

## Modification of Polysulfones by Click Chemistry: Zwitterionic Graft Complex and Their Antiprotein Fouling Property

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**ABSTRACT:** A new nonspecific protein antifouling polysulfone (PSU) material has been elaborated by grafting zwitterionic sulfobetaine (SB) complex by the means of PSU azidation (PSU-N<sub>3</sub>) followed by “Click-Chemistry” with acetylenic zwitterionic SB monomer (*N,N*’-diethyl-*N*-propargyl-*N*-(3-sulfopropyl) ammonium (DEPAS)). Azidation (PSU-N<sub>3</sub>) and functionalization (PSU-*g*-DEPAS) PSU materials were characterized by <sup>1</sup>H NMR, Fourier transform infrared spectroscopy, and thermogravimetric analysis. Contact angle and protein adsorption on PSU-*g*-DEPAS and unmodified PSU membrane were studied. The results show that the chemical modification of PSU improved both the hydrophilicity and antiprotein adsorption ability. This new fouling-resistance to protein material has potential for biomaterials applications. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2015**, *132*, 41327.

**KEYWORDS:** biomaterials; membranes; synthesis and processing

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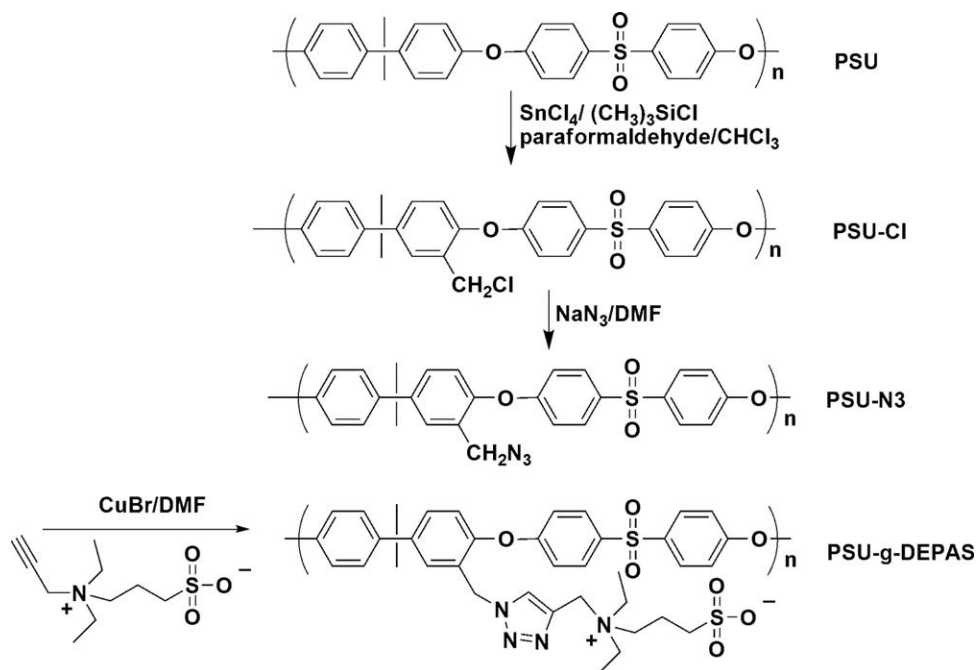
### INTRODUCTION

