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Excellent Biocompatible Polymeric Membranes Prepared via Layer-By-Layer Self-Assembly

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ABSTRACT: Excellent biocompatible polymeric membranes were prepared by combining the antifouling property of poly(ethylene glycol) methyl ether (mPEG) and the anticoagulant property of poly(sodium *p*-styrene sulfonate) (PSS). Block copolymers of poly(ethylene glycol) methyl ether-*b*-poly(sodium *p*-styrene sulfonate) (mPEG-*b*-PSS) with different chain lengths were synthesized by ATRP using mPEG macroinitiator. The copolymers were then used to modify polyethersulfone (PES) membrane via layer-by-layer (LBL) self-assembly technology. The chemical compositions, surface morphologies and hydrophilicity of the modified membranes were characterized, indicating that the mPEG-*b*-PSS copolymers were successfully deposited on the membranes surfaces. Then, the blood compatibility and cytocompatibility of the modified membranes were systematically investigated. The results indicated that the mPEG-*b*-PSS copolymers could improve the hydrophilicity and the resistance to protein adsorption, and had great effect on suppressing platelet adhesion, prolonging clotting times, and improving cytocompatibility. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41245.

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INTRODUCTION

Good biocompatibility, including blood compatibility and cytocompatibility, is highly desirable for blood-contacting devices, such as hemodialyzer, artificial organs, and blood purification instruments.[1–3] Controlling the surface property of such biomaterials is of crucial importance for their design of biomedical materials and blood-contacting devices.[4–6] In blood-material interactions, protein adsorption on material surfaces is the first event of many undesired biological reactions and responses, followed which were platelet adhesion and activation of coagulation pathways, and then leading to thrombus formation.[7–10] For the biomaterials that will be in contact with human body only for a short period of time, superlow or nonfouling property (prevention of nonspecific protein adsorption) of the materials can help to improve the biocompatibility in return. Thus, antifouling property is very important in designing biomedical materials.

In recent years, many hydrophilic polymers including poly(acrylic acid) (PAA),[11] poly(*N*-vinylpyrrolidone) (PVP),[12] polyethylene glycol (PEG) or its derivatives,[13–16] and zwitterionic polymers[17–19] were utilized to modify materials with enhanced hydrophilicity and antifouling property, since these polymers can form a hydration layer via electrostatic interaction, hydrogen bond or ionization.[20,21] PEG and its derivatives are widely used in preparing antifouling materials since they possess many required physical and biochemical properties, such as biocompatibility, nontoxicity, nonantigenicity, and miscibility with many solvents.[15,22] On the other hand, many studies have been reported on developing new synthetic anticoagulants or antithrombogenic materials with heparin-like structure to mimic the activity of heparin.[23,24] Most of these materials are composed of ionic polymers containing sulfate, sulfamide and carboxylic acid groups, since it is believed that the anticoagulant activity of heparin is caused by the presence of these ionic functional groups.[25,26] Poly(sodium *p*-styrene sulfonate) (PSS) and sodium *p*-styrene sulfonate based copolymers own sulfonic acid groups and have been used in many applications, such as surface modification, proton exchange membranes, ion exchange resins, polymeric stabilizers for emulsion polymerization.[27–30] Due to

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

the sulfonic acid groups in the PSS chain, we can deduce that PSS could improve the blood compatibility (especially the anticoagulant property) of modified materials. Herein, it is practicable to endow membranes with antifouling property and anticoagulant property by combining PEG and PSS.

In order to improve the biocompatibility of biomedical materials, surface modification is an applicable choice to manipulate and control the surface property.[31] Many surface modification techniques including UV grafting, coating, surface-initiated atom transfer radical polymerization (SI-ATRP) have been employed to immobilize hydrophilic groups, negative charged groups, biological macromolecules (such as bovine serum albumin, collagen and heparin), and planting endothelial cell onto the surfaces.[12,20] For both fundamental and applied studies in the biomedical field, the surface design and control of substrates with nanometer- or micrometer-sized multilayers are of considerable interest,[32] which can be realized by layer-by-layer (LBL) assembly. LBL assembly has become a versatile technique for fabricating tailored thin films of diverse composition, and the multilayer is usually assembled through noncovalent electrostatic and hydrogen-bonding interactions.[33–35]

In this study, we aimed to fabricate excellent biocompatible membranes via a simple and efficient method. Thus, layer-by-layer (LBL) self-assembly technology was chosen, and the cationic polyelectrolytes used in the LBL were poly(ethylene glycol) methyl ether-*b*-poly(sodium *p*-styrene sulfonate) (mPEG-*b*-PSS) block copolymers with different PSS chain lengths, which were synthesized via atom transfer radical polymerization (ATRP). Though similar copolymers had been synthesized in previous reports,[27,30] the biocompatibility was not investigated. The membrane blood compatibility, including antifouling property (prevention of nonspecific protein adsorption), platelet adhesion, activated partial thromboplastin time (APTT) and thrombin time (TT), and the cytocompatibility, including MTT assay and cell morphology, were systematically investigated. The effect of the PSS chain length on the membrane performances was also studied.

EXPERIMENTAL

Materials

Polyethersulfone (PES, Ultrason E6020P, BASF, Germany), poly (ethylene glycol) methyl ether (mPEG5000, Aldrich, China), 2-bromoisobutyryl bromide (BiBB, 97%, Aladdin), 4-dimethylaminopyridine (DMAP, 99%, Aladdin), sodium *p*-styrene sulfonate (NaSS), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, 99%, Sigma-Aldrich), ethyl 2-bromoisobutyrate (EbiB, 98%, Aladdin), sodium chloride (NaCl, 99%, Aladdin), sodium dihydngen phoshate (NaH_2PO_4, 99%, Aladdin), dibasic sodium phosphate (Na_2HPO_4, 99%, Aladdin) and polyethyleneimine (PEI, 50%, $M_w = 70,000$ g/mol, Aladdin) were used as received. Dichloromethane (CH_2Cl_2, 99.5%, Aladdin) and triethylamine (TEA, 99.5%, Alladin) were distilled before use. Copper (I) bromide (CuBr, 98%, Aladdin) was purified according to the reported procedures.[36] Isotonic phosphate-buffered saline solution (PBS, pH 7.4) was prepared by NaCl, NaH_2PO_4, and Na_2HPO_4. Bovine serum albumin (BSA, fraction V, ≥95%), bovine serum fibrinogen (BFG, ≥75%) and bovine serum γ-globulins (99%) were obtained from Sigma-Aldrich of America. Micro BCA™ protein assay reagent kits were purchased from PIERCE of America. APTT and TT reagent kits were purchased from SIEMENS. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich of America. Deionized water was used throughout the studies.

