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Applying Thermosettable Zwitterionic Copolymers as General Fouling-Resistant and Thermal-Tolerant Biomaterial Interfaces

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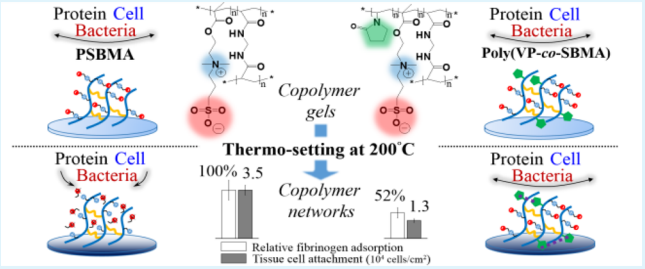
Supporting Information

ABSTRACT: We introduced a thermosettable zwitterionic copolymer to design a high temperature tolerance biomaterial as a general antifouling polymer interface. The original synthetic fouling-resistant copolymer, poly(vinylpyrrolidone)-*co*-poly(sulfobetaine methacrylate) (poly(VP-*co*-SBMA)), is both thermal-tolerant and fouling-resistant, and the antifouling stability of copolymer coated interfaces can be effectively controlled by regulating the VP/SBMA composition ratio. We studied poly(VP-*co*-SBMA) copolymer gels and networks with a focus on their general resistance to protein, cell, and bacterial bioadhesion, as influenced by the thermosetting process. Interestingly, we found that the shape of the poly(VP-*co*-SBMA) copolymer material can be set at a high annealing temperature of 200 °C while maintaining good antifouling properties. However, while the zwitterionic PSBMA polymer gels were bioinert as expected, control of the fouling resistance of the PSBMA polymer networks was lost in the high temperature annealing process. A poly(VP-*co*-SBMA) copolymer network composed of PSBMA segments at 32 mol % showed reduced fibrinogen adsorption, tissue cell adhesion, and bacterial attachment, but a relatively higher PSBMA content of 61 mol % was required to optimize resistance to platelet adhesion and erythrocyte attachment to confer hemocompatibility to human blood. We suggest that poly(VP-*co*-SBMA) copolymers capable of retaining stable fouling resistance after high temperature shaping have a potential application as thermosettable materials in a bioinert interface for medical devices, such as the thermosettable coating on a stainless steel blood-compatible metal stent investigated in this study.



KEYWORDS: *zwitterionic copolymer, temperature tolerance, fouling-resistant, thermosetting, charge neutrality, sulfobetaine methacrylate*

INTRODUCTION

Antifouling polymer materials have been widely studied in the design of bioinert interfaces to prevent nonspecific protein adsorption, cell adhesion, and bacterial attachment for various biomedical applications,[1−8] such as blood-contacting implanted devices,[9−11] drug (or gene) delivery carriers,[12,13] filtration membranes,[14] and diagnostic biosensors,[15,16] as well as coatings for ship hulls.[17,18] In general, good nonspecific protein resistance is the key to bioinert polymer interfaces. To achieve a well-defined protein-resistant surface, the molecular design of the antifouling material must meet three prerequisites: (i) it must not be hydrophobic, (ii) it must be electrically neutral, and (iii) it must contain hydrogen bond acceptors instead of donors. These guidelines have been used for the design of an antifouling formulation for medical applications with a new chemical structure that has nonspecific protein resistance.[19] Zwitterionic materials possess all three of these required molecular characteristics; phosphobetaine (PB), carboxylbetaine (CB), and sulfobetaine (SB) are well-known zwitterionic materials used in bioinert polymeric interfaces for resistance to general biofoulants that each represent one of these prerequisites.[20−28]

Poly(sulfobetaine methacrylate) (PSBMA) has become the most widely studied zwitterionic polymer due to its robust antifouling properties and ease of synthesis.[10,15,22,29] Various techniques have been applied to improve its applicability while maintaining the versatile functionality of antifouling PSBMA materials, such as thermoresponsive hydrogels,[30] hydrophobic-driven assembled copolymers, and ionic-anchoring copolymers.[10] Until now, the preparation, grafting, and coating of zwitterionic PSBMA interfaces have all been performed at temperatures below 100 °C, which yields excellent antifouling properties when used for a specific biofouling-resistant target. However, the thermal tolerance of PSBMA materials and whether their antifouling properties can be maintained when placed in contact with a complex medium such as human blood

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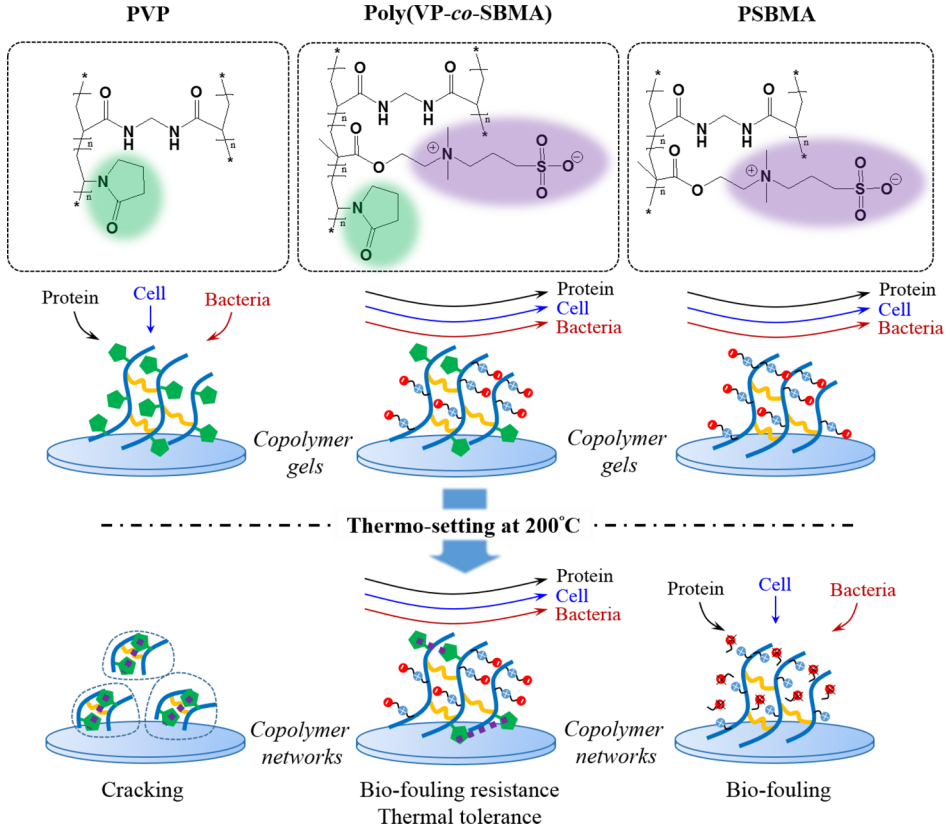
Scheme 1. Molecular Structure and Thermosetting Illustration of PVP, Poly(VP-*co*-SBMA), and PSBMA Copolymer Network Gels via Annealing Procedure at 200 °C



or tissue after being subjected to a high temperature process is unclear.

Medical devices such as surgical knives, artificial arms, metal stents, and implanted metals must be sterilized at 121 °C.[31] However, while a temperature higher than 121 °C would remove most bacteria that cause inflammation, the antifouling materials must now withstand high temperature sterilization while maintaining their antifouling properties to prevent thrombosis. Poly(vinylpyrrolidone) (PVP) is one solution; it has been reported to have good thermal stability up to 130 °C,[32] to become cross-linked at 200 °C to form a thin film on a surface,[33] and to have a low single protein adsorption level. However, although PVP resists fouling by single proteins, it is not suitable for application in a complex medium such as 100% human blood, tissue cells, or bacteria.

In this study, we introduced a combination of thermally stable PVP and durable antifouling PSBMA to form a new class of thermosettable and fouling-resistant zwitterionic copolymer interfaces with thermal tolerance, as shown in Scheme 1. This study systematically clarified the effects of thermal treatment on the biofouling resistance of poly(vinylpyrrolidone)-*co*-poly(sulfobetaine methacrylate) (poly(VP-*co*-SBMA)) copolymer gels without thermosetting and copolymer networks with thermosetting. The effect of regulating the VP/SBMA ratio and applying the thermal annealing treatment on the surface composition, hydrophilicity, swelling behavior, zeta (ζ) potential, and degree of shaping of poly(VP-*co*-SBMA) are discussed. We demonstrated the biocompatibility of copolymer gels and networks by human fibrinogen adsorption, platelet activation, erythrocyte hemolysis, tissue cell adhesion, and bacterial attachment. The results indicate that the thermal-induced, cross-linkable PVP segments in the poly(VP-*co*-SBMA) copolymer gel are amenable to thermosetting and have good mechanical properties, and by controlling the ratio of PVP to PSBMA, the prepared poly(VP-*co*-SBMA) copolymer networks could tolerate temperatures up to 200 °C while maintaining excellent general resistance to plasma proteins, blood cells, mammalian cells, and bacteria.

EXPERIMENTAL SECTION

Materials. *N*,*N*,*N*′,*N*′-Tetraethylmethylenediamine (TEMED), ammonium persulfate (APS), *N*,*N*′-methylenebis(acrylamide) (MBAA), 2-(methacryloyloxy)ethyl]trimethylammonium chloride (TMA), 3-sulfopropyl methacrylate potassium salt (SA), diiodomethane, and 1-vinyl-2-pyrrolidone (VP) were purchased from Sigma-Aldrich. Human plasma fibrinogen (fraction I) was purchased from Sigma Chemical Co. [2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide (sulfobetaine methacrylate, SBMA) was purchased from Monomer-Polymer & Dajac Laboratories, Inc. Deionized water (DI water) was purified by the Millipore water purification system with a minimum resistivity of 18.0 MΩ·m.

