# Bioadhesive Control of Plasma Proteins and Blood Cells from Umbilical Cord Blood onto the Interface Grafted with Zwitterionic Polymer Brushes

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# Bioadhesive Control of Plasma Proteins and Blood Cells from Umbilical Cord Blood onto the Interface Grafted with Zwitterionic Polymer Brushes

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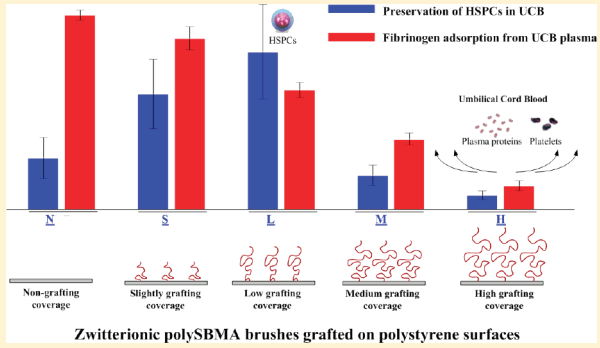
**ABSTRACT:** In this work, bioadhesive behavior of plasma proteins and blood cells from umbilical cord blood (UCB) onto zwitterionic poly(sulfobetaine methacrylate) (polySBMA) polymer brushes was studied. The surface coverage of polySBMA brushes on a hydrophobic polystyrene (PS) well plate with surface grafting weights ranging from 0.02 mg/cm$^2$ to 0.69 mg/cm$^2$ can be effectively controlled using the ozone pretreatment and thermal-induced radical graft-polymerization. The chemical composition, grafting structure, surface hydrophilicity, and hydration capability of prepared polySBMA brushes were determined to illustrate the correlations between grafting properties and blood compatibility of zwitterionic-grafted surfaces in contact with human UCB. The protein adsorption of fibrinogen in single-protein solutions and at complex medium of 100% UCB plasma onto different polySBMA brushes with different grafting coverage was measured by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies. The grafting density of the zwitterionic brushes greatly affects the PS surface, thus controlling the adsorption of fibrinogen, the adhesion of platelets, and the preservation of hematopoietic stem and progenitor cells (HSPCs) in UCB. The results showed that PS surfaces grafted with polySBMA brushes possess controllable hydration properties through the binding of water molecules, regulating the bioadhesive and bioinert characteristics of plasma proteins and blood platelets in UCB. Interestingly, it was found that the polySBMA brushes with an optimized grafting weight of approximately 0.1 mg/cm$^2$ at physiologic temperatures show significant hydrated chain flexibility and balanced hydrophilicity to provide the best preservation capacity for HSPCs stored in 100% UCB solution for 2 weeks. This work suggests that, through controlling grafting structures, the hemocompatible nature of grafted zwitterionic polymer brushes makes them well suited to the molecular design of regulated bioadhesive interfaces for use in the preservation of HSPCs from human UCB.



Zwitterionic polySBMA brushes grafted on polystyrene surfaces

## INTRODUCTION

Nonfouling polymer brushes have an important medical application, providing blood-contacting interfaces to prevent thrombogenicity by resisting nonspecific plasma protein-fouling.[1−7] It is generally acknowledged that the water molecules around the pendant groups of the nonfouling chains play a key role in providing interfacial resistance to nonspecific protein adsorption.[8−10] A set of functional properties is generally used to guide the design of new nonfouling polymer brushes in contact with human blood: they should be hydrophilic and electrically neutral, and they should be hydrogen bond acceptors but not hydrogen bond donors.[11] PEG-based materials are most commonly used as synthetic nonfouling surfaces for resisting protein adsorption.[11,12] Despite the fact that PEG exhibits excellent nonfouling capability, it faces the problem of blood compatibility and stability.[13−15] Recently, zwitterionic brushes containing biomimetic structures such as phosphobetaine, sulfobetaine, and carboxybetaine have received increased attention for use in a new generation of nonfouling surfaces.[5,16−23] An important characteristic of a zwitterionic structure is that it should have both a positively and a negatively charged moiety within the

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Table 1. Characteristic Data of polySBMA Brushes Grafted on PS-*g*-polySBMA

| | reaction conditions | | characterization of polySBMA brushes | | | | bioadhesion of polySBMA brushes | |
|---|---|---|---|---|---|---|---|---|
| | | | | | rms roughness[b] (nm) | | | |
| sample ID | SBMA (wt %) | methanol/$H_2O$ (v/v) | grafting weight[a] (mg/cm$^2$) | contact angle (deg) | dry | hydration | fibrinogen[c] (%) | $R_{CD34+}$[d] (%) |
| N | | | 0.00 ± 0.000 | 82 ± 1 | 3.0 ± 0.4 | 3.1 ± 0.5 | 100.0 ± 2.6 | 18 |
| S | 5 | 3/1 | 0.02 ± 0.014 | 62 ± 1 | 2.5 ± 0.3 | 2.8 ± 0.4 | 88.0 ± 5.9 | 40 |
| L | 10 | 3/1 | 0.10 ± 0.018 | 41 ± 3 | 4.7 ± 0.5 | 7.4 ± 0.6 | 61.6 ± 3.9 | 55 |
| M | 20 | 2/1 | 0.35 ± 0.012 | 32 ± 3 | 5.6 ± 0.5 | 4.2 ± 0.5 | 36.2 ± 3.1 | 12 |
| H | 30 | 1/1 | 0.56 ± 0.021 | 24 ± 2 | 7.0 ± 0.6 | 2.2 ± 0.3 | 12.2 ± 2.6 | 5 |

[a]The surface grafting weight of polySBMA brushes on the PS plate was determined by the extent of weight increase compared to the virgin PS plate based on the total surface area of the 24-well PS plate in contact with the reaction solution. [b]rms roughness of grafted polymer brush on the PS-*g*-polySBMA surfaces that was determined by bio-AFM in the dry state under atmospheric environment and in the hydrated state under a PBS solution at 37 °C. [c]Relative protein adsorption of fibrinogen on these PS-*g*-polySBMA surfaces from 100% UCB plasma solution. [d]The number ratio of $CD34^+$ cells ($R_{CD34+}$) was defined as the ratio of the number of cells expressing CD34 at a certain time to the initial number of cells expressing CD34.