Synthesis of the Block mPEG-*b*-PSS Copolymers

Poly(ethylene glycol) methyl ether-*b*-poly(sodium *p*-styrene sulfonate) (mPEG-*b*-PSS) block copolymers were synthesized by ATRP method as shown in Scheme S1. The macroinitiator brominated poly(ethylene glycol) methyl ether (mPEG-Br) was synthesized as follows:[37,38] mPEG (10.0 g, 2.0 mmol) and DMAP (1.0 g, 8.2 mmol) were mixed with TEA (2.0 mL, 14.0 mmol) in 80 mL of CH_2Cl_2 in a three-neck round-bottom flask equipped with a condenser and magnetic stirrer. After cooling to 0°C, BiBB (3 mL, 24.3 mmol) in 37 mL of CH_2Cl_2 was added dropwise into the solution under nitrogen over 30 min, and then the temperature was allowed to rise to room temperature. After stirring for another 24 h, the solvent was removed by a rotary evaporator. The crude mPEG-Br was precipitated in cold absolute ethyl ether, recrystallized in absolute ethanol for three to four times, and dried *in vacuo* at room temperature for 48 h.

The mPEG-*b*-PSS copolymers with different PSS chain lengths were then synthesized by ATRP as follows: mPEG-Br (1.0 mmol), NaSS (50/100/400 mmol), PMDETA (173.0 mg, 1.0 mmol) and CuBr (143.0 mg, 1.0 mmol) were dissolved in water/methanol (3/1, v/v %) solution in a Schlenk flask. Then the flask was subjected to three freeze-pump-thaw cycles and sealed off *in vacuo* before being kept in 30°C. When the reaction was conducted for 12 h, the polymerization was terminated by exposure to air, and the crude product was concentrated by a rotary evaporator at 40°C. Then the product was dissolved in water and precipitated into THF (this precipitation clean-up was repeated for three times) to remove the unreacted initiator, monomer and ligand. The resulting polymer was purified by dialysis with water followed by freeze-drying. Additionally, PSS homopolymer was also synthesized by using EbiB as the ATRP initiator.

Polymer Multilayers Prepared by LBL Assembly

Polymer multiplayers were deposited on PES membrane by LBL assembly. The concentrations of polyelectrolytes solutions were 3 mg/mL both for PEI and mPEG-*b*-PSS copolymers (or PSS) with 0.5*M* NaCl. Since PES membrane is negatively charged, the first layer adsorbed is a polycation,[39] and PEI was chosen due to its high degree of branching and high surface charge density to promote anchoring of the first layer onto the PES membrane.[40] In a typical procedure, PES membrane was dipped into a polyelectrolyte solution for 15 min at room temperature, then washed with deionized water to remove loosely bound polyelectrolyte, and then dipped into the next polyelectrolyte solution. This cycle was repeated until the desired numbers of layers were achieved. The $(PEI/PSS100)_n$ multilayer deposited membrane represented that the membrane was modified with PEI and mPEG-*b*-PSS100 block copolymer by LBL; and so did the

others. In particular, $(PEI/PSS0)_n$ multilayer represented that PSS was used in the LBL.

To measure the thickness of the polymer multilayer, spectroscopic ellipsometry was utilized and silicon wafer was chosen as the substrate due to its smooth surface. The pretreatment of silicon wafer was carried out according to the published procedure.[41] The polymer multilayer was deposited on the silicon wafer at the same condition as that for the PES membrane.

Characterizations

Polymer Characterization. ^{1}H NMR measurements were recorded on a Bruker AVII-400 MHz spectrometer (Bruker Co., Germany) with deuterated water for all the synthesized polymers. The molecular weight was measured on a gel permeation chromatography (GPC) instrument, which was performed on a Waters-1515 against polyethylene oxide (PEO) standards using water (proper ionic strength) as an eluent. The sample concentration was 2 to 3 mg/mL, and the flow rate was 1000 mL/min at 40°C. Elemental analysis based on the determination of carbon (C), hydrogen (H) and sulfur (S), was performed by means of a CARLO ERBA 1106 elemental analyzer (Italy), with a carrier gas (He, at a flow rate of 100 mL/min) at a combustion temperature of 1000°C using the solid samples. The sodium in the copolymers was removed via ion exchange column prior to elemental analyses.

Surface Characterization. Ellipsometry measurements were performed on a spectroscopic Sentech SE 850 ellipsomter (Sentech, Japan). Spectroscopic data were acquired between 400 and 800 nm with a 2 nm increment, and the thicknesses were extracted with the integrated software by fitting with a classical wavelength dispersion model. Scanning electron microscopy (SEM) images for platelet and cell morphology were observed by using JSM-7500F scanning microscope (JEOL, Japan) with the voltage of 5 kV. X-ray Photoelectron Spectroscopy (XPS) was used to characterize the surface compositions of the samples. A Kratos AXIS ULTRA^{DLD} XPS Instrument employing Al Kα excitation radiation was used. Water contact angles (WCAs) were measured and calculated in static mode on a contact angle goniometer (Dataphysics OCA20, Germany) equipped with a video capture at ambient temperature. One drop of water (3 μL) was dropped on the surface of the membrane with an automatic piston syringe and photographed. Three spots were performed for each sample.

