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Hemocompatible Control of Sulfobetaine-Grafted Polypropylene Fibrous Membranes in Human Whole Blood via Plasma-Induced Surface Zwitterionization

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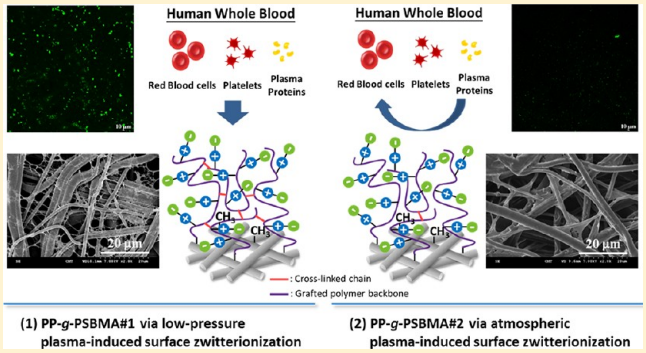
ABSTRACT: In this work, the hemocompatibility of zwitterionic polypropylene (PP) fibrous membranes with varying grafting coverage of poly(sulfobetaine methacrylate) (PSBMA) via plasma-induced surface polymerization was studied. Charge neutrality of PSBMA-grafted layers on PP membrane surfaces was controlled by the low-pressure and atmospheric plasma treatment in this study. The effects of grafting composition, surface hydrophilicity, and hydration capability on blood compatibility of the membranes were determined. Protein adsorption onto the different PSBMA-grafted PP membranes from human fibrinogen solutions was measured by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies. Blood platelet adhesion and plasma clotting time measurements from a recalcified platelet-rich plasma solution were used to determine if platelet activation depends on the charge bias of the grafted PSBMA layer. The charge bias of PSBMA layer deviated from the electrical balance of positively and negatively charged moieties can be well-controlled via atmospheric plasma-induced interfacial zwitterionization and was further tested with human whole blood. The optimized PSBMA surface graft layer in overall charge neutrality has a high hydration capability and keeps its original blood-inert property of antifouling, anticoagulant, and antithrmbogenic activities when it comes into contact with human blood. This work suggests that the hemocompatible nature of grafted PSBMA polymers by controlling grafting quality via atmospheric plasma treatment gives a great potential in the surface zwitterionization of hydrophobic membranes for use in human whole blood.



INTRODUCTION

The development of hemocompatible surfaces/interfaces resisting the adsorption of plasma proteins and the adhesion of blood cells is highly desirable for important biomedical applications as blood-contacting devices used such as hemodialysis system, blood cell filters, antithrombogenic implants, drug carriers, and biosensors.[1−9] It is well-known that nonspecifically adsorbed plasma proteins on general hydrophobic surfaces interact in a series of reactions leading to plasma clotting.[10,11] Among plasma proteins, fibrinogen plays a leading role in mediating surface-induced adhesion and activation of platelets in human blood plasma. However, only a few synthesized protein-resistant materials are regarded as reliable blood-contacting systems. Zwitterionic materials containing the pendant groups of phosphobetaine, sulfobetaine, and carboxylbetaine have received growing attention for use as blood-inert surfaces because of their excellent inhibition in plasma protein adsorption.[6−8,12−23] A general molecular characteristic of zwitterionic betaines is that they have both cationic and anionic charged moieties on the same side chain, maintaining overall charge neutrality. In recent years, zwitterionic poly(sulfobetaine methacrylate) (PSBMA) has been widely studied due to its ease of synthesis. Previous works showed that the surfaces grafted PSBMA brushes are ideal for resisting nonspecific protein adsorption when the surface density and chain length of zwitterionic groups are controlled. It was also reported that the surfaces grafted with PSBMA brushes provided an effective and stable blood-inert nature of a zwitterionic surface for uses in human plasma solution.[13,19,23]

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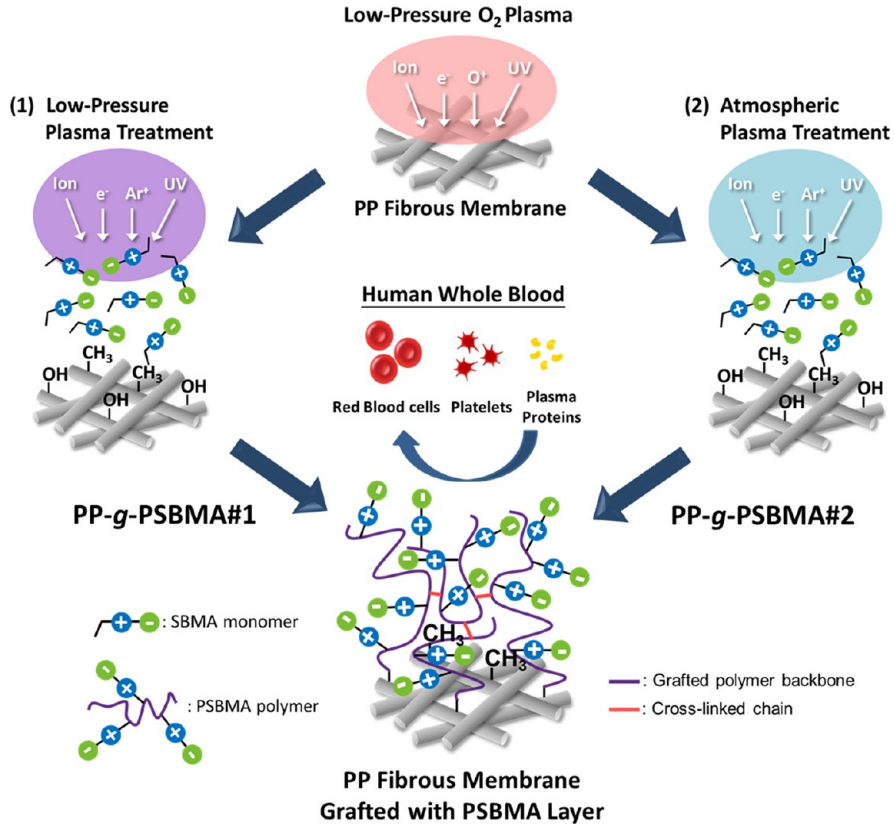

Figure 1. Schematic plasma-induced surface zwitterionization process: (1) low-pressure treatment and (2) atmospheric plasma treatment.

Several strategies have been adopted to improve the protein resistance of membranes, such as blending zwitterionic betaines containing polymers before membrane formation or surface grafting of zwitterionic moiety.[25−28] Polymer blending before membrane formation is a much more preferable method in engineering process, but limited zwitterionic moiety can be incorporated into the membrane so that the improvement of membrane fouling from blood-based fluids is also limited. Surface grafting is considered as one of the promising modifications to incorporate zwitterionic functionalities onto a wide range of hydrophobic membrane materials for improved hemocompatibility. However, it is always a challenge to control the surface grafted with a highly polar structure of zwitterionic layer from the highly hydrophobic and chemical-inert surface of a propylene- or fluoro-polymer membrane. The surface zwitterionization was demonstrated by using ozone surface activation and surface-initiated atom transfer radical polymerization (ATRP).[8,23,24] A well-packed zwitterionic PSBMA grafted surface can be controlled through this technique with highly efficient in improving protein resistance. As compared to surface grafting via ATRP, plasma-induced surface zwitterionization provides several distinct advantages, such as high grafting yield and short modification time. Surface zwitterionization by plasma treatment is easy to operate and suitable for biomedical membrane modification in the dry and clean process. However, the surface grafting quality is always the major concern.

