Dendritic Saccharide Surfactant Polymers as Antifouling Interface Materials to Reduce Platelet Adhesion

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Received August 25, 2005; Revised Manuscript Received December 7, 2005

Here, we report on the synthesis of dendritic saccharide surfactant polymers as antifouling interface materials to reduce platelet adhesion. An acetal-protected poly(amidoamine) (PAMAM) dendron (**5**, $G = 2$) was first synthesized by using aminoacetaldehyde dimethyl acetal (**1**) as the starting material to provide a monovalent focal structure with dimethyl acetal-protected aldehyde functionality. Maltose dendron (M4, **6**) was obtained by reacting the peripheral amine groups of acetal-dendron (**5**) with maltonolactone. The dendritic surfactant polymers (**9**) were then synthesized via a two-step method by sequential addition of maltose dendron and hexanal to react with the amine groups on the poly(vinylamine) (PVAm) backbone. Surface activity of the amphiphilic glycopolymers at the air/water interface was demonstrated by reduction in water surface tension. Adsorption of the amphiphilic glycopolymers at the solid/water interface was examined on octadecyltrichlorosilane (OTS)-coated coverslips by water contact angle measurements. A nanoscale understanding of surface-induced self-assembly of the dendritic surfactant polymer on highly oriented pyrolytic graphite (HOPG) was gained using AFM operated in fluid tapping mode. A lateral ordering of adsorbing surfactant polymer was visualized with a pattern in strands 60° out of alignment. The static platelet adhesion tests show that the hexyl side chains can facilitate adsorption of the surfactant polymers onto hydrophobic substrates, while the maltose dendron side chains can provide a dense canopy of protective glycocalyx-like layer as an antifouling interface to reduce platelet adhesion.

Introduction

Synthetic biomaterials, particularly polymers, are widely used for biomedical applications, including cardiovascular devices, artificial organs, and biosensors.[1,2] However, exposure of the foreign biomaterials to blood initiates nonspecific interactions with proteins and cells. This "biofouling" includes the spontaneous adsorption of plasma proteins followed by adhesion and activation of platelets, leukocytes, and bacteria, if present, which results in well-documented problems of thrombosis, excessive wound healing, and infection.[3−5] The interface between a biomaterial and the biologic environment has central importance to these problems, because it controls the protein and cell−surface interactions. Consequently, the most common approach for improving blood compatibility has been to modify the interfacial properties of the existing biomedical devices.[6,7] This has included covalently coupled coatings, physical adsorption, and high energy physiochemical treatments.[8−10] Even though some surface treatments have improved blood compatibility, major advances in clinical applications have yet to be realized.

Our approach to this problem is to design interface materials based on mimicking the cell surface glycocalyx, which is a complex coating of highly glycosylated molecules that dominate the interface between a cell and its environment. The glycocalyx provides a physical basis for maximizing entropic repulsion that minimizes nonspecific interactions, including undesirable cell and molecular adhesions.[11−14] Biomimicry of the cell glycocalyx is a strategy for creating a hydrated saccharide layer as a biocompatible interface for existing biomaterials.[15−17] One advantage of using saccharides is that they have no amphiphilic or surface-active character and do not exhibit entropic aggregation transitions (no cloud point) in water as does poly(ethylene glycol) (PEG).[18] In our previous studies, saccharides, including dextran and oligomaltose, were attached directly to a poly(vinylamine) (PVAm) backbone, to create a comblike surfactant polymer that undergoes stable surface assembly on hydrophobic substrates.[14,19,20] While this approach has proven relatively successful in suppressing protein and platelet adhesion, direct attachment of saccharides to the polymer backbone limits the density and spatial arrangement of saccharides that can be accommodated. The rigid rod conformation of pendant saccharides also requires the spatial presentation to be designed so as to minimize underlying defect areas that could provide adhesion sites for proteins and platelets.

Here, we report on the synthesis of a dendritic saccharide surfactant polymer as a novel surface-assembling, biomimetic interface material. To address the critical issue of controlled spatial arrangement of the saccharides, glycodendrons were designed and attached to a PVAm backbone to create a canopy of protective glycocalyx-like saccharide over a biomaterial surface, eliminate potential defect areas, and provide quantitative control over the biomimetic structure and its properties of inhibiting protein and platelet adhesion.