same side chain segment while maintaining overall charge neutrality. Previous works showed that zwitterionic brushes grafted to surfaces are ideal for resisting nonspecific protein adsorption when the surface density and chain length of zwitterionic groups are controlled. Poly(sulfobetaine methacrylate) (polySBMA), with a methacrylate main chain and an analogue of the taurine betaine pendant group ($CH_2CH_2N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$), has become the most widely studied zwitterionic brush due to the ease of synthetic preparation.[6,24−26] Previous studies showed that controlling the surface grafting coverage of zwitterionic polySBMA layers is important for modified substrates with a blood-compatible interface.[6,24] Our study showed that it is important to maintain the charge neutrality of grafted zwitterionic polymer brushes to minimize the electrostatic interactions with plasma proteins and blood cells.[25] We also reported that surfaces grafted with polySBMA brushes provide an effective and stable nonfouling quality to zwitterionic surfaces used in human blood.[26]

Plasma proteins and blood cells are the primary components of blood and function as signaling and self-sustaining processes in the human body. Hematopoietic stem and progenitor cells (HSPCs) are multipotent blood cells that can be found in peripheral blood or umbilical cord blood (UCB). HSPCs are capable of producing every type of mature blood cell (i.e., thrombocytes, erythrocytes, and leukocytes). In general, the population and concentration of plasma proteins and blood cells are quite different in circulating blood compared to peripheral blood and UCB.[27−29] There are 15−75 times more HSPCs in UCB than in peripheral blood.[30] Thus, UCB is an attractive candidate for HSC transplantation, which is commonly performed to treat patients suffering from hematological disorders and malignant diseases after chemotherapy.[29,30] However, the surfaces of nonfouling brushes used in preserving HSPCs in UCB have not yet been extensively studied. In our previous investigation, it was found that the expression ratio of surface markers (CD34) on HSPCs stored in amphiphilic PEGylated flasks was significantly higher than when stored in polystyrene tissue culture flasks.[31] The balance of hydrophobic and hydrophilic domains distributed on the surfaces greatly influences the viability of HSPCs from UCB when stored in such flasks. However, it is still unclear whether HSPCs can be well preserved when stored in zwitterionic flasks that have regulated interfacial hydrophilicity via surface coverage with nonfouling polymer brushes.

In this work, a hydrophobic polystyrene (PS) well plate grafted with zwitterionic polySBMA brushes was studied as an example of a tunable bioadhesive interface placed in contact with plasma proteins and blood cells from human UCB *in vitro*. It is important to understand the effects of both grafting coverage and the structure of polySBMA brushes on the hydration properties and bioadhesive characteristics of zwitterionic PS surfaces, as the results from such studies would directly enable the design of zwitterionic flasks for use in specific blood storage. In the present work, we developed a systematic surface grafting control of PS well plates with polySBMA brushes via ozone pretreatment and thermal-induced radical graft polymerization. The surface grafting structures of polySBMA brushes in a dry state and a hydrated state on PS plates were examined. The adsorption of plasma proteins and the adhesion of platelets onto the zwitterionic PS plate surfaces from UCB plasma were demonstrated, and the number of surviving HSPCs from 100% UCB stored in such well plates was determined. This study introduces a fundamental understanding of how the structures governing the fouling-resistance properties of plasma proteins and the level of zwitterionic grafting coverage affect the hydrophilicity of the grafted brush surface. The study also provides new insight into the preservation of highly valuable blood cells from UCB, such as HSPCs.

## MATERIALS AND METHODS

**Materials.** Twenty-four-well cell culture plates made of nontreated PS were purchased from Falcon (351147) and were used as received. The [2-(methacryloyloxy)ethyl] dimethyl(3-sulfopropyl)ammonium hydroxide (sulfobetaine methacrylate, SBMA) macromonomer was purchased from Monomer-Polymer & Dajac Laboratories, Inc. Methanol (99%) was obtained from Sigma-Aldrich and was used as a solvent for thermal-induced radical graft polymerization. Fibrinogen (fraction I from human plasma) was purchased from Sigma Chemical Co. Umbilical cord blood and plasma solution were obtained from the Blood Services Foundation of Kaohsiung Medical Hospital. RepCell and HydroCell flasks were purchased from CellSeed Inc. Phosphate buffered saline (PBS) was purchased from Sigma-Aldrich. Deionized water used in the experiments was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩ.

**Preparation of PolySBMA Brushes.** The PS plate surface in each well was pretreated with a continuous stream of $O_3/O_2$ mixture at a flow rate of 6 L/min for 30 min and an ozone concentration of approximately 46 mg/L at 25 °C, which was generated from a custom-built ozone generator (Model OG-10PWA, Ray-E Creative Co., Ltd., Taiwan). After ozone treatment, the 24-well PS plate was cooled

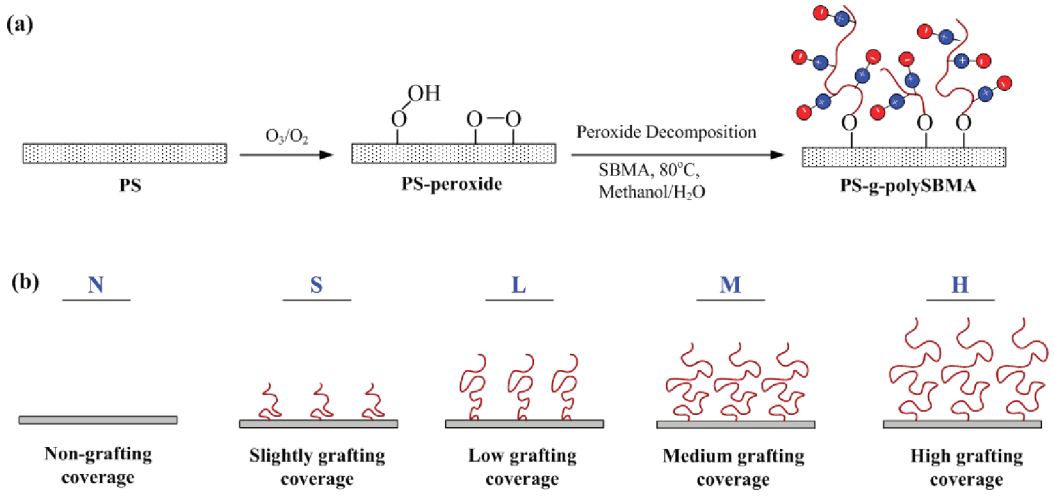


**Figure 1.** Schematic illustration of (a) the preparation process of the PS-*g*-polySBMA via thermal-induced radical graft-polymerization and (b) the grafting structures of polySBMA brushes grafted on PS surfaces.