Blood Compatibility

Plasma Collection. Healthy human fresh blood (man, 26 years old) was collected by using vacuum tubes (6 mL, Terumo Co.), containing citrate/phosphate/dextrose/adenine-1 mixture solution (CPDA-1) as the anticoagulant (anticoagulant to blood ratio, 1:9).[42] The blood was centrifuged at 1000 rpm (or at 4000 rpm) for 15 min to obtain platelet-rich plasma (PRP) (or platelet-poor plasma, PPP).

All the blood compatibility experiments were performed in compliance with the relevant laws and institutional guidelines.[43]

Protein Adsorption Experiment. Protein adsorption experiments were carried out with BSA and BFG solutions to evaluate the antifouling property of the modified membranes. The protein (BSA or BFG) was dissolved in PBS with a concentration of 1 mg/mL. The membranes with an area of 1 × 1 cm^2 were placed in individual wells of 24-well tissue culture plate and equilibrated with PBS overnight, and then incubated in the protein solution at 37°C for 2 h under static conditions. After the adsorption process, the membrane was gently rinsed with PBS three times and then immersed in 2 wt % aqueous sodium dodecyl sulfate (SDS) solution at 37°C for 1 h under agitation to remove the proteins adsorbed onto the membrane. The amount of protein was quantified by Micro BCA^{TM} protein assay reagent kits (detection limits: 0–20 μg/mL), and the absorbance at 562 nm of protein solution was recorded on an UV-1750 UV-Vis spectrophotometer (Shimadzu, Japan). Independent measurements were performed in triplicate samples and the total amounts of the adsorbed proteins were calculated from the concentration of the standard protein solution.

Platelet Adhesion. According to the method reported previously,[44] the membranes (1 × 1 cm^2 each piece) were placed in individual wells of 24-well tissue culture plate with PBS overnight and equilibrated at 37°C for 1 h. Then the PBS was removed and 0.4 mL of fresh PRP was introduced. The membrane was incubated with PRP at 37°C for 2 h under static conditions. Then the PRP was decanted off and the membrane was rinsed three times with PBS. Finally, the membrane was treated with 2.5 wt % glutaraldehyde in PBS at 4°C for about 1 day. To prepare SEM sample, the membrane was washed with PBS, subjected to a series of graded alcohol-PBS solutions (25%, 50%, 70%, 75%, 90%, 95%, and 100%) and isoamyl acetate-alcohol solutions (25%, 50%, 75%, and 100%) for 15 min each time. The critical point drying of the specimens was done with liquid CO_2. The specimen was sputter-coated with a gold layer and examined by SEM. The number of the adherent platelets on the membrane was calculated according to five SEM images at 1000× magnification from different places on the same membrane.

Clotting Times. Activated partial thromboplastin time (APTT) and thrombin time (TT) were measured by an automated blood coagulation analyzer CA-50 (Sysmex Co., Japan), and platelet-poor plasma (PPP) was used in the experiment.[12] APTT was measured as follows: the membrane with an area of 0.5 × 0.5 cm^2 (four pieces) was immersed in 0.2 mL PBS (pH 7.4) for 1 h, then the PBS was removed and 100 μL of fresh PPP was introduced. After incubating at 37°C for 30 min, 50 μL of the incubated PPP was added into a test cup, followed by the addition of 50 μL of APTT agent (incubated 10 min before use). After incubating at 37°C for 3 min, 50 μL of 25 m*M* $CaCl_2$ solution was added, and then the APTT was measured. For TT, 100 μL of fresh PPP was introduced and the sample was incubated at 37°C for 30 min. Then 50 μL of the incubated PPP was added into a test cup, followed by the addition of 100 μL of TT agent (incubated 10 min before use), and then the TT was measured. At least three measurements were averaged to get a reliable value.

Cytocompatibility

Cell Morphology on the Membrane. For cell morphology observation, endotheliocytes were seeded onto the membrane at

Table I. Reaction Conditions and Average Molecular Weights for mPEG-*b*-PSS Copolymers Synthesized by ATRP

Sample	Macroinitiator/ NaSS/CuBr/PMDETA	M_n[a] (g/mol)	M_n[b] (g/mol)	M_w/M_n[b]	PSS[b] (wt %)
mPEG			6,113	1.13	0
mPEG-Br			6,912	1.09	0
mPEG-*b*-PSS50	1/50/1/1	9,715	8,253	1.21	48
mPEG-*b*-PSS100	1/100/1/1	12,921	11,212	1.24	60
mPEG-*b*-PSS400	1/400/1/1	21,280	19,262	1.28	77
PSS[c]	1/100/1/1		21,697	1.19	100

[a] Determined by elemental analysis measurement.
[b] Determined by GPC measurement with reference to PEO standards.
[c] The ATRP initiator was ethyl 2-bromoisobutyrate (EbiB).

a density of approximately 2.5×10^4 cells/cm^2. After 6 days, the seeded membrane was immediately rinsed with PBS and fixed with 2.5 wt % glutaraldehyde in PBS at 4°C for 1 day. Then, the sample was subjected to a drying process by immersing them in a series of graded alcohol-PBS solutions (25%, 50%, 70%, 90%, 95%, and 100%) and isoamyl acetate-alcohol solutions (25%, 50%, 75%, and 100%) for 15 min each time. The critical point drying of the specimens was done with liquid CO_2. The specimen was sputter-coated with a gold layer and examined by SEM.

MTT Assay. After cell culture for 2, 4, and 6 days, the viability of the endotheliocytes was determined by MTT assay. Endotheliocytes were seeded onto the membrane at a density of approximately 2.5×10^4 cells/cm^2. The same cells cultured in wells without membrane sample were served as the control in this study. After the determined time intervals, 45 μL of the MTT solution (1 mg/mL in the test medium) was added to each well and incubated for 4 h at 37°C. Mitochondrial dehydrogenases of viable cells cleave selectively to the tetrazolium ring, yielding blue/purple formazan crystals. Then 400 μL of ethanol was added to dissolve the formazan crystals. Therefore, the quantity of the formazan crystals dissolved in the ethanol can reflect the level of the cell metabolism. The dissolvable solution was jogged homogeneously in about 15 min by a shaker. The solution of each sample was aspirated into a microtiter plate and the optical density of the formazan solution was read on the Microplate reader (Model 550, Bio-Rad) at 492 nm. All the experiments were repeated three times, and the results were expressed as mean ± SD.