Polysulfone (PSU) has been used in bioapplications such as filtration<sup>1,2</sup>, hemodialysis,<sup>3</sup> ultrafiltration,<sup>4,5</sup> and bioreactor technology due to its excellent mechanical strength, chemical resistance, and thermal stability. However, serious membrane fouling always happened and anticoagulants are needed during hemodialysis due to the intrinsic hydrophobic properties and inadequate blood compatibility of PSU. Various approaches had been studied to reduce or minimize membrane fouling by improving the hydrophilicity of the membrane such as physical blending,<sup>6,7</sup> chemical grafting,<sup>8</sup> and surface modifications. For example, Xu and coworkers<sup>9</sup> synthesized an amphiphilic polymer of PSU-graft-poly(ethylene glycol) methyl ether methacrylate (PSU-*g*-POEM), and then blended it with polyethersulfone (PES) to tune the hydrophobicity and fouling-labile properties of PES membranes. Ishihara et al.<sup>10,11</sup> modified PSU membranes with phospholipid polymers and demonstrated reduced protein adsorption and low platelet adhesion relative to PSU. Mayes et al.<sup>12</sup> grafted the amphiphilic copolymers poly(ethylene glycol) (PEG) on PSU side chains and displayed the graft copolymer preferentially enhances wettability, porosity, and protein resistance compared to unmodified PSU membranes.

Compared with above methods, the copper (I)-catalyzed 1,3-dipolar cycloaddition of azides and alkynes, also known as “click” chemistry, is recently often used in the field of polymer

science to modify polymers/copolymers and complex architectures owing to its quantitative yields, high functional group tolerance, and mild reaction conditions.<sup>13–15</sup> For example, Timur, Yagci and coworkers<sup>16,17</sup> synthesized two well-defined amphiphilic graft copolymer PSU with hydrophilic poly(acrylic acid) (PAA) and PEG side chains by click chemistry. The PAA grafted PSU displayed increased hydrophilicity and reduced protein fouling and cell adhesion properties with respect to the unmodified PSU precursor. The obtained PEG grafted PSU demonstrated exhibited bioinert character compared to the unmodified PSU.

Sulfobetaine (SB) is a zwitterionic molecule containing both cationic and anionic groups on the same monomers, its polymer has demonstrated better antifouling performance and stability over PEG.<sup>18–21</sup> For example, Zhao and coworkers<sup>8</sup> grafted zwitterionic polymer of poly(sulfobetainemethacrylate) (PSBMA) on PSU membrane via surface-initiated atom transfer radical polymerization and demonstrated enhanced protein antifouling properties and good blood compatibility. Our previous studies also showed polyurethane membrane grafted with SB materials enhance their biocompatibility.<sup>22,23</sup> It can be expected, therefore, that the surface functionalization of hydrophobic PSU with zwitterionic SB is highly desirable to develop superior antifouling PSU materials and may have potential applications in biomedical field. To the best of our knowledge,



**Scheme 1.** Overall process for zwitterionic functionalization of PSU by click chemistry.

there are no reports on fabricating antifouling PSU membrane grafted with SB molecules by click chemistry.

As part of our continuous interest in developing synthetic methods for the preparation and modification of various polymer materials,<sup>22–25</sup> we report herein modification of PSU by this modular approach to yield zwitterionic structure as shown in Scheme 1. The chemical structures changes of functionalized PSU membrane were investigated. The nonspecific protein-fouling resistances were evaluated against the bovine serum albumin (BSA).

## EXPERIMENTAL

### Materials

PSU (PSU Udel P1700LCD) was purchased from Solvay Advanced Polymers. Paraformaldehyde, chlorotrimethylsilane, tin (IV) chloride, sodium azide ( $\text{NaN}_3$ ), 2, 2'-dipyridyl, and CuBr were purchased from Sinopharm (Shanghai, China). *N,N'*-diethyl-*N*-propargyl-*N*-(3-sulfopropyl) ammonium (DEPAS) were synthesized according to previously reported procedures by us.<sup>25</sup>

### Characterization

The chemical structures were characterized by Fourier transform infrared-attenuated total reflectance (FTIR, Tensor 27, Bruker).  $^1\text{H}$  NMR (400 MHz) spectrum was recorded on a Bruker Avance-400 (400 MHz) spectrometer. Surface elemental analysis of membranes was carried out by VG Multilab 2000 X-ray photoelectron spectrometer (XPS) with the Aluminum  $K\alpha$  as X-ray optical source (15 kV, 10 mA) and an initiation angle of  $90^\circ$ . SEM measurements were carried out on a SIRION 200 field emission scanning electron microscope. Contact angle measurements of a drop of water (2  $\mu\text{L}$ ) on the membranes were carried