Preparation of PVP, PSBMA, and Poly(VP-*co*-SBMA) Copolymer Gels and Networks. The cross-linker MBAA was dissolved in DI water at 1.6 wt % and stirred for 6 h at 50 °C. SBMA and VP at varying molar ratios were added to the MBAA solution, and the mixture was stirred for 10 min or until completely dissolved. The copolymeric reaction of the hydrogels was initiated by 0.2 wt % APS and 0.2 wt % TEMED, and the solution was immediately transferred to a container to form a layer with a thickness of 0.2 mm. After polymerization for 3 h at 25 °C in a drybox under a nitrogen atmosphere, the gels were immersed in water for 48 h to remove chemical impurities. All gels were made in disk form with a diameter of 10.0 mm (10 mm biopsy punch, Acuderm Inc.) and stored in DI water at 4 °C until ready to use. The prepared gels were later dried in an evaporating oven to remove the extra water to form a consistent shape.

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Finally, the polymer and copolymer networks were annealed at 200 °C to fix their shape by thermosetting.

The chemical structures of the prepared gels and networks were determined with Fourier transform infrared spectroscopy (FT-IR) (PerkinElmer Spectrum One). The spectra were captured by 32 scans to reduce the noise in the curve, as shown in Figure 1. X-ray photoelectron spectroscopy (XPS) (Thermal Scientific K-Alpha spectrometer) was performed to confirm that the resultant material interfaces had the correct chemical compositions using a monochromated Al K X-ray source of 1486.6 eV as the excitation energy. The photoelectron signals of the XPS were measured with a hemispherical analyzer with a pass energy between 50 and 150 eV and detected at a takeoff angle of 45° in relation to the sample surface. All the spectra were referenced by a binding energy (BE) of C 1s to 284.6 eV. Unifit 2012 (Unifit Scientific Software, Germany) was used to deconvolute the peaks via Shirley background subtraction and Lorentzian fitting function. The fitting was set with 0.1−2 of full width at half-maximum for the peaks and used 10 times for integration. The quantification data were used to determine the composition of the copolymer gels and networks by calculations based on the spectral area sulfur-to-nitrogen (S/N) ratio (S 2p of the SBMA side groups and N 1s of the VP groups) at BEs of approximately 399 and 168 eV, respectively, as summarized in Table 1.

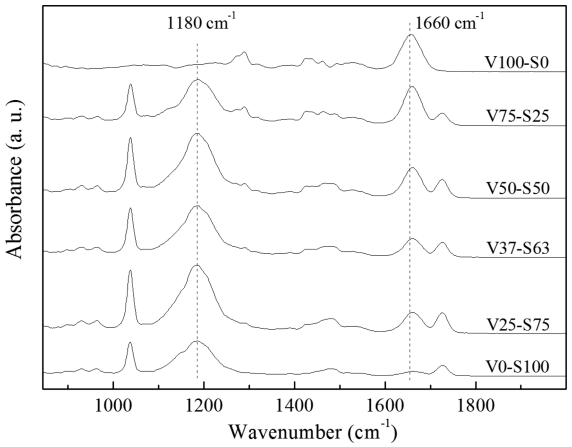

Figure 1. FT-IR spectra of PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) gels without thermosetting.

Characterization of PVP, PSBMA, and Poly(VP-*co*-SBMA) Copolymer Gels and Networks. The weight and volume swelling ratios were used to determine the hydrophilicity and water content of the hydrogels. The weight swelling ratio was measured gravimetrically by the difference between dried and wet copolymer gels or networks using the equation $(W_w - W_d)/W_d$. The wet weights (W_w's) of the as-prepared gels and networks were measured before they were dried in a vacuum at 40 °C for 3 days, after which their dry weights (W_d's) were measured by an identical procedure. We determined the volume swelling ratio similarly to the swelling ratio, using $(V_w - V_d)/V_d$, where V_d and V_w are the volume of dried disks and the diameter of swollen disks, respectively, measured using a caliper (minimum scale is 0.1 mm) for the diameter. The degree of shaping of the copolymer gels and networks is defined as the difference in volume between the copolymer gels and copolymer networks divided by the volume of the copolymer gels.

$$\text{degree of shaping (\%)} = (V_{gels} - V_{networks})/V_{gels} \cdot 100\%$$

where V_{gels} is the volume of the copolymer gels and $V_{networks}$ is the volume of the copolymer networks.

A dynamic mechanical analyzer (DMA 7e, PerkinElmer) was used to carry out the compressional tests of the gels with and without thermosetting. The stress−strain curves are shown in the Supporting Information, Figure S1, to demonstrate the amount of shaping by comparing the slopes of the curves. These curves were obtained from three repeated samplings. All the gels and networks were compressed to obtain the failure strain and stress. Young's moduli were measured by the fitting slope of the first 10% strain in the curves, and all results are summarized in Table 2.

The oil-phase contact angles were measured with an angle meter (Automatic Contact Angle Meter, Model CAVP, KyowDa Interface Science Co., Ltd., Japan) at 25 °C, using diiodomethane as the dropping solution, where the copolymer gels and networks were first submerged in water medium and then diiodomethane was slowly dropped onto their surfaces at three different sites. The average of the angle values was calculated from five independent samples.

The ζ potential of the copolymer gels and networks was measured by dynamic light scattering (Beckman Coulter Delsa Nano S). The prepared gels and networks were first dried in a lyophilizer for 24 h and ground into a white powder. The samples were then dissolved in a pH 7.4 phosphate buffered saline (PBS) solution to form a cloudy suspension. After standing for 5 h, the upper solution was used for the ζ potential measurement. TMA and SA gels with the same composition as the previous copolymer gels were made and used as the positive and negative references, respectively, for the ζ potential.

Human Plasma Protein Adsorption, Human Blood Platelets, and Whole Blood Cell Adhesion. The adsorption of fibrinogen, a human plasma protein, was evaluated by enzyme-linked immunosorbent assay (ELISA) to improve the sensitivity of the target proteins. Human blood platelets and whole blood cell attachments were tested using blood samples from healthy volunteers. The detailed protocols for human protein adsorption and human blood cell attachment were explained further in earlier publications.[10,14,29]

Red Blood Cell Hemolysis. Hemolysis assay was used to estimate the disruption of red blood cells (RBCs), thereby determining the degree of nonfouling of the polymeric gels and networks, and the

Table 1. Characteristic Data of Poly(VP-*co*-SBMA) Copolymer Gels and Networks

		poly(VP-*co*-SBMA) gels without thermosetting[b]					poly(VP-*co*-SBMA) networks with thermosetting[b]				
sample ID	comonomer molar ratio[a] [VP]/[SBMA]	N(3°)/S	N(4°)/S	(VP)/(SBMA)	swelling ratio	contact angle	N(3°)/S	N(4°)/S	(VP)/(SBMA)	swelling ratio	contact angle
V100-S0	100/0	–	–	100/0	9.19	152	–	–	–	–	–
V75-S25	75/25	4.35	1.01	81/19	3.01	153	–	–	–	–	–
V50-S50	50/50	2.17	1.02	68/32	2.41	155	2.38	1.08	70/30	2.05	147
V37-S63	37/63	0.65	0.99	39/61	2.26	159	0.72	1.05	42/58	1.94	143
V25-S75	25/75	0.36	1.03	26/74	1.99	164	0.44	1.12	31/69	1.83	141
V0-S100	0/100	0	1.01	0/100	1.62	165	0	1.16	0/100	1.47	139

[a]Reaction molar ratio of comonomers to initiator was fixed at 90 to 1. The total reactant mass percentage was 20 wt % in the prepared reaction solution. [b]The mole mass ratio of PVP to SBMA, (VP)/(SBMA), in the poly(VP-*co*-SBMA) copolymer gels and networks was determined by XPS in the dry state from the spectral area ratio of the atomic percentages based on the N 1s of the pyrrolidone group from PVP segments and S 2p of the sulfonate group from PSBMA segments at the BEs of 399 and 168 eV, respectively.

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Table 2. Mechanical Data of Poly(VP-*co*-SBMA) Copolymer Gels and Networks

	poly(VP-*co*-SBMA) gels without thermosetting			poly(VP-*co*-SBMA) networks with thermosetting		
sample ID	modulus (MPa)	fracture strain (%)	fracture stress (MPa)	modulus (MPa)	fracture strain (%)	fracture stress (MPa)
V100-S0	0.09 ± 0.1	49 ± 3.4	0.16 ± 0.1	–	–	–
V50-S50	0.12 ± 0.1	42 ± 2.5	0.31 ± 0.2	0.18 ± 0.1	38 ± 2.3	0.34 ± 0.3
V0-S100	0.14 ± 0.2	43 ± 3.1	0.32 ± 0.0	0.05 ± 0.0	54 ± 1.1	0.11 ± 0.2

processes of isolation, purification, and quantitation of the RBCs are described in more detail in a previous work.[30]

Cell Attachment Assay. Human HT-1080 fibroblasts (ATCC, Manassas, VA) modified with stable luciferase and EGFP expression by using the plasmid pAAV-luciferase-EGFP were used to examine the attachment behavior of cells on the polymeric gel surfaces. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. A cell suspension at 2×10^4 cells/mL was prepared, and 1 mL of the cell suspension was put onto either the copolymer gels or network disks, which were then incubated for 3 days at 37 °C/5% CO_2. A Nikon TS100 microscope with a 10× objective lens and a blue excitation fluorescence (450−490 nm) filter was used to observe the cells after proliferation, and the fluorescence images were used to quantify the amount of cell attachment on the surfaces of the six independent disks (n = 6 in total) for each gel substrate, and the average result was reported.