RESULTS AND DISCUSSION

Synthesis of mPEG-*b*-PSS Copolymers by ATRP

In this study, three block copolymers (mPEG-*b*-PSS50, mPEG-*b*-PSS100, and mPEG-*b*-PSS400) with different chain lengths were synthesized by ATRP using the same macroinitiator mPEG-Br. The chain lengths of the PSS in the copolymers were controlled by adjusting the amount of the monomer used in the polymerization, while the length of mPEG was kept constant (mPEG5000). The reaction conditions and the average molecular weights for the copolymers are shown in Table I. The ATRP method provides the mPEG-*b*-PSS copolymers with controlled molecular weights (M_n) and narrow polydispersities (M_w/M_n = 1.2–1.3). The narrow polydispersities indicate the well-controlled polymerization using the ATRP method. The ^{1}H NMR spectra of mPEG, mPEG-Br and mPEG-*b*-PSS100 are shown in Supporting Information Figure S1.

Surface Characterization

Thickness of Polymer Multilayer Measured by Spectroscopic Ellipsometry. In order to measure the thickness of the polymer multilayer, silicon wafer was chosen as the substrate; since PES membrane sample could not be used for spectroscopic ellipsometry, and its polarized light could not reach the requirement of the instrument. The thickness of the samples with different bilayer numbers were measured by spectroscopic ellipsometry, as shown in Supporting Information Figure S2. The thickness of one bilayer was about 3.1 nm for the $(PEI/PSS)_n$ deposited membranes, and the thickness showed evident linear relation with the bilayer numbers.

Chemical Compositions of Membrane Surfaces. XPS was utilized to track the surface chemical compositions, and the spectra of PES, PES/$(PEI/PSS100)_3$ and PES/$(PEI/PSS100)_{12}$ are shown in Figure 1 and Table II. After depositing the polymer

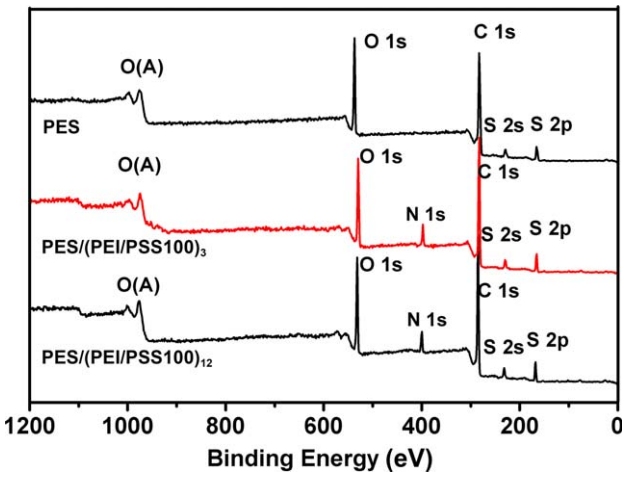

Figure 1. XPS spectra for the pristine PES, $(PEI/PSS100)_3$ and $(PEI/PSS100)_{12}$ modified membranes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Elemental Surface Compositions of the Membranes Determined by XPS

	Atom quantity percentage (%)			
Sample	C	N	O	S
PES	66.08		22.86	11.06
PES/(PEI/PSS100)$_3$	65.43	4.97	20.33	9.27
PES/(PEI/PSS100)$_{12}$	64.33	6.81	19.69	9.17

multilayers on PES membranes, the element *N* was detected for PES/(PEI/ PSS100)$_3$ and PES/(PEI/PSS100)$_{12}$ membranes and the content increased with the increase of bilayer number from 4.97 and 6.81% as shown in Table II. Simultaneously, the contents of the element C, O, and S slightly decreased with the increase of the bilayer number. Though the content changes were not obvious for C, O, and S, the content change for N was obvious, indicating that the PEI and mPEG-*b*-PSS copolymers (or PSS) were successfully deposited onto the PES membrane and the thicknesses of the multilayers were increased with the bilayer numbers. The thicknesses of the multilayers were also measured by spectroscopic ellipsometry and exhibited similar results as shown in Supporting Information Figure S2.

Hydrophilicity of the Membranes. Water contact angle (WCA) is usually used to characterize the hydrophilicity of membrane surface, which provides wettability property of the membrane surface.[45] As shown in Figure 2, the WCA for PES membrane was about 75° and the WCAs for the (PEI/PSS0)$_n$ multilayer modified membranes were about 52°. For the (PEI/PSS50)$_n$ and (PEI/PSS100)$_n$ multilayer modified membranes with different bilayer numbers, the WCAs were about 35°. For the (PEI/PSS400)$_n$ multilayer modified membranes, the WCAs were about 50°. These results indicated that the (PEI/PSS0)$_n$ multilayer could improve the hydrophilicity of the membranes; after that mPEG was introduced in the copolymers, the multilayer could further improve the hydrophilicity, and the multilayer with shorter PSS chains showed better hydrophilicity. Thus, mPEG in the block copolymers could efficiently improve the hydrophilicity of the membranes.

