ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

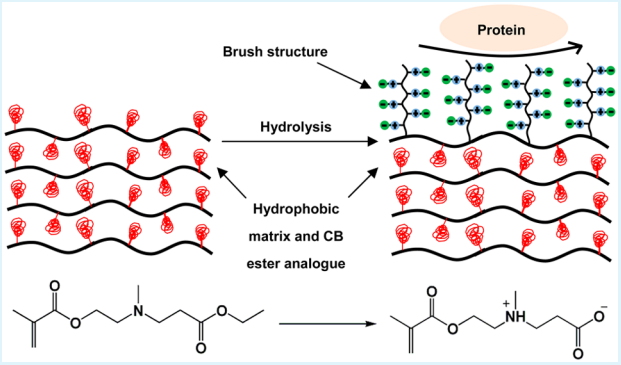
Development of Robust and Recoverable Ultralow-Fouling Coatings Based on Poly(carboxybetaine) Ester Analogue

Guangzhi Wang,[†] Longgang Wang,[†] Weifeng Lin,[†] Zhen Wang,[†] Juan Zhang,[†] Fangqin Ji,[†] Guanglong Ma,[†] Zhefan Yuan,[†] and Shengfu Chen*[,†,‡]

[†]Key Laboratory of Biomass Chemical Engineering of Ministry of Education, College of Chemical and Biological Engineering, Zhejiang University, Hangzhou, Zhejiang 310027, China

[‡]Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, Jiangsu Key Laboratory of Biomedical Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210046, China

ABSTRACT: Polyurethane with zwitterionic side chains (PCB-ester-PU) based on a poly(carboxybetaine) ester analogue is developed for marine coatings and biomedical applications by introducing dihydroxy-terminated PCB-ester$(OH)_2$ with different polymerization as the macrodiol, 4,4′-diphenylmethane diisocyanate (MDI) as the diisocyanate, and 1,4-butanediol (1,4-BD) as the chain extender. Robust coatings are obtained and exhibit long-term excellent resistance to nonspecific protein adsorption, bacterial adhesion, and human umbilical vein endothelial cell (HUVEC) attachment after hydrolysis. Tests of adhesion on different substrates and film hardness indicate that the material possesses far more stable mechanic properties than hydrogel coatings. Moreover, such a resistance can be generated not only by alkaline solution, but also by a physiological buffer (such as phosphate-buffered saline (0.15 M pH 7.4 PBS)) or by steam in an autoclave. Ultimately, its excellent long-term nonfouling property, its healing capability through self-regeneration and superior mechanic properties (such as hardness and elasticity), and its good adhesiveness as a paint on both polar and nonpolar substrates make this material an ideal candidate as a coating for marine and medical devices.



KEYWORDS: *polyurethane, carboxybetaine, nonfouling, hydrolysis, zwitterionic polymers, coatings*

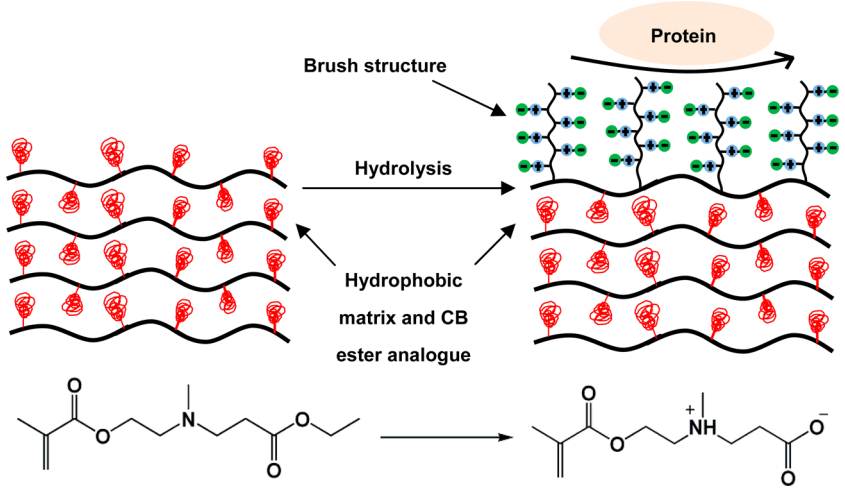
1. INTRODUCTION

"Biological fouling", initiated by the undesired nonspecific protein and other biomacromolecule adsorption on surfaces, is a great challenge in many applications, ranging from biomedical devices to marine coatings. These unwanted fouling will either cause implantable medical device failure and adverse outcomes to the patients[1,2] or generate a severe fuel penalty[3,4] and greenhouse gas emissions[5] caused by the colonization of unwanted marine organisms on the submerged surfaces. Instead of today's methods through leaching problematic bioactive gradients, such as rapamycin in drug-polymer coated stent[6,7] or copper ions and biocides in marine coatings[8] to suppress biofouling formation, a new method was proposed to directly reduce "biological fouling" through preventing nonspecific protein and other biomacromolecule adsorption on surfaces at the initiate stage.[1,2] Thus, two main classes of nonfouling materials were investigated to directly reduce "biological fouling": poly(ethylene glycol) (PEG)-based materials[9,10] and zwitterionic-based materials,[11,12] including poly(2-methacryloyloxyethyl phosphorylcholine) (pMPC),[13−15] poly(sulfobetaine) (pSB),[16−18] and poly(carboxybetaine) (pCB).[19−21] Encouraging results for the resistance to both protein molecules and large organism on various surfaces were achieved.[22−27] Polysulfobetaine-grafted surfaces formed by surface-initiated atom transfer radical polymerization (SI-ATRP) showed excellent resistance to a typical marine organism *Ulva*, which indicates the potential of polyzwitterions as environmentally benign, ultralow-fouling marine coatings.[24] However, all these nonfouling coatings are very vulnerable, because of their extremely high capability of hydration, which leads these nonfouling coatings to be hydrogel and hinders their long-term applications, because of very low mechanic properties and the penetration of reactive molecules. The resistance could be lost due to detachment or scratches caused by physical impact or degradation in water containing reactive environments, such as water, oxygen, and possible catalytic ions. Thus, the hydrophobic zwitterionic precursor-based nonfouling materials, silicone hydrogel[28] and drug-loaded wound dressing,[29] were developed in our recent works to improve mechanic strength and slow the degradation by increasing the hydrophobicity of polymer matrix and reducing the contact between the polymer matrix with reactive mediums. Poly(carboxybetaine) ester

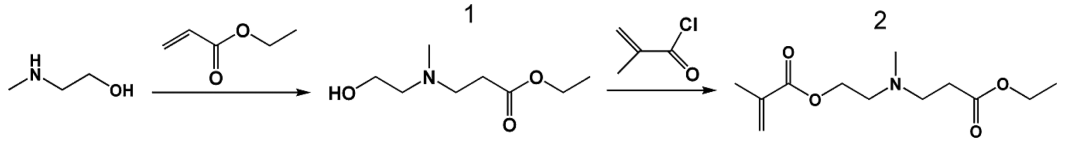
Received: December 16, 2014
Accepted: July 10, 2015
Published: July 10, 2015