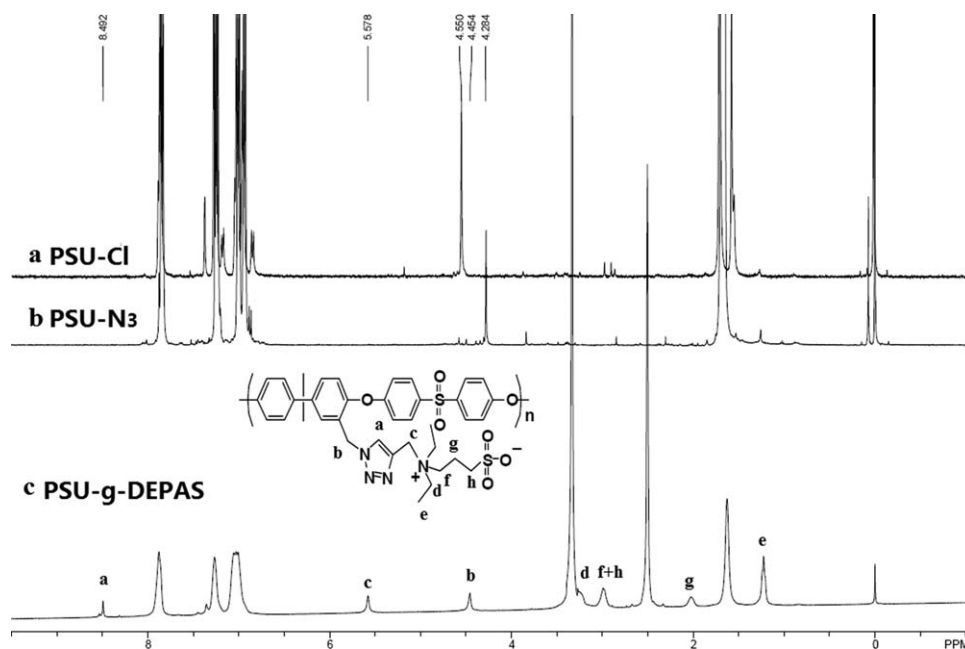
out using the sessile drop method with a CAM 100 KSV (KSV, Finland). Recording the drop profile with a CCD camera allowed monitoring changes in contact angle. TG analyses of polymer samples were done using a TA instrument 2100 thermal analyzer having a 951 TG module. TG studies were done in  $\text{N}_2$  atmosphere in the temperature range of 40–700°C using a heating rate of 10°C/min. Molecular weights ( $M_n$ ,  $M_w$ ) and polydispersity index ( $M_w/M_n$ ) were determined relative to a calibration with polystyrene standards, by using gel permeation chromatography (GPC; Waters 1525/2414, Waters Instrument, MA) equipped with Empower software at ambient temperature. Freshly distilled THF served as the mobile phase and was delivered at a flow rate of 1.0 mL/min.

### Chloromethylation and Azidation of PSU

All of the polymers preparation procedures are represented in Scheme 1. Chloromethylation of PSU and azidation of PSU were performed according to the reported methods.<sup>16,17</sup>