Experimental Section

Materials. Aminoacetaldehyde dimethyl acetal, ethylenediamine, and hexanal were purchased from Aldrich and used as received. Methyl acrylate (Aldrich Chem.) with the inhibitors removed by washing with 10% aqueous NaOH solution was redistilled under reduced pressure. Dimethyl sulfoxide (DMSO, Aldrich Chem.) was freshly distilled before use. Methanol-soluble maltonolactone was prepared according to the

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10.1021/bm050611p CCC: $33.50
Published on Web 03/04/2006

literature method by oxidizing maltose monohydrate (Aldrich Chem.) with I_2 in a KOH methanol solution. Poly(vinylamine) (PVAm, Mn 1.63×10^4, Mw/Mn 1.34) was synthesized according to the literature method by free radical polymerizaton of *N*-vinylformamide (Aldrich Chem.) using *a*,*a*′-azoisobutyronitrile (AIBN, Aldrich Chem.) as the initiator.

Methods. ^{1}H NMR spectra were recorded on a 300 MHz Varian Gemini-300 spectrometer. Samples were prepared with concentrations of about 10 mg/mL, and 64 scans were collected at room temperature using the solvent peak as the reference. MALDI-MS mass spectrometry was performed on a BrukerBiflex III MALDI-TOF mass spectrometer by dissolving the sample and the matrix of 2,5-dihydroxybenzoic acid in 1:1 (v/v) ethanol and water, and mass spectra were typically accumulated from 200 laser shots. Molecular weight of PVAm was measured by gel permeation chromatography (GPC) on a Waters 2690 Alliance system equipped with a SynChropak CATSEC300 column (Eprogen Inc., Darien, IL) and a Mini Dawn light scattering detector. It was carried out at room temperature with an aqueous solution of 0.2 M $NaNO_3$ containing 0.1% trifluoroacetic acid as eluent at a flow rate of 0.5 mL/min. The specific refractive index increment (d*n*/d*c*) for PVAm was determined as 0.182 on a Waters 410 differential refractometer. The water contact angle was measured with a Rame−Hart contact angle goniometer by the sessile drop method. The receding angle (θ_R) was obtained by decreasing the volume of the water drop from 10 to 2 μL. Surface tensions of aqueous surfactant polymer solutions were measured by the Du Nouy ring method, using a Sigma 703 surface tensiometer at 25 °C.

Synthesis of Acetal-Protected PAMAM Dendron 2 (*G* = 0.5). A solution of aminoacetaldehyde dimethyl acetal (**1**) (8.4 g, 80 mmol) in methanol was added dropwise to a solution of methyl acrylate (20.6 g, 240 mmol) in methanol, under a bubbling stream of nitrogen and magnetic stirring, over a period of 2 h. The final mixture was stirred for 30 min at 0 °C and then allowed to warm to room temperature and stirred for a further 24 h. The solvent was removed under reduced pressure at 40 °C using a rotary evaporator, and the resulting colorless oil was dried under vacuum at 50 °C overnight to give the final product (19.7 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 2.47 (t, J = 7.1 Hz, 4H), 2.59 (d, J = 5.2 Hz, 2H), 2.86 (t, J = 6.9 Hz, 4H), 3.35 (s, 6H), 3.67 (s, 6H), 4.38 (t, J = 5.2 Hz, 1H).

Synthesis of Acetal-Protected PAMAM Dendron 3 (*G* = 1). A solution of **2** (G = 0.5) (8.3 g, 30 mmol) in methanol was carefully added to a vigorously stirred solution of ethylenediamine (45 g, 750 mmol) in methanol at 0 °C. The rate of addition was such that the temperature did not rise above 40 °C. After complete addition, the mixture was stirred for 4 days at room temperature. The solvent was then removed under reduced pressure, maintaining the temperature no higher than 40 °C, using a rotary evaporator. The excess ethylenediamine was removed using an azeotropic mixture of toluene and methanol (9:1, v/v). The remaining toluene was removed by azeotropic distillation using methanol. Finally, the remaining methanol was removed under vacuum to give the amine terminated dendron **3** as a colorless oil (7.6 g, 76%). ^{1}H NMR (S2, 300 MHz, $CDCl_3$), δ: 1.46 (s, 4H), 2.36 (t, J = 5.7 Hz, 4H), 2.58 (d, J = 4.8 Hz, 2H), 2.83 (m, 8H), 3.30 (q, J = 5.7 Hz, 4H), 3.36 (s, 6H), 4.44 (t, J = 4.8 Hz, 1H), 7.32 (b, 2H).