Blood Compatibility of the Modified PES Membranes

Antifouling Property. To measure the antifouling property of the modified membrane, BSA adsorption and BFG adsorption were studied, and the results are shown in Figure 3. The adsorbed amount of BSA for the pristine PES membrane was about 22 μg/cm^2. For the (PEI/PSS0)$_n$ multilayer assembled membranes, the adsorbed amounts were about 15 μg/cm^2 for all the membranes with different bilayer numbers. For the (PEI/PSS50)$_n$ and (PEI/PSS100)$_n$ multilayer assembled membranes, the adsorbed amounts of BSA were about 2 μg/cm^2 for all the membranes with different bilayer numbers, indicating that even 3 bilayers could efficiently decrease the BSA adsorption. However, the (PEI/PSS400)$_n$ multilayer assembled membranes with more bilayers exhibited lower adsorbed amount of BSA. Similar results were found for the BFG adsorption for all the membranes, for which the adsorbed amounts of BFG for the pristine PES, (PEI/PSS0)$_n$, (PEI/PSS50)$_n$, (PEI/PSS100)$_n$, and (PEI/PSS400)$_n$ were about 16, 13, 1.5, 1.5, and 4 μg/cm^2, respectively. Thus, we could make a conclusion that the (PEI/PSS50)$_n$ and (PEI/PSS100)$_n$ multilayer modified membranes were antifouling. In our previous study,[46] poly(sulfobetaine methacrylate) (PSBMA) was grafted from polysulfone (PSf) membrane, and the BSA and BFG adsorption for the modified membranes were

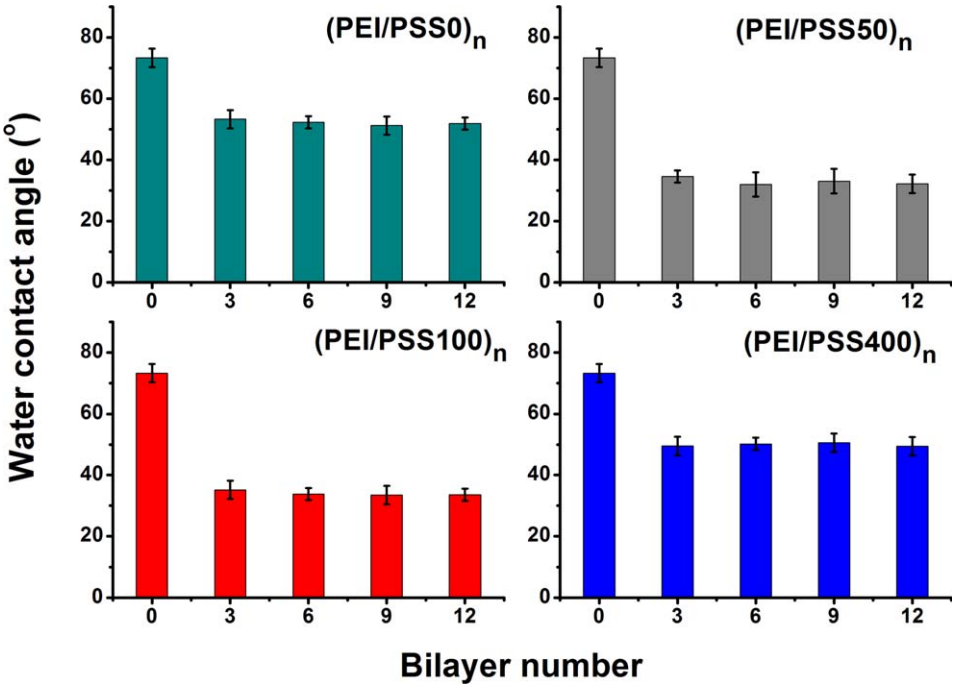

Figure 2. Water contact angles (WCAs) of the membranes with different bilayer numbers. Values are expressed as mean ± SD, $n = 3$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

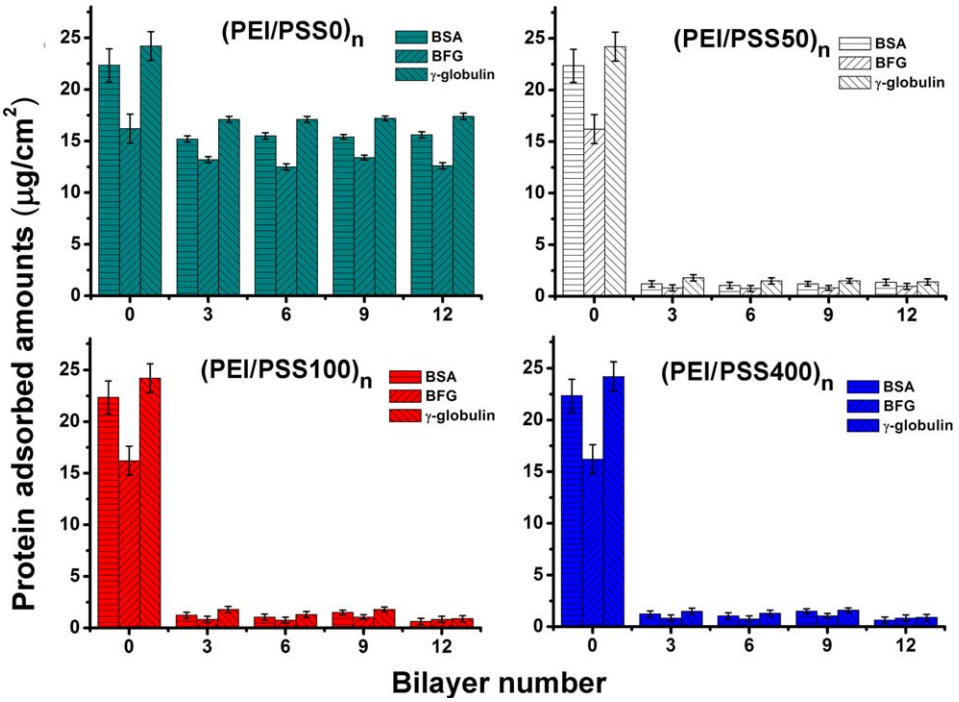

Figure 3. Protein adsorptions by membranes with different bilayer numbers. Values are expressed as means ± SD, $n = 3$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

about 3 and 2 $\mu g/cm^2$, respectively. Thus, the prepared membranes showed similar protein adsorption results.

