellent antifouling properties. It should be noted that almost no platelets adhered to the PSTG4-grafted PVC film, as shown in Figure 7(f); this may have been because it had the best hydrophilicity and protein adsorption resistance.

CONCLUSIONS

We developed a simple and effective coating strategy to enhance the antifouling properties and long-term stability of PVC-based medical films. First, free amino groups were introduced onto the PVC film surface by a wet chemical modification with ethylenediamine as the nucleophilic reagent. Mixed-charge terpolymers (PSTGs) were efficiently immobilized onto the surface of the PVC film through a combination of the reaction between the amino groups on the PVC surface and the epoxy pendants in the GMA moieties of PSTG and the self-crosslinking reaction between the epoxy groups. The static contact angle, AFM, ATR–FTIR spectroscopy, and XPS results all confirmed the formation of coatings on the PVC films. Compared with the ungrafted PVC, all of the PSTG-grafted PVC films exhibited antifouling properties, as shown by significantly less protein adsorption and platelet adhesion compared to that of the bare PVC film. A significantly high density of PSTG on the PVC surface was obtained through a combination of the dependence of the graft density on the polymer concentration and the epoxy group density of the PSTG chains, which showed better antifouling properties. Such a simple and robust method for coating PVC films with a mixed-charge copolymer is promising for the fabrication of blood-compatible medical devices.

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