Synthesis of Acetal-Protected PAMAM Dendron 4 (*G* = 1.5). A solution of **3** (G = 1) (7.5 g, 22.5 mmol) in methanol was added dropwise to a solution of methyl acrylate (10.32 g, 120 mol) in methanol, under bubbling a stream of nitrogen and magnetic stirring, over a period of 1 h. The final mixture was stirred for 1 h at 0 °C and then allowed to warm to room temperature and stirred for a further 24 h. The solvent was removed under reduced pressure at 40 °C using a rotary evaporator. The resulting colorless oil was dried under vacuum at 50 °C overnight to yield the final product (14.8 g, 97%). ^{1}H NMR (S3, 300 MHz, $CDCl_3$), δ: 2.36 (m, J = 6.3 Hz, 4H), 2.44 (t, J = 6.9 Hz, 8H), 2.55 (t, J = 6.0 Hz, 4H), 2.63 (t, J = 5.1 Hz, 2H), 2.77 (t, J = 6.6 Hz, 8H), 2.86 (t, J = 6.3 Hz, 4H), 3.29 (q, J = 5.1 Hz, 4H), 3.36 (s, 6H), 3.68 (s, 12H), 4.44 (t, J = 5.1 Hz, 1H), 7.15 (b, 2H).

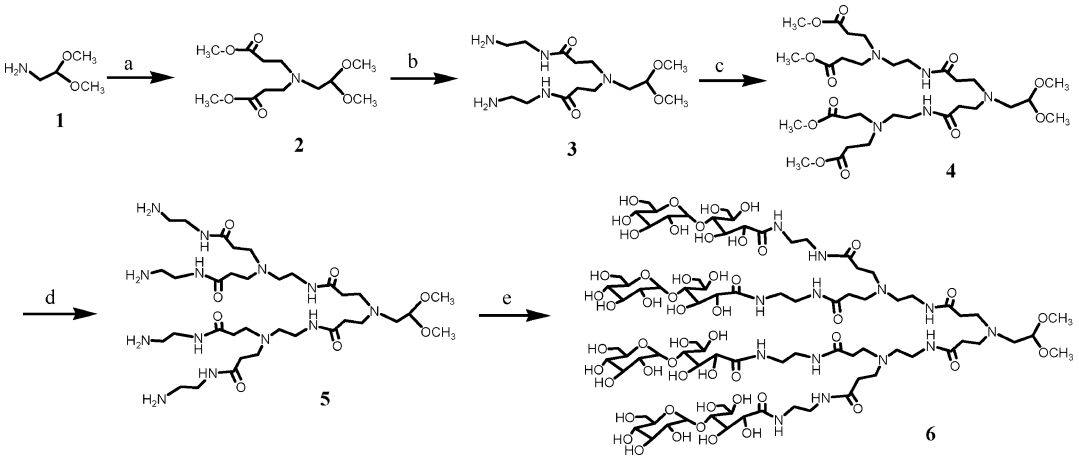
Synthesis of Acetal-Protected PAMAM Dendron 5 (*G* = 2). A solution of **4** (G = 1.5) (13.9 g, 20 mmol) in methanol was added carefully to a vigorously stirred solution of ethylenediamine (150 g, 2.5 mol) in methanol at 0 °C. The rate of addition was such that the temperature did not rise above 40 °C. After the mixture was stirred for 4 days at room temperature, the solvent was removed under reduced pressure, maintaining the temperature no higher than 40 °C, using a rotary evaporator. The excess ethylenediamine was removed using an azeotropic mixture of toluene and methanol (9:1, v/v). The remaining toluene was removed by azeotropic distillation using methanol. Finally, the remaining methanol was removed under vacuum to give the tetraamine terminated dendron **7** as a yellowish oil (15.6 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.88 (s, 8H), 2.36 (t, J = 5.7 Hz, 12H), 2.52 (t, J = 5.7 Hz, 4H), 2.58 (d, J = 5.1 Hz, 2H), 2.73 (t, J = 5.7 Hz, 8H), 2.82 (t, J = 6.0 Hz, 12H), 3.27 (m, 12H), 3.35 (s, 6H), 4.44 (t, J = 5.1 Hz, 1H), 7.62 (t, J = 5.4 Hz, 4H), 7.99 (t, J = 5.7 Hz, 2H). MALDI-MS calculated for $C_{34}H_{71}N_{13}O_8$ $[M + Na]^{1+}$ m/z = 813.01, found 813.19.