Previous studies show that the control of surface grafting coverage is important for zwitterionic membrane with a hemocompatible surface.[10,19] It was reported that the zwitterionic PSBMA-grafted membrane provides good resistance of plasma protein adsorption, while surface grafting of PSBMA layer is fully covered. As shown in our previous work, zwitterionic betaines generate a tightly bound, structured water layer around the zwitterionic head groups via electrostatically induced hydration. Importantly, the charge neutrality of grafted zwitterionic PSBMA brush is a key factor to keep minimizing the electrostatic interactions with plasma proteins and blood cells.[13,23] However, two important issues of surface zwitterionization are still unclear: (i) how grafting structures of PSBMA layer on membrane surface can be controlled via plasma-induced interfacial copolymerization and (ii) how the charge bias of PSBMA layer deviated from electrical balance of positively and negatively charged moieties influences the membrane blood compatibility. The results from such studies would directly enable the rational design of zwitterionic membranes for use in blood-contacting applications. In this study, a model membrane system can be developed with a combination of the excellent mechanical bulk properties of hydrophobic polypropylene (PP) materials and the good antithrombogenic surface characteristics of zwitterionic PSBMA layers. In the present work, we demonstrated a systematic surface grafting control of PP membranes with PSBMA via plasma-induced surface zwitterionization, which was carried out by low-pressure and atmospheric plasma treatments. The effects of different plasma treatments on the grafting structures of these zwitterionic membranes with various grafting coverage and surface charge neutrality were examined in detail. The correlation of hydration capacity and protein adsorption was discussed to illustrate the adhesion of platelets onto the PSBMA-grafted membrane surface from human blood. The anticoagulant activity of the membranes in recalcified plasma-clotting tests from a platelet-poor plasma (PPP) solution and the attachment of blood cells onto membrane surface from 100% human whole blood were also

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demonstrated. This study not only demonstrates a plasma-induced surface zwitterionization that achieves a effective hemocompatible membrane surface in complex media such as human plasma and whole blood, but also provides a fundamental understanding of the correlations between interfacial charge neutrality and blood compatibility.

MATERIALS AND METHODS

Materials. Polypropylene (PP) fibrous membranes with an average pore size of 3 μm, a thickness of about 220 μm, and a diameter of 293 mm were purchased from the Membrane Solutions LLC. (MFPP293300) and were used as received. [2-(Methacryloyloxy)-ethyl] dimethyl(3-sulfopropyl)-ammonium hydroxide (sulfobetaine methacrylate, SBMA) macromonomer was purchased from Monomer-Polymer & Dajac Laboratories, Inc. Methanol was obtained from Sigma-Aldrich and was used as a solvent for the plasma-induced graft-polymerization. Fibrinogen (fraction I from human plasma) was purchased from Sigma Chemical Co. Human blood and plasma solution were obtained from the Taiwan Blood Services Foundation. Phosphate buffer saline (PBS) was purchased from Sigma-Aldrich. Deionized water used in the experiments was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩ m.

Surface Zwitterionization. A schematic illustration of the plasma-induced surface zwitterionization on PP membranes under the control of low-pressure or atmospheric plasma treatment is shown in Figure 1. The PP membrane was first treated by low-pressure plasma with an oxygen flow rate of 30 sccm and input power of 50 W controlled by a 13.56 MHz RF generator (Cesar 136, Dressler). The post-treated PP membrane with a surface area of approximately 0.4 cm^2 was incubated in a methanol solution containing 30 wt % SBMA monomer. After the SBMA-coated PP membrane at was dried at 25 °C for 24 h, the membrane coated with the SBMA monomer layer of ~20 mg/cm^2 was then followed by low-pressure or atmospheric plasma treatment with an argon flow rate of 10 slm and input power of 150 W controlled by a 13.56 MHz RF generator. After plasma treatment, the zwitterionic PP membrane was transferred into purified methanol and was then extracted with deionized water and methanol, respectively, each for 60 min in the ultrasonic device to strip off PSBMA homopolymers and unreacted monomers. The residual solvent was removed in a vacuum oven under reduced pressure for 1 day and dried in a freeze-dryer at −45 °C for 1 day. In this study, all membranes after plasma treatment were cleaned using the same postwash procedures. The surface grafting yield (mg/cm^2) of the zwitterionic PP membrane is defined as the weight difference between the modified PP membrane and the virgin PP membrane divided by the surface area of the virgin PP membrane.

Surface Characterization. The chemical composition of surface-modified PP fibrous membranes with grafted PSBMA layer was characterized using by X-ray photoelectron spectroscopy (XPS). XPS analysis was performed using a Thermal Scientific K-Alpha spectrometer equipped with a monochromated Al K X-ray source (1486.6 eV photons). The energy of emitted electrons was measured with a hemispherical energy analyzer at pass energies ranging from 50 to 150 eV. All data were collected at a photoelectron takeoff angle of 45° with respect to the sample surface. The binding energy (BE) scale is referenced by setting the peak maximum in the C 1s spectrum to 284.6 eV. The high-resolution C 1s spectrum was fitted using a Shirley background subtraction and a series of Gaussian peaks. Water contact angles were measured with a goniometer (Automatic Contact Angle Meter, model FTA1000, First Ten Ångstroms Co, Ltd., U.S.) at 25 °C. The DI water was dropped on the sample surface at 10 different sites. The surface morphology of virgin and these surface-modified PP membranes were examined by scanning electron microscopy (SEM). All membrane samples were sputter-coated with gold prior to observation under Hitachi model S3000 SEM operating at an accelerating voltage of 7 keV.

Protein Adsorption on the PSBMA-Grafted PP Membranes. In this study, the adsorption of human fibrinogen on the membranes was evaluated using the enzyme-linked immunosorbent assay (ELISA) according to the standard protocol described briefly below. First, the membranes with a surface area of 0.4 cm^2 were placed in individual wells of a 24-well tissue culture plate, and each well was equilibrated with 1000 μL of PBS for 60 min at 37 °C. Next, the membranes were soaked in 500 μL of fibrinogen solution with a concentration of 1 mg/mL. After 180 min of incubation at 37 °C, the membranes were rinsed five times with 500 μL of PBS and then incubated in bovine serum albumin (BSA, purchased from Aldrich) for 90 min at 37 °C to block the areas unoccupied by protein. The membranes were rinsed with PBS five more times, transferred to a new plate, and incubated in 500 μL of PBS solution. The membranes were incubated with a primary monoclonal antibody that reacted with the fibrinogen for 90 min at 37 °C and then were blocked with 10 mg/mL BSA in PBS solution for 24 h at 37 °C. The membranes were subsequently incubated with the secondary monoclonal antibody, horseradish peroxidase (HRP)-conjugated immunoglobulins, for 60 min at 37 °C. The membranes were rinsed five times with 500 μL of PBS and transferred into clean wells, followed by the addition of 500 μL of PBS containing 1 mg/mL chromogen of 3,3′,5,5′-tetramethylbenzidine, 0.05 wt % Tween 20, and 0.03 wt % hydrogen peroxide. After incubation for 20 min at 37 °C, the enzyme-induced color reaction was stopped by adding 500 μL of 1 mmol/mL H_2SO_4 to the solution in each well, and finally the absorbance of light at 450 nm was determined by a microplate reader. Protein adsorption on the membranes was normalized with respect to that on the virgin PP membrane as a reference. These measurements were carried out six times for each membrane (n = 6).