quickly in an ice box at 4 °C, purged with argon for 10 min, and kept in methanol before reaction. The surface-induced thermal graft polymerization was then applied to the ozone-treated PS plates. A range of SBMA content from 5 wt % to 30 wt % in a mixed-solvent of methanol and deionized water solvent was used to demonstrate the graft-polymerization properties of each PS plate with varying grafting coverage and varying structures of the polySBMA brushes. The different reaction solutions used for the surface zwitterionization of the PS plates are summarized in Table 1. A prepared reaction solution of 0.5 mL was added to each ozone-treated well in a 24-well PS plate at 80 °C for 24 h. After the reaction, the polySBMA-grafted PS plate was transferred to a container with purified methanol, and the plate was then extracted with deionized water and methanol. The residual solvent was removed in a vacuum oven under reduced pressure. In this study, all plates that underwent surface modification were subjected to the same washing conditions.

**Surface Characterization of PolySBMA Brushes.** The surface composition of the grafted polySBMA brushes was characterized by X-ray photoelectron spectroscopy (XPS). XPS analysis was performed using a Thermal Scientific K-Alpha spectrometer equipped with a monochromated Al K X-ray source (1486.6 eV photons). The energy of the emitted electrons was measured with a hemispherical energy analyzer at pass energies ranging from 50 to 150 eV. All data were collected at a photoelectron takeoff angle of 45° with respect to the sample surface. The binding energy (BE) scale is referenced by setting the peak maximum in the C 1s spectrum to 284.6 eV. The high-resolution N 1s and S 2p spectrum was fitted using a Shirley background subtraction and a series of Gaussian peaks. The surface grafting weight of polySBMA brushes on the PS plate was determined by the difference in weight compared to the unmodified PS plate and based on the total surface area of the 24-well PS plate in contact with the reaction solution. Prior to the weight measurements, the PS plates were dried in a freeze-dryer at −45 °C for 1 day. Water contact angles were measured with a goniometer (Automatic Contact Angle Meter, Model FTA1000, First Ten Ångstroms Co, Ltd. USA) at 25 °C. The deionized water was dropped on the sample surface at 10 different sites, and the surface contact angle was measured over a duration of 10 s. In this study, the hydration capacity ($mg/cm^2$) of the prepared surface is defined as the difference in wet weight between the polySBMA grafted PS well and the unmodified PS well divided by the total surface area of the unmodified PS well. The surface morphology of the grafted polySBMA brushes in dry and hydrated states was examined by bioatomic force microscopy (bio-AFM). All AFM images were acquired with a JPK Instruments AG multimode NanoWizard (Germany). The instrument is equipped with a NanoWizard scanner and can be operated in air or liquid. For tapping-mode AFM, a commercial Si cantilever (TESP tip) from JPK was used with a resonant frequency of approximately 320 kHz. The relative humidity was less than 40%.

**Protein Adsorption on the PolySBMA Grafted PS Well Plate.** The adsorption of human fibrinogen on the polySBMA-grafted PS well plates was evaluated using an enzyme-linked immunosorbent assay (ELISA) according to the standard protocol described briefly below. First, each polySBMA-grafted well of a 24-well PS plate was equilibrated with 1000 μL of PBS for 60 min at 37 °C. For the test of single protein adsorption, the wells were soaked in 500 μL of fibrinogen solution at a concentration of 1 mg/mL. For the test of plasma protein adsorption, the wells were soaked in 500 μL of 100% platelet poor plasma (PPP) solution from umbilical cord blood (UCB). PPP was prepared by centrifugation of the UCB at 3000 rpm for 10 min. After 180 min of incubation of the fibrinogen solution at 37 °C or the PPP solution at 4 °C, the wells were rinsed five times with 500 μL of PBS and then incubated in bovine serum albumin (BSA, purchased from Aldrich) at a concentration of 1 mg/mL for 90 min at 37 °C to block the areas unoccupied by protein. The wells were rinsed with PBS five more times and incubated in a 500 μL PBS solution. The wells were incubated with a primary monoclonal antibody that reacted with the fibrinogen for 90 min at 37 °C and were then blocked with 10 mg/mL BSA in PBS solution for 24 h at 37 °C. The wells were subsequently incubated with the secondary monoclonal antibody, horseradish peroxidase (HRP)-conjugated immunoglobulins, for 60 min at 37 °C. The wells were rinsed five times with 500 μL of PBS before adding 500 μL of PBS containing 1 mg/mL chromogen consisting of 3,3′,5,5′-tetramethylbenzidine, 0.05 wt % Tween 20, and 0.03 wt % hydrogen peroxide. After incubation for 20 min at 37 °C, the enzyme-induced color reaction was stopped by adding 500 μL of 1 mol/L $H_2SO_4$ to the solution in each well, and finally, the absorbance of light at 450 nm was determined with a microplate spectrophotometer. Protein adsorption on the polySBMA-grafted well surfaces was normalized with respect to that on the unmodified PS. These measurements were carried out six times for each well ($n = 6$).

**Blood Platelet Adhesion on the PolySBMA Grafted PS Well Plate.** Platelet-rich plasma (PRP) containing approximately $1 \times 10^5$ cells/mL was prepared by centrifugation of 100% UCB at 1200 rpm for 10 min. The platelet concentration was determined by microscopy (NIKON TS 100F). To test the activated platelet adhesion on the polySBMA-grafted PS plates, 200 μL of the PRP was placed on the well surface in each well plate and incubated for 120 min at 37 °C. After the wells were rinsed twice with 1000 μL of PBS, they were incubated with 2.5% glutaraldehyde of PBS for 48 h at 4 °C to fix the adhered platelets and adsorbed proteins. They were then rinsed two times with 1000 μL of PBS and gradient-dried with 0%, 10%, 25%, 50%, 75%, 90%, and 100% (v/v) ethanol in PBS for 20 min at each


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step, with a final air drying step. Finally, each well was cut and sputter-coated with gold prior to observation with a JEOL JSM-5410 SEM operating at 7 keV.