Synthesis of Maltose Dendron 6 (Maltose Tetramer, M4). A solution of PAMAM dendron (G = 2, **5**) (158 mg, 0.2 mmol) in methanol was added to a solution of maltonolactone (544 mg, 1.6 mmol) in DMSO under magnetic stirring at 60 °C for 24 h. The mixture was then cooled to room temperature and precipitated by ethyl ether. The raw product was obtained by centrifugation and dialyzed against water with poly(carbonate) membrane of Mw cutoff 1000, followed by lyophilizing to give the acetal-protected maltose tetramer (**6**) (212 mg, 49%). ^{1}H NMR (300 MHz, D_2O/DMSO 2:5, v/v), δ: 2.20−2.38 (b, 12H), 2.40−2.60 (overlap with DMSO, 6H), 2.70−2.90 (b, 12H), 3.00−4.06 (m, 74H), 4.42 (b, 1H), 4.84 (s, 4H). MALDI-MS calculated for $C_{82}H_{151}N_{13}O_{52}$ $[M + Na]^{1+}$ m/z = 2175.15, found 2176.22.

Synthesis of PVAm Glycopolymer 8 (PVAm-M4). Acetal-protected maltose dendron **6** (M4) was deprotected in situ by hydrolysis for the conjugation with the amine groups on the PVAm backbone by the reductive amination according to the literature method. First, **6** (84 mg, 0.04 mmol) was dissolved in water in a 25 mL flask at room temperature, and the pH value was adjusted to 1 by adding a small amount of TFA. The flask was put in a 55−60 °C water bath and kept stirring for 1 h. A solution of PVAm (4.3 mg, 0.1 mmol of NH_2) in MilliQ water was then added, and the resulting solution was cooled to room temperature. The pH was carefully adjusted to 5−6 with 50 mM $NaHCO_3$ aqueous solution. A solution of $NaCNBH_3$ (25 mg, 0.4 mmol) in water was added. The mixture was kept stirring at room temperature for another 24 h. The reaction solution was dialyzed against water using cellulose membranes with Mw cutoff 3500. The solid final product was obtained by lyophilizing (48 mg, 37%). ^{1}H NMR (300 MHz, D_2O), δ: 1.5−2.1 (−C***H***$_2$− of PVAm backbone), 3.0−4.4 (−C***H***− of all maltose C***H***'s and C***H***$_2$'s except the ones at the glycosidic linkage), 5.15 (−C***H***− at the glycosidic linkages).

Synthesis of Dendritic Surfactant Polymers 9 (PVAm-M4/Hex). Dendritic surfactant polymers PVAm-M4/Hex (**9**) were synthesized via a two-step method by sequential addition of maltose dendron and hexanal to react with the amine groups on PVAm backbone. In a typical example, the first step is the same as the synthesis of PVAm-M4 (**8**). M4 (**6**) (84 mg, 0.04 mmol) was dissolved in water in a 25 mL flask at room temperature, and the pH value was adjusted to 1 by adding a small amount of TFA. The flask was put in a 55−60 °C water bath and kept stirring for 1 h. A solution of PVAm (4.3 mg, 0.1 mmol of NH_2) in MilliQ water was then added, and the resulting solution was cooled to room temperature. The pH was carefully adjusted to 5−6 with 50 mM $NaHCO_3$ aqueous solution. A solution of $NaCNBH_3$ (25 mg, 0.4 mmol) in water was added, and the mixture was kept stirring at room temperature for 24 h. For the second step, a solution of hexanal (6.0 mg, 0.06 mmol) in ethanol was added, and the reaction was carried out at room temperature for another 24 h. Finally, the reaction solution was dialyzed against water using cellulose membranes with Mw cutoff

Scheme 1. Synthesis of Acetal-Protected Maltose Dendrons **6** (M4)[a]



[a] (a) $CH_2{=}CH_2COOCH_3$, MeOH, room temperature, 24 h; (b) $H_2NCH_2CH_2NH_2$, MeOH, room temperature, 4 days; (c) $CH_2{=}CH_2COOCH_3$, MeOH, room temperature, 24 h; (d) $H_2NCH_2CH_2NH_2$, MeOH, room temperature, 4 days; (e) maltose lactone, DMSO, 60 °C, 24 h.

3500. The solid final product was obtained by lyophilizing (43 mg, 46%). ^{1}H NMR (300 MHz, D_2O/DMSO 2:5, v/v), δ: 0.85 (−C***H***$_3$ of hexyl groups, C***H***$_3$(CH$_2$)$_4$CH$_2$NH−), 1.10 (−(C***H***$_2$)$_4$− of hexyl groups, CH$_3$(C***H***$_2$)$_4$CH$_2$NH−), 2.2−2.4 (−C***H***$_2$–CONH− of PAMAM dendron), 2.6−2.8 (−C***H***$_2$–N− of PAMAM dendron), 3.0−4.0 (−CONH−C***H***$_2$− of PAMAM dendron, −C***H***− of all maltose C***H***'s and C***H***$_2$'s except the ones at the glycosidic linkage), 4.88 (−C***H***− at the glycosidic linkages).