### Click Functionalization of PSU

PSU- $\text{N}_3$  (1.0 g), *N,N'*-diethyl-*N*-propargyl-*N*-(3-sulfopropyl) ammonium (DEPAS; 3.5 g, 1.5 mmol), 2, 2'-dipyridyl (0.15 mmol), CuBr (0.15 mmol) were added into a Schlenk tube and were dissolved in 50 mL of DMF. The mixture was purged with  $\text{N}_2$  for 30 min to remove the dissolved oxygen, and the temperature was maintained at 80°C for 12 h. After completion of reaction, the mixture was poured into methanol and then washed with dilute ammonia. The obtained product (PSU-g-DEPAS) was dried in vacuum at room temperature for 24 h. FTIR (ATR):  $\nu(\text{SO}_3^-) = 1040 \text{ cm}^{-1}$ .  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  8.49 (s, triazole-H), 7.88 (br, Ar-H), 7.36 (br, Ar-H), 7.06–7.01 (m, Ar-H), 5.58 (s,  $-\text{CH}_2-$ ), 4.56 (s,  $-\text{CH}_2-$ ), 3.27–3.24 (m,  $\text{CH}_2\text{N}^+(\text{CH}_3)_2\text{CH}_2$ ), 2.99 (br,



**Figure 1.**  $^1\text{H}$  NMR spectra of (a) PSU-Cl (in  $\text{CDCl}_3$ ), (b) PSU- $\text{N}_3$  (in  $\text{CDCl}_3$ ), and (c) PSU-g-DEPAS (in  $\text{DMSO}-d_6$ ).

$-\text{CH}_2\text{CH}_2\text{SO}_3^-$ ), 2.02 (br,  $-\text{CH}_2\text{CH}_2\text{SO}_3^-$ ), 1.63 (s,  $-\text{CH}_3$ ), 1.22 (s,  $-\text{NCH}_2\text{CH}_3$ ).

#### Membranes Preparation

The PSU and PSU-g-DEPAS membranes were prepared by spin coating coupled with a liquid-liquid phase separation method at room temperature.

#### Protein Static Adsorption on the Membranes Surface

Protein solutions with concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were freshly prepared by dissolving BSA (PI = 4.9) into 0.1M phosphate buffer solution (PBS, pH = 7.4). The membrane samples with the size of  $2 \times 2 \text{ cm}^2$  were put into vials filled with 10 mL protein solution. The vial was then maintained at  $25^\circ\text{C}$  for 24 h with interval waving to reach an adsorption-desorption equilibrium. Next, equilibrium concentrations of BSA(C) were measured by UV-vis spectrophotometer at 280 nm, and the adsorbed mass of protein per unit volume of membrane ( $Q \mu\text{g}/\text{cm}^2$ ) was calculated by the equation:  $Q = (C_0 - C)/S$ , where C and  $C_0$  are the equilibrium and initial concentrations, respectively, S is the area of the membrane. The final results were averaged from three measurements for each kind of polymer membrane.

## RESULTS AND DISCUSSION

#### Syntheses of Polymers

Chloromethylation of PSU and azidation of PSU was performed according to the reported methods.<sup>16,17</sup> Then, alkyne-DEPAS was clicked to the PSU main chain. The overall process is summarized in Scheme 1.

The related products of each step were characterized by the  $^1\text{H}$  NMR spectra (Figure 1). The percent of chloromethylation was estimated to be 19 mol % from the area of the  $\text{CH}_2$  protons at 4.55 ppm relative to that of the reference peak at 7.88

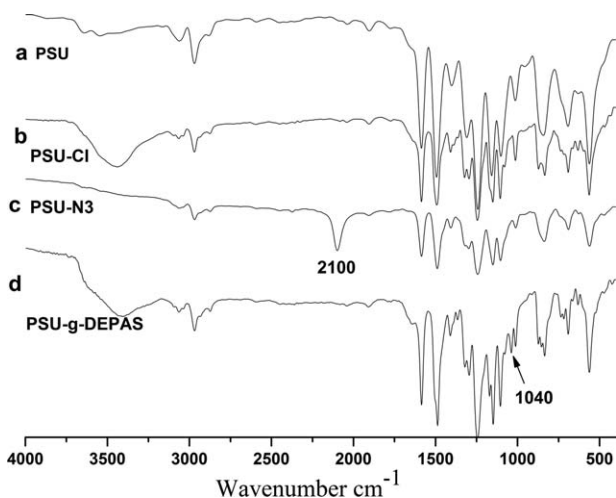
ppm, which ascribed to the four aromatic protons of metasubstituted phenyl ring adjacent to the sulfonyl group [Figure 1(a)]. After azidation, the signal of the  $\text{CH}_2$  protons at 4.55 ppm was moved to 4.47 ppm demonstrating the formation of PSU- $\text{N}_3$ . After treatment with acetylenic SB monomer via click reaction, the methylene protons at 4.46 ppm were completely shifted to 5.58 ppm indicating that the click reaction proceeded in almost a quantitative yield, which are similar with the reported results.<sup>16,17,26</sup> And a new signal peak at 8.49 ppm corresponding to the triazole protons appeared further confirms the successful click process [Figure 1(c)]. At the same time, the appearance of the total protons (1.22–3.27 ppm) corresponding to the DEPAS protons confirms the successful grafting process [Figure 1(c)].