DOI: 10.1021/acsami.5b05162
ACS Appl. Mater. Interfaces 2015, 7, 16938−16945

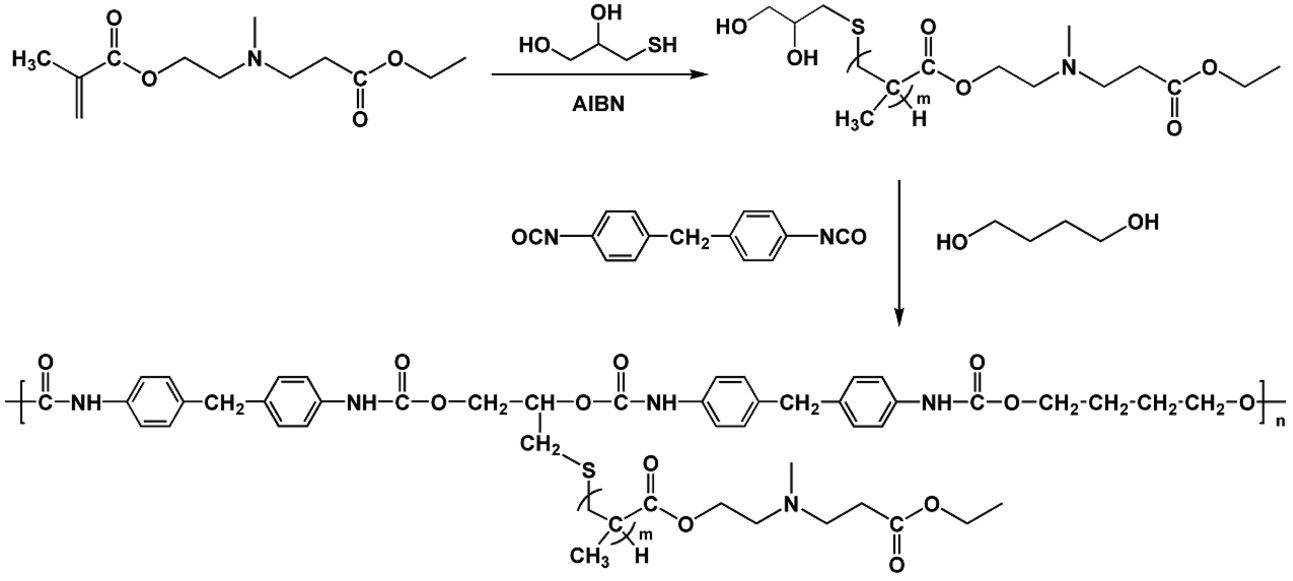
Scheme 1. Descriptive Mechanism of High Resistance to Protein Adsorption, Bacterial Adhesion, and Mammalian Cell Attachment of the PUs by Slow and Controlled Hydrolysis of CB-Ester Incorporated into the Side Chains of It



Scheme 2. Synthesis of the Carboxybetaine Ester Analogue (Compound 2)



Scheme 3. Synthesis Process of Polyurethane with Poly(carboxybetaine) Ester Analogue Side Chains (PCB-Ester-PU)



analogue is such a hydrophobic zwitterionic precursor, of which the nonfouling property could be obtained through surface hydrolysis. However, our these newly developed materials could not reach the ultralow-fouling level, which is a critical level that is highly required by biomedical and marine coatings, even when additional nonfouling monomers (e.g., *N*-vinylpyrrolidone (NVP) or poly(ethylene glycol) methacrylate (PEGMA)) were incorporated. Moreover, those nonfouling monomers (NVP and PEGMA) also increase the undesired water content in polymer matrix, which could cause a negative impact on their mechanic properties and long-term durability. Until now, there has not been any nonfouling material that can be directly used for robust coatings or paints with both excellent nonfouling property and good mechanic properties, such as strength, hardness, and adhesion to substrates. Thus, it should be a necessary step to develop robust nonfouling coatings for biomedical device and marine coatings.

In this work, polyurethane (PU) with brush chains of the poly(carboxybetaine) ester analogue, which is a hydrophobic zwitterionic precursor before pH 7.4, because of the tertiary amine groups that are surrounded by bulky hydrophobic groups,[30] was developed to solve the dilemma between ultralow fouling and highly hydrophobic matrix (Scheme 1). This new PU (PCB-ester-PU), formed by dihydroxy-terminated poly(carboxybetaine) ester analogue (PCB-ester$(OH)_2$) side chains, 1,4-butanediol and 4,4′-diphenylmethane diisocyanate, ex-

DOI: 10.1021/acsami.5b05162
ACS Appl. Mater. Interfaces 2015, 7, 16938−16945

Table 1. Detailed Compositions of PCB-Ester-PUs

sample	CB-ester/3-mercapto-1,2-propanediol (molar ratio)	Mn of PCB-ester	PDI of PCB-ester	PCB-ester(OH)$_2$/MDI/1,4-BD ratio	PCB-ester content (wt %)
PCB-ester-PU1	2:1	1960	1.204	1:2:1	41.0
PCB-ester-PU2	5:1	3099	1.327	1:2:1	63.5
PCB-ester-PU3	10:1	4959	1.513	1:2:1	77.7
PCB-ester-PU4	20:1	7878	1.709	1:2:1	87.4
PCB-ester-PU5	10:1	4959	1.513	1:4:3	63.8

hibited excellent resistance to nonspecific protein adsorption, bacterial adhesion and HUVEC cell attachment after a quick hydrolysis. Furthermore, the coating showed long-term stable and self-recoverable resistance against scratches and strong adhesion strength measured according to ISO 4624 on polar stainless steel and nonpolar propylene. In short, this excellent nonfouling coating is no longer delicate one for most applications, which might be an ideal candidate for medical devices and marine coatings.

2. MATERIALS AND METHODS

2.1. Materials. 2-(Methylamino) ethanol (≥99%), ethyl acrylate (≥99%), triethylamine (≥99.5%), methacryloyl chloride (≥95%), 3-mercapto-1,2-propanediol (≥95%), 2,2′-azobis(2-methylpropionitrile) (AIBN, ≥98%), and 1,4-butanediol (1,4-BD) were from Aladdin (Shanghai). 4,4′-Diphenylmethane diisocyanate (MDI, ≥98%) and dibutyltindilaurate (DBT) were obtained from Alfa Aesar (Tianjing, PRC). Horseradish peroxidase (HRP)-conjugated goat antihuman lgG(H + L) was purchased from Beijing Biosynthesis Biotechnology Co. Physiological phosphate buffered saline (PBS, pH 7.4) was prepared by dissolving NaCl, KCl, Na_2HPO_4, and KH_2PO_4 in pure water. Tetrahydrofuran (THF) and ethyl acetate were dried over 4A molecular sieves prior to use. All other chemicals were of reagent grade.