Adsorption of Dendritic Surfactant Polymers on OTS-Coverslips. Octadecyltrichlorosilane (OTS)-coated glass coverslips were used as a hydrophobic substrate for surfactant polymer adsorption. Each surfactant was first dissolved in water with the concentration of 0.5 mg/mL, and the OTS-coverslips were submerged in the solution for 24 h. The solution was removed, and the coverslips were washed with water three times and air-dried at room temperature overnight prior to measurement of water contact angle.

Surface-Induced Self-Assembly Studied by AFM. The surface-induced assembly of surfactant polymers at a solid surface/water interface was studied by atomic force microscopy (AFM). AFM imaging of surfactants on highly oriented pyrolytic graphite (HOPG) under aqueous medium was performed using a Nanoscope III Multimode AFM (Digital Instruments, Santa Barbara, CA) equipped with a glass fluid cell attachment. Silicon nitride (Si_3N_4) cantilevers (Nanoprobes, Digital Instruments) having integrated Si_3N_4 tips and a nominal spring constant of 0.58 N/m were used. A freshly cleaved HOPG substrate was first imaged in water as a reference. Surfactant solution (0.1 mg/mL) was flowed into the fluid cell without withdrawing the AFM probe. Images were obtained using tapping mode with scan rate of 1−5 Hz, typically applying tip forces of 3−5 nN.

Platelet Adhesion. Static platelet adhesion and activation tests were performed on OTS-coverslips (ϕ 12 mm) and dendritic surfactant polymers-coated OTS-coverslips. Whole blood (9 mL) was drawn from healthy, medication-free human donors into a 10-mL tube containing 1 mL of 3.8% sodium citrate anticoagulant, centrifuged at 800 rpm for 15 min to collect platelet-rich plasma (PRP). The platelet density was about $3.6 \times 10^5/\mu$L determined by Coulter A^c.T diff (Beckman Coulter, Schaumburg, IL). The test coverslips were put in a 24-well culture plate and hydrated by adding 0.5 mL of PBS for 15 min, and then were incubated with 0.5 mL of diluted PRP with about 3.0×10^4 platelets/well for 1 h. The PRP suspension was removed, and the coverslips were gently washed with PBS. Adherent platelets were fixed by adding 0.5 mL of 1% PFA in PBS and incubating at room temperature for 1 h, followed by washing with PBS. The fixed platelets were stained by addition of 8 μL of fluorescein isothiocyanate-tagged anti-CD41a (FITC-anti-CD41a) in 0.5 mL of staining media (2% fetal bovine serum and 0.01% $CaCl_2$ in PBS), followed by 1 h of incubation in the dark. The coverslips were rinsed with PBS, mounted on a coverslide face-up using Crystal Mount. The images were collected with a Spot RT chilled CCD camera and analyzed using MetaMorph software (Universal Imaging Corp.) on a Nikon Diaphot epifluorescence inverted microscope. All coverslips were run in triplicate, and multiple fields were examined for each coverslip.

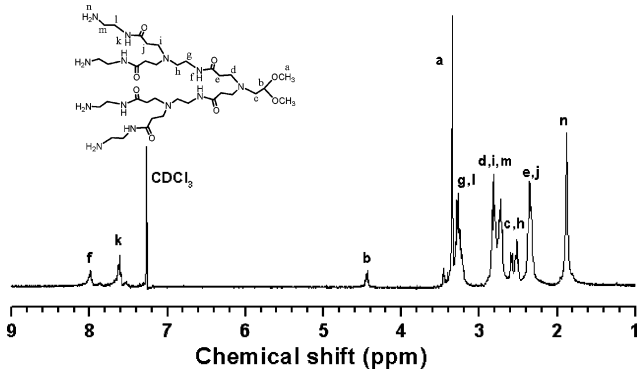


Figure 1. ^{1}H NMR spectrum of acetal-protected PAMAM dendron **5** ($G = 2$) in $CDCl_3$.