As the PSS and proteins were negatively charged in PBS (pH = 7.4) and electrostatic repulsion was existed between membrane surfaces and proteins, the membranes with long PSS chain length should be more antifouling (lower protein adsorption). However, the results of the protein adsorption experiments were opposite. The seemingly inconsistent results could be explained by the interface interaction between the membrane surface and protein. There are many factors that affect the protein adsorption amounts and the interaction between membrane surface and protein, such as surface hydrophilicity (or hydrophobicity), surface charged character, surface free energy and topological structure, solution environment (e.g. pH, ionic strength, and protein concentration), and protein characters.[44] According to the water contact angle results, the hydrophilicity of the membranes was decreased with the increase of the PSS chain length. For the protein adsorption experiments, the adsorbed amounts onto the membranes were also increased with the PSS chain length, which was in agreement with decreased hydrophilicity of the membrane surfaces. Therefore, the hydrophilicity (or hydrophobicity) had greater effect than the electrostatic repulsion on the protein adsorption results.

The above results indicated that $(PEI/PSS0)_n$ multilayer could improve the resistance to protein adsorption; after that mPEG was introduced in the block copolymers, the $(PEI/PSS)_n$ multilayer could further improve the antifouling property, and the multilayers with shorter PSS chains (the PEG chain is relatively longer) exhibited better antifouling property. Thus, the PSS chain lengths had effect on the antifouling property and it was important to control the copolymer composition. In other words, short PSS chains (such as mPEG-*b*-PSS50 and mPEG-*b*-PSS100) had excellent antifouling property, and long PSS chains (such as mPEG-*b*-PSS400) had relatively poor antifouling property. These results were in consistent with the results of the hydrophilicity measurement and the mPEG in the block copolymers could efficiently decrease the protein adsorption of the membranes

Platelet Adhesion. The adhesion and activation of platelets on a membrane surface were then used to evaluate the blood coagulation of the modified membranes. The activated platelets can activate many kinds of coagulation factors, which then result in thrombus on the material surface.[47] Therefore, *in vitro* platelet adhesion test can be carried out to investigate the blood compatibility of material surfaces. Once the platelets were activated, "pseudopodia" would stretch out, accompanied by the aggregation of the platelets.[48] The typical SEM images of the platelets adhering on different modified membranes are shown in Figure 4. For the pristine PES membrane, numerous platelets aggregated and accumulated on the surface (about 3.87×10^7 cell/cm^2), and spread in flattened and irregular shapes; moreover, pseudopodia were observed. After depositing the polymer multilayers, the numbers of adherent platelets were significantly decreased as shown in Figure 4(b). For the $(PEI/PSS0)_n$ multilayer assembled membranes, few platelets were observed on the surfaces and the pseudopodia were not obvious. The $(PEI/PSS50)_n$, $(PEI/PS100)_n$, and $(PEI/PSS400)_n$ multilayer assembled membranes also showed fewer amounts of adhered platelets compared with that of the pristine PES membrane, and the amounts decreased with the increase of the PSS chain length for

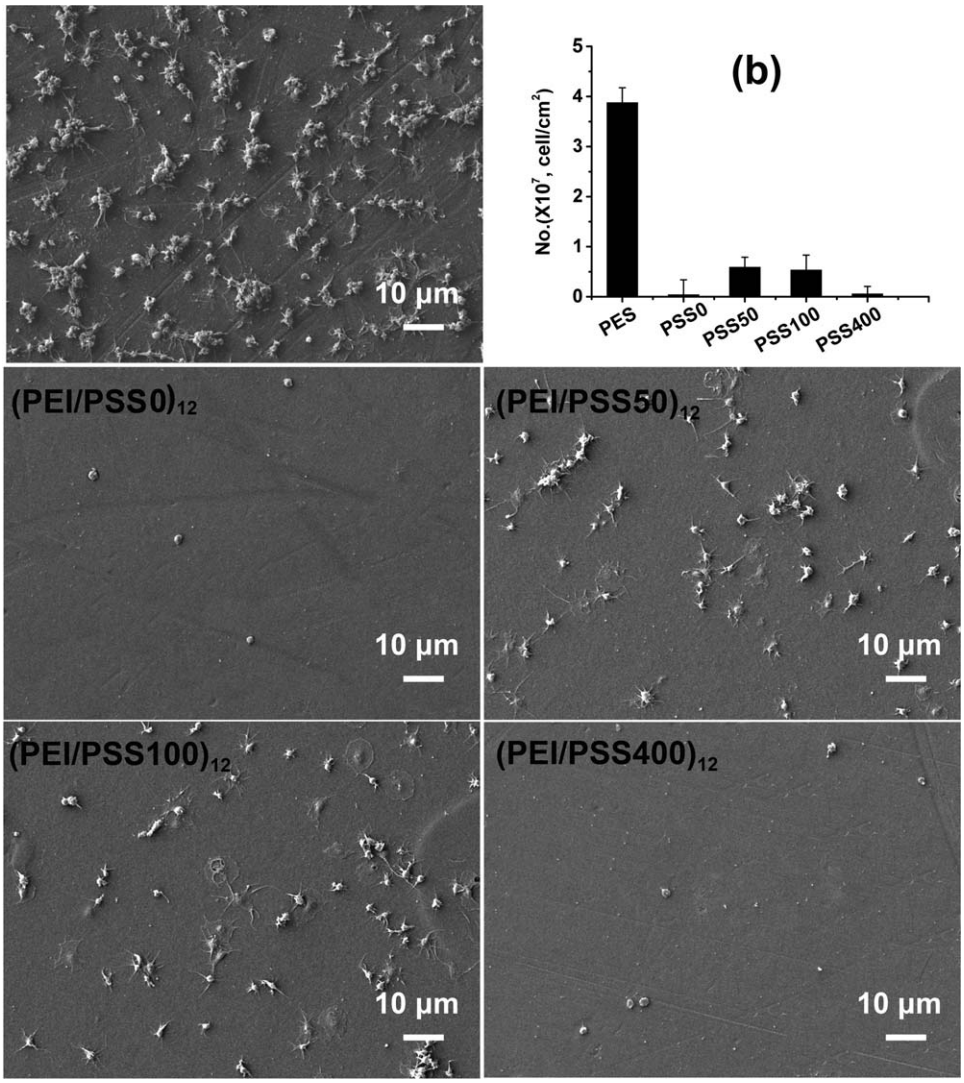

Figure 4. SEM images of the platelets adhering onto the membranes with the magnification of ×1000 (b is the number of the adhering platelets onto the membranes from platelet-rich plasma estimated by SEM images).

the $(PEI/PS100)_n$ and $(PEI/PSS400)_n$ multilayer modified membranes. These results indicated that the PSS and mPEG-*b*-PSS copolymers with long PSS chains could suppress platelet adhesion, and the PSS in the block copolymers could efficiently suppress the platelet adhesion for the modified membranes.