Bacteria Adhesion Assay. *Escherichia coli* (*E. coli*) was used to investigate the bacterial adhesion behavior on the surface of poly(VP-*co*-SBMA) copolymer gels without thermosetting and copolymer networks with thermosetting. The *E. coli* cultures were incubated in the medium (3.0 mg/mL beef extract and 5.0 mg/mL peptone) at 37 °C and shaken at 100 rpm for 12 h to reach the stationary phase, then plated on either the prepared copolymer gel or network at a concentration of 106 cells/mL. The samples were cleaned before use by submerging the prepared copolymer gels or networks in 75 wt % ethanol for 1 h and washed three times with PBS solution. Each gel was then put into the wells of a 24-well plate, to which 24 mL of bacteria suspension was added, with 1 mL in each well. The samples were then incubated in an oven for 24 h at 37 °C. After incubation, the bacterial suspensions were removed from the wells and then all samples were washed with PBS three times to remove the bacteria that had accumulated but not adhered on the surfaces. Live/Dead Baclight was added to the samples to stain the adhering bacteria, and the stained samples were washed with PBS three times and then observed with a fluorescence microscope using 450−490 nm excitation, an Olympus BX51 CCD camera, and a 10× objective lens.

Fibrinogen Adsorption, Whole Blood Cell Attachment, and Stability Tests of Stainless Steel Disks by Coating Poly(VP-*co*-SBMA) Gels/Networks. We purchased 316 L type stainless steel disks (SUS 316 L) from the Walsin Lihwa Corp. (Taiwan) (5 mm diameter, 1 mm thick). These stainless steel disks were first cleaned three times with ethanol and DI water. Then, the disks were placed into an ultraviolet ozone cleaning oven for 20 min to remove surface impurities. The cleaned stainless steel disks were immediately transferred to a container filled with the reacting gel solution. After polymerization for 3 h at 25 °C, the disks were coated with the gel layer to a thickness of 0.2 mm. These coated disks were cut into disk-form gels with a diameter of 10 mm using a biopsy punch. The coated disks were dried and annealed at 200 °C to form the coated networks. All the samples were stored at 4 °C before use in experiments. The biofouling tests on the gel-coated stainless steel disks, including fibrinogen adsorption and whole blood cell adhesion, followed the above procedures. The stability of coated gels and networks was confirmed by time-dependent fibrinogen adsorption. The original, gel-coated, and network-coated stainless steel disks were immersed in a PBS solution, and fibrinogen adsorption was measured at 0 h, 12 h, 1 day, 7 days, 14 days, and 30 days.

RESULTS AND DISCUSSION

Six cross-linked (co)polymer gels of PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) were prepared using two different VP to SBMA monomer ratios via thermally induced copolymerization, as shown in Table 1. The VP segment was introduced in the poly(VP-*co*-SBMA) network for thermal tolerance, and the SBMA segment functioned as the fouling resistance component in the copolymer network. An increase in the VP content might strengthen the cross-linking capability of the copolymer network; however, the formation of the VP radical state is required at a high annealing temperature of 200 °C because the antifouling characteristics of SBMA might be reduced or become unstable at that temperature. Thus, the optimum composition ratio of PVP/PSBMA was determined by balancing the thermal tolerance and the fouling resistance of the poly(VP-*co*-SBMA) copolymer gels and networks, as shown in Scheme 1. In addition, substances with different capacities for biofouling, such as plasma proteins, blood cells, tissue cells, and bacteria, were tested in contact with the various poly(VP-*co*-SBMA) copolymer interfaces to determine the fouling resistant properties as influenced by the thermosetting control. To demonstrate the application of the designed thermosetting materials, the poly(VP-*co*-SBMA) copolymer gels and networks were applied as a thermal coating annealed at 200 °C on the stainless steel surface of metal stents to demonstrate whether they were compatible with human blood.

Characterization of Poly(VP-*co*-SBMA) Copolymer Gels and Networks with Thermosetting Control. Chemical characterization of the prepared poly(VP-*co*-SBMA) copolymer samples was analyzed by Fourier transform infrared spectroscopy (FT-IR) and X-ray photoelectron spectroscopy (XPS). In general, spectroscopy was used to detect specific chemical functionality in the infrared absorption spectrum to definitively discriminate asymmetric groups. While the overall molar percentage of a specific compound increased, the relative peak intensity of the infrared spectrum might have positive correlations, as shown in the FT-IR spectra of PVP, poly(VP-*co*-SBMA), and PSBMA (co)polymer gels and networks in Figure 1. The stretching bands of amide carbonyl groups (N—C═O) at 1660 cm^{-1} from PVP segments and the sulfonate groups ($-SO_3$) at 1180 cm^{-1} from PSBMA segments indicated the presence of VP and SBMA in the prepared copolymer gels of V25-S75, V37-S63, V50-S50, and V75-S25. We found that the decreased intensity of N—C═O adsorption and the increased intensity of $-SO_3$ adsorption were associated with an increase in the amount of SBMA segments in the poly(VP-*co*-SBMA) gels. The FT-IR spectra showed that the VP/SBMA composition in the copolymer networks was successfully determined by controlling the SBMA concentration from 25 to 100 wt % in the reaction solution.

A stable and defined shape is critical for the development of a thermoset biomaterial coating for medical devices that has a defined geometry. In this study, the swelling ratio in aqueous

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solution was used to determine the shaping properties of the poly(VP-*co*-SBMA) copolymer gels and networks with thermosetting control. Figure 2 shows the weight and volume

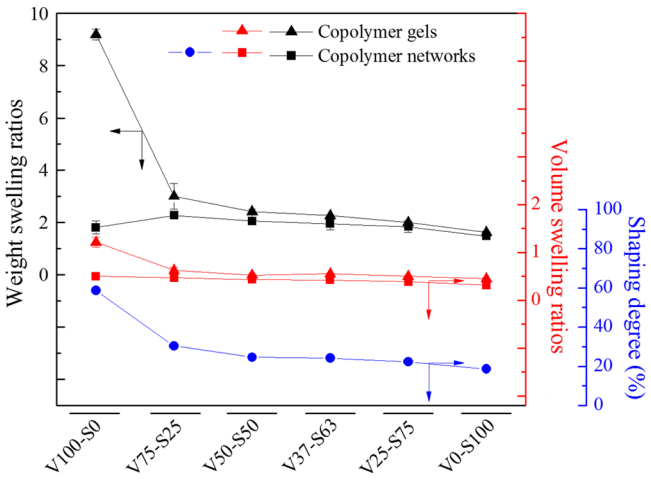

Figure 2. Weight, volume swelling ratios, and shaping degrees of PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) (co)polymer gels without thermosetting and networks with thermosetting.

swelling ratios of the prepared copolymer gels hydrated with DI water at 25 °C. In the first stage of copolymer gel preparation, before thermosetting, 1.6 wt % MBAA in DI water was used for cross-linking different molar ratios of VP and SBMA with a total solid content of 20 wt %. We found that the weight swelling ratio of a PVP gel is higher than that of a PSBMA gel, indicating that the PVP gel either had a greater capacity for interchain spacing or more flexible mobility of the polymer segments for hydration by water molecules. The measurements of the volume swelling ratio indicated that the PSBMA gels hydrated to equilibrium showed less of a shape deviation when compared to their dry state than the PVP gels did. The prepared poly(VP-*co*-SBMA) copolymer gels showed swelling behavior similar to the zwitterionic PSBMA gel. The degree of shaping was defined as the percentage of the extra-swelling volume of the hydrated (co)polymer networks with thermosetting control. A high degree of shaping of the V100-S0 network indicates that the PVP segments contain thermally induced cross-linking at the thermosetting temperature of 200 °C. Comparing the higher degree of shaping of the poly(VP-*co*-SBMA) copolymer networks to that of a pure PSBMA network indicated that the PVP segments also functioned as a cross-linking element in the copolymer networks. The results revealed that the thermosetting property of poly(VP-*co*-SBMA) might provide a potential way to counter the deformation of biomaterials by high temperature sterilization or an annealing process.

In general, fouling-resistant polymer gels are highly hydrophilic, making them very fragile with poor mechanical properties. Thus, good control over the mechanical properties is critical in the development of biocompatible hydrogels for tissue engineering. The mechanical properties are expressed by both Young's moduli (slope of the first 10% in the strain−stress curve) and the fracture strain/stress that represents the elasticity and strength of a gel. Figure S1 in the Supporting Information shows the strain−stress curves for PVP, PSBMA, and poly(VP-*co*-SBMA) gels to illustrate the shaping properties and mechanical strength of the gels. The mechanical data are summarized in Table 2. The results show that the evaluated modulus of the copolymer gels without thermosetting increased when SBMA segments were present in the PVP gels (0.09−0.14 MPa), and that the shape deformation of the PSBMA gel (V0-S100) was less than that of the PVP (V100-S0) and poly(VP-*co*-SBMA) gels (V50-S50). The moduli of the copolymer networks (V50-S50*) subjected to thermosetting at 200 °C explicitly increased from 0.12 to 0.18 due to the formation of cross-linked network structures between the PVP segments, as shown by the increased in the fracture stress of V50-S50* from 0.31 to 0.34, suggesting that the copolymer networks subjected to thermosetting treatments had enhanced mechanical strength. The low fracture strain of V50-S50* indicates less shape deformation than V0-S100*. The modulus and fracture stress results were associated with the swelling ratio and degree of shaping. It was shown that the incorporation of PVP segments into the copolymer networks could function as thermally induced cross-linking elements which reduced the network deformation and improved the compressive strength. This result indicates that the shapeable poly(VP-*co*-SBMA) copolymer networks that have good mechanical strength might be useful as interfacial coatings for medical devices, especially those that require annealing at high temperature.