**Preservation of Hematopoietic Stem and Progenitor Cells Stored in Umbilical Cord Blood.** A sample of human umbilical cord blood was collected using vacuum tubes (Venoject II, Terumo, Co.) containing EDTA·2Na. Human UCB was injected into each well of the polySBMA grafted PS plates and was stored in a refrigerator for two weeks at 4 °C. After slowly shaking the well plates for 60 s, 1 mL of the UCB was sampled and analyzed using flow cytometry (Coulter EPICS XL; Beckman-Coulter Co.) to measure surface markers of hematopoietic stem and progenitor cells.[32−34] The number of HSPCs was found by examining $CD34^+$ cells. This was performed by incubating the cells with anti-CD34 antibody and anti-CD45 antibody and following the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines for flow cytometry.[35]

## RESULTS AND DISCUSSION

**Surface Zwitterionization and Characterization.** In the present work, a set of well-controlled grafted surfaces with polySBMA polymer brushes was prepared via thermal-induced radical graft polymerization. This work focused on the bioadhesive control of plasma proteins and blood cells in contact with polySBMA-grafted PS plates via adjusting the grafting coverage of the polySBMA polymer brushes by varying their concentration in the reaction solution. The reaction solutions used for the surface graft polymerization of the PS well plates are shown in Table 1. As shown in Figure 1a, the processes involved in the surface graft polymerization of the PS well plate can generally be divided into two stages. The first stage is to activate the reactive sites of the peroxide on the PS plate via ozone treatment. In this stage, the amount of peroxide is controlled by the ratio of $O_3/O_2$ content and the duration of ozone treatment. The peroxide concentration of ozone-treated PS was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) following procedures reported in the literature. An ozone pretreatment time of 30 min was used to produce a peroxide content of approximately 2.85 nmol/cm$^2$ distributed on the PS plate surface. In general, an increase in ozone pretreatment time can result in an increase in the amount of peroxide produced on the surface of the PS plate, but too large an increase in ozone pretreatment duration may cause unfavorable etching or degradation on the PS surface. The second stage is to copolymerize the zwitterionic monomer of SBMA onto the PS well plates via thermal-induced radical graft-polymerization. In this stage, the decomposition of peroxides on the ozone-treated PS plates is controlled by the temperature in the reaction solution. In this study, a graft polymerization time of 24 h at 80 °C was assumed to be sufficient for almost total decomposition of the peroxides and complete copolymerization with SBMA monomer.[36,37]

The grafting density, chain length, and chain conformation of the grafted zwitterionic polymer brushes are the primary factors determining surface hydration and the ability to bind water molecules. To control the surface grafting coverage of polySBMA brushes on PS well plates, the content of SBMA monomer in the reaction solution was controlled within a range from 5 wt % to 30 wt %, as measured by surface grafting weight. Figure 2 shows the effect of surface grafting weight of polySBMA on the change of surface contact angle and the hydration capacity of the modified PS well plates. The reduced values of water contact angle in Figure 2 indicate that the surface hydrophilicity of modified PS plates increases with increasing surface grafting weight. A water contact angle as low

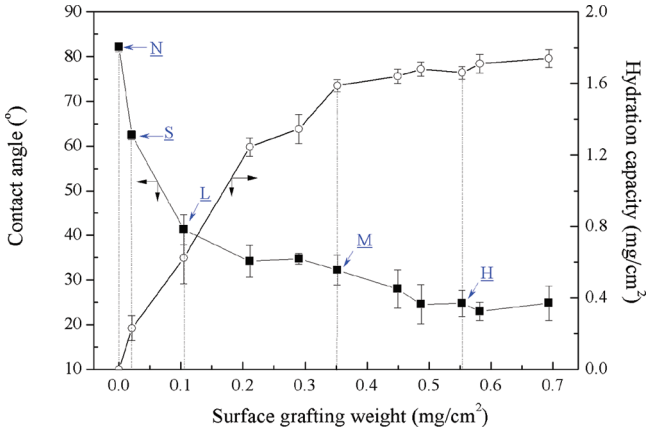


**Figure 2.** Changes in the water contact angle and hydration capacity for the prepared PS-*g*-polySBMA as a function of the surface grafting weight of polySBMA brushes on the PS surface. These measurements were carried out 6 times for each well ($n$ = 6).

as approximately 25° was achieved on the PS well plate grafted with polySBMA brushes (PS-*g*-polySBMA), indicating an obvious increase in hydrophilicity compared to the unmodified PS surface, whose water contact angle was approximately 82°. The decrease in the water contact angle along with the increase in surface grafting weight of the PS-*g*-polySBMA is attributed to the increased surface coverage of hydrophilic polySBMA brushes on the hydrophobic PS well plate. There also appeared to be almost no progressive change in the water contact angle as the grafting weight reached above 0.45 mg/cm$^2$ for the PS-*g*-polySBMA. This result suggests that grafting the polySBMA brushes beyond the above-mentioned weight results in full surface coverage and uniformity, allowing for complete water−surface contact. In this study, the hydration capacity (mg/cm$^2$) of the prepared polymer brushes was evaluated by the difference in wet weight between the polySBMA grafted PS well plate and the unmodified PS well plate divided by the total surface reaction area of the unmodified PS well plate. The measured quantity of hydration capacity is proportional to the amount of binding water molecules around the zwitterionic pendant group of the polySBMA brushes.[38] An increase in the grafting weight and, thus, the thickness of the polySBMA brush resulted in an increase in the surface hydration. The results in Figure 2 indicate that the hydration capacity of the PS-*g*-polySBMA is approximately 1.74 mg/cm$^2$. To investigate how grafting coverage and the structure of polySBMA brushes are associated with the hydration properties and hemocompatible characteristics of the PS-*g*-polySBMA surface, five samples with different grafting weight were defined as no (N), slight (S), low (L), medium (M), and high (H) grafting coverage of polySBMA brushes on PS well plates, as shown in Figure 1b.