Blood Platelet Adhesion on the PSBMA-Grafted PP Membranes. The PP membranes of 0.4 cm^2 surface area were placed in individual wells of a 24-well tissue culture plate, and each well was equilibrated with 1000 μL of phosphate buffered solution (PBS) for 2 h at 25 °C. Blood was obtained from a healthy human volunteer. Platelet-rich plasma (PRP) containing about 1×10^5 cells/mL was prepared by centrifugation of the blood at 1200 rpm for 10 min. The platelet concentration was determined by a microscopy (NIKON TS 100F). For the test of unactivated platelet adhesion on the membranes, 200 μL of the PRP was directly placed on the PP surface in each well of the tissue culture plate and incubated for 120 min at 37 °C. For the test of activated platelet adhesion on the membranes, 200 μL of the PRP, first recalcified by the addition of calcium (1 M $CaCl_2$, 5 μL), was placed on the PP surface in each well of the tissue culture plate and incubated for 120 min at 37 °C. After the PP membranes were rinsed twice with 1000 μL of PBS, they were immersed into 2.5% glutaraldehyde of PBS for 48 h at 4 °C to fix the adhered platelets and adsorbed proteins, then rinsed three times with 1000 μL of PBS and gradient-dried with ethanol in 0% v/v PBS, 10% v/v PBS, 25% v/v PBS, 50% v/v PBS, 75% v/v PBS, 90% v/v PBS, and 100% v/v PBS for 20 min in each step and dried in air. Finally, the samples were sputter-coated with gold using a Fine Coat Ion Sputter JFC-1100 before scanning with a Hitachi model S3000 SEM operating at an accelerating voltage of 7 keV.

Plasma-Clotting Time of the PSBMA-Grafted PP Membranes. The anticoagulant activities of the prepared membranes were evaluated by testing plasma-clotting time in human plasma. Prior to the test, the PSBMA-grafted PP membranes with 0.4 cm^2 were placed into the 24-well plate. Human normal plasma (platelet poor) was prepared from anticoagulated human blood from three donors by centrifugation (1200 rpm for 10 min at 25 °C followed by 3000 rpm for 10 min at 25 °C). Plasma was recalcified to 20 mM $CaCl_2$ by the addition of calcium from a 1 M stock solution and shaken for 30 s at 37 °C. Next, 0.5 mL of plasma was immediately added to each well in a 24-well plate (Falcon, nontissue culture treated polystyrene). The clotting time of the plasma was determined as the time where the onset of the absorbance transition occurred by reading the absorbance at 660 nm using the PowerWave microplate spectrophotometer with programmed temperature control at 37 °C. Each clotting time is reported as the average value of repeated measurements of six samples (n = 6).

Whole Blood Cell Attachment on the PSBMA-Grafted PP Membranes. The PSBMA-grafted PP membranes with 0.4 cm^2 surface area were placed in individual wells of a 24-well tissue culture

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Table 1. Surface Zwitterionization of the PP Membranes

surface zwitterionization	the conditions of zwitterionization			surface grafting yield (mg/cm^2)	surface contact angle (deg)
	PSBMA reaction amount (mg/cm^2)	temperature (°C)	grafting time (s)		
zwitterionization-1[a]	20	<50	0−120	0.00−0.82	38−135
zwitterionization-2[b]	20	<50	0−120	0.00−3.07	38−138

[a]Membranes prepared by plasma-induced surface zwitterionization using low-pressure plasma treatment. [b]Membranes prepared by plasma-induced surface zwitterionization using atmospheric plasma treatment.

plate, and each well was equilibrated with 1000 μL of PBS for 24 h at 37 °C. 250 mL of fresh whole blood obtained from five healthy human volunteers was mixed with 35 mL of citrate phosphate dextrose adenine-1 (CPDA-1). 1000 μL of whole blood was placed on the substrate surface in each well of the tissue culture plate and incubated for 2 h at 37 °C. After the membranes were rinsed twice with 1000 mL of PBS, they were immersed in 300 μL of 4.0% formaldehyde in PBS for 15 min at 4 °C to fix the adhered blood cells, and then rinsed three times with 1000 μL of PBS. Blood cells attached to the sample surfaces were stained with 3 μL of CD3-FITC, CD14-FTIC, and CD45-FITC in 270 μL of PBS with 2.5% glutaraldehyde at 4 °C for 15 min. After being washed with PBS three times, the morphology of adhered blood cells on the substrates in PBS was observed from Nikon A1R laser scanning confocal microscope (LSCM, A1R, Nikon, Japan) images at a 200× magnification from five different places on the same chip. During observation, the images were taken at λ_{ex} = 488 nm/λ_{em} = 520 nm for detection of the FITC dye. The confocal images were taken in z-steps of 1 μm to depths of 30 μm from the optical horizontal sections of three different areas on each membrane. Image Analysis Software (SimplePCI, Version 5.3.1, Hamamatsu Co.) calculated the number of adhered blood cells, which were captured from confocal images of 200× magnification.

RESULTS AND DISCUSSION

Surface Zwitterionization and Characterization. As illustrated in Figure 1, an integrated process of plasma-induced surface zwitterionization was developed to modify the hydrophobic and chemical-inert PP fibrous membrane with a well-controlled PSBMA-grafted layer. The coating quality of SBMA monomer on the hydrophobic PP surface was found to result in dramatically poor spatial distribution and to form the droplet shrinkage of SBMA due to the large difference in surface polarity between SBMA and PP. Thus, the virgin PP membrane was pretreated by low-pressure plasma with oxygen and followed by the incubation of DI water at 60 °C to generate the hydroxyl groups, resulting in an increase in the diiodomethane contact angle from 38° to 103° and the chemical compatibility between SBMA monomer and PP membrane surfaces. Next, the surface coverage of PSBMA-grafted layer on PP (PP-*g*-PSBMA) membranes can be first regulated by the amount of uniform-coated SBMA monomer and then followed by the control of plasma treating time under low-pressure or atmospheric pressure conditions. From general understanding of gas plasma treatment and surface-induced copolymerization, the possible mechanism of the surface grafting reaction could be divided into two reaction stages. In the initial stage, energetic species (Ar metastables and ions) and UV radiation from plasma treatment could generate initiator radicals both in SBMA monomer and on PP membrane surface. Two types of the addition reactions may occur. First, the copolymerization of initiator radicals on PP membrane surface to SBMA monomer will result in the immobilization of PSBMA layers from the PP membrane surface. Second, the polymerization of initiator radicals in SBMA monomer to SBMA monomer will result in the formation of PSBMA homopolymer on the PP membrane surface. In the subsequent stage, continued surface plasma treatment induces the scission of chemical bonding in PSBMA polymer chains and the generation of free radicals in both PSBMA polymer brushes and PSBMA homopolymer. Next, the cross-linking reaction by recombination of free radicals might occur between PSBMA homopolymer chains and PP membrane surface, PSBMA polymer brushes, or PSBMA homopolymer chains. Thus, the grafting qualities of PSBMA layer on the PP-*g*-PSBMA membrane surface might be different from the surface zwitterionization control between low-pressure and atmospheric plasma treatments. The effects of different plasma treatments on the grafting structures of these zwitterionic membranes with various grafting coverage and surface charge neutrality of PSBMA layers were examined.