Clotting Times. The APTT and TT tests are widely used for clinical detection of the abnormality of blood plasma and for the primary screening of the anticoagulative chemicals and they are recently applied in the evaluation of the *in vitro* antithrombogenicity of biomaterials.[49,50] The results of APTT and TT for the modified membranes are shown in Figure 5 and Supporting Information Figure S3. It was found that the APTTs for the $(PEI/PSS0)_n$ multilayer modified membranes were prolonged about 40 s compared with that for pristine PES membrane (53 s), which indicated that the $(PEI/PSS)_n$ multilayer could obviously improve the anticoagulation of the membranes. Furthermore, when the $(PEI/mPEG\text{-}b\text{-}PSS)_n$ multilayer was deposited onto PES membranes, the APTTs were different for the block copolymers with different PSS chain lengths. The APTTs for the $(PEI/PSS50)_n$, $(PEI/PSS100)_n$, and $(PEI/PSS400)_n$ multilayer modified membranes were about 74 s, 75 s, and 88s, respectively. These results indicated that PSS and mPEG-*b*-PSS copolymers could improve the anticoagulation property of the membranes, and the copolymer with longer chain of PSS segment showed better anticoagulation property.

However, the TTs for the modified membranes increased slightly compared with the APTT as shown in Supporting Information Figure S3. According to the studies,[51,52] the APTT reflects the level of blood coagulation factors (V and X) in plasma in endogenous pathway of coagulation. TT reflects the level of common pathway of coagulation. Thus, the $(PEI/PSS)_n$ multilayer modified membranes had effect on endogenous pathway of coagulation, attributing to the reaction or combination between the coagulation factors (V and X) in plasma and the hydrophilic surfaces.[12]

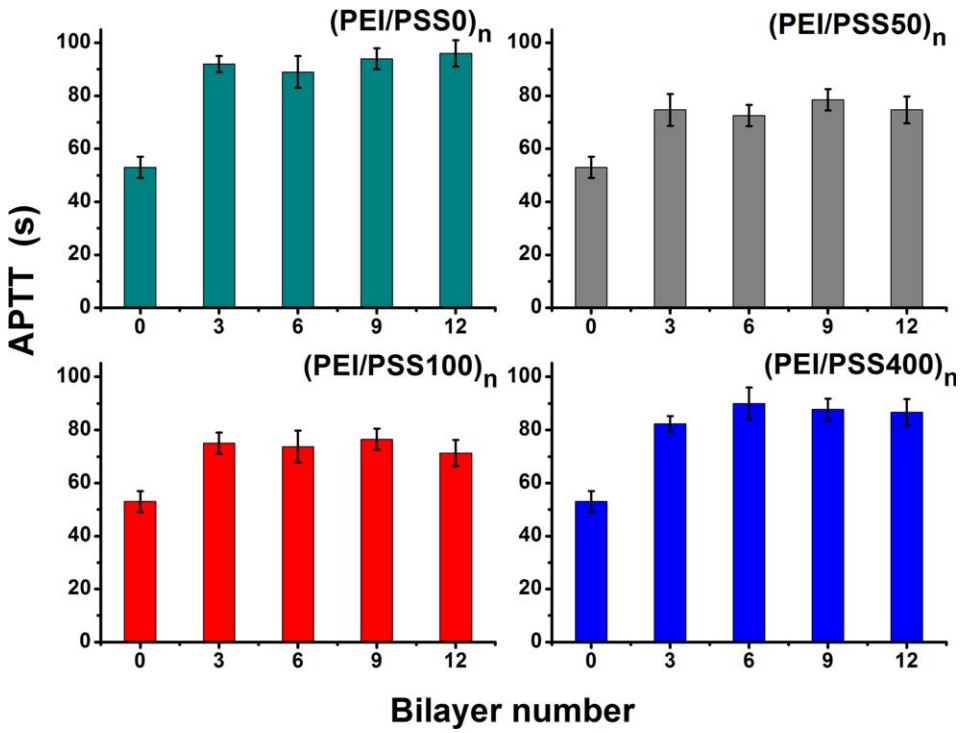

Figure 5. Activated partial thromboplastin times (APTTs) for the modified membranes. Values are expressed as means ± SD, $n = 3$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In addition, the enhancement in anticoagulant activity might be resulted from the partial contributions of the increased surface hydrophilicity, decreased protein adsorption, and suppressed platelet adhesion.[53,54] The previous experiments showed that mPEG in the block copolymers could efficiently improve the hydrophilicity and decrease the protein adsorption for the modified membranes; while PSS in the block copolymers could efficiently suppress the platelet adhesion. Thus, the anticoagulant property