To quantitatively determine the VP/SBMA composition, the SBMA mole fraction in poly(VP-*co*-SBMA) copolymer gels and networks in the dry state was determined by XPS from the spectral area ratio of the atomic percentages based on the N 1s of the pyrrolidone group from PVP segments and S 2p of the sulfonate group from PSBMA segments at BEs of 399 and 168 eV, respectively. Figure 3 shows the VP and SBMA composition of the N 1s and S 2p core-level spectra on thermoset PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) gel and network interfaces. In the N 1s spectrum, the doublet peak signal of the N element was clearly visible, and the analysis result represents the secondary amide group (2°N) of MBAA, the tertiary amine group (3°N) of VP, and the quaternary amine group (4°N) of SBMA at binding energies of 399.56, 399.2, and 402 eV, respectively. In this study, we define the secondary amide (2°N) as the reference peak to process the curve-fitting to estimate the relative amount of tertiary amine in the prepared samples. The N 1s signal of V0-S100 (containing MBAA and PSBMA) showed two clear peaks at 399.2 and 402 eV representing secondary amine of MBAA and quaternary amine of PSBMA. Here, the 399.2 eV peak of V0-S100 was used as the basis of the binding energy to fit the other samples as shown in Figure 3. Therefore, we regarded that the deconvolution of secondary amine and tertiary amine peaks in the N 1s peak provides a reasonable approach to determine the relative composition of VP and SBMA segments in the prepared gels and networks. To identify the quantitative composition of VP/SBMA and the charge neutrality of the zwitterionic PSBMA segments in the poly(VP-*co*-SBMA) copolymer gels and networks, [3°N]/[S] and [4°N]/[S] ratios were estimated based on the deconvolution peak areas. From the XPS analysis, 3°N was found only in PVP (V100-S0), and S was found only in PSBMA (V0-S100). Thus, the 3°N/S ratio was an appropriate index to indicate the comonomer composition of the [VP]/[SBMA] ratio in the prepared poly(VP-*co*-SBMA) samples. The results, summarized in Table 1, showed that [VP]/[SBMA] approaches the theoretical

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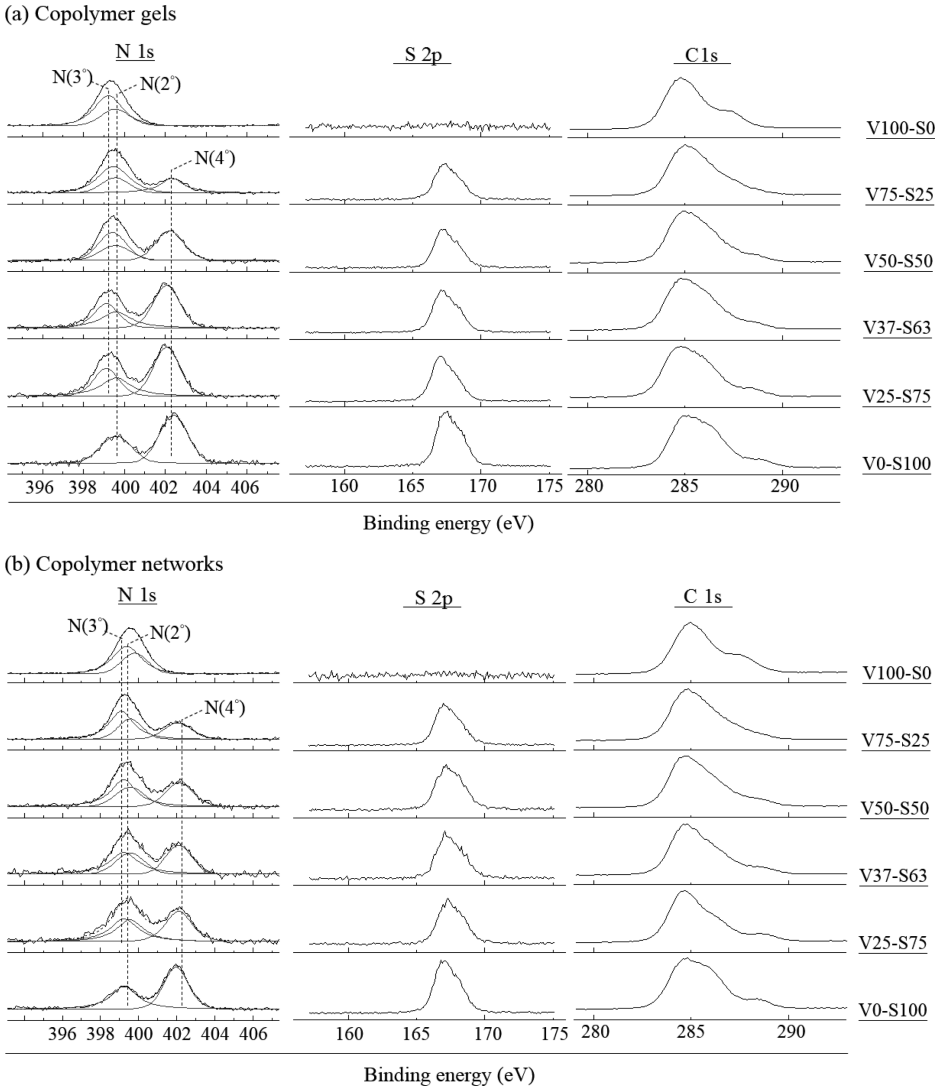

Figure 3. XPS analysis of PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) (co)polymer (a) gels without thermosetting and (b) networks with thermosetting.

comonomer ratio with an increase of the SBMA monomer concentration in the reaction solution, indicating that the reactivity of the VP monomer was higher than that of SBMA during the thermally induced polymerization.

In our previous studies,[8,10] the zwitterionic polymer PSBMA gel was identified as an ideal biofouling-resistant material, because of its overall charge neutrality resulting from a balance of positively charged quaternary amine groups ($-N(CH_3)_2^+-$) and negatively charged sulfonate groups ($-SO_3^-$). Therefore, it was important to analyze the deviation from charge neutrality in the poly(VP-*co*-SBMA) networks composed of zwitterionic PSBMA segments resulting from the 200 °C thermal annealing process. From the XPS analysis, the 4°N peak can be identified as positively charged groups of $-N(CH_3)_2^+-$ and the S 2p peak can be identified as negatively charged groups of $-SO_3^-$ in the PSBMA segments. Thus, the 4°N/S ratio is a good indicator to demonstrate the charge neutrality of zwitterionic SBMA moieties in the copolymer networks associated with thermosetting. Table 1 shows that the poly(VP-*co*-SBMA) gels prepared without thermosetting had a 4°N/S ratio of approximately 1.01 ± 0.02 (0.99−1.03), indicating almost equal amounts of $-N(CH_3)_2^+-$ and $-SO_3^-$ which maintained a charge neutrality of a zwitterionic nature. However, the poly(VP-*co*-SBMA) networks prepared with thermosetting at the high annealing temperature of 200 °C had a 4°N/S ratio of approximately 1.15 ± 0.55 (1.05−1.16), indicating a positive charge bias from zwitterionic neutrality. Partial decomposition of the sulfonic acid groups ($-SO_3^-$) at high temperature might result in an unbalanced charge bias of the PSBMA segments. Especially in the thermoset sample of V0-S100 (4°N/S ∼ 1.15), the high positive charge bias in the PSBMA polymer networks might lead to a loss of biofouling resistance. That the increase of PVP segments enhances the thermal tolerance of poly(VP-*co*-SBMA) networks with a much lower $-SO_3^-$ decomposition than that of PSBMA polymer networks is an important finding. Regulation of the VP composition in the poly(VP-*co*-SBMA) networks shows control of the charge neutrality (4°N/S ∼ 1.05) in the V37-S75 sample prepared with thermosetting at 200 °C, which might make it possible to formulate a bifunctional zwitterionic copolymer that has thermal tolerance and biofouling resistance.

Human Blood Compatibility of Poly(VP-*co*-SBMA) Copolymer Gels and Networks. The control of protein adsorption resistance of a material interface is critical to

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controlling the hemocompatibility of a biomaterial for medical use. It is acknowledged that there is a strong correlation between platelet adhesion, erythrocyte attachment, and blood clotting as a result of the adsorption of human fibrinogen (FN). Previous studies showed that adsorption of a nonspecific monolayer of proteins from human plasma usually occurs at hydrophobic or charged interfaces. Most studies also showed that enhancing the hydrophilicity or water hydration of a noncharged interface provides an effective reduction of nonspecific protein adsorption on the target materials used in a blood-contacting system.[10,15,29,30] ELISA with monoclonal antibodies was used to determine the specific fibrinogen adsorption onto different poly(VP-*co*-SBMA) copolymer gel surfaces at 37 °C. Figure 4 shows that zwitterionic PSBMA

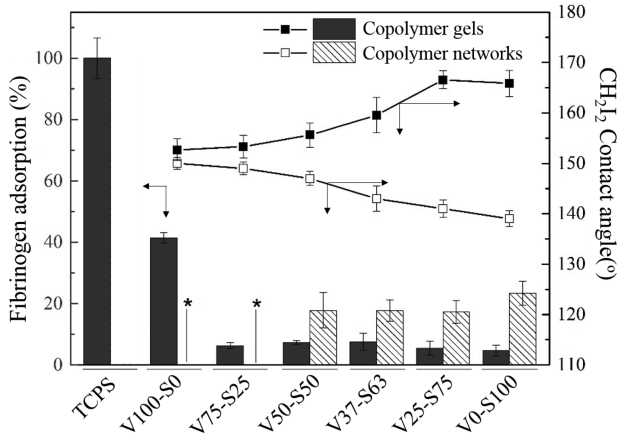

Figure 4. Human fibrinogen adsorption and diiodomethane contact angles of PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) (co)polymer gels without thermosetting and networks with thermosetting, using tissue culture polystyrene (TCPS) as positive control. Asterisks (*) indicate V100-S0 and V75-S25 networks cracked after the thermosetting.