To achieve the desired grafting coverage of PSBMA layer on PP membrane surface, the plasma treating time for resulting membranes was adjusted in the range of 120 s under the defined control of SBMA monomer coating of about 20 mg/cm^2 for each plasma-induced surface zwitterionization. In this work, two types of plasma treatment of surface zwitterionization for PP fibrous membranes are summarized in Table 1. The surface grafting yield (mg/cm^2) of the modified PP membrane is defined as the weight difference between the modified PP membrane and the virgin PP membrane divided by the surface area of the virgin PP membrane. It was found that a high efficient grafting copolymerization was obtained using plasma-induced surface zwitterionization. As shown in Figure 2, the grafting yield of grafted PSBMA layers on the surface of PP membranes increased as the plasma treating time increased from 15 to 120 s. It provides a significant insight that plasma-induced surface zwitterionization can be performed at an efficient grafting time, as listed in Table 1, as compared to surface-initiated atom transfer radical polymerization. The

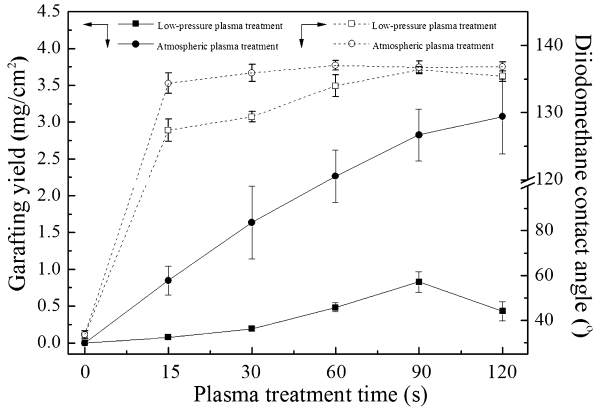

Figure 2. Changes in the surface grafting yield and diiodomethane contact angle of PSBMA-*g*-PP fibrous membranes as a function of plasma treatment time during low-pressure and atmospheric plasma treatment, respectively.

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increased values of diiodomethane contact angle in Figure 2 indicate that the surface hydrophilicity of PP-*g*-PSBMA membranes enhances with increasing surface coverage of zwitterionic grafted PSBMA polymer on the hydrophobic PP membranes. The diiodomethane contact angle of the PP-*g*-PSBMA membrane surface was as high as about 138°, indicating a significant enhancement in hydrophilicity as compared to the virgin PP membrane surface. It was also found that there was an almost unchanged value of the diiodomethane contact angle at the grafting yield above 0.5 mg/cm^2. This indicates that grafted PSBMA polymer on the PP membrane surface approaches its respective saturated coverage with steady diiodomethane contact angle. The grafting yield of grafted PSBMA layers on the surface of PP membranes for atmospheric plasma treatment is obviously higher than that for low-pressure plasma treatment, which might be due to the formation of a PSBMA-grafted polymer that was preferable on the PP membrane via the moderate plasma energy of atmospheric plasma-induced surface zwitterionization.

Surface morphology of the zwitterionic membranes is associated with the surface coverage of grafted PSBMA polymer on the PP membrane, which was observed by SEM. In Figure 3,

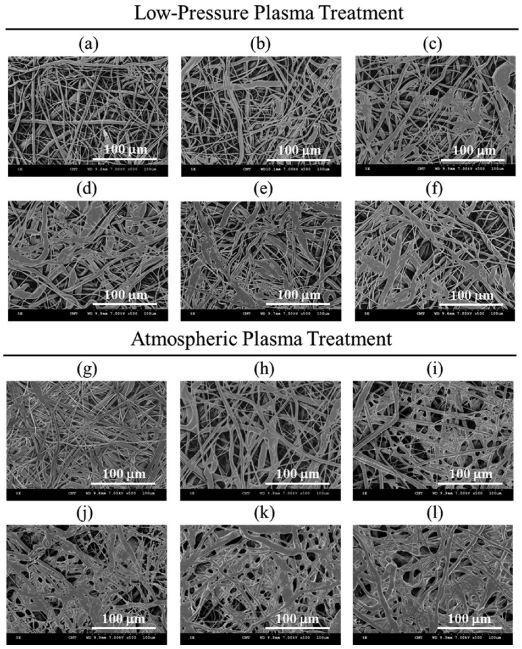

Figure 3. SEM images of surface morphology of the modified PP fibrous membrane with grafted PSBMA (a and g) virgin PP fibrous membrane; (b–f) the PP-*g*-PSBMA#1 membranes with low-pressure plasma treatment times of 15, 30, 60, 90, and 120 s; (h–l) the PP-*g*-PSBMA#2 membranes with atmospheric plasma treatment times of 15, 30, 60, 90, and 120 s. All images with magnification of 500×.

the surface grafting coverage of PSBMA layer on the PP-*g*-PSBMA fibrous membrane revealed an obvious change as the plasma treating time was increased from 15 to 120 s. According to the surface images in Figure 3b–f using low-pressure plasma treatment and that in Figure 3h–l using atmospheric plasma treatment, it clearly indicated that the increase of diiodomethane contact angle on the membrane surface was due to the increase in surface coverage of grafted PSBMA polymer. Grafting yield and surface contact angle of PSBMA-grafted layer obtained from the measurements in Figure 2 showed an increase value as the plasma treating time increased, which was consistent with the results of change in morphology on the PP-*g*-PSBMA membrane surface from SEM observation. As compared to low-pressure plasma treatment, atmospheric plasma-induced zwitterionization obtained the relatively higher grafting yield shown in Figure 2, and the formation of an obvious skin layer on PP fibers was observed in Figure 3h–l. The results indicated that the grafting efficiency of PSBMA polymer from PP fibrous membranes via atmospheric plasma is better than that via traditional low-pressure plasma during the plasma-induced surface zwitterionization.