2.2. Synthesis of Carboxybetaine Ester Analogue (CB-Ester). A two-step synthesis process was conducted (Scheme 2). First, 2-(methylamino) ethanol was added dropwise into ethyl acrylate slowly at 0 °C and the mixture was stirred overnight at room temperature to form a colorless oil compound (compound **1**). The compound then was dissolved in anhydrous tetrahydrofuran, followed by the addition of triethylamine. After adding methacryloyl chloride dropwise at 0 °C, the reaction was carried out overnight at room temperature.

The mixture was then purified by vacuum filtration through an alkaline alumina column and condensed by rotary evaporation at 30 °C to remove THF. After dissolved by ethyl acetate, the resulting liquid was extracted by a saturated sodium carbonate solution and then a saturated sodium chloride solution, respectively. The solvent then was dehydrated by anhydrous sodium sulfate and evaporated by rotary evaporation at 30 °C; a light yellow viscous liquid was obtained. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 6.11 (m, 1H), 5.57 (m, 1H), 4.25 (t, 2H), 4.14 (q, 2H), 2.79 (t, 2H), 2.73 (t, 2H), 2.48 (t, 2H), 2.33 (s, 3H), 1.95 (s, 3H), 1.26 (t, 3H). The yield of the resulting compound (compound 2) was ∼90%, with a purity of ∼99% by ^{1}H NMR.

2.3. Synthesis of PCB-Ester(OH)$_2$ with Different Degree of Polymerization. The CB-ester monomer was used to the synthesis of PCB-ester(OH)$_2$ by free radical polymerization using AIBN as an initiator (Scheme 3). To control the different degrees of polymerization, 3-mercapto-1,2-propanediol was introduced as a chain transfer reagent. In our experiment, CB-ester and 3-mercapto-1,2-propanediol was added into a Pyrex vial, according to the molar ratio of 10:1. The amount of AIBN added was 0.01 wt %, based on monomers. After the elimination of oxygen by bubbling with nitrogen gas for ∼30 min, polymerization was carried out in THF at 60 °C for 15 h. After removal of THF by rotary evaporation and redissolution in ethyl acetate, the product was precipitated into hexane, centrifuged and dried under vacuum at 40 °C. The number-average molecular weight (M_n) and polydispersity index (PDI) of PCB-ester(OH)$_2$ determined by gel permeation chromatography (GPC) are 4959 g/mol and 1.51, respectively. To clarify the influence of side chain polymerization to nonspecific protein resistance ability of the new material, by controlling the molar ratio of CB-ester and chain transfer reagent, 3-mercapto-1,2-propanediol, we synthesized PCB-ester(OH)$_2$ with different degrees of polymerization.

2.4. Synthesis of Polyurethane with PCB Side Chains (PCB-Ester-PU). A two-step condensation polymerization was performed in THF under a nitrogen atmosphere to synthesize linear PCB-ester-PU by introducing dihydroxy-terminated PCB-ester(OH)$_2$ with different polymerization as the macrodiol, MDI as the diisocyanate, and 1,4-BD as the chain extender. Briefly, PCB-ester(OH)$_2$ and MDI were added to 30 mL dried THF, according to a molar ratio of 1:2, and stirred for 1 h at 60 °C to form a low-molecular-weight prepolymer. Subsequently, 1,4-BD and DBT were added as the chain extender and catalyst respectively, and the mixture was allowed to react at 65 °C for another 3 h. Scheme 3 gives a detailed illustration of the synthesis process of polyurethane with zwitterionic side chains. The resulting polymers were precipitated in deionized water and centrifuged. After redissolved in THF, the polymers were casted into a Teflon mold at 40 °C for 24 h to evaporate the solvent. The final products (thickness of 0.25−0.5 mm) were taken from the mold, cut into appropriate sizes, and then selectively hydrolyzed with 0.1 M NaOH at room temperature for further characterization.

By these means, we synthesized PCB-ester-PU materials with PCB side chains of different degrees of polymerization; the detailed compositions of PCB-ester-PUs are listed in Table 1. The PCB-ester content in PCB-ester-PUs increase from 41.0% (w) to 87.4% (w), while the ratio of PCB-ester(OH)$_2$/MDI/1,4-BD is maintained at 1:2:1. To compare chain length effect with the density of brush on the resistance to protein adsorption, PCB-ester-PU5 was prepared to match PCB-ester-PU2 at an equal amount of PCB-ester(OH)$_2$ by adjusting the PCB-ester(OH)$_2$/MDI/1,4-BD ratio to 1:4:3.

2.5. Gel Permeation Chromatography (GPC). The number-average and weight-average molecular weights (M_n and M_w) and molecular weight distribution (M_w/M_n) of PCB-ester(OH)$_2$ were determined by GPC (Schambeck SFD GmbH, RI2000, Germany) at 30 °C. The mobile phase used was DMF with a flow rate of 1.0 mL/min.

2.6. Attenuated Total Reflectance Infrared (ATR-IR) Analysis. ATR-IR characterization of PCB-ester-PU was carried out using a Nicolet Nexus 670 FTIR spectrometer and a ZnSe crystal at a 45° angle of incidence.

2.7. Static Contact Angle Measurement. Static contact angles were measured using a Krüss DSA-10 contact angle goniometer (Hamburg) at room temperature and ambient humidity. All measurements were carried out by placing a 10 μL drop of liquid onto the surface of each fully cured sample using a 1000 μL screw-top syringe.

2.8. Protein Adsorption Measurement (ELISA). Nonspecific protein binding to different PCB-ester-PUs and tissue culture polystyrene (TCPS) controls was determined by quantifying HRP-conjugated anti-IgG adsorption. First, the samples were incubated with 1 μg mL^{-1} anti-IgG for 1.5 h, followed by five rinses with phosphate-buffered saline (PBS). The PCB-ester-PU films and TCPS controls were then removed and placed in 24-well plates. Next, 1 mL of 1 μg mL^{-1} *o*-phenylenediamine (OPD) in 0.1 M citrate−phosphate buffer (pH 5.0), containing 0.03% hydrogen peroxide was then added. Enzyme activity was stopped by adding an equal volume of 2 N H_2SO_4

DOI: 10.1021/acsami.5b05162
ACS Appl. Mater. Interfaces 2015, 7, 16938−16945

after 15 min. The tangerine color (i.e., the relative protein adsorption) was measured at 492 nm.