The process was also monitored by FTIR spectroscopy (Figure 2). Figure 2(c) exhibited a new characteristic absorption band of azide groups at  $2100 \text{ cm}^{-1}$  which confirms the successful azidation of PSU-Cl. As seen in Figure 2(d), the peak at  $2100 \text{ cm}^{-1}$  attributed to the azide groups completely disappeared after click reaction. Furthermore, we can see that a new peak appears at  $1040 \text{ cm}^{-1}$  corresponding to the  $-\text{SO}_3^-$ . These evidences clearly demonstrate the introduction of SB structures into PSU pendent line.

It can also be seen from Table I that the higher number-average and weight-average molecular weights were obtained after “click” reaction. Nevertheless, molecular weight of PSU-g-DEPAS as estimated by  $^1\text{H}$ -NMR analysis strongly corresponds to the PSU- $\text{N}_3$  polymer (Table I).

#### Thermal Behaviors of Modified PSU

The thermal behaviors of pristine and modified PSU series were investigated via TG. The TG of these polymers is plotted in Figure 3. The temperature of maximum rate of mass loss ( $T_{\text{max}}$ )



**Figure 2.** FT-IR spectra of PSU(a), PSU-Cl(b), PSU-N<sub>3</sub>(c), and PSU-g-DEPAS(d).

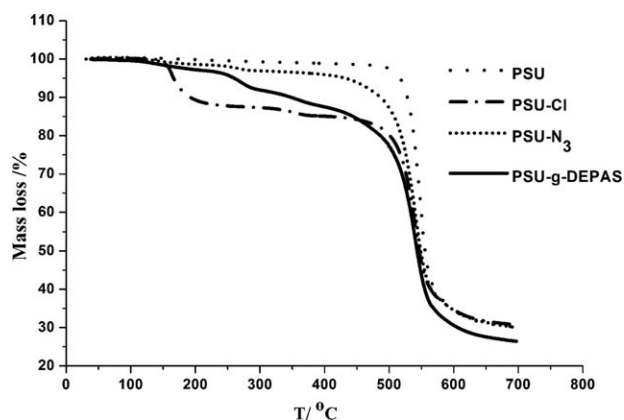
was determined from differential thermogravimetric trace (DTG; Table II). From the TG traces, we can see that the modified PSU samples are thermally less stable than the pristine PSU, most probably correspond to the bulky pendant groups along the polymer backbone. The thermal degradation of modified PSU derivatives takes place in multi stages with different mass losses according to the varied substituent (Table II). Also, it is observed that, in the case of modified PSU, the most important mass loss occurs in the range of 400–700°C ascribed to thermal degradation of PSU chain. For the PSU-CH<sub>2</sub>Cl, a DTG peak at a low temperature in the range of 150–250°C was observed, which due to the loss of chloromethylene groups (CH<sub>2</sub>Cl) according to the published results.<sup>26,27</sup> A major mass loss (54%) is observed in the third stage of decomposition occurring between 440 and 700°C, which is due to breakdown of the polymer backbone. Regarding to PSU-N<sub>3</sub>, about 2% mass loss occurs between 245 and 290°C, which ascribes to the decomposition of N<sub>3</sub> side. And the result is similar with the reported work.<sup>26</sup> A major mass loss (65%) occurs between 410 and 700°C, which attribute to the breakdown of the polymer backbone. For the modified PSU (PSU-g-DEPAS), three decomposition steps were observed, the first step takes place in the range 225–300°C having 5% mass loss. In the second stage, about 3% mass loss can be observed. The third step is the main