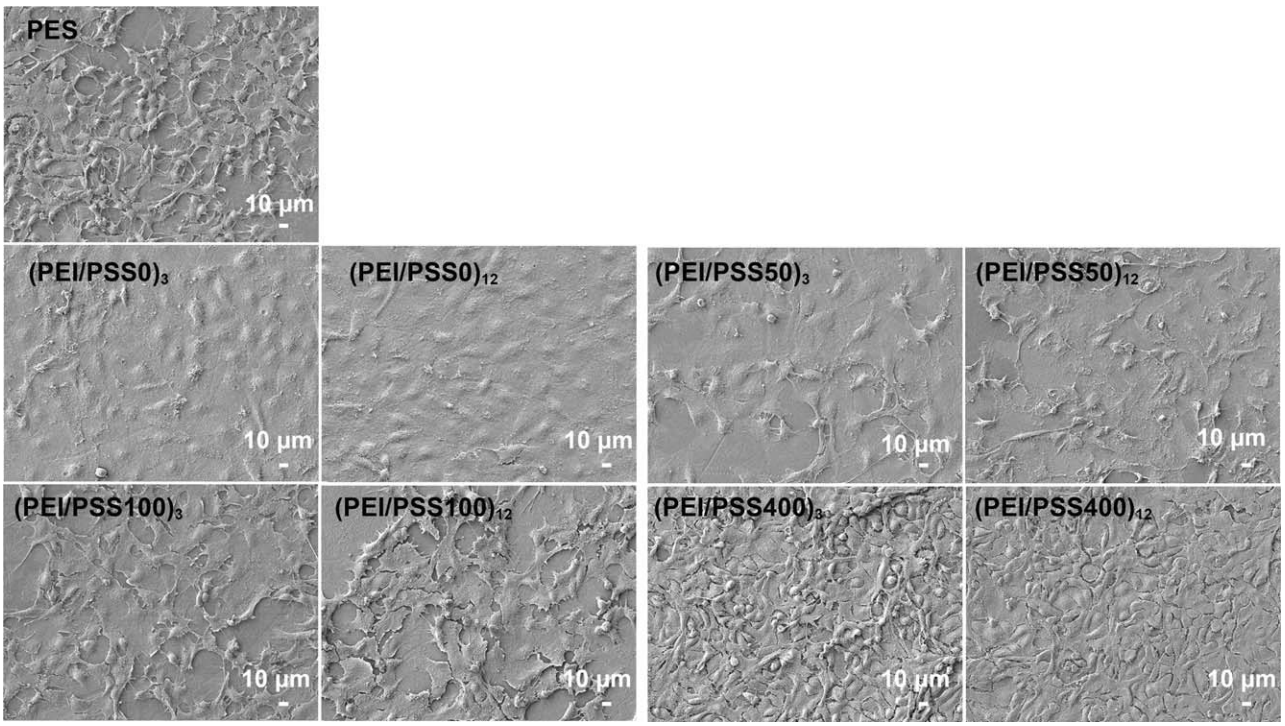

Figure 6. SEM images of endotheliocytes cultured on the membranes after 6 days. Magnification: ×300.

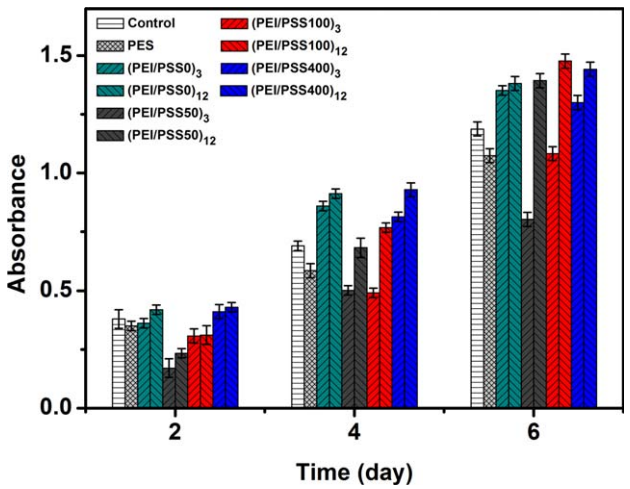

Figure 7. MTT-tetrazolium assay. Formazan absorbance expressed as a function of time from endotheliocytes seeded onto different membranes and the controls. Values are expressed as mean ± SD, $n = 3$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of the modified membranes by the mulitilayers combining mPEG and PSS (mPEG-*b*-PSS block copolymer) was significantly improved.

Cytocompatibility

Generally speaking, the cells will undergo their morphological changes to stabilize the cell-material interface after contacting biomaterials. The whole process of adhesion and spreading consists of cell attachment, filopodial growth, cytoplasmic webbing, flattening of the cell mass and the ruffling of peripheral cytoplasm progressing in a sequential fashion.[55,56] The cell morphologies after culturing for 6 days are shown in Figure 6. The endotheliocytes extended pseudopodia to adhere onto the materials. On the $(PEI/PSS)_n$ multilayer modified membranes, the adhering amounts of the endotheliocytes were larger than that on pristine PES membrane. Moreover, the mPEG-*b*-PSS block copolymer with longer PSS chains showed better cell adhesion.

In addition, the formazan absorbance indicated that the endotheliocytes seeded onto the control sample (the polystyrene cell-culture plate), and the membranes were able to convert the MTT into a blue formazan product. As shown in Figure 7, the viability of the cells on the modified membranes was better than that on the control sample. Moreover, the $(PEI/PSS)_n$ multilayer with longer PSS chain showed better viability. Therefore, it could be concluded that the $(PEI/PSS)_n$ multilayer would improve the cytocompatibility, and the $(PEI/PSS)_n$ multilayer with longer PSS chain had better cytocompatibility.

CONCLUSIONS

Block copolymers of poly(ethylene glycol) methyl ether-*b*-poly(-sodium *p*-styrene sulfonate) (mPEG-*b*-PSS) were synthesized via ATRP method. The copolymers were then used for the modification of polyethersulfone (PES) membrane by layer-by-layer (LBL) assembly technology to improve the biocompatibility. The modified membranes showed improved antifouling property, blood compatibility (suppressed platelet adhesion and prolonged clotting time) and cytocompatibility compared with the pristine PES membrane. The results indicated that the membranes modified by the mPEG-*b*-PSS copolymers with shorter PSS chains showed better antifouling property, and the mPEG-*b*-PSS copolymers with longer PSS chains showed better biocompatibility (blood compatibility and cytocompatibility). Thus, the antifouling property, blood compatibility and cytocompatibility for the membranes were successfully combined by the mPEG-*b*-PSS block copolymers, and the properties could be adjusted by varying the PSS chain lengths in the mPEG-*b*-PSS copolymers (the length of mPEG was constant). The LBL self-assembly modified membranes with integrated antifouling property and anticoagulant property provide wide choice for specific applications such as hemodialysis, hemofiltration, plasma separation, and so on.

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