2.9. Bacterial Adhesion Test. *Staphylococcus aureus* was used to determine the bacterial adhesion properties of the novel PCB-ester-PU material (PCB-ester-PU3). First, the bacteria were cultured overnight at 37 °C on sterile LB agar plates. Next, a 60 mL flask containing sterile LB medium was incubated with a single colony (acquired from the starter cultures) at 37 °C and 200 rpm for 12 h. The culture then was centrifuged at 4750 rpm and 4 °C for 20 min after being decanted into two 50 mL centrifuge tubes. The supernatant was discarded and each cell pellet was resuspended in 30 mL of sterile PBS. After washing the cells twice, the bacterial concentration was spectrophotometrically adjusted to 1×10^6 cells/mL in sterile LB culture medium, which was then used to measure bacterial adhesion. Prior to testing, the PCB-ester-PU films and TCPS controls were pretreated by immersion in PBS for 10 min and then placed in the bacterial suspension for 1 h, washed with sterile PBS and then stained with Live/Dead BackLight bacterial viability kits. The results were directly observed using a CCD camera mounted on a Nikon Eclipse Ti series microscope with a 40× lens.

2.10. HUVEC Cell Surface Attachment Assay. PCB-ester-PU3 of appropriate size and tissue culture polystyrene (TCPS) were placed individually in the wells of 24-well plates. After being irradiated with UV light for 30 min, HUVEC cells were seeded onto the samples at a concentration of 10^5 cells/mL in supplemented RPMI 1640 medium. After growing for 24 h at 37 °C, 5% CO_2, and 100% humidity, the cells were dyed by FDA (fluorescein diacetate) for 5 min and the samples were photographed at 20× magnification on a Nikon Eclipse Ti series microscope. Each measurement had three replicate wells.

2.11. Pull-Off Test for Adhesion and Determination of Film Hardness. The pull-off test of PCB-ester-PU coating (PCB-ester-PU3) on different substrates was conducted according to international standard ISO 4624. First, the polymer was dissolved in THF and painted on different substrates to obtain stable coatings (on one or both sides) after drying. Then, dollies were bonded directly to the surface of the coated, cured panel using a cyanoacrylate adhesive. After curing of the adhesive, the bonded dolly assemblies were placed in a suitable tensile tester. By applying a tensile stress which was increased at a substantially uniform rate in a direction perpendicular to the plane of the coated substrate, we measured the tensile stress necessary to break the coating/substrate bond. For each substrate, six repetitions were performed and the mean value was reported. The detailed steps of pull-off test for adhesion are given in ISO 4624. The bonded dolly assemblies are shown in Figure 1.

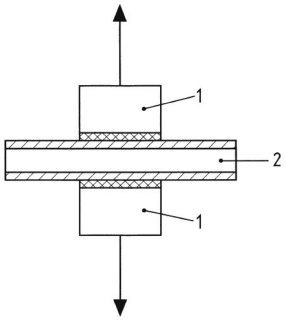


Figure 1. Illustration of test assembly. [Legend: 1, dolly coated with adhesive; 2, substrate painted on one or both sides with PCB-ester-PU coating.]

Apart from adhesion strength, film hardness is also important for the mechanic property of coatings. Increasing of film hardness will reduce friction or collision damage of coatings. Determination of film hardness by pencil test was conducted according to international standard ISO 15184.

2.12. Self-Regeneration Test. Self-regeneration of the new PCB-ester-PU material (PCB-ester-PU3) was measured by observing the surface adsorption of FITC (fluorescein iso-thiocyanate) labeled BSA (albumin from bovine serum) after different hydrolysis time. First, FITC-labeled BSA was prepared via the following method: (1) dissolve 100 mg BSA in 50 mL buffer (pH 9) and dissolve 2 mg FITC in 2 mL of DMSO (dimethyl sulfoxide); (2) blend the two solutions with stirring and put it in darkness for 8 h; and (3) remove unreacted FITC of the mixed solution by dialysising in PBS (pH 7.4) more than 4 times.

The polymer coated on the surface of a glass slide was immersed into 0.1 M NaOH for 3 h of hydrolysis. At the moments of 1.5 h and 10 min before the hydrolysis termination, respectively, the sample was taken out and carefully washed with PBS, a straight line was softly scratched on it to remove the hydrolyzed layer, and then the sample was immediately placed back into the immersion. A new straight line was also scratched onto the sample, as done previously, when the hydrolysis time was over; as a result, three scratched lines on the surface were obtained. The sample was put into FITC-labeled BSA for 20 min and photographed at 4× magnification on a Nikon Eclipse Ti series microscope.

2.13. Steam Hydrolysis and Long-Term Nonfouling Performance. To explore the hydrolysis property of the material (PCB-ester-PU3) under the condition of high-temperature steam, we put the polymer films in 120 °C steam for 20 min and then tested its nonspecific protein adsorption by quantifying HRP-conjugated anti-IgG adsorption (ELISA). To test its hydrolysis property in saline solution and long-term nonfouling performance, the polymer films (PCB-ester-PU3) were directly hydrolyzed in PBS for 30 days. A sample that was hydrolyzed in 0.1 M NaOH for 0.5 h and then PBS for 30 days was also prepared, for comparison. Nonspecific protein adsorption of long-term samples was also tested by ELISA. The detailed experimental procedures of ELISA were shown in section 2.8, and all the data were relative to tissue culture polystyrene (TCPS) controls.

3. RESULTS AND DISCUSSION

3.1. Surface Characterization of PCB-Ester-PU Material. Stable coatings of the hydrophobic PCB-ester-PUs were obtained on both polar stainless steel and nonpolar propylene substrates. According to our previous reports,[28,29] CB-ester could be hydrolyzed gradually to a protein-resistant zwitterionic format in basic NaOH solution. The chemical structure of the new material (PCB-ester-PU3) was confirmed by attenuated total reflectance−infrared (ATR-IR) spectroscopy. The red curve in Figure 2 demonstrates the formation of urethane bonds and the incorporation of CB-ester into the side chains of PUs by converting regular hydrophilic zwitterions to the hydrophobic esterified format. The blue curve shows the

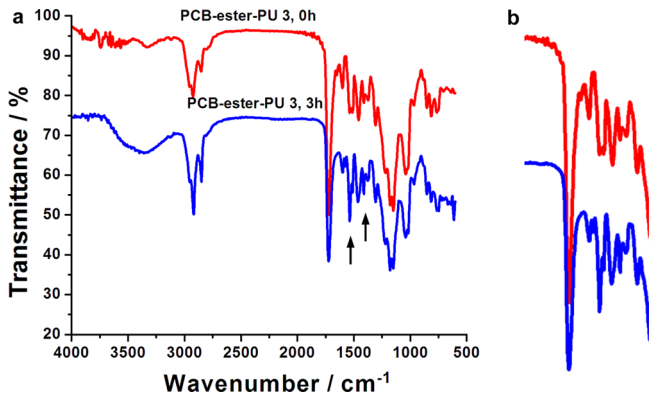


Figure 2. (a) ATR-IR spectra of PCB-ester-PU3 before and after 3 h hydrolysis; (b) magnified spectra in the wavenumber region between 2000 cm^{-1} to 1250 cm^{-1} to give detailed illustration of the new peaks observed at 1561 cm^{-1} and 1398 cm^{-1}.