The composition and structure of the polySBMA brushes on the PS surface were characterized by XPS and AFM. Figure 3 showed the typical XPS spectrum of the unmodified PS surface and the PS-*g*-polySBMA surface. For sample N on the unmodified PS well plate, there is evidence of secondary amine groups that indicate the presence of a positive surface charge. The presence of the grafted polySBMA brushes could be ascertained from the tertiary amine groups and the sulfonate groups, as evidenced by the binding energies of the $-CH_2\underline{N}(CH_3)_2CH_2-$ at 402 eV and $-CH_2\underline{S}O_3$ at 167 eV, respectively, which appear on the PS-*g*-polySBMA. It was determined that both the intensity of the binding energies at


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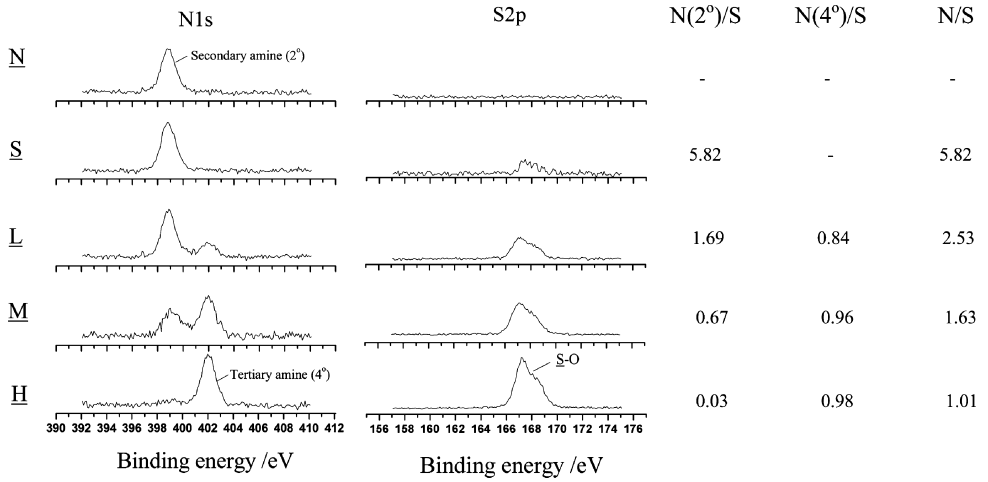


**Figure 3.** XPS spectra of the modified PS surfaces with polySBMA brushes in the N 1s and S 2p regions of samples N, L, S, M, and H.

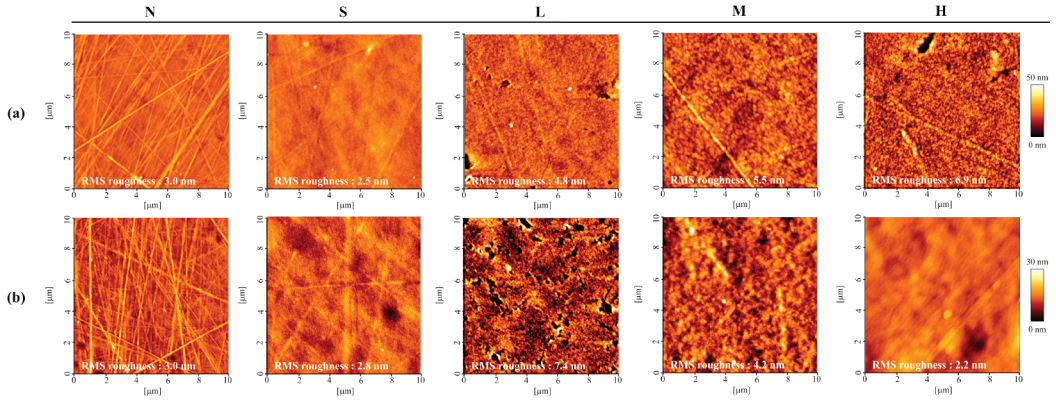


**Figure 4.** Tapping-mode bio-AFM images of surface morphology and rms roughness of the PS-*g*-polySBMA: surface images in (a) dry state and (b) hydrated state of samples N, L, S, M, and H. The dimensions of the scan images are 10.0 $\mu$m × 10.0 $\mu$m.

402 and 167 eV and the ratio of N/S decreased as the surface grafting weight of the polySBMA brushes was increased from 0.02 mg/cm$^2$ to 0.56 mg/cm$^2$. The results indicate that the expansion of the grafted polySBMA polymer depends on the content of SBMA monomer in the reaction solution during thermal-induced radical graft-polymerization. Beyond a grafting weight of 0.56 mg/cm$^2$, the ratio of N/S approaches 1.0, thus indicating the formation of a homogeneous zwitterionic brush distribution, as evidenced on the PS-*g*-polySBMA surface of sample H with overall surface charge neutrality. Figure 4 shows the bio-AFM images of the conformational structure of the grafted polymer brush on the PS-*g*-polySBMA surfaces that were observed in the dry state under atmospheric conditions and in the hydrated state under a PBS solution at 37 °C. It was interesting to observe that these zwitterionic surfaces of samples S through H display different characteristics in the dry state and in the hydrated state, whereas the unmodified PS surface images of sample N keep the same surface morphology. The results indicate that the surface roughness of PS-*g*-polySBMA is associated with the surface grafting weight of the polySBMA brushes. For the samples observed in a dry state, the increase of surface roughness along with the increase in the weight of the polySBMA brushes is attributed to the increased thickness and spreading of zwitterionic polymer brushes on the hydrophobic PS well plate. However, when the samples were observed in a hydrated state, the surface roughness of PS-*g*-polySBMA exhibited a maximum for sample L, at a surface grafting weight of the polySBMA brushes about 0.10 mg/cm$^2$. An obvious increase in the surface roughness of PS-*g*-polySBMA (sample L) from a dry state (~4.7 nm) to a hydrated state (~7.4 nm) showed that surface grafted polySBMA brushes could keep their well-extended conformation in PBS due to the excellent hydration. For PS-*g*-polySBMA sample H, the decreased surface roughness from a dry state (~6.9 nm) to a hydrated state (~2.2 nm) indicated high surface packing of the hydrated polySBMA brushes on the PS surface. The AFM results suggest that the grafting structure of the polySBMA brushes is strongly associated with the surface coverage.