**Table I.** Molecular Weight Characteristics of the Products at Different Modification Steps

	Mn <sup>a</sup>	Mw <sup>a</sup>	Mw/Mn <sup>a</sup>	Mn <sup>b</sup>
PSU	64710	94150	1.46	-
PSU-Cl	82280	128000	1.56	-
PSU-N <sub>3</sub>	85760	128200	1.49	-
PSU-g-DEPAS	-	-	-	125010

<sup>a</sup> Determined by GPC using polystyrene standards.

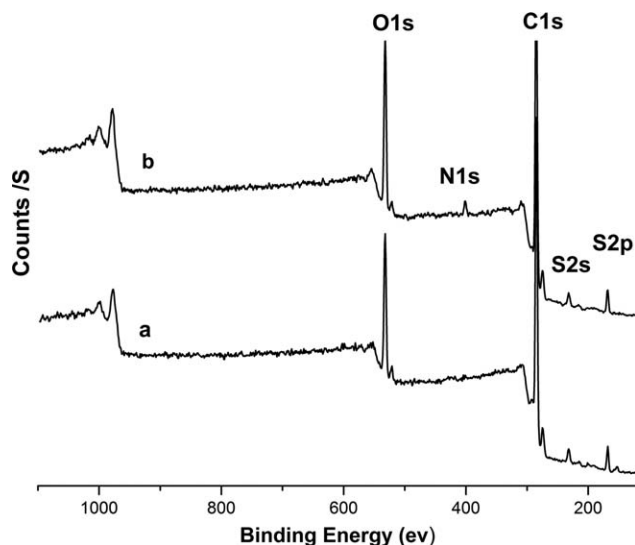
<sup>b</sup> Determined by <sup>1</sup>H-NMR analysis.



**Figure 3.** TG curves of pristine and modified PSU.

**Table II.** Thermal Properties of Pristine and Modified PSU

Sample	Mass loss/(TG temp range/ °C)		
	Stage 1	Stage 2	Stage 3
PSU	-	-	70(485-700)
PSU-Cl	15 (150-230)	2 (310-380)	54 (440-700)
PSU-N <sub>3</sub>	-	2 (245-290)	65 (410-700)
PSU-g-DEPAS	5 (225-300)	3 (330-385)	60 (445-700)