DOI: 10.1021/acsami.5b05162
ACS Appl. Mater. Interfaces 2015, 7, 16938−16945

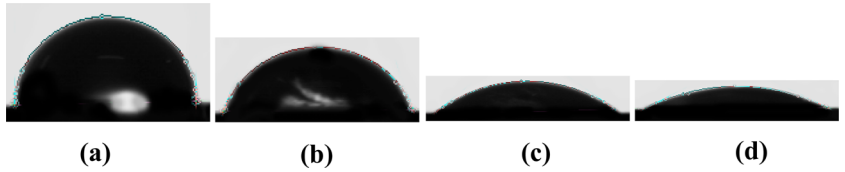


Figure 3. Typical advancing water contact angles of PCB-ester-PU3 (a) before hydrolysis, (b) after 3 h of hydrolysis, (c) after 6 h of hydrolysis, and (d) after 12 h of hydrolysis using 0.1 M NaOH. Here, the advancing angles for panels a–d were 90.2° ± 2°, 70.3° ± 1°, 37.4° ± 2°, and 26.8° ± 1°, respectively.

spectrum of PCB-ester-PU3 after 3 h hydrolysis in 0.1 M NaOH. The new peaks observed at 1561 and 1398 cm^{-1} were assigned to COO^- groups, indicating the hydrolysis of CB-ester and the formation of zwitterionic groups. Furthermore, an additional broad shoulder observed at 3393 cm^{-1} was probably due to an increase in hydration caused by the formation of hydrophilic zwitterionic groups. The surface hydrophilicity of the polymer (PCB-ester-PU3) was further characterized by static water contact angle measurements. Figure 3 shows that the hydrophilicity of the PU surfaces improved because of the increase in hydrolysis time and the observed contact angles decreased from 90.2° ± 2° before hydrolysis to 26.8° ± 1° after 12 h of hydrolysis using 0.1 M NaOH. The results indicate that zwitterionic segments occurred near the surface of the PU films via the hydrolysis of CB-ester incorporated into the side chains of PU and the zwitterionic units increased after hydrolysis.

3.2. Evaluation of Protein Adsorption. The protein adsorption of these novel PCB-ester-PU materials containing CB-ester analogues before and after hydrolysis was evaluated using HRP-conjugated anti-IgG. The amount of anti-IgG adsorption on different films was determined by monitoring the increase in tangerine color intensity at 492 nm that is caused by the reaction of HRP with OPD, relative to TCPS. The resistance to HRP-conjugated anti-IgG was improved dramatically from PCB-ester-PU1 to PCB-ester-PU3 and decreased slightly at PCB-ester-PU4 after 3 h of hydrolysis in 0.1 N NaOH (Figure 4a), which is the best hydrolysis condition in this system. This trend of resistance is mainly caused by the increase of the CB-ester content and the formation of longer zwitterionic polymer brush after hydrolysis of PCB-ester side chain. It is worthwhile to emphasize that the nonspecific adsorption of HRP-conjugated anti-IgG on PCB-ester-PU3 and PCB-ester-PU4 was 0.38% ± 0.15% and 0.8% ± 0.32% of the amount on TCPS, respectively. Moreover, the fibrinogen, a well-known "soft" blood plasma protein for easy adsorption on various surfaces through weak nonspecific interactions, adsorption on PCB-ester-PU3 is 0.45% of the amount on TCPS. These results indicated that the protein adsorption is below or close to the general definition of ultralow fouling, which <0.5% of the monolayer. This excellent resistance reaches a comparable level of the most highest quality of zwitterionic polymer-coated surfaces obtained by SI-ATRP.[14,21,22] The slight decrease in the resistance to protein of PCB-ester-PU4 mainly was caused by the low degree of polymerization of the PU chain due to the interruption of the linear PU backbone formation by the longer PCB-ester side chains. The zwitterionic PCB chain formed might detach from the surface without enough long hydrophobic chains in a polymer matrix. In fact, the lowest mechanical property of PCB-ester-PU4 among all PCB-ester-PUs was also evidence of the low degree of polymerization. Beside the PCB-ester content, the chain length and arrangement of zwitterionic polymer brush play a very important role in this excellent

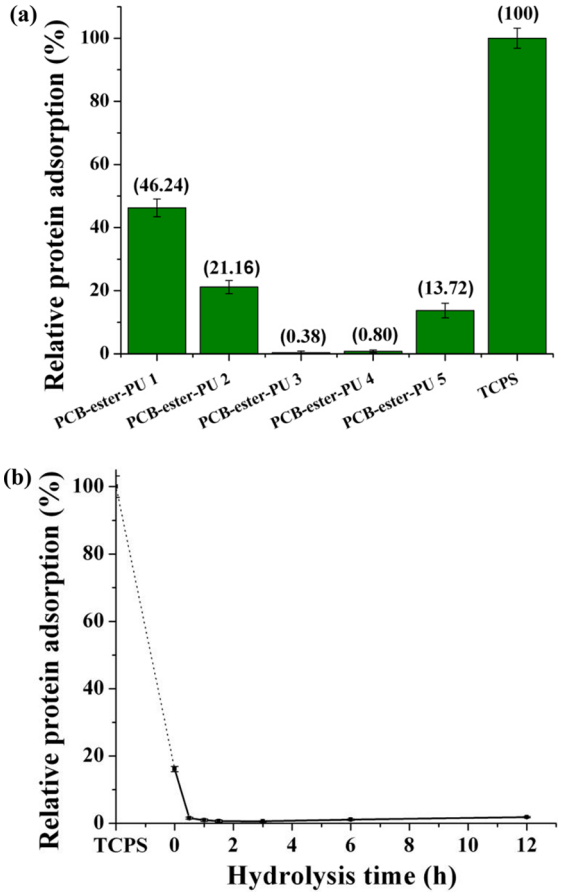


Figure 4. (a) HRP-conjugated IgG adsorption on PCB-ester-PU1–5 and TCPS, hydrolyzed for 3 h in 0.1 M NaOH, normalized to TCPS. (b) HRP-conjugated IgG adsorption versus hydrolysis time of PCB-ester-PU3 film, normalized to TCPS.

resistance to protein adsorption. For example, both PCB-ester-PU2 and PCB-ester-PU5 have very similar PCB-ester contents, but PCB-ester-PU5 shows better resistance than PCB-ester-PU2, which indicates that a certain chain length is required for covering defects due to the polydispersity of PCB-ester$(OH)_2$. It is believed that the final PCB zwitterionic polymer brush generated from high PCB-ester content matrix must be rather highly dense to cover most defects to prevent protein adsorption, which should be the reason why PCB-ester-PU3 reaches the ultralow fouling level.