**Correlation between Surface Hydration and Grafting Structure of the polySBMA Brushes with Protein Adsorption and Platelet Adhesion.** In general, the ability of a surface to resist plasma protein adsorption, such as fibrinogen, is a prerequisite for that surface to resist blood platelet adhesion.[39,40] Therefore, the adsorption of fibrinogen to a material interface usually provides a good indication of the surface hemocompatibility. The formation of a bound water layer was considered to be critical in repelling plasma proteins and generating the antithrombogenic surface.[9,10,38] Recent studies have shown that the protein-resistance properties of zwittteroinic polymer brushes are attributed to their strong hydration capabilities and conformational structures.[10,22,25] It is accepted that the formation of a hydration layer around the zwitterionic pendant group of the polymer chains results in a


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hemocompatible character. In general, whereas hydrophilic and neutral nonfouling polymer brushes form a hydration layer via hydrogen bonds, zwitterionic polySBMA brushes form a hydration layer via electrostatic interactions.[25] Therefore, it is expected that polySBMA brushes are capable of binding significant quantities of water, which is correlated with their blood compatibility. The evaluation of protein adsorption from single fibrinogen solution on the hydrated surfaces should be considered to be an indicator not only of surface hydrophilicity and hydration capability but also of the surface grafting structure of a hydrated layer on the prepared surface.[41] In this study, the grafting structures of polySBMA brushes associated with protein adsorption on a PS-*g*-polySBMA surface were systematically identified by AFM analysis. The surface grafting weight of the polySBMA brushes was also measured, and it was found to increase from 0.02 mg/cm$^2$ to 0.56 mg/cm$^2$.

Figure 5 shows that the dependence of the relative fibrinogen adsorption and surface roughness of the PS-*g*-polySBMA on the surface grafting weight of polySBMA brushes can be controlled by the surface coverage of zwitterionic polymer brushes. The increase in grafting weight from increased thickness of the polySBMA brushes resulted in the reduced adsorption of human fibrinogen and increased hydration capacity, as indicated in Figure 2. Single protein resistance and hydration capacity were maximized when the grafting weight of polySBMA brushes was above 0.48 mg/cm$^2$. Furthermore, the relative protein adsorption on PS-*g*-polySBMA was effectively reduced to ~10% of that of an unmodified PS surface as the hydration capacity increased above 1.74 mg/cm$^2$. The results support that the protein-resistance properties of zwittteroinic polySBMA brushes are attributed to their strong hydration capabilities. Horbett et al. showed that the adhesion and activation of platelets from the bloodstream might be correlated with the adsorption of proteins on surfaces.[39,40] Even a small amount of plasma proteins on a surface can lead to the thrombogenic reaction. Figure 6 shows SEM photographs with image magnification of 1000× of the prepared substrates in contact with 100% PRP solution form human UCB for 120 min at 37 °C in vitro. The SEM results showed the formation of thrombosis on the unmodified PS surface; sample N displayed a full-scale platelet adhesion at the PRP contact side. However, there clearly appeared to be some degree of inactivated platelet adhesion on PS-*g*-polySBMA surfaces of sample S, L, and M, whereas the relative adsorbed amount of single fibrinogen is above ~20% that of an unmodified PS surface. On the basis of previous reports from Horbett et al., it is believed that reducing plasma protein adsorption levels to below 10 ng/cm$^2$ on biomaterial surfaces can effectively prevent the adhesion and activation of platelets from the bloodstream.[39,40] It was shown that no platelets adhered and no platelets were activated on PS-*g*-polySBMA surface sample H with high grafting coverage (~0.56 mg/cm$^2$) and overall charge neutrality (N/S ~ 1.0). The results extended the previous hypothesis that even a small amount of fibrinogen on a surface can lead to the adhesion of platelets from a plasma solution of human UCB.

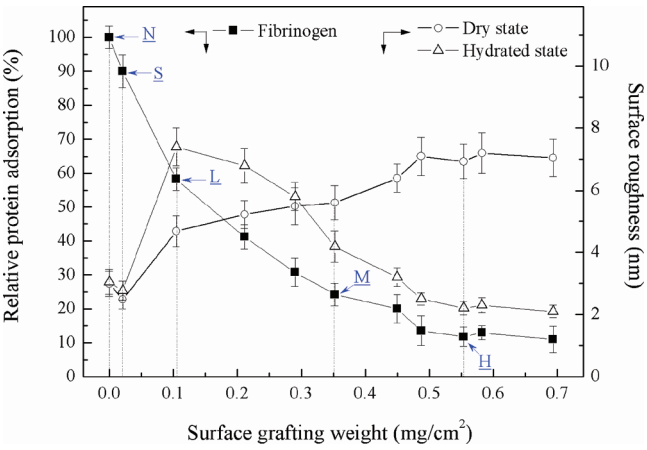


**Figure 5.** Changes in the relative protein adsorption of fibrinogen and surface roughness of polymer brushes in dry and hydrated states for the prepared PS-*g*-polySBMA as a function of the surface grafting weight of polySBMA brushes on the PS surface. Each experimental data point represents an average of six samples. As a reference, the amount of adsorbed fibrinogen to PS is 336 ng/cm$^2$, as determined from radiolabeled experiments.[43].

In this work, it was also found that a reduction in nonspecific fibrinogen adsorption and platelet adhesion was strongly correlated with the variation of the grafting structure of polySBMA brushes on the PS surface. In Figure 5, the results showed that the increase in surface grafting weight of polySBMA brushes from 0.02 to 0.7 mg/cm$^2$ gave the zwitterionic polymer brushes more surface coverage for hydrophobic PS substrates, as evidenced by the increase in surface roughness of grafted brushes in the dry state. At surface grafting weights below 0.28 mg/cm$^2$, the maximal increase in the surface roughness of sample L in the PBS solution indicated that enough of a hydrated surface existed between grafted brushes to allow for a well-extended conformation of individual polySBMA chains, but there was partial exposure to the hydrophobic domains of the PS substrate to induce fibrinogen adsorption and platelet adhesion. Interestingly, the decreased change in the surface roughness of sample H from the dry state to the hydrated state promoted the complete coverage of hydrated polySBMA brushes on the PS surface, indicating the formation of a tightly compacted layer from the zwitterionic sulfobetaine groups for resisting fibrinogen adsorption and platelet adhesion. The study shows for the first time that surface- grafted polymer brushes of polySBMA with high grafting coverage and overall charge neutrality can be used to achieve high blood compatibility while in contact with 100% human UCB plasma solution.