network gels (V0-S100) without thermosetting had the expected high resistance toward fibrinogen adsorption, while polystyrene-based TCPS (tissue culture polystyrene) surfaces showed high fibrinogen adsorption due to hydrophobically driven interactions. PVP polymer gels (V100-S0) showed a clear reduction in the relative protein adsorption of 60% compared to the TCPS surface. A significant decrease of the relative protein adsorption to below 10% was observed for poly(VP-*co*-SBMA) gel surfaces of V75-S25, V50-S50, V37-S63, and V25-S75. The resistance to fibrinogen adsorption on these copolymer gel surfaces was comparable to that on the surface of a PSBMA gel, even at the low composition of 20 mol % PSBMA as in the V75-S25 sample. In general, surface hydrophilicity is associated with the nonspecific adsorption of proteins. Figure 4 shows the effect of the SBMA composition in poly(VP-*co*-SBMA) copolymer gels on the correlation of the diiodomethane contact angle related to protein adsorption with thermal treatment control. PSBMA gel surfaces with a higher contact angle than that of PVP gel surfaces showed better protein resistance resulting from greater hydrophilicity of the zwitterionic interfaces. The increased contact angle also indicated an enhancement in the hydrophilicity of prepared poly(VP-*co*-SBMA) copolymer networks with an increase in the SBMA molar ratio. However, we found that thermosetting at 200 °C reduced the contact angle of PSBMA networks (V0-S100), indicating an obvious decrease in gel interfacial hydrophilicity after the thermal treatment process. Thus, protein adsorption on the V0-S100 surface at an annealing temperature of 200 °C is over 20% higher than that on a PSBMA network without thermosetting, which might be due to the partial decomposition of the sulfonic acid groups ($-SO_3^-$) at high temperature resulting in an unbalanced charge bias of the PSBMA segments, as supported by the XPS analysis. Most importantly, the increase of VP segments enhanced the protein resistance of poly(VP-*co*-SBMA) networks due to a much lower decomposition of $-SO_3^-$ than for the PSBMA polymer networks. However, there is a composition limitation for the VP segments in the poly(VP-*co*-SBMA) networks for thermal tolerance with cracking, such as in V100-S0 and V75-S25. Herein, the composition of VP and SBMA segments in the copolymer network with approximately 40 mol % VP in the V37-S36 sample is controlled to obtain high fibrinogen resistance without thermal cracking and with controllable zwitterionic charge neutrality.

Figure 5 shows a set of confocal laser scanning microscopic (CLSM) images of human platelet adhesion and erythrocyte attachment on prepared copolymer gel and network surfaces at a magnification of 1000×. The prepared samples came into contact with a platelet-rich-plasma (PRP) solution, which was separated from human whole blood by centrifugation at 1200 rpm for 10 min at 37 °C. Figure 5a clearly shows that the platelets showed almost no adhesion to the zwitterionic PSBMA gel surfaces compared to PVP gels without thermosetting. The large amount of platelet adhesion on the PVP gel surfaces is due to the activation of the interface by adsorbed fibrinogen. However, PSBMA network surfaces subjected to thermosetting also lost platelet resistance after the annealing process at 200 °C. We found that the platelet resistance for thermoset poly(VP-*co*-SBMA) network surfaces was well preserved, especially for V37-S63. This observation strongly correlated with the fibrinogen adsorption in Figure 4, which confirms the previous hypothesis that protein adsorption above a certain level might lead to the adhesion and activation of platelets from human plasma on a surface. Based on protein adsorption and platelet adhesion described above, we could clearly see that poly(VP-*co*-SBMA) copolymer networks with a tunable molar ratio of zwitterionic SBMA segments could be bifunctional, with tolerance to high temperature shaping and good hemocompatibility when in contact with human plasma.

Whole human blood is a complex solution, containing a huge number of amino acids, proteins, platelets, erythrocytes, and leukocytes. Therefore, resistance of a material interface to interlinked blood cell attachment, activation, and clotting in 100% whole blood is a challenge. Erythrocytes are the major cell type in human whole blood. Human whole blood was tested in contact with the prepared poly(VP-*co*-SBMA) gel and network surfaces, as shown in Figure 5b. For PVP and PSBMA polymer gels without thermosetting, the V100-S0 surface showed a large amount of erythrocyte attachment as well as platelet adhesion, while the V0-S100 surface showed the expected resistance to adhesion by blood cells. V50-S50, V37-S63, and V25-S75 poly(VP-*co*-SBMA) copolymer gels also had good fouling-resistant interfaces with respect to erythrocyte attachment. However, fibrinogen adsorption and platelet activation induced clotting on the interface with interlinked erythrocyte attachment on thermoset V0-S100 PSBMA polymer networks. The results confirmed that the zwitterionic interface of the PSBMA polymer network subjected to thermal

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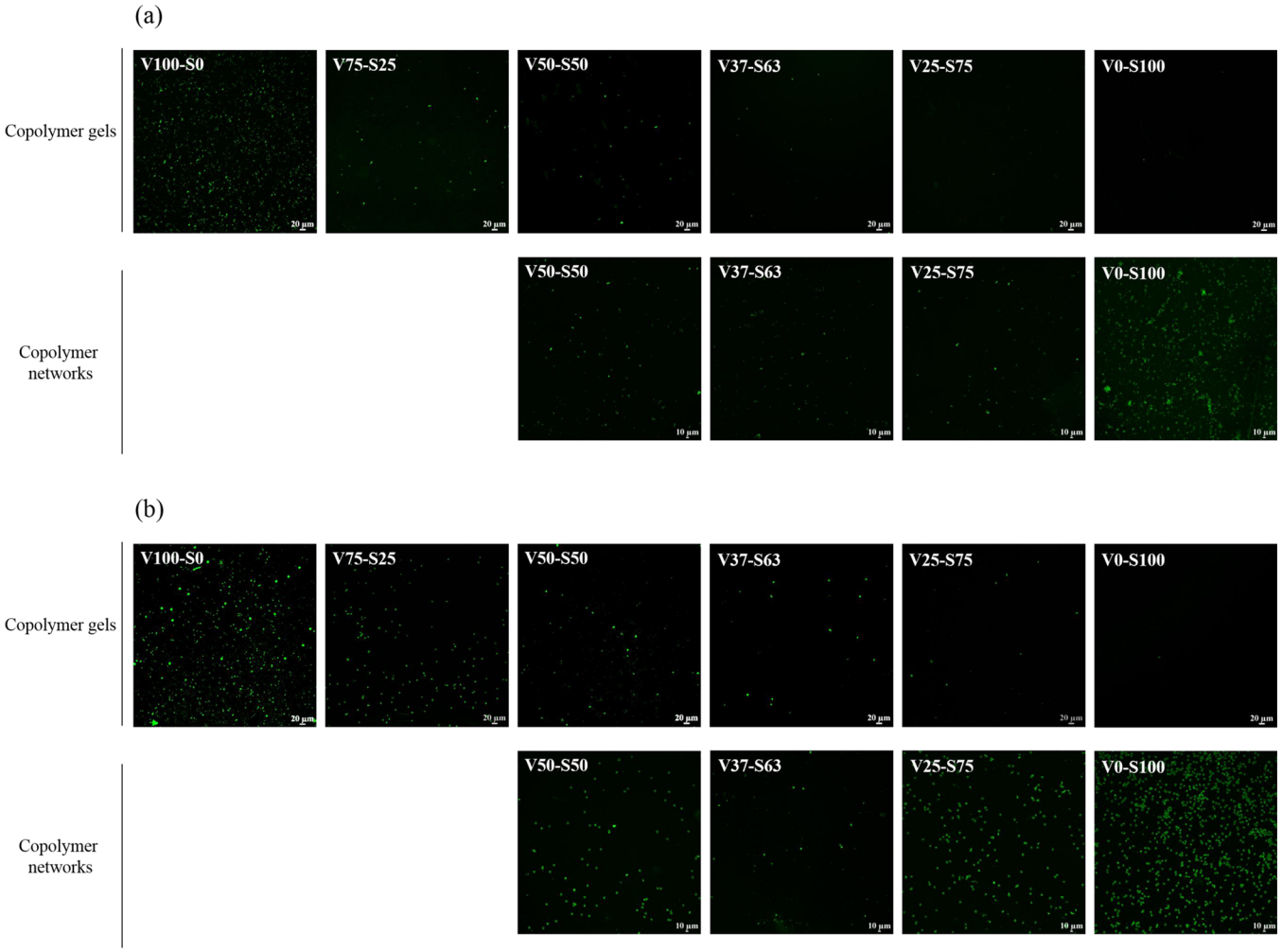

Figure 5. CLSM images of (a) human platelet adhesion and (b) erythrocyte attachment observed on PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) (co)polymer gels without thermosetting and networks with thermosetting. All images are at a magnification of 1000×.

shaping at 200 °C lost its blood-inert properties when in contact with human blood. For the poly(VP-*co*-SBMA) copolymer networks, the amount of erythrocyte attachment depends on the magnitude of the charge bias of the copolymer interface as determined by the ratio of the atomic percentage of N(4°)/S measured by XPS, as shown in Table 1. The results indicated that the charge neutrality of the zwitterionic polymer interface resulted in minimum electrostatic interaction with the blood cells. Thermoset V37-S63 poly(VP-*co*-SBMA) copolymer networks had a controllable overall charge balance of N(4°)/S ∼ 1.05, which produced good hemocompatibility in human whole blood, while maintaining the thermal tolerance of the PVP segments. This outcome represents a breakthrough in the potential for the molecular design of thermosettable blood-inert materials with zwitterionic interfaces.

The red blood cell (RBC) hemolysis assay was performed to evaluate the antihemolytic activity and blood compatibility of the prepared copolymer gels and networks. When red blood cells encounter an incompatible environment, such as a hydrophobic surface or a nonisotonic environment, the blood cell membrane might be destroyed and the hemoglobin released from the disrupted membrane. The amount of released hemoglobin can be easily determined by measuring the absorbance at 541 nm to determine the extent of hemolysis. DI water (+) and PBS (−) were used as the positive and negative controls for RBC hemolysis, as shown in Figure 6. All of the thermoset poly(VP-*co*-SBMA) copolymer gels and networks showed a safe level of hemolysis of 2% or lower, indicating good antihemolytic activity of the copolymer interface in contact with the blood.