Figure 4b shows the protein adsorption versus hydrolysis time of PCB-ester-PU3, as shown, the best resistance (~0.4%), relative to TCPS, was obtained after 1.5–3.0 h of hydrolysis in 0.1 M NaOH (pH 13), which was in agreement with the measurement by Lin et al.[28] As the hydrolysis time increased, the protein adsorption gradually increased, which is possibly because the harsh strongly alkaline conditions caused damage

DOI: 10.1021/acsami.5b05162
ACS Appl. Mater. Interfaces 2015, 7, 16938–16945

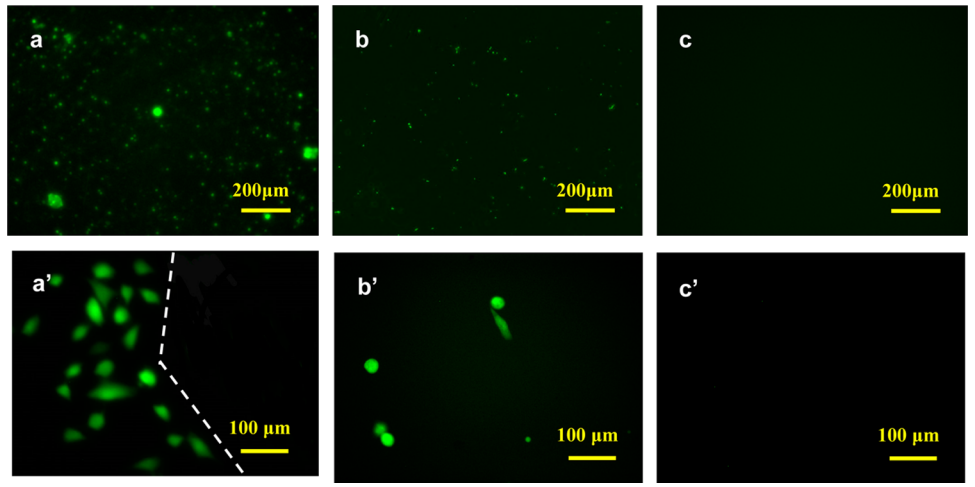


Figure 5. Bacterial adhesion and HUVEC cells attachment on the surfaces of (a and a′) TCPS, (b and b′) PCB-ester-PU3 before hydrolysis, and (c and c′) PCB-ester-PU3 after 3 h of hydrolysis using 0.1 M NaOH. The boundaries of hydrolyzed PCB-ester-PU3 in panel a′ are marked with dotted lines; HUVEC cells grew healthily on TCPS on the left-hand side of the boundaries, and no cell was found on hydrolyzed PCB-ester-PU3 on the right-hand side.

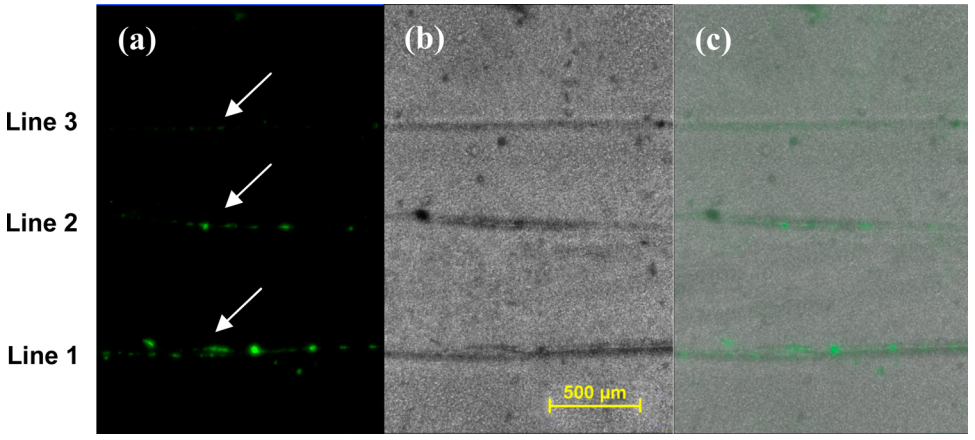


Figure 6. Microscopic images of FITC labeled BSA on the surfaces of PCB-ester-PU3 after 0, 10 min, and 1.5 h of hydrolysis using 0.1 M NaOH: (a) fluorescent photograph, (b) normal photograph, and (c) merger of panels a and b.

of the structure and degradation of the polymer after an excessively long hydrolysis time. The hydrophobic matrix incorporated into the PU chains slowed the degradation rate of these polymers in bulk format and resulted in long periods of protein resistance. After all, these results indicated that we successfully developed a new material with long-lasting excellent protein adsorption resistance.

3.3. Bacterial Adhesion and HUVEC Cells Attachment. Consequently, the excellent protein-resistant PCB-ester-PU3 also shows excellent resistance to bacterial adhesion and cell attachment. Bacterial adhesion onto surfaces and the subsequent formation of a biofilm can cause a severely negative impact on many biomedical applications. It is clearly shown that hardly any *Staphylococcus aureus* (*S. aureus*) was found on PCB-ester-PU3 after 3 h of hydrolysis, whereas a large amount of *S. aureus* was observed on the surfaces of TCPS (Figure 5). Moreover, the adhesion of *S. aureus* on fresh PCB-ester-PU3 also is quite low, which suggests that the early hydrolysis of PCB-ester could also generate resistance to bacterial adhesion, in agreement with the protein resistance on fresh PCB-ester-PU3 (see Figure 4b). On the other hand, similar results of HUVECs attachment were observed. No HUVEC was observed on PCB-ester-PU3 after 3 h of hydrolysis, and very few HUVECs on fresh PCB-ester-PU3 were observed, whereas many HUVECs on the TCPS were observed under good attachment and spreading conditions (see Figure 5a′, left.). In fact, the right-hand side of Figure 5a′ was covered by hydrolyzed PCB-ester-PU3, indicating that no toxicity molecules were leached out during incubation. This result gives further evidence of good cell compatibility and low cytotoxicity of PCB-ester-PUs. In short, this high resistance to both bacterial adhesion and mammalian cell attachment and good cell compatibility offer great potential for biomedical applications.

3.4. Results of Self-Regeneration. Moreover, the resistance of PCB-ester-PU can be recovered through self-regeneration after being damaged by the unwanted scratches. The self-regeneration of PCB-ester-PU3 is illustrated by FITC-labeled BSA adsorption. As shown in Figure 6, three straight lines, marked as line 1 (fresh scratch), line 2 (10 min of hydrolysis), and line 3 (1.5 h of hydrolysis) were observed under the microscope. The strong adsorption of FITC-labeled BSA (line 1) on the freshly damaged surface indicates that the material has very low resistance to protein adsorption before regeneration. The surface adsorption of FITC-labeled BSA on the scratched line (line 2) was reduced remarkably after 10 min

DOI: 10.1021/acsami.5b05162
ACS Appl. Mater. Interfaces 2015, 7, 16938−16945

of hydrolysis in 0.1 N NaOH, which illustrates that the material could possess nonfouling property after a quick hydrolysis to form zwitterionic segments. At the end, the protein adsorption was very little and difficult to observe after 1.5 h of hydrolysis (line 3). The zwitterionic polymer brush-covered surface was formed through self-regeneration by quick hydrolysis from hydrophobic matrix. In short, the PCB-ester-PU could heal the resistance of the damaged surface through regeneration.