**Preservation of Hematopoietic Stem and Progenitor Cells.** The bioadhesive control of blood cells from human blood onto the material interface is a critical issue for improved preservation or separation processes in blood treatments and medical therapy. It was generally believed that a material surface grafted with nonfouling polymer brushes provides a bioinert interface to maintain normal and stable function in blood cells.[25,37,41] Our previous hypothesis proposed that the surfaces must allow for the control of bioadhesive and bioinert properties from hydrophobic–hydrophilic structures in order to preserve the survival of stem cells in human blood.[31] This hypothesis was tested in this study. Hematopoietic stem and progenitor cells from human UCB were used to investigate the capability of blood stem cells stored in five different well plates of PS-*g*-polySBMA (sample N, S, L, M, and H) with controllable surface coverage of grafted polySBMA brushes. Human UCB was stored at 4 °C in each well plate for two weeks to determine whether HSPCs can survive without differentiation. In general, the number of blood cells can be measured using flow cytometry analysis of surface antigens on


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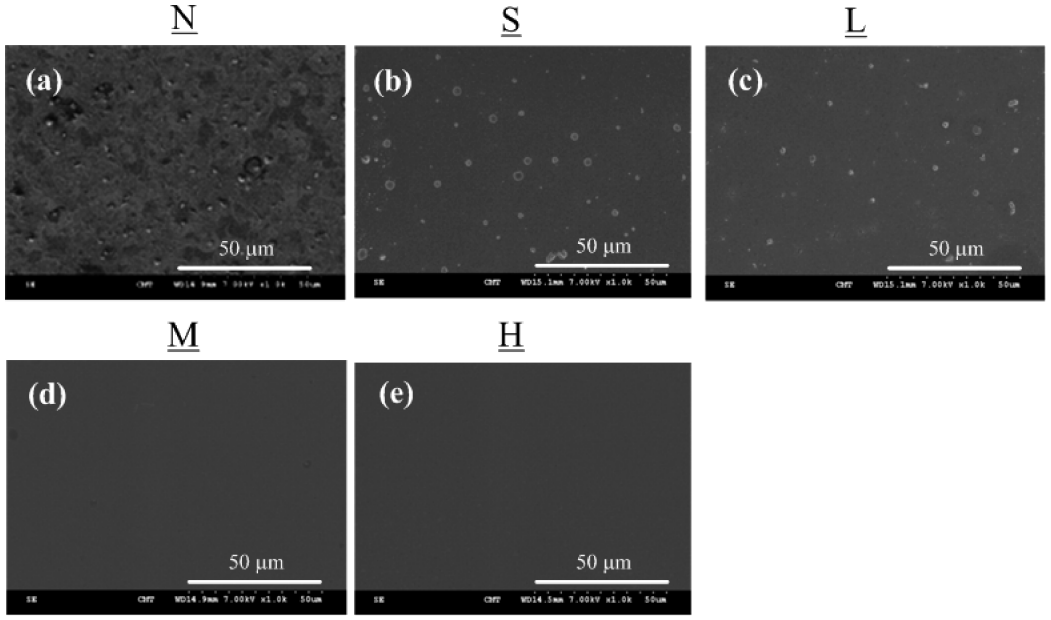


**Figure 6.** SEM photographs of human blood cells adhered onto the PS-*g*-polySBMA surface of (a) sample N, (b) sample L, (c) sample S, (d) sample M, and (d) sample H. All images are at a magnification of 1000×.

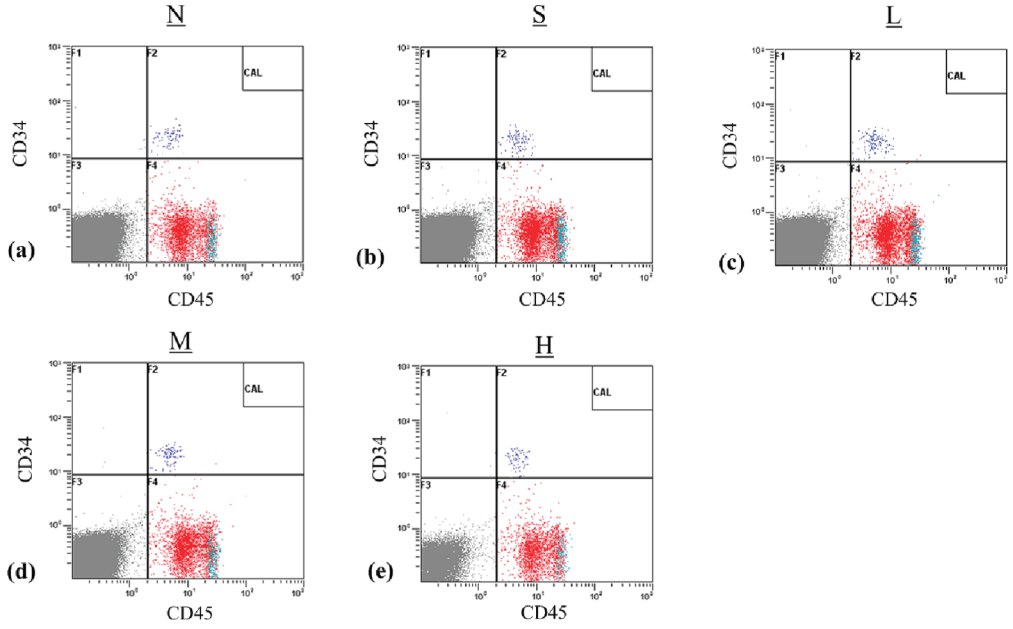


**Figure 7.** Flow-cytometric scattergrams of UCB with anti-CD45 antibody conjugated with FITC and anti-CD34 antibody conjugated with PE show the fluorescent intensity for the PS-*g*-polySBMA surface of (a) sample N, (b) sample L, (c) sample S, (d) sample M, and (d) sample H.