Correlation of Grafting Structure, Hydration Capability, and Charge Neutrality of the PSBMA-Grafted PP Membranes with Protein Adsorption. Previous molecular simulation studies showed that the water molecules around the zwitterionic pendant groups of the nonfouling chains play a key role in providing interfacial resistance to nonspecific protein adsorption.[18,29–31] The general concepts of the functional groups that create the protein-resistant surfaces conform to hydrophilic, electrically neutral, and hydrogen-bond acceptors rather than hydrogen-bond donors as criteria of antifouling.[3,32] Therefore, the evaluation of protein adsorption on the zwitterionic membrane surfaces should be considered not only the surface hydrophilicity and hydration capability but also the surface charge neutrality of grafted zwitterinoic PSBMA layer on the prepared membranes. In general, fibrinogen adsorption on membrane surfaces is considered as a key event to induce a full scale blood platelet adhesion, leading to thrombosis and embolism at the blood-contacting side of material interfaces in the bloodstream.[11,33,34] Thus, the measurement of fibrinogen adsorption on the prepared membranes may provide a good indication to correlate with membrane hemocompatibility. In this study, grafting structures of PSBMA layer on PP surface were identified by XPS analysis. Figure 4 shows the surface composition of C 1s, N 1s, and S 2p core-level spectra on the virgin PP membrane and the PSBMA-grafted PP membranes. The C 1s core-level spectrum of the membranes was curve-fitted with peak components for the [C–C, C–H], [C–O], and [C═O] species, at the binding energies of about 284.6, 286.5, and 288.8 eV, respectively. The characterization results of grafted PSBMA layer on the PP membranes are summarized in Table 2. The theoretical peak component area ratio of [C–C, C–H]/[C═O] for the chemical structure of SBMA monomer is 23.0:1. From composition analysis summarized in Table 2, the contribution of the [C–C, C–H]/[C═O] ratio should be considered not only the PP and PSBMA polymer components but also the cross-linking structures in grafted PSBMA layer. For the cases of PP-*g*-PSBMA membranes with saturated coverage of grafted PSBMA polymer, the [C–C, C–H]/[C═O] ratio is mainly attributed form the top coverage layer of PSBMA on the PP surfaces due to the limitation of sampling depth analyzed by XPS. It was found that the range of [C–C, C–H]/[C═O] ratios of the PP-*g*-PSBMA#2 remained between 22.5 and 28.4 from atmospheric plasma-induced surface zwitterionization, which is close to the theoretical value of 23.0, with the increase of plasma treating time to 90 s, indicating a limited cross-linking reaction occurs between PSBMA chains or between PSBMA chains and PP membrane surface. Oppositely, the [C–C, C–H]/[C═O] ratio of the PP-*g*-PSBMA#1 using low-pressure plasma-induced surface zwitterionization is deviated above 23.0, which indicates the cross-linking reaction occurs between PSBMA chains or between PSBMA chains and PP membrane surface. However, the [C–C, C–H]/[C═O] ratio

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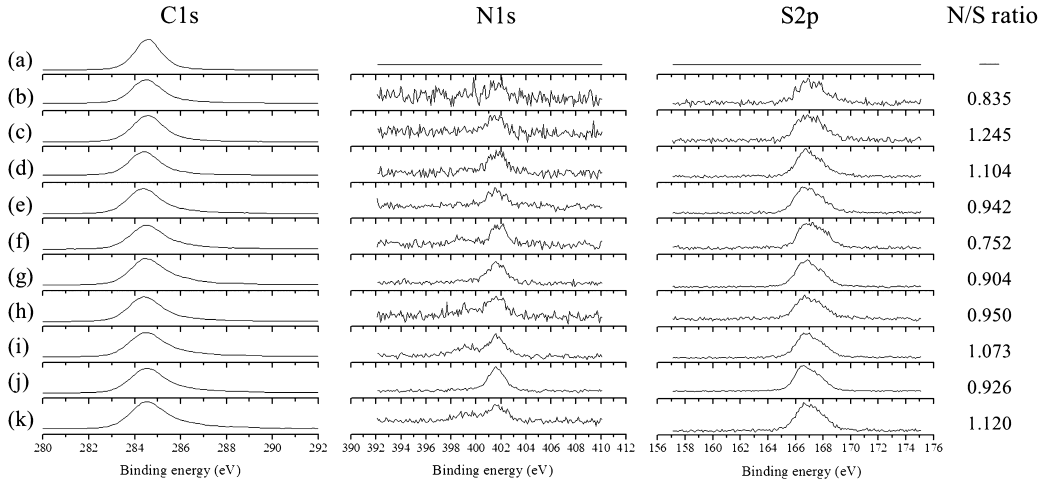

Figure 4. XPS spectra of the modified PP fibrous membranes with PSBMA in C1s, N1s, and S2p regions: (a) virgin PP fibers; (b–f) the PP-*g*-PSBMA#1 membranes with low-pressure plasma treatment times of 30, 60, 90, and 120 s; (f–k) the PP-*g*-PSBMA#2 membranes with atmospheric plasma treatment times of 15, 30, 60, 90, and 120 s, respectively.

Table 2. Surface Characterization of PSBMA Composition on the PP Membranes

		compositions of grafting PSBMA layer (mol %)		
samples	plasma treatment time (s)	[C–C, C–H]	[C=O]	[C–C, C–H]/[C=O]
SBMA[a]		92.00	4.00	23.00
PP-*g*-PSBMA #1[b]	15	89.04	2.57	34.65
	30	91.09	2.76	33.00
	60	89.45	2.37	37.74
	90	83.96	3.23	25.99
	120	84.29	4.61	18.28
PP-*g*-PSBMA #2[c]	15	81.26	2.86	28.41
	30	85.35	3.10	27.53
	60	83.34	3.58	23.28
	90	82.85	3.68	22.51
	120	79.91	5.70	14.02

[a]Theoretical compositions of SBMA monomer. [b]Membranes prepared by plasma-induced surface zwitterionization using low-pressure plasma treatment. [c]Membranes prepared by plasma-induced surface zwitterionization using atmospheric plasma treatment.

of the PP-*g*-PSBMA#1 decreased to ~18.28 and that of PP-*g*-PSBMA#2 decreased to ~14.02 as the plasma treating time increased to 120 s, which indicates the longer plasma treatment at both low and atmospheric pressure resulted in the higher chemical degradation of PSBMA structure. Thus, the optimized plasma treating time, such as 90 s for the case of PP-*g*-PSBMA#1 and 60 s for the case of PP-*g*-PSBMA#2, is required to control the grafting structure of the brush-like PSBMA layer on the target membrane surface.