Fibroblast Cell Attachment on Poly(VP-*co*-SBMA) Copolymer Gels and Networks. Adhesion of tissue cells or visceral organs after surgery is an important clinical issue for wound healing, tissue engineering, and regeneration medicine.[34] Thus, preventing the attachment and spreading of cells might be the key to better biomaterials or medical devices for implantation. Figure 7 shows the quantitative results for human fibroblast (HT1080) adhesion to the poly(VP-*co*-SBMA) copolymer gel and network surfaces, compared to the TCPS positive control surfaces, as measured by the standard assay. For the prepared copolymer gels without thermosetting, a decrease of attached HT1080 cells was observed with an increase of PSBMA segments in the poly(VP-*co*-SBMA) copolymer gels. No attached cells were observed on the zwitterionic PSBMA polymer gel surfaces. However, thermoset PSBMA network surfaces showed different results for cell attachment. We also observed good cell adhesion resistance as well as blood inertness for the thermosettable poly(VP-*co*-

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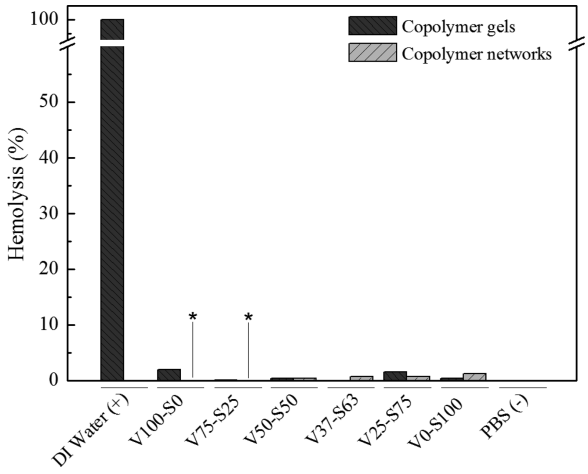

Figure 6. Hemolysis of RBC solution in the presence of PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) (co)polymer gels without thermosetting and networks with thermosetting, using tissue culture polystyrene (TCPS) as positive control. Asterisks (∗) indicate V100-S0 and V75-S25 networks cracked after the thermosetting.

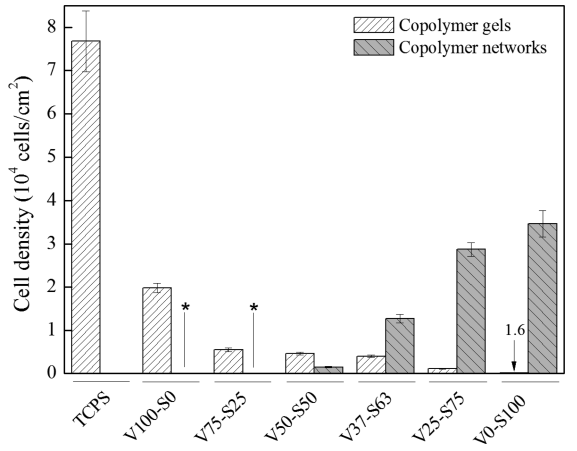

Figure 7. Statistical analysis of HT1080 cell attachment on the surfaces of PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) (co)polymer gels without thermosetting and networks with thermosetting, using tissue culture polystyrene (TCPS) as positive control. Cell culture was performed at an initial concentration of 2×10^4 cells/mL. Asterisks (∗) indicate V100-S0 and V75-S25 networks cracked after the thermosetting.

SBMA) copolymer networks with the overall charge neutrality specifically set by PSBMA segments modulated by a particular PVP composition.

Antibacterial Efficacy of Poly(VP-*co*-SBMA) Copolymer Gels and Networks. Bacterial infection is a major cause of failure in general surgery. Thus, the suppression of bacterial attachment and biofilm formation on the surface of an implant is critical to suppressing host defenses and preventing side effects.[35,36] In general, *E. coli* is associated with bacterial infections. Figure 8 shows a set of qualitative images of *E. coli* attached to poly(VP-*co*-SBMA) copolymer gels and networks. The Live/Dead BacLight assay was used to characterize the long-term accumulation of *E. coli* on the prepared sample surfaces for 24 h at 37 °C. It was clear that bacteria adhered to the PVP polymer gel surface. Increased attachment of *E. coli* with an increase in the mass composition of PSBMA segments in copolymer gels without thermosetting on the poly(VP-*co*-SBMA) copolymer gel surface was observed. Zwitterionic PSBMA gel surfaces showed perfect resistance to bacterial adhesion as well as to protein adsorption, blood cell attachment, and tissue cell adhesion. Most importantly, the bacteria observed on these gel surfaces were all active, indicating that the PVP or PSBMA segments were nontoxic to the accumulated *E. coli*. However, a red color indicated the adhesion of dead bacteria on the thermosetting PSBMA polymer networks. The result supported the idea that some $-SO_3^-$ decomposition in the sulfobetaine groups at high temperature resulted in a positive charge bias of the PSBMA segments, as indicated by XPS analysis and shown in Figure 3. Thus, the zwitterionic groups of sulfobetaine pendent ($-CH_2CH_2N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$) became partially converted to positively charged groups containing quaternary ammonium ($-CH_2CH_2N^+(CH_3)_2-$) in the PSBMA segments. In general, a cationic surface can kill approaching bacteria or inhibit their growth on the material substrates. As the PVP segments in the thermosetting poly(VP-*co*-SBMA) copolymer networks increased, the thermal tolerance of the zwitterionic sulfobetaine pendent group at high annealing temperature increased. This hypothesis is supported by the observation that no cells or dead *E. coli* were attached to the network surfaces of thermoset V50-S50 and V37-S63. The results also support that *E. coli* attachment was correlated with protein adsorption and cell adhesion on the material interface of poly(VP-*co*-SBMA) copolymer gels and networks.

ζ Potentials of Poly(VP-*co*-SBMA) Copolymer Gels and Networks. Measurement of the ζ potential is a recognized method to verify the positive and negative charge variance on a material interface. We used dynamic light scattering to measure the ζ potential values of the copolymer gels and networks to confirm the surface charge variance attributed to the thermosetting treatment, as shown in Figure 9. TMA and SA polymer gels were prepared as the ζ potential reference for a controlled interface with a positive charge or a negative charge, respectively. The experiment was performed at pH 7.4 in PBS solution to prevent interference. A positive ζ potential was observed in SBMA-rich copolymer networks (V25-S75 and V0-S100) after the thermosetting procedure. The results indicate the formation of a positively charged interface of copolymer networks due to the degradable loss of SO_3^- groups during annealing at 200 °C, which is consistent with the XPS analysis and fouling resistance results. Importantly, the results indicate that PVP segments in copolymer networks cause thermal tolerance that prevents the degradation of SBMA functional groups, such as for V50-S50. The results also support the observation that charge neutrality of prepared poly(VP-*co*-SBMA) copolymer gels and networks maintains good fouling resistance to proteins, cells, and bacteria.

Clinical Aspects of the Thermosettable Poly(VP-*co*-SBMA) Networks Applied to a Hemocompatible Metal Stent. Implantation of a stainless steel based metal stent is currently an important therapeutic approach to treat vascular infarction by enlarging a blood vessel. High temperature sterilization of the metal stent is required to prevent infection and inflammation. In this study, we included experiments to demonstrate an application for our designed thermosetting materials (Figure 10). A challenging issue in stent therapy is *restenosis*, which means the artery becomes narrowed again within months of the procedure. To prevent this issue, a good solution is coating the metal stent interface with a

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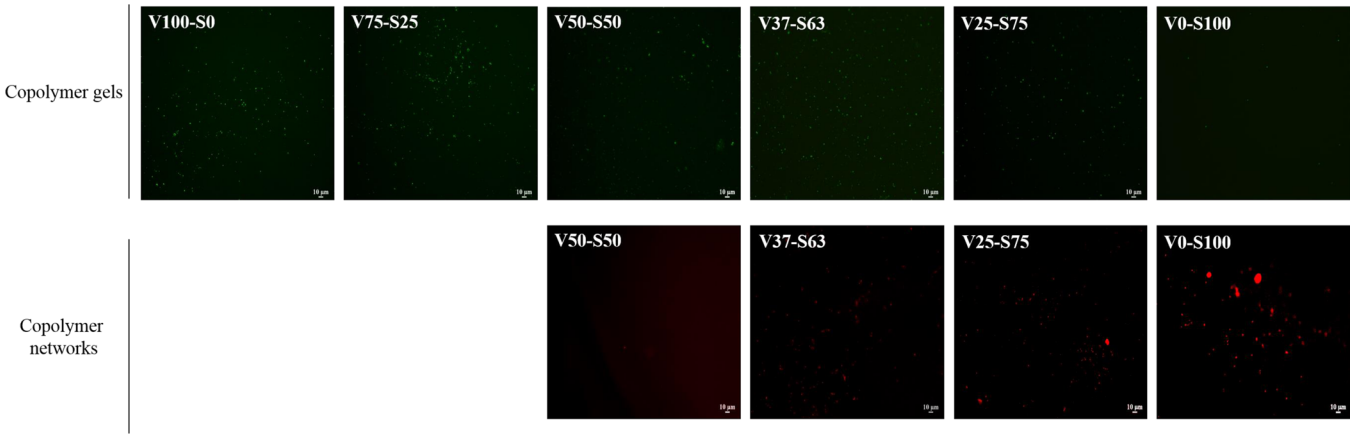

Figure 8. Fluorescence microscopic images of *E. coli* attachment on the surfaces of PVP (V100-S0), poly(VP-*co*-SBMA) (V75-S25, V50-S50, V37-S63, V25-S75), and PSBMA (V0-S100) (co)polymer gels without thermosetting and networks with thermosetting. All images are at a magnification of 1000×.