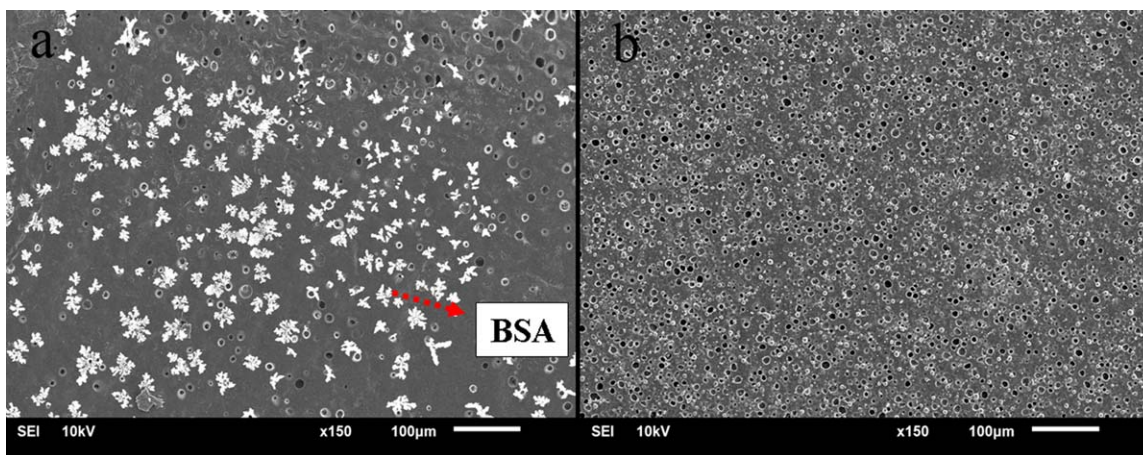


**Figure 4.** XPS wide-scan spectra of pristine PSU (a) and PSU-g-DEPAS (b) membrane surfaces.

**Table III.** Water Contact Angle and Adsorption Capacities of BSA on PSU and PSU-g-DEPAS Membranes

	Static water contact angle/ (°)	Adsorption amount/ (μg·cm <sup>-2</sup> )
PSU	104.8	72
PSU-g-DEPAS	84.3	17





**Figure 5.** SEM images of the surface of pristine PSU membrane (a) and PSU-g-DEPAS (b) after BSA adsorptions. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

degradation stage, the mass loss is 60%, and represents a typical decomposition process of the PSU chain.

#### XPS Analysis of Membrane Surface

Surface membrane composition was determined by XPS analysis. The XPS spectra of pristine PSU membrane and PSU-g-DEPAS membrane were displayed in Figure 4. Compared with the spectrum of pristine PSU membrane, a new peak at 404 eV appeared in that of PSU-g-DEPAS membrane, which was assigned to N 1 s signal. Table III summarizes the atomic percentage of the pristine and modified membranes. The increase of surface elemental nitrogen content (Table III) indicates that SB groups have been grafted the PSU materials successfully, which is similar as the reported work.<sup>28</sup>

#### Hydrophilicity

The hydrophilicity of the membrane surface was generally characterized by static contact angle measurement. The static water contact angle values of pristine PSU and PSU-g-DEPAS membranes are listed in Table III. Compared with PSU, the contact angle of the PSU-g-DEPAS membranes significantly decreased to 84°. The SB-functionalized membrane exhibited improved hydrophilicity than the pristine membrane.

#### Protein Adsorption

To demonstrate the protein resistance of SB functionalized PSU membranes, the BSA protein adsorption tests on surfaces of pristine PSU membranes and PSU-g-DEPAS membranes were performed. As shown in Table III, the BSA adsorption amount for the PSU membrane was higher (about 72  $\mu\text{g}/\text{cm}^2$ ) than that for the SB functionalization membranes PSU-g-DEPAS (about 17  $\mu\text{g}/\text{cm}^2$ ), which indicates the SB functionalization display PSU membrane excellent nonspecific protein antifouling property. This result is consistent with that of the membrane hydrophilicity as stated above.

Figure 5 showed the SEM images of surfaces of pristine and zwitterionic membrane after adhesion tests. As shown in Figure 5(a), many BSA were adhered on the surface of pristine PSU membrane. However, there were only very few BSA adhered on

the PSU-g-DEPAS membrane surface [Figure 5(b)], which indicates the SB functionalization offers PSU membrane excellent nonspecific protein antifouling property.

#### CONCLUSIONS

In summary, a new SB grafted PSU has been successfully synthesized by click chemistry. The obtained SB grafted PSU displayed increased hydrophilicity properties. And the PSU-g-DEPAS membranes display excellent nonspecific protein antifouling property. This material could be an alternative in biomedical fields.

#### ACKNOWLEDGMENTS

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#### AUTHOR CONTRIBUTIONS

Canhong Xu performed the majority of the experiments, Dr Gu supervised the experiments and wrote the manuscript, Dr Xu and Dr Yang provided reagents and technical assistance, Huan Xia and Jiehao Du assisted to complete the experiments, Dr Huang and Dr Liu analyzed data and prepared of the manuscript, Dr Zhou and Dr Bai contributed insightful discussions.

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