Previous studies suggested that the formation of the bounded water layer on a highly hydrated surface was considered as a crucial issue to repel plasma proteins and generate the antithrombogenic surface.[23,24,30] In general, surface structures of grafted hydrophilic polymer, such as chain length and chain conformation, are associated with the capacity of water molecules on the hydrated surface. In this study, hydration capacity (mg/cm^2) of the prepared membrane is defined as the difference in wet weight between the PSBMA grafted PP membrane and the virgin PP membrane divided by the total surface area of the virgin PP membrane. In Figure 2, it should be noted that the change in surface contact angle is usually not a good indication of the degree of hydration on the highly hydrophilic surface coverage. The surface grafting coverage and surface thickness of the grafted PSBMA polymer are associated with the capacity of water molecules on the hydrated surface. Figure 5 shows that the dependence of the relative fibrinogen adsorption and hydration capacity of the treated PP membranes on the surface grafting yield of PSBMA layer can be controlled by the plasma treatment time from 0 to 120 s. It was found that there was no hydration on the virgin PP membrane with high hydrophobic property of the fibrous structures. In general, the increase in grafting yield associated with the increase in the thickness of the PSBMA layer resulted in the increased quantity of hydration capacity, which depends on the contribution of binding water molecules around the zwitterionic structure of the PSBMA brushes or captured water molecules in the confined space between cross-linked PSBMA chains. Results in Figure 5 indicated that reduction in nonspecific fibrinogen adsorption exhibited a positive correlation with the variation of the hydration capacity of PP-*g*-PSBMA membranes. Protein resistance and hydration capacity were maximized when the plasma treatment time of grafted PSBMA was 90 s for the case of PP-*g*-PSBMA#1 and 60 s for the case of PP-*g*-PSBMA#2, respectively, which is consistent with the XPS analysis of grafting structures of PSBMA layer on PP membranes summarized in Table 2. The relative protein adsorption on PP-*g*-PSBMA#2 is effectively reduced to about 5% of that on virgin PP as the hydration capacity increases to 4.8 mg/cm^2. However, the relative protein adsorption on PP-*g*-PSBMA#1 is only reduced to the limitation at 65% of that on virgin PP, even as the hydration capacity increased to 4.2 mg/cm^2. The results may be related to the types of water molecules in the hydrated layer and the grafting structure of the PSBMA layer on the PP membrane surface. For the case of PP-*g*-PSBMA#2, a plasma treatment time of 120 s with a surface contact angle similar to the case at 60 s generated relatively less protein resistance by ~50%, and the hydration capacity was reduced to 2.6 mg/cm^2.

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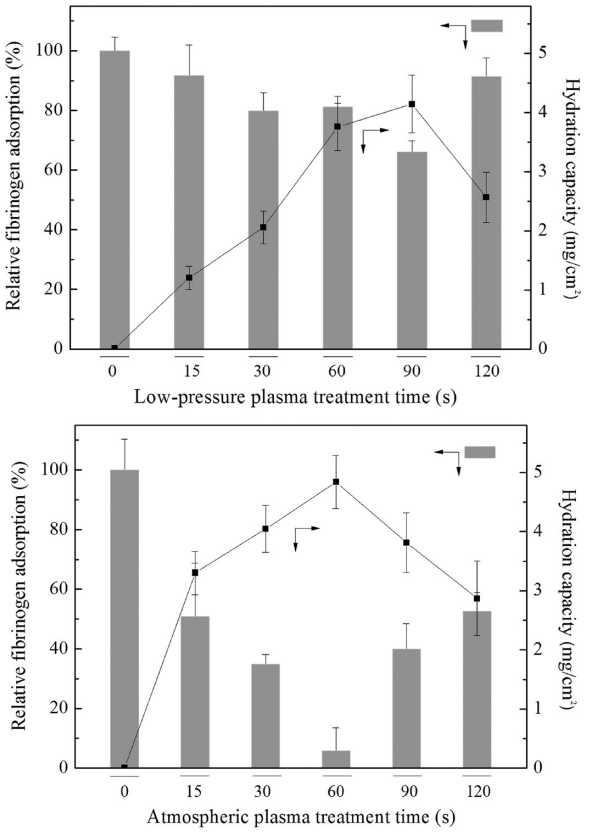

Figure 5. Changes in the relative fibrinogen adsorption and hydration capacity of the PP-*g*-PSBMA membranes with times of 0, 15, 30, 60, 90, and 120 s with (a) low-pressure plasma treatment and (b) atmospheric plasma treatment.

The results may be associated with the chemical degradation of the grafted PSBMA layer on the PP membrane, which is revealed by the XPS measurements.

Interestingly, the results showed that the hydration capacity of PP-*g*-PSBMA#1 is higher than that of PP-*g*-PSBMA#2 at the same PSBMA grafting yield of about 0.6 mg/cm^2, which is due to that the confined space for captured water molecules in the grafting structure of network-like PSBMA layer is more than that of brush-like PSBMA layer. However, it should be noted that the reduction of relative fibrinogen adsorption on the PP-*g*-PSBMA#2 membranes is more than that on PP-*g*-PSBMA#1. This might be due to that the contribution of binding water molecules around the brush-like structure of the zwitterionic PSBMA layers on the PP-*g*-PSBMA#2 membranes dominates the protein-resistant performance. The results confirmed previous statements that hydration plays a key role in the resistance of zwitterionic surfaces to nonspecific protein adsorption. Our previous study also showed that it is important to minimize the electrostatic interactions of PSBMA surfaces with plasma proteins by controlling the charge neutrality of the grafted zwitterionic PSBMA brushes.[13,23,24] In Figure 5, the inflection point in the dependence of relative fibrinogen adsorption and hydration capacity on plasma treatment time may be attributed to the overall charge balance in the grafted PSBMA layer on the PP membrane surface. A good index for the charge neutrality of the PSBMA-grafted PP membranes is defined as the compositional ratio of positively and negatively charged moieties in the PSBMA layer, which was determined by the ratio of the atomic percentages of nitrogen and sulfur (N/S ratio), as shown in Figure 4. It was found that reduction in nonspecific fibrinogen adsorption exhibited a positive correlation with the variation of the N/S ratio of PP-*g*-PSBMA membranes. The results showed that the charge bias of PP-*g*-PSBMA#1 with N/S ratio of about 1.00 ± 0.25 is higher than that of PP-*g*-PSBMA#2 with N/S ratio of about 1.00 ± 0.07 at the same range of the plasma treatment time from 15 to 90 s. Thus, the observed results clearly illustrate that the resistance of relative fibrinogen adsorption on the PP-*g*-PSBMA#2 membranes is higher than that on PP-*g*-PSBMA#1. However, the N/S ratio of PP-*g*-PSBMA#2 membrane increased to 1.12 as the plasma treatment time was further increased to 120 s, resulting in low protein resistance and reduced hydration capacity. It was shown in Figures 4 and 5 that even a slight charge bias in the grafted PSBMA layer can induce electrostatic interactions between proteins and the zwitterionic membrane surfaces, leading to surface protein adsorption. These results suggest that the general evaluation of protein adsorption on the PP-*g*-PSBMA membrane surfaces should consider not only the grafting structure and hydration capacity, but also the charge neutrality of the grafted PSBMA polymer layer.