3.5. Methods of Surface Activation and Long-Term Nonfouling Performance of the Material. In fact, such a resistance can not only be generated by 0.1 N NaOH solution, but also by physiological PBS solution and by steam in an autoclave. The protein adsorption of PCB-ester-PU3 could be reduced to 4.6% relative adsorption of TCPS after 30 days in PBS (Figure 7, sample a). This indicated that the generation of

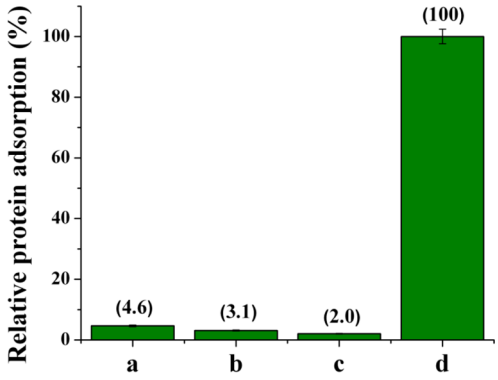


Figure 7. Relative protein adsorption of (a) hydrolysis in PBS for 30 days, (b) hydrolysis in 0.1 M NaOH for 0.5 h and then PBS for 30 days, (c) hydrolysis in 120 °C steam for 20 min, and (d) TCPS.

a nonfouling property could occur under mild conditions (the physiological condition or seawater), suggesting that there is no limitation for applying this method for large objects, such as maritime boat immersed in slightly basic seawater, of which the pH value is in the range of 8.0−8.5. Moreover, the protein adsorption after 30 days could be even lower than 3.1% relative adsorption of TCPS when the PCB-ester-PU3 is pretreated in 0.1 M NaOH for 0.5 h (Figure 7, sample b), which indicated that the resistance can be maintained very well for long-term application. Taken together with the accelerated hydrolysis process of PCB-ester-PU3 in 0.1 M NaOH (Figure 4), it is suggested that the resistance of PCB-ester-PU3 could be maintained much longer than 30 days in PBS, which is an ideal long-term property to meet the requirement for maritime application. Furthermore, it is found that the protein adsorption of PCB-ester-PU3 is only 2.0% relative adsorption of TCPS after 20 min of hydrolysis in steam (at 120 °C) in an autoclave (Figure 7, sample c). This high-temperature steam hydrolysis opens a pathway for biomedical coating through compatibility with traditional sterilizing methods. Furthermore, the PCB-ester-PU3 does not show swelling and obvious degradation of the mechanic properties after these three hydrolysis methods. All the results suggest that these PCB-ester-PU coatings are robust in both resistances for long-term application.

3.6. Results of Pull-Off Strength and Film Hardness. Furthermore, the mechanic properties are far more stable than other hydrogel coatings. Figure 8a shows that the coating of PCB-ester-PU3 could adhere onto stainless steel very tightly and not be shedding, even curved to a rather large angle. Figures 8b and 8c showed the coating on stainless steel before and after the pull-off test, respectively. The pencil hardness of coating reaches to H, according to international standard ISO 15184, which is the generally acceptable hardness for industrial coatings. Moreover, the maximum load applied at the point of coating failure was recorded by tensile machine and is reported in Table 2. The pull-off test results showed the adhesion

Table 2. Results of Pull-Off Test and Film Hardness

substrate	pull-off strength (MPa)	film hardness
stainless steel	1.62 ± 0.18	H
polypropylene	0.83 ± 0.04	H

strength of 1.62 ± 0.18 MPa on steel and 0.83 ± 0.04 MPa on polypropylene without surface pretreatment and additional binding agents, respectively, which indicates that the material have good adhesion properties on both polar stainless steel and nonpolar propylene surfaces. This result offers great possibility for the facile use of this material for ultralow-fouling coatings on biomedical devices and the submerged surfaces of boat.

4. CONCLUSION

In summary, a new polyurethane material containing poly(carboxybetaine) ester analogue side chains, showing ultralow fouling to protein adsorption, bacterial adhesion, and HUVEC cell attachment, has been developed and characterized. The zwitterionic brush generated from interfacial hydrolysis of ester groups in the hydrophobic side chains of PU dramatically reduce the protein adsorption to the level of the zwitterionic polymer brush prepared by SI-ATRP, which is the commonly believed best method to attain a nonfouling surface. Its superior mechanical properties (such as hardness and elasticity), good adhesiveness as a paint on both polar and nonpolar substrates,

Figure 8. (a) Photograph of PU coating on steel. (b, c) Also shown are photographs of the sample (b) before and (c) after the pull-off test.

DOI: 10.1021/acsami.5b05162
ACS Appl. Mater. Interfaces 2015, 7, 16938−16945

and, furthermore, the excellent long-term nonfouling capability along with the healing process through self-regeneration provide great potential of this material for application as a coating for medical and marine devices.

It should be pointed out that this is still an initial step toward marine antifouling coatings, since the complexity of the environment and the variety of fouling organisms is much more challenging than what we expected. Our preminary results showed that PCB-ester-PU3 coatings have a remarkable resistance to the settlement of diatoms after hydrolysis in 0.1 M NaOH for 5 min. This result suggests that the polymer has fairly good resistance to certain marine species and will be reported in the future.

AUTHOR INFORMATION

Corresponding Author

*E-mail: schen@zju.edu.cn.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors appreciate financial support from the National Nature Science Foundation of China (Nos. 21174127, 21474085), the Ph.D. Programs Foundation of Ministry of Education of China (No. 20110101110034), Zhejiang Provincial Natural Science Foundation of China (No. LZ13E030001), and the Department of Education of Zhejiang Province (No. Z200804487).

REFERENCES

(1) Horbett, T. A. The Role of Adsorbed Proteins in Animal Cell Adhesion. *Colloids Surf., B* **1994**, *2*, 225−240.

(2) Ratner, B. D.; Bryant, S. J. Biomaterials: Where We Have Been and Where We Are Going. *Annu. Rev. Biomed. Eng.* **2004**, *6*, 41−75.

(3) Callow, J. A.; Callow, M. E. Trends in the Development of Environmentally Friendly Fouling-Resistant Marine Coatings. *Nat. Commun.* **2011**, *2*, Article No. 244 (DOI: 10.1038/ncomms1251).

(4) Schultz, M. P. Effects of Coating Roughness and Biofouling on Ship Resistance and Powering. *Biofouling* **2007**, *23*, 331−341.

(5) Poloczanska, E. S.; Butler, A. J. Biofouling and Climate Change. In *Biofouling*; Dürr, S., Thomason, J. C., Eds.; Wiley−Blackwell: New York, 2010; Chapter 23, pp 333−347.