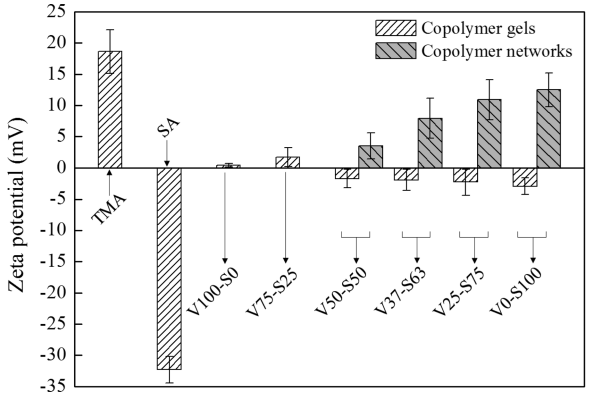

Figure 9. ζ potential analysis of copolymer gels and networks in pH 7.4 PBS solution. TMA and SA polymer gels are used as the ζ potential reference of positive charge and negative charge.

hemocompatible layer. Here, we applied a poly(VP-*co*-SBMA) coating on the surface of stainless steel stents to test the prepared thermoset samples in contact with human blood. The thermosettable poly(VP-*co*-SBMA) gels and networks were applied as shown in Figure 10A and sterilized simultaneously at 200 °C during the annealing process. The optimized hemocompatible V37-S63 copolymer was selected for application onto the stainless steel stents and compared with reference coatings of V0-S100 and V100-S0. The protein adsorption results showed that only the coating with V37-S63 gels and networks maintained the best fouling resistance to fibrinogen adsorption after the thermosetting process at 200 °C, as shown in Figure 10B. A critical desirable requirement for metal stents is stable hemocompatibility in undiluted human whole blood. Figure 10C shows CLSM images of blood cells attached to stainless steel surfaces coated with V100-S0, V37-S63, and V0-S100 copolymer gels and networks for 120 min at 37 °C. The results show that relatively lower blood cell attachment was observed on interfaces coated with V37-S63 copolymer compared to V100-S0 and V0-S100 interfaces. The extent of the blood cell attachment clearly indicates that excellent blood compatible interfaces of stainless steel stent can be achieved by the coating the surface with thermosettable V37-S63 copolymer networks. Moreover, an important requirement for stent therapy is long-term stability after a stent is implanted into a human. A 1 month stability test of the poly(VP-*co*-SBMA) gels and networks coating on the stainless steel interfaces of metal stents is shown in Figure 10D. The stainless steel surfaces coated with V37-S63 copolymer gels/networks were immersed in PBS during 0 h, 12 h, 1 day, 7 days, 14 days, and 30 days, and subsequently the relative fibrinogen adsorption on surfaces was measured with ELISA at 37 °C. After the thermosetting control, the copolymer networks of V37-S63 formed a robust and cross-linked coating layer on the stainless steel surface resulting in a stable protein resistance in the long-term durability test for 1 month. However, it was observed that the samples coated with V37-S63 copolymer gels lost protein resistance stability after the PBS incubation at 12 h. After 30 days, these gel coated samples without thermosetting treatment performed a similar level of protein adsorption as compared with virgin samples, indicating all of the gel coatings physically detached from the stainless steel surfaces. The results show that the coating samples of V37-S63 copolymer gels lose 80% relative protein resistance after 14 days while the thermosettable copolymer networks maintain stable resistance to protein adsorption through 30 days, which indicates a favorable potential for the application of a coating to metal stents for vascular disease therapy. Blood-inert stent interfaces with a thermosettable coating of zwitterionic poly(VP-*co*-SBMA) copolymer networks can be presently prepared clinically, showing the applicability of polymeric biomaterials that have a required annealing process in real-world situations.

CONCLUSION

A formulation of PVP and PSBMA segments of thermosettable zwitterionic copolymers for general fouling resistance was proposed and demonstrated. We found that the PSBMA polymer gels showed good interfacial resistance to proteins, cells, and bacteria as expected, but lost their antifouling properties if thermosetting at a high annealing temperature of 200 °C was applied. Interestingly, poly(VP-*co*-SBMA) copolymer networks with a carefully balanced molar ratio of PVP/PSBMA were shown to have thermally tolerant bioinert interfaces to fibrinogen, platelets, erythrocytes, fibroblasts, and *E. coli*. XPS analysis showed that the decomposition of the sulfonic acid groups ($-SO_3^-$) at 200 °C resulted in an unbalanced charge bias of the PSBMA segments, which formed a positively charged interface on PSBMA polymer networks to induce serious biofouling but were bactericidal. However,

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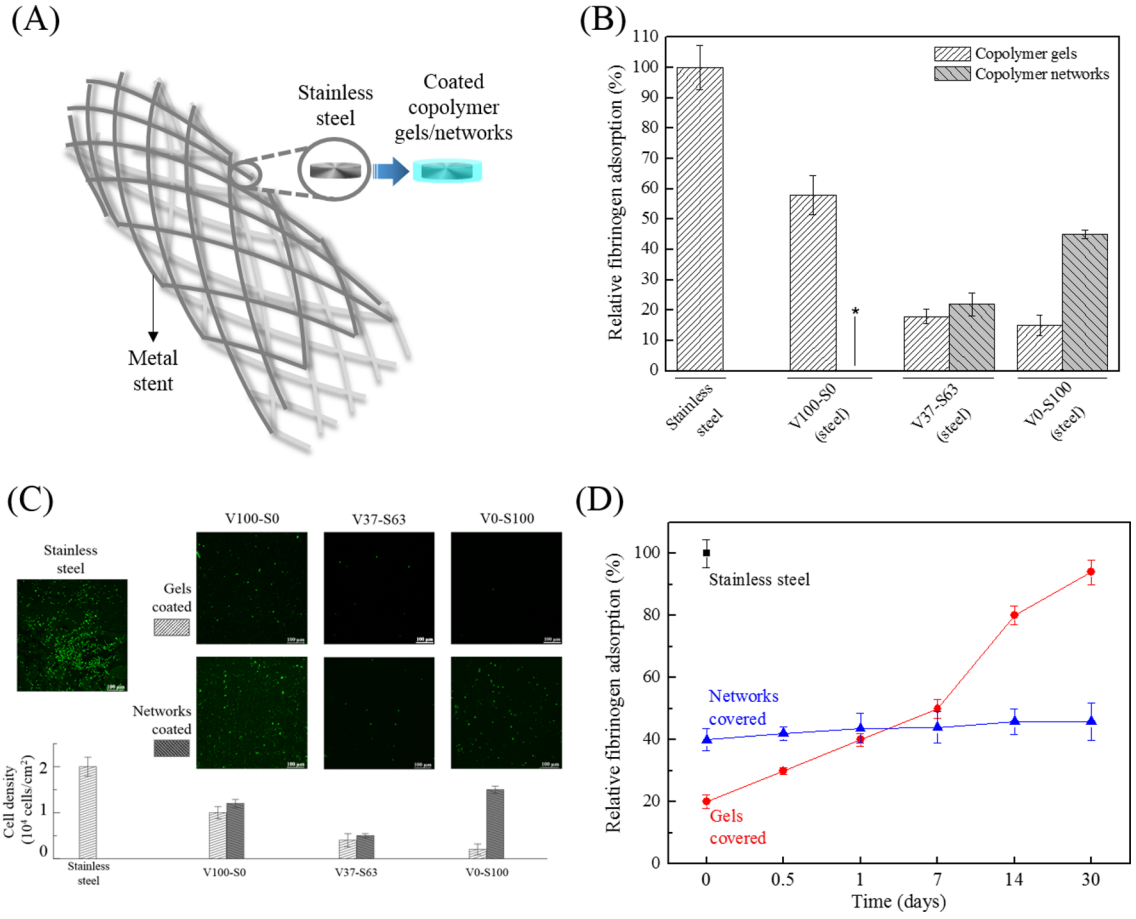

Figure 10. Application aspect of poly(VP-*co*-SBMA) coating on the stainless steel interfaces of metal stent. (A) Stainless stain disks coated with copolymer gels and networks. (B) Relative fibrinogen adsorption on various copolymer coated surfaces of V100-S0, V37-S63, and V0-S100 determined from ELISA with pristine stainless steel as a reference. (C) CLSM images of human blood cells attached onto various copolymer coated surfaces and their quantitative analysis of the changes in blood cell attachment. (D) Protein-resistant stability of copolymer gels and networks coated stainless steel stents in 30 days.

$-SO_3^-$ decomposition was reduced by including PVP segments in the thermosettable copolymers to maintain zwitterionic charge neutrality. Overall, the compositions of poly(VP-*co*-SBMA) copolymer networks containing a molar mass of PSBMA segments ranging from 31 to 62 mol % are bioinert, have a high thermal tolerance and good mechanical properties, and are potentially applicable as thermosettable antifouling coatings for the next generation of medical devices.

The highlighted principal results of this study are listed. First, a new class of thermosettable zwitterionic materials can be prepared by combining PVP and PSBMA segments as cross-linkable copolymer networks with good mechanical properties and low shape deformation, which are suitable for coating. Second, thermally tolerant zwitterionic materials to a temperature of 200 °C can be obtained by introducing PVP segments into PSBMA networks to reduce the thermal degradation of zwitterionic sulfobetaine groups to maintain electrical neutrality and fouling resistance. Third, well-controlled resistance of poly(VP-*co*-SBMA) copolymer gels and networks to protein, tissue cells, and bacteria can be achieved by a low PSBMA copolymer composition of 32 mol %. Fourth, poly(VP-*co*-SBMA) copolymer interfaces with good hemocompatibility should be produced by a high PSBMA copolymer composition of 61 mol %. Fifth, thermosettable poly(VP-*co*-SBMA) copolymer networks can be practically applied as a blood compatible coating on the stainless steel surface of metal stents used in vascular infarction therapy.