Human Blood Compatibility of the PSBMA-Grafted PP Membranes. In general, hydrophobic membranes in contact with human blood will lead to the adsorption of plasma protein such as fibrinogen and the clotting factors on those membrane surfaces.[5,11,20,35] An introduced full-scale blood platelet adhesion then results in the formation of thrombosis and embolism at the blood contact side of membrane surfaces from the bloodstream. Previous studies showed that the adhesion and activation of platelets from the bloodstream might be correlated with the adsorption of plasma proteins on surfaces. Thus, the human platelet adhesion test has already become a recognized technique to estimate the hemocompatibility of a prepared membrane surface.[24,30,36−38] Figure 6 shows SEM images, at a magnification of 2000×, of the prepared membranes via both low-pressure and atmospheric plasma treatment in contact with recalcified PRP solution for 120 min at 37 °C in vitro. The SEM results showed the formation of thrombosis on the virgin PP fibrous membranes with full-scale platelet activation at the blood-contact site, as shown in Figure 6a and g. Results showed that platelet activation and thrombosis formation were remarkably suppressed on both types of the PP-*g*-PSBMA#1 (Figure 6b−f) and PP-*g*-PSBMA#2 (Figure 6h−l) as compared to the virgin PP membranes. It should be noted that inhibition in platelet adhesion and activation exhibited a positive correlation with the variation of nonspecific fibrinogen adsorption associated with the N/S ratio of PP-*g*-PSBMA membranes. Similar to the results in fibrinogen resistance, the inhibition of relative platelet activation on the PP-*g*-PSBMA#2 membranes is more effective than that on PP-*g*-PSBMA#1. Importantly, the results in Figures 5 and 6 showed that lower fibrinogen adsorption on the prepared membranes resulted in the less thrombogenicity induced by platelet adhesion, indicating a strong correlation between fibrinogen adsorption and platelet activation on membrane surfaces. It was found that the excellent performance of the prepared PP-*g*-PSBMA#2 membrane with the plasma treatment time at 60 s (Figure 6j) in no obvious adhesion and activation of blood platelets is due to its ability to highly resist nonspecific protein adsorption from human blood plasma. The results suggest that the well-hydrated surface of brush-like

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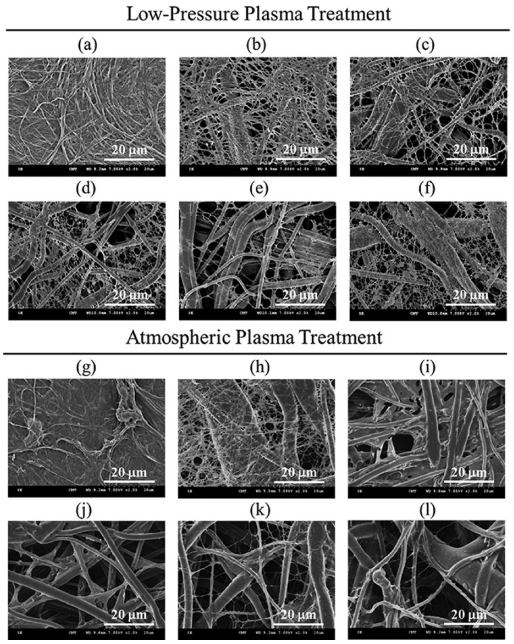

Figure 6. SEM images of platelets adhered onto the surface of the modified PP fibrous membranes with grafted PSBMA (a and g) virgin PP fibrous membrane; (b–f) the PP-*g*-PSBMA#1 membranes with low-pressure plasma treatment times of 15, 30, 60, 90, and 120 s; (h–l) the PP-*g*-PSBMA#2 membranes with atmospheric plasma treatment times of 15, 30, 60, 90, and 120 s. All images with magnification of 2000×.

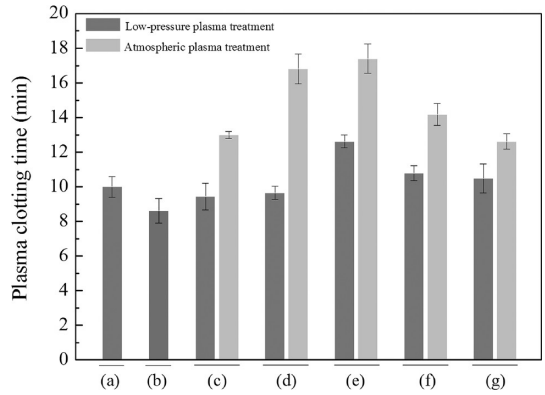

Figure 7. Plasma clotting time of recalcified platelet-poor plasma and in the presence of modified PP fibrous membranes with grafted PSBMA: (a) blank PS wells; (b) virgin PP fibers; (c) 15 s; (d) 30 s; (e) 60 s; (f) 90 s; and (g) 120 s of low-pressure and atmospheric plasma treatment, respectively. Clotting time for blank PS wells was about 10 min at 37 °C. Each clotting time is an average value of six samples.

PSBMA-grafted PP membranes with surface charge neutrality modified by atmospheric plasma-induced zwitterionization could efficiently reduce protein adsorption, suppress platelet adhesion/activation, and increase its hemocompatibility.

Nonspecifically adsorbed plasma proteins interact in a serious of reactions leading to plasma clotting, which was further tested to estimate the blood compatibility of the prepared membranes in this study. The prepared membranes of PP-*g*-PSBMA#1 and PP-*g*-PSBMA#2 were directly incubated with human plasma to inspect the effects of direct-contact activation on membrane-induced plasma clotting as evaluated by their recalcified-plasma-clotting times. In this work, 0.4 cm^2 of each modified membranes was incubated with 0.5 mL of recalcified human PRP solution in a 24-well plate at physiologic temperature of 37 °C. The plasma clotting for the recalcified plasma solutions in blank PS wells was determined to have an upper limit of plasma-clotting time of about 10 min at 37 °C for the protocol used. As shown in Figure 7, the clotting time decreased to ~8.5 min when the recalcified PRP solution was added to the hydrophobic PP fibrous membrane. The result indicates that hydrophobic membrane surface is highly activating plasma clotting through the intrinsic coagulation pathway. It was found that the average clotting time increased with the increase of membrane hydration capacity, which is attributed to the grafting structure and charge neutrality of grafted PSBMA layers on the PP membrane surface.

In Figure 7, almost no significant difference in plasma clotting time of the absence or presence of PP-*g*-PSBMA#1 membranes was observed at the membrane hydration capacity above 1.0 mg/cm^2. The results indicate that the network-like PSBMA cross-linking layer on PP-*g*-PSBMA#1 membrane surface does not activate plasma clotting through the intrinsic coagulation pathway. However, it is interesting to observe that when the recalcified PRP solution was added to the PP-*g*-PSBMA#2 membrane, the average clotting time increased from 13 to 18 min at the membrane hydration capacity above 3.0 mg/cm^2. The clotting time of PRP was prolonged and increased with the hydration capacity of the grafted PSBMA polymer, indicating an anticoagulant activity of brush-like PSBMA layer on PP-*g*-PSBMA#2 membranes with balanced charge neutrality. Plasma-clotting time and anticoagulant activity were maximized when the atmospheric plasma treatment time was at 60 s, which clearly indicates that the PP-*g*-PSBMA membrane has a charge-balanced dependence on anticoagulant activity or contact activation to prevent or activate, respectively, plasma clotting in human blood. The inflection point in the dependence of anticoagulant activity on PP-*g*-PSBMA membranes may be due to the formation of the bounded water layer on a well-hydrated zwitterionic PSBMA layer with overall charge neutrality. Thus, the general concept for preparing antithrombogenic membranes from hydrophobic PP fibrous materials could be performed in the ease of atmospheric plasma-induced surface zwitterionization, but it should concern the issue of surface grafting coverage and structures of zwitterionic nonfouling nature in human blood.