Results and Discussion

Synthesis of Acetal-Protected PAMAM Dendron and Saccharide Dendron. To create the glycodendritic structure for the surfactant polymers, a new strategy was used to synthesize an acetal-protected poly(amidoamine) (PAMAM) dendron and saccharide dendron, as shown in Scheme 1. Aminoacetaldehyde dimethyl acetal (**1**) was used as the starting material to provide a monovalent focal structure with dimethyl acetal-protected aldehyde functionality. The acetal-protected poly(amidoamine) (PAMAM) dendron **5** ($G = 2$) was built up according to the literature method,[21] using reiterative cycles of Michael addition with methyl acrylate to give ester-terminated half generations **2** ($G = 0.5$) and **4** ($G = 1.5$) and amidation reaction with ethylenediamine to give amine-terminated full generations **3** ($G = 1$) and **5** ($G = 2$). From the NMR spectrum of **5**, as shown in Figure 1, the corresponding proton signals of the methyloxyl, acetal, amine groups, amide groups, and various methylene groups were clearly observed, with their integration matching the structure of **5** very well. The structure of **5** was also confirmed by MALDI-MS with a $[M + Na]^+$ peak at $m/z = 813.19$ (calculated mass 813.00).

Maltose was chosen as a model to synthesize the saccharide dendron, although this method is applicable to a wide range of

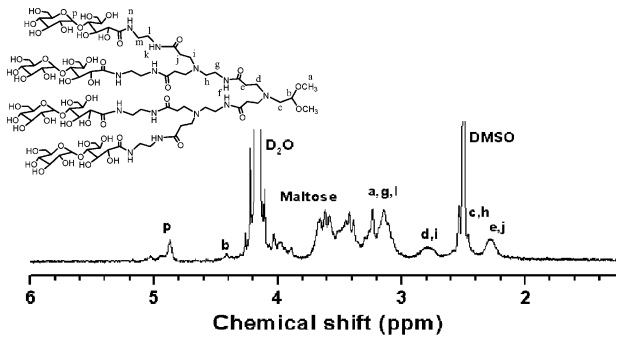
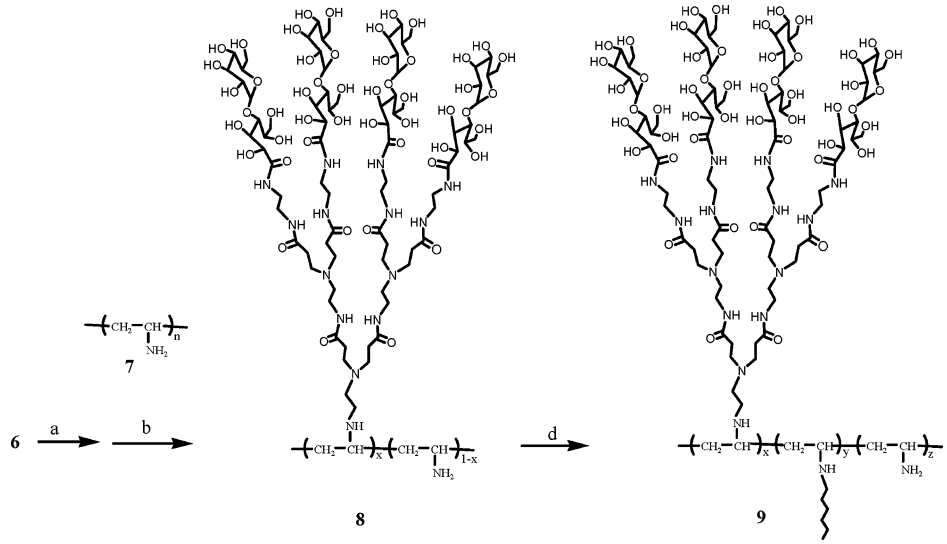


Figure 2. ^{1}H NMR spectrum of acetal-protected maltose dendron **6** (M4) in D_2O/DMSO (2:5, v/v).

saccharides. To produce the functionality to react with amine groups, maltose was oxidized by iodine to give maltonolactone according to the literature,[22] which was then used in excess to react with the peripheral amine groups of **5** to give maltose dendron **6** (maltose tetramern, M4). The structure of **6** was confirmed by MALDI-MS with a $[M + Na]^+$ peak at m/z = 2176.22 (calculated mass 2174.15). From the NMR spectrum of **6**, as shown in Figure 2, the corresponding proton signals from maltose and PAMAM dendron were clearly observed. The results show that the acetal-saccharide dendron M4 was well constructed.