the cells bound to each fluorescent-labeled antibody. HSPCs are known to express several characteristic surface markers (e.g., $CD34^+$) and lose this expression after differentiation.[35,43] Figure 7 shows flow-cytometric scattergrams of cord blood cells after lysis of erythrocytes. HSPCs were analyzed by counting $CD45^+CD34^+$ cells following ISHAGE guidelines and indicated by a blue color.[35] A strong correlation was found between the surface grafting structures of polySBMA brushes and the number of $CD45^+CD34^+$ cells from cord blood in well plates after 14 days of storage. The highest number of $CD45^+CD34^+$ cells was observed in UCB stored in the sample L well. Sample M and sample H, having higher surface grafting weights than sample S and sample L, showed a lower number of $CD45^+CD34^+$ cells. Figure 8 shows the quantity of $CD34^+$ cells in UCB stored in prepared PS-*g*-polySBMA well plates after cultivation for 14 days at 4 °C. RepCell flasks are commonly believed advantageous for the preservation of stem cells, which can be used as a positive control. HydroCell flasks are commercially available bioinert flasks used for a negative control. The number of $CD34^+$ cells in umbilical cord blood stored in the RepCell flasks is 220 ± 30 cells/20 μL, and the number of cells in HydroCell flasks is 80 ± 25 cells/20 μL after preservation for two weeks at 4 °C. Plasma protein adsorption of fibrinogen on these PS-*g*-polySBMA surfaces from 100% UCB plasma solution was also tested. Similar to the results of single fibrinogen adsorption in Figure 5, enhanced protein resistance in UCB plasma solution was observed as the grafting weight increased. In this study, the ratio of $CD34^+$ cells


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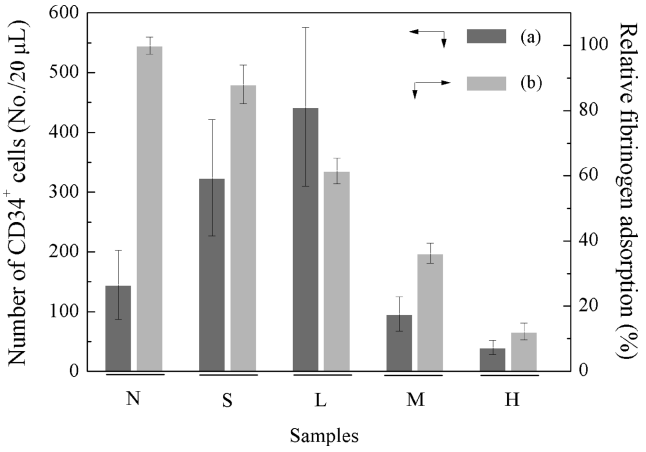


**Figure 8.** Number of $CD34^+$ cells in UCB stored in samples N, L, S, M, and H for 14 days at 4 °C and relative fibrinogen adsorption on each sample surface from 100% UCB plasma solution for 2 h at 4 °C. Data are expressed as the mean ± standard deviation (SD) of three independent measurements using three different samples of UCB.

($R_{CD34+}$) was defined as the ratio of the number of cells expressing CD34 to the initial number of cells expressing CD34 at a specific time, as listed in Table 1. It was observed that the $R_{CD34+}$ of the unmodified PS well plates (sample N) significantly decreased to less than 20% after 14 days of storage. In contrast, the $R_{CD34+}$ of the polySBMA-grafted well plates (sample S and sample L) was more than 40% after 14 days, whereas sample L had a ratio more than three times higher than that of sample N. However, the capacity for preserving HSPCs decreased as the grafting weight of polySBMA brushes increased from 0.34 mg/cm$^2$ to 0.56 mg/cm$^2$, which was the case for the $R_{CD34+}$ of sample M and sample H, which was 12% after 14 days of UCB storage. This difference could be due to the formation of a strong protein-resistance surface from the highly grafted polySBMA brushes for reducing the attachment of HSPCs. Importantly, it was shown that there was an optimal surface coverage of grafted polySBMA brushes on the PS-*g*-polySBMA surface of sample L for the preservation of a relatively high number of these survival HSPCs. As indicated in Figure 6, the highest capacity of grafted zwitterionic interface for the preservation of HSPCs might be attributable to the high surface roughness as well as flexible chain mobility of polySBMA brushes in the hydrated state. Partial hydrophobic domains distributed on the PS-*g*-polySBMA surface of sample L also involved bioadhesive interaction for the preservation of HSPCs in cord blood. The results confirmed the previous hypothesis of a balanced arrangement of bioadhesive and bioinert properties in hydrophobic−hydrophilic interfacial structures is crucial in preserving the viability of HSPCs. As a result, it was concluded that the hydrated polySBMA brushes on the PS well plate surfaces with high surface coverage led to low adsorption of plasma proteins but highly flexible chains enable the preservation of HSPCs in human UCB.

## CONCLUSIONS

In this study, a tunable bioadhesive polystyrene interface of grafted polySBMA brushes was observed under a range of surface grafting conditions, and the hemocompatible quality of this interface was evaluated using human umbilical cord blood. The surface hydration and grafting structures in aqueous media were determined to illustrate the hydrated chain conformation of prepared polySBMA brushes, which strongly depends on the brush density. We found that polySBMA brushes of higher surface grafting weights had enhanced interfacial hydration capacity in aqueous solution, resulting in an increase in their resistance to platelet adhesion and a reduction of protein adsorption in human UCB. The results showed that PS grafted polySBMA brushes exhibited a modifiable bioadhesive activity that allowed for the preservation of HSPCs in 100% human UCB, depending on the grafting weights of the polymer brushes. The grafted polySBMA brushes with a grafting weight of approximately 0.1 mg/cm$^2$ presented a ratio of $CD34^+$ greater than 50% in human UCB stored for 14 days, which was attributed to the formation of a balanced bioadhesive/bioinert interface consisting of highly flexible chains of hydrated polySBMA that were immobilized on a hydrophobic PS surface. This study shows that the creation and modification of bioadhesive interfaces for plasma proteins and blood cells can be achieved on hydrophobic surfaces that are grafted with hydrophilic polySBMA brushes and that the surface coverage and morphology of zwitterionic nonfouling polymers are important conditions to consider in applications involving human blood.

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