Stable hemocompatibility of biomedical membranes used in contact with human whole blood is highly desirable for hemodialysis devices or blood filters.[5] In general, a multistep process of plasma protein adsorption, blood platelet adhesion, and blood cell attachment strongly affects the different stages of thrombotic response induced by the blood-contacting membranes. In this study, the extent of blood cell attachment from human whole blood was further observed by direct blood–membrane contact for the inspection of overall hemocompatibility of the PP fibrous surfaces grafted with different coverage of PSBMA layers. Figure 8 shows LSCM images of blood cells that attached to the PSBMA-grafted surfaces by contact of the prepared membranes with 100% human whole blood for 120 min at 37 °C in vitro. Attachment of blood cells on membrane surfaces from human whole blood is considered to be a challenging process, comparable to that from PRP solution. The LSCM results show that a relatively low amount of blood cell attachment was observed on the membrane surfaces of PP-*g*-PSBMA#2 (Figure 8h–l) from whole blood as compared to

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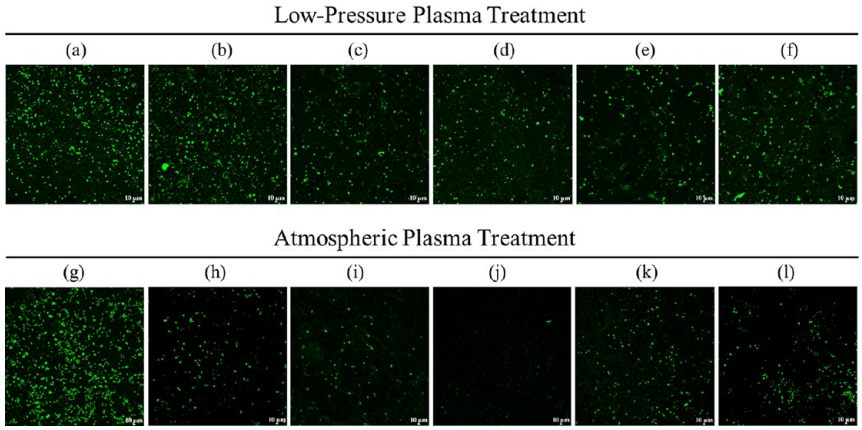

Figure 8. LSCM images of whole blood cells adhered onto the surfaces of the modified PP fibrous membranes with grafted PSBMA (a and g) virgin PP fibrous membrane; (b–f) the PP-*g*-PSBMA#1 membranes with low-pressure plasma treatment times of 15, 30, 60, 90, and 120 s; (h–l) the PP-*g*-PSBMA#2 membranes with atmospheric plasma treatment times of 15, 30, 60, 90, and 120 s. All images are shown at a magnification of 200×.

that of the full-scale attached blood cells on the hydrophobic PP fibrous surface (Figure 8a and g) and the membrane surfaces of PP-*g*-PSBMA#1 (Figure 8b–f). It clearly shows that the excellent blood–inert membrane surface of the PP-*g*-PSBMA#2 in Figure 8j can be achieved by atmospheric plasma-induced surface zwitterionization, which was also shown in Figure 6j to completely resist platelet adhesion and activation. Statistical quantitative data of relative blood cell attachment on prepared membrane surfaces are presented in Figure 9 via the

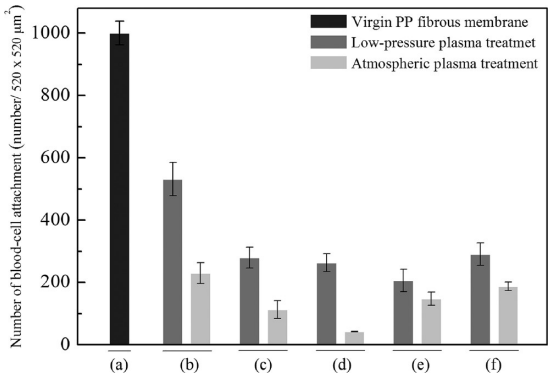

Figure 9. Number of blood-cell attachment and in the presence of modified PP fibrous membranes with grafted PSBMA: (a) blank PS wells; (b) 15 s; (d) 30 s; (e) 60 s; (f) 90 s; and (g) 120 s of low-pressure and atmospheric plasma treatment, respectively.

image analysis using SimplePCI software. Blood-cell attachment from human whole blood is quite similar to plasma protein adsorption from PPP solution and blood platelet adhesion from PRP solution onto the surfaces of PSBMA-grafted PP membranes. The resistance of blood-cell attachment on the PP-*g*-PSBMA#2 membranes is higher than that on PP-*g*-PSBMA#1. Importantly, the PP-*g*-PSBMA#2 membrane with the plasma treatment time at 60 s in Figure 5, Figure 6j, and Figure 8j showed that the strong resistance of blood cell attachment as well as plasma protein adsorption and blood platelet adhesion were observed. These results indicate that the PP membrane prepared by atmospheric plasma-induced surface zwitterionization is able to achieve an effective nonfouling membrane in human whole blood when the surface structure and charge neutrality of grafted PSBMA layer are properly controlled.

CONCLUSIONS

In this work, PSBMA-grafted PP fibrous membranes with controllable grafting qualities and blood compatibility were obtained via plasma-induced surface zwitterionization. The correlation of surface grafting structures, charge neutrality, hydrophilicity, and hydration capability with blood compatibility of the membranes was systematically determined. Blood compatibility of the zwitterionic PP membranes was carefully evaluated by plasma protein adsorption, platelet adhesion, plasma-clotting time, and whole blood-cell attachment. The present study has shown that brush-like PSBMA layer on the PP membrane surface using the atmospheric plasma technique is a promising approach to the preparation of membrane with limited cross-linking, low chemical degradation, and high balanced charge neutrality of zwitterionic PSBMA-grafted structure, which is different from the general observation using low-pressure plasma treatment. The results showed that the zwitteironic PP membrane exhibited an antifouling character for plasma-protein and blood-platelet resistance that depended on the surface grafting coverage and structures of the PSBMA layer on PP membranes. Importantly, the membrane grafted with the optimized hydration of PSBMA-grafted layer presented excellent blood-inert property and anticoagulant activity in human blood, which was attributed to the formation of a strong interfacial hydration layer due to the binding of water molecules around un-cross-linked and electrical neutral PSBMA chains. This study suggests that the hemocompatible zwitterinoic PP membranes by controlling grafting hydrated PSBMA structures from plasma-induced surface zwitterionization give them great potential in the ideal design of antithrombogenic membranes for use in human whole blood.

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Notes

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