Synthesis of Dendritic Surfactant Polymers. Poly(vinylamine) (PVAm) was used as the backbone polymer and was prepared by free radical polymerization of *N*-vinylfromamide according to the literature method.[19] The molecular weight (Mn 1.63×10^4, Mw/Mn 1.34) was determined by gel permeation chromatography (GPC) with a cationic column and a laser scattering detector. Dendritic surfactant polymers were synthesized via a two-step method[23] by the sequential addition of M4 (**6**) and followed by hexanal (molar feed ratio of M4/hexanal 2:3) to react with the amine groups on the PVAm backbone, as shown in Scheme 2. Acetal-protected M4 was first hydrolyzed with 1% aqueous trifluoroacetic acid (TFA) at 55 °C for 1 h, to provide the fully deprotected aldehyde-fuctionalized maltose dendron in situ,[24] which was ready to react with the amine groups on PVAm (**7**) to give glycopolymers **8** (PVAm-M4) via reductive amination using sodium cyanoborohydride ($NaCNBH_3$). The grafting percentage of M4 on PVAm in the first step was 12.5 mol %, as calculated from the purified products of **8** by comparing the NMR peaks between glycosidic protons

Scheme 2. Synthesis of Dendritic Surfactant Polymers **9** (PVAm-M4/Hex)[a]



[a] (a) 1% TFA (v/v), 55 °C, 1 h; (b) PVAm (**7**), $NaHCO_3$, $NaCNBH_3$, pH 6.0, room temperature, 24 h; (c) hexanal, ethanol, room temperature, 24 h.

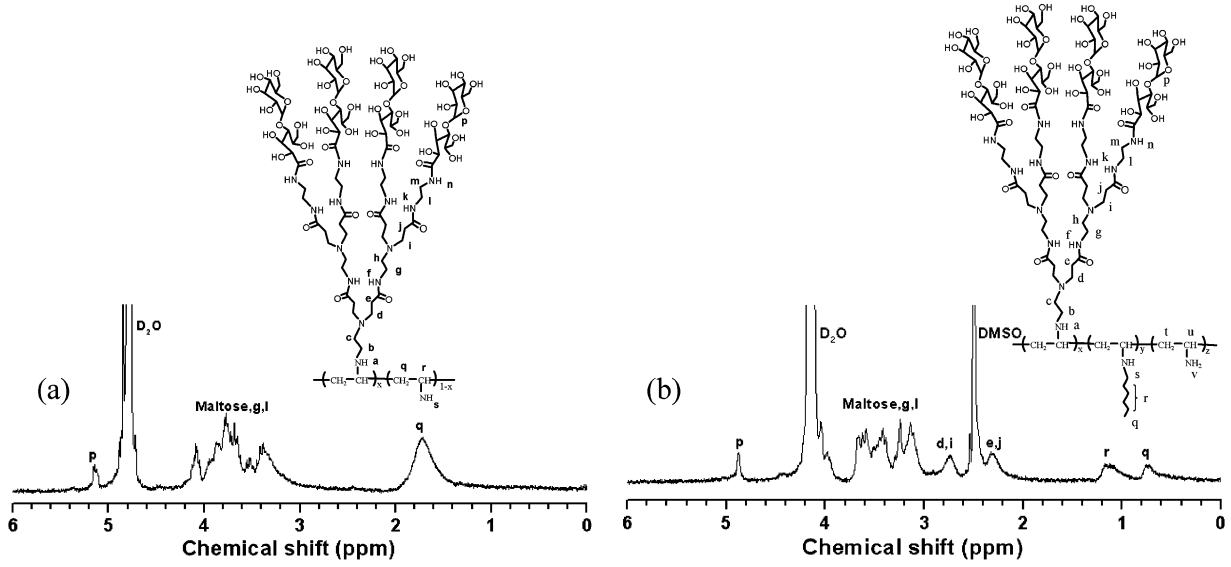


Figure 3. ^{1}H NMR spectrum of (a) PVAm-M4 and (b) PVAm-M4/Hex in D_2O/DMSO-d_6 (2:5, v/v).

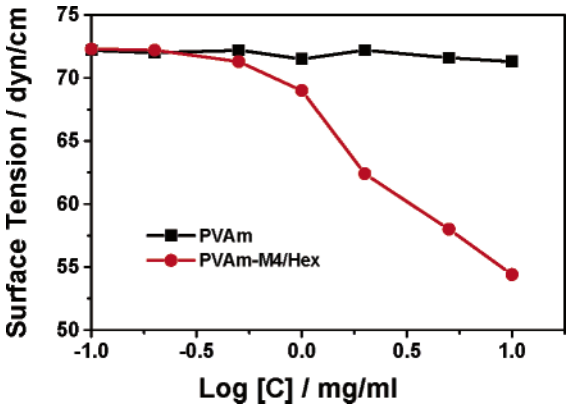


Figure 4. Surface tension of branched maltose surfactant polymers in aqueous solutions.