■ ASSOCIATED CONTENT

Supporting Information

Figure with mechanical properties of copolymer gels and networks; chemical mechanism of PVP at 200 °C; photos of stainless steel disks coated with copolymer gels and networks; thermogravimetric analysis of poly(VP-*co*-SBMA) copolymer. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b01756.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Hoffman, A. S. *Biomaterials: Interfacial Phenomena and Applications*; Cooper, S. L., Peppas, N. A., Hoffman, A. S., Ratner, B. D., Eds.; Advances in Chemistry Series 199; American Chemical Society: Washington, DC, 1982; Chapter 1, pp 3−8.
(2) Ratner, B. D.; Hoffman, A. S.; Schoen, F. J.; Lemons, J. E. *Biomaterials Science, an Introduction to Materials in Medicine*, 2nd ed.; Elsevier: Amsterdam, 2004.
(3) Ratner, B. D. The Catastrophe Revisited: Blood Compatibility in the 21st Century. *Biomaterials* **2007**, *28*, 5144−5147.
(4) Ratner, B. D.; Bryant, S. J. Biomaterials: Where We Have Been and Where We Are Going. *Annu. Rev. Biomed. Eng.* **2004**, *6*, 41−75.
(5) Jiang, S.; Cao, Z. Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications. *Adv. Mater.* **2010**, *22*, 920−932.
(6) Chen, S.; Jiang, S. A New Avenue to Nonfouling Materials. *Adv. Mater.* **2008**, *20*, 335−338.
(7) Xu, F. J.; Neoh, K. G.; Kang, E. T. Bioactive Surfaces and Biomaterials via Atom Transfer Radical Polymerization. *Prog. Polym. Sci.* **2009**, *34*, 719−761.
(8) Sin, M.-C.; Chen, S.-H.; Chang, Y. Hemocompatibility of Zwitterionic Interfaces and Membranes. *Polym. J.* **2014**, *46*, 436−443.
(9) Zhang, Z.; Zhang, M.; Chen, S.; Horbett, T. A.; Ratner, B. D.; Jiang, S. Blood Compatibility of Surfaces with Superlow Protein Adsorption. *Biomaterials* **2008**, *29*, 4285−4291.
(10) Chang, Y.; Shih, Y.-J.; Lai, C.-J.; Kung, H.-H.; Jiang, S. Blood-Inert Surfaces via Ion-Pair Anchoring of Zwitterionic Copolymer Brushes in Human Whole Blood. *Adv. Funct. Mater.* **2013**, *23*, 1100−1110.
(11) Liu, L.; Chen, G.; Chao, T.; Ratner, B. D.; Sage, E. H.; Jiang, S. Reduced Foreign Body Reaction to Implanted Biomaterials by Surface Treatment with Oriented Osteopontin. *J. Biomater. Sci. Polym. Ed.* **2008**, *19*, 821−835.
(12) Hamidi, M.; Azadi, A.; Rafiei, P. Hydrogel Nanoparticles in Drug Delivery. *Adv. Drug Delivery Rev.* **2008**, *60*, 1638−1649.
(13) Cao, Z.; Yu, Q.; Xue, H.; Cheng, G.; Jiang, S. Nanoparticles for Drug Delivery Prepared from Amphiphilic PLGA Zwitterionic Block Copolymers with Sharp Contrast in Polarity between Two Blocks. *Angew. Chem.* **2010**, *122*, 3859−3864.
(14) Chang, Y.; Chang, W.-J.; Shih, Y.-J.; Wei, T.-C.; Hsiue, G.-H. Zwitterionic Sulfobetaine-Grafted poly(vinylidene fluoride) Membrane with Highly Effective Blood Compatibility via Atmospheric Plasma-Induced Surface Copolymerization. *ACS Appl. Mater. Interfaces* **2011**, *3*, 1228−1237.
(15) Chang, Y.; Liao, S.-C.; Higuchi, A.; Ruaan, R.-C.; Chu, C.-W.; Chen, W.-Y. A Highly Stable Nonbiofouling Surface with Well-Packed Grafted Zwitterionic Polysulfobetaine for Plasma Protein Repulsion. *Langmuir* **2008**, *24*, 5453−5458.
(16) Yang, W.; Bai, T.; Carr, L. R.; Keefe, A. J.; Xu, J.; Xue, H.; Irvin, C.; Chen, S.; Wang, J.; Jiang, S. The Effect of Lightly Crosslinked Poly(carboxybetaine) Hydrogel Coating on the Performance of Sensors in Whole Blood. *Biomaterials* **2012**, *33*, 7945−7951.
(17) Zhang, Z.; Finlay, J.; Wang, L.; Gao, Y.; Callow, J.; Callow, M. E.; Jiang, S. Polysulfobetaine-Grafted Surfaces as Environmentally Benign Ultralow Fouling Marine Coatings. *Langmuir* **2009**, *25*, 13516−13521.
(18) Ekblad, T.; Bergström, G.; Ederth, T.; Conlan, S. L.; Mutton, R.; Clare, A. S.; Wang, S.; Liu, Y.; Zhao, Q.; D'Souza, F.; et al. Poly(ethylene Glycol)-Containing Hydrogel Surfaces for Antifouling Applications in Marine and Freshwater Environments. *Biomacromolecules* **2008**, *9*, 2775−2783.
(19) Holmlin, R. E.; Chen, X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. Zwitterionic SAMs That Resist Nonspecific Adsorption of Protein from Aqueous Buffer. *Langmuir* **2001**, *17*, 2841−2850.
(20) Ishihara, K.; Ueda, T.; Nakabayashi, N. Preparation of Phospholipid Polymers and Their Properties as Polymer Hydrogel Membranes. *Polym. J.* **1990**, *22*, 355−360.
(21) Iwasaki, Y.; Ishihara, K. Phosphorylcholine-Containing Polymers for Biomedical Applications. *Anal. Bioanal. Chem.* **2005**, *381*, 534−546.
(22) Chang, Y.; Chen, S.; Zhang, Z.; Jiang, S. Highly Protein-Resistant Coatings from Well-Defined Diblock Copolymers Containing Sulfobetaines. *Langmuir* **2006**, *22*, 2222−2226.
(23) Zhang, Z.; Chao, T.; Chen, S.; Jiang, S. Superlow Fouling Sulfobetaine and Carboxybetaine Polymers on Glass Slides. *Langmuir* **2006**, *22*, 10072−10077.
(24) 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.
(25) Kostina, N. Y.; Rodriguez-Emmenegger, C.; Houska, M.; Brynda, E.; Michálek, J. Non-Fouling Hydrogels of 2-Hydroxyethyl Methacrylate and Zwitterionic Carboxybetaine (meth)acrylamides. *Biomacromolecules* **2012**, *13*, 4164−4170.
(26) Zhang, L.; Cao, Z.; Bai, T.; Carr, L.; Ella-Menye, J.-R.; Irvin, C.; Ratner, B. D.; Jiang, S. Zwitterionic Hydrogels Implanted in Mice Resist the Foreign-Body Reaction. *Nat. Biotechnol.* **2013**, *31*, 553−556.
(27) Bai, T.; Liu, S.; Sun, F.; Sinclair, A.; Zhang, L.; Shao, Q.; Jiang, S. Zwitterionic Fusion in Hydrogels and Spontaneous and Time-Independent Self-Healing under Physiological Conditions. *Biomaterials* **2014**, *35*, 3926−3933.
(28) Bai, T.; Sun, F.; Zhang, L.; Sinclair, A.; Liu, S.; Ella-Menye, J.-R.; Zheng, Y.; Jiang, S. Restraint of the Differentiation of Mesenchymal Stem Cells by a Nonfouling Zwitterionic Hydrogel. *Angew. Chem., Int. Ed.* **2014**, *53*, 12729−12734.
(29) Shih, Y.-J.; Chang, Y. Tunable Blood Compatibility of Polysulfobetaine from Controllable Molecular-Weight Dependence of Zwitterionic Nonfouling Nature in Aqueous Solution. *Langmuir* **2010**, *26*, 17286−17294.
(30) Chang, Y.; Yandi, W.; Chen, W.-Y.; Shih, Y.-J.; Yang, C.-C.; Chang, Y.; Ling, Q.-D.; Higuchi, A. Tunable Bioadhesive Copolymer Hydrogels of Thermoresponsive poly(N-Isopropyl Acrylamide) Containing Zwitterionic Polysulfobetaine. *Biomacromolecules* **2010**, *11*, 1101−1110.
(31) Ananthanarayan, R.; Paniker, C. K. J. *Text Book of Microbiology*, 7th ed.; Orient Longman Private: Chennai, India, 2006.
(32) Liu, X.; Sun, K.; Wu, Z.; Lu, J.; Song, B.; Tong, W.; Shi, X.; Chen, H. Facile Synthesis of Thermally Stable Poly(N -Vinylpyrrolidone)-Modified Gold Surfaces by Surface-Initiated Atom Transfer Radical Polymerization. *Langmuir* **2012**, *28*, 9451−9459.
(33) Telford, A. M.; James, M.; Meagher, L.; Neto, C. Thermally Cross-Linked PNVP Films as Antifouling Coatings for Biomedical Applications. *ACS Appl. Mater. Interfaces* **2010**, *2*, 2399−2408.
(34) Balakrishnan, B.; Mohanty, M.; Umashankar, P. R.; Jayakrishnan, A. Evaluation of an in Situ Forming Hydrogel Wound Dressing Based on Oxidized Alginate and Gelatin. *Biomaterials* **2005**, *26*, 6335−6342.
(35) Costerton, J. W.; Cheng, K. J.; Geesey, G. G.; Ladd, T. I.; Nickel, J. C.; Dasgupta, M.; M, T. J. Bacterial Biofilms In Nature And Disease. *Annu. Rev. Microbiol.* **1987**, *41*, 435−464.
(36) Chapman, R. G.; Ostuni, E.; Liang, M. N.; Meluleni, G.; Kim, E.; Yan, L.; Pier, G.; Warren, H. S.; Whitesides, G. M. 271 Polymeric Thin Films That Resist the Adsorption of Proteins and the Adhesion of Bacteria. *Langmuir* **2001**, *17*, 1225−1233.

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