(6) Sousa, J. E.; Serruys, P. W.; Costa, M. A. New Frontiers in Cardiology Drug-Eluting Stents: Part I. *Circulation* **2003**, *107*, 2274−2279.

(7) Suzuki, T.; Kopia, G.; Hayashi, S.-i.; Bailey, L. R.; Llanos, G.; Wilensky, R.; Klugherz, B. D.; Papandreou, G.; Narayan, P.; Leon, M. B.; Yeung, A. C.; Tio, F.; Tsao, P. S.; Falotico, R.; Carter, A. J. Stent-Based Delivery of Sirolimus Reduces Neointimal Formation in a Porcine Coronary Model. *Circulation* **2001**, *104*, 1188−1193.

(8) Yebra, D. M.; Kiil, S.; Dam-Johansen, K. Antifouling Technology—Past, Present and Future Steps Towards Efficient and Environmentally Friendly Antifouling Coatings. *Prog. Org. Coat.* **2004**, *50*, 75−104.

(9) Chapman, R. G.; Ostuni, E.; Takayama, S.; Holmlin, R. E.; Yan, L.; Whitesides, G. M. Surveying for Surfaces that Resist the Adsorption of Proteins. *J. Am. Chem. Soc.* **2000**, *122*, 8303−8304.

(10) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. A Survey of Structure-Property Relationships of Surfaces that Resist the Adsorption of Protein. *Langmuir* **2001**, *17*, 5605−5620.

(11) Jiang, S.; Cao, Z. Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications. *Adv. Mater.* **2010**, *22*, 920−932.

(12) Chen, S.; Jiang, S. A New Avenue to Nonfouling Materials. *Adv. Mater.* **2008**, *20*, 335−338.

(13) Chen, S.; Zheng, J.; Li, L.; Jiang, S. Strong Resistance of Phosphorylcholine Self-Assembled Monolayers to Protein Adsorption: Insights into Nonfouling Properties of Zwitterionic Materials. *J. Am. Chem. Soc.* **2005**, *127*, 14473−14478.

(14) Konno, T.; Hasuda, H.; Ishihara, K.; Ito, Y. Photo-Immobilization of a Phospholipid Polymer for Surface Modification. *Biomaterials* **2005**, *26*, 1381−1388.

(15) Goda, T.; Konno, T.; Takai, M.; Moro, T.; Ishihara, K. Biomimetic Phosphorylcholine Polymer Grafting from Polydimethylsiloxane Surface Using Photo-Induced Polymerization. *Biomaterials* **2006**, *27*, 5151−5160.

(16) Zhang, Z.; Chao, T.; Chen, S. F.; Jiang, S. Y. Superlow Fouling Sulfobetaine and Carboxybetaine Polymers on Glass Slides. *Langmuir* **2006**, *22*, 10072−10077.

(17) Chang, Y.; Chen, S. F.; Zhang, Z.; Jiang, S. Y. Highly Protein-Resistant Coatings from Well-defined Diblock Copolymers Containing Sulfobetaines. *Langmuir* **2006**, *22*, 2222−2226.

(18) Zhang, Z.; Chen, S. F.; Chang, Y.; Jiang, S. Y. Surface Grafted Sulfobetaine Polymers via Atom Transfer Radical Polymerization as Superlow Fouling Coatings. *J. Phys. Chem. B* **2006**, *110*, 10799−10804.

(19) Zhang, Z.; Cheng, G.; Carr, L. R.; Vaisocherova, H.; Chen, S. F.; Jiang, S. Y. The Hydrolysis of Cationic Polycarboxybetaine Esters to Zwitterionic Polycarboxybetaines with Controlled Properties. *Biomaterials* **2008**, *29*, 4719−4725.

(20) Yang, W.; Xue, H.; Li, W.; Zhang, J. L.; Jiang, S. Y. Pursuing "Zero" Protein Adsorption of Poly(carboxybetaine) from Undiluted Blood Serum and Plasma. *Langmuir* **2009**, *25*, 11911−11916.

(21) Ladd, J.; Zhang, Z.; Chen, S.; Hower, J. C.; Jiang, S. Zwitterionic Polymers Exhibiting High Resistance to Nonspecific Protein Adsorption from Human Serum and Plasma. *Biomacromolecules* **2008**, *9*, 1357−1361.

(22) Cheng, G.; Zhang, Z.; Chen, S.; Bryers, J. D.; Jiang, S. Inhibition of Bacterial Adhesion and Biofilm Formation on Zwitterionic Surfaces. *Biomaterials* **2007**, *28*, 4192−4199.

(23) Cheng, G.; Li, G.; Xue, H.; Chen, S.; Bryers, J. D.; Jiang, S. Zwitterionic Carboxybetaine Polymer Surfaces and Their Resistance to Long-term Biofilm Formation. *Biomaterials* **2009**, *30*, 5234−5240.

(24) Zhang, Z.; Finlay, J. A.; Wang, L. F.; Gao, Y.; Callow, J. A.; Callow, M. E.; Jiang, S. Y. Polysulfobetaine-Grafted Surfaces as Environmentally Benign Ultralow Fouling Marine Coatings. *Langmuir* **2009**, *25*, 13516−13521.

(25) Cheng, G.; Xue, H.; Zhang, Z.; Chen, S.; Jiang, S. A Switchable Biocompatible Polymer Surface with Self-Sterilizing and Nonfouling Capabilities. *Angew. Chem., Int. Ed.* **2008**, *47*, 8831−8834.

(26) Cao, B.; Tang, Q.; Cheng, G. Recent Advances of Zwitterionic Carboxybetaine Materials and Their Derivatives. *J. Biomater. Sci., Polym. Ed.* **2014**, *25*, 1502−1513.

(27) Coneski, P. N.; Wynne, J. H. Zwitterionic Polyurethane Hydrogels Derived from Carboxybetaine-Functionalized Diols. *ACS Appl. Mater. Interfaces* **2012**, *4*, 4465−4469.

(28) Lin, W.; Zhang, J.; Wang, Z.; Chen, S. Development of Robust Biocompatible Silicone with High Resistance to Protein Adsorption and Bacterial Adhesion. *Acta Biomater.* **2011**, *7*, 2053−2059.

(29) Ji, F.; Lin, W.; Wang, Z.; Wang, L.; Zhang, J.; Ma, G.; Chen, S. Development of Nonstick and Drug-Loaded Wound Dressing Based on the Hydrolytic Hydrophobic Poly(carboxybetaine) Ester Analogue. *ACS Appl. Mater. Interfaces* **2013**, *5*, 10489−10494.

(30) Zhang, J.; Wang, Z.; Lin, W.; Chen, S. Gene Transfection in Complex Media Using PCBMAEE-PCBMA Copolymer with Both Hydrolytic and Zwitterionic Blocks. *Biomaterials* **2014**, *35*, 7909−7918.

DOI: 10.1021/acsami.5b05162
ACS Appl. Mater. Interfaces 2015, 7, 16938−16945