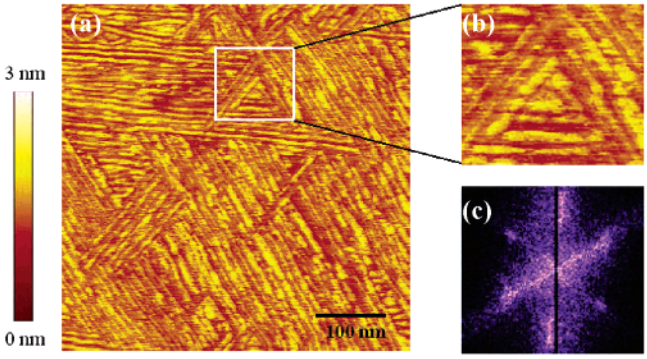


Figure 5. Tapping-mode AFM images acquired in situ of the self-assembly of dendritic surfactant polymers. (a) Adsorption of PVAm-M4/Hex in water on HOPG at room temperature after 2 h (image size: 0.5 μm × 1 μm); (b) the zoom-in AFM image; (c) the hexagonal pattern in the two-dimensional Fourier transform.

(5.1−5.2 ppm from M4) and methylene protons (1.4−2.0 ppm from PVAm) (Figure 3a). In the second step, hexanal was reacted with the remaining amine groups to generate the dendritic surfactant polymer **9** (PVAm-M4/Hex). Purification was performed by exhaustive dialysis against water with membranes of Mw cutoff 3500. The ^{1}HNMR spectrum of purified **9** (Figure 3b) shows proton peaks derived from M4 and Hex groups, confirming the expected composition for the dendritic surfactant polymers. The Hex grafting percentage in the final polymers was 50.2 mol %, as calculated from the second step by comparing the NMR peaks between glycosidic protons (from M4) and methyl protons (0.7−0.9 ppm from Hex). Thus, the molar ratio of M4/Hex on the PVAm backbone was close to 1:4.

Surface-Active Properties at the Air/Water Interface. Surface-active properties of the surfactant polymers at the air/water interface were determined from surface tension measurements using the Du Nouy ring method, as shown in Figure 4. PVAm showed negligible surface activity, as indicated by no decrease in surface tension with increasing PVAm concentration. In contrast, PVAm-M4/Hex exhibits significant surface-active behavior, as indicated by a substantial decrease in surface tension with increasing surfactant concentration. The lowest surface tension achieved within the measured concentration range is about 54 dyn/cm, a decrease of 18 dyn/cm as compared to pure water. No critical micelle phenomenon was observed, which is consistent with previous studies on comblike surfactant polymers.[19]

Surface-Active Properties at the Solid/Water Interface. Surface-active properties of the surfactant polymers at the solid/water interface were measured using contact-angle goniometry. In the presence of a hydrophobic solid surface under water, surfactants can minimize interfacial energy by adsorbing at the solid/water interface. To test this, PVAm-M4/Hex was adsorbed on a model hydrophobic substrate consisting of an octadecyltrichlorosilane (OTS) self-assemble monolayer deposited on glass coverslips. Significant reduction in the water contact angle was observed with the average receding contact angle 48° for PVAm-M4/Hex, as compared to the contact angle 105° for OTS-coverslips.

A nanoscale understanding of surface-induced self-assembly of the dendritic surfactant polymer on highly oriented pyrolytic graphite (HOPG) was gained using an atomic force microscopy (AFM) operated in fluid tapping mode. Freshly cleaved HOPG is atomically flat and highly hydrophobic and is a good model substrate for assisting the molecular self-assembly.[25] The AFM image for self-assembling PVAm-M4/Hex on HOPG in water was obtained in situ. After 2 h of adsorption, a lateral ordering of adsorbing surfactant polymer was visualized, as shown in Figure 5a. There was an adsorption pattern of the surfactant polymer in strands 60° out of alignment (Figure 5b). This ordering was verified by the two-dimensional Fourier transform of the image, showing an epitaxial growth pattern with hexagonal angular dependence (Figure 5c). This pattern is consistent with a mechanism by which hexyl side chains adsorb in registry with the graphite atoms, constrains the polymer backbone to the surface, and orients the maltose dendron side chains away from the surface.[15] Complete monolayer coverage of the HOPG substrate was observed within the 24 h adsorption period after. The thickness of the adsorbed surfactant polymers is about 1 nm, which indicates a single monolayer on the surface. These results infer that the dendritic surfactant polymers are effective in creating a glycocalyx-like hydrated layer on the hydrophobic surface through hydrophobic interaction.

Platelet Activation and Adhesion. To examine the static platelet adhesion and activation, tests were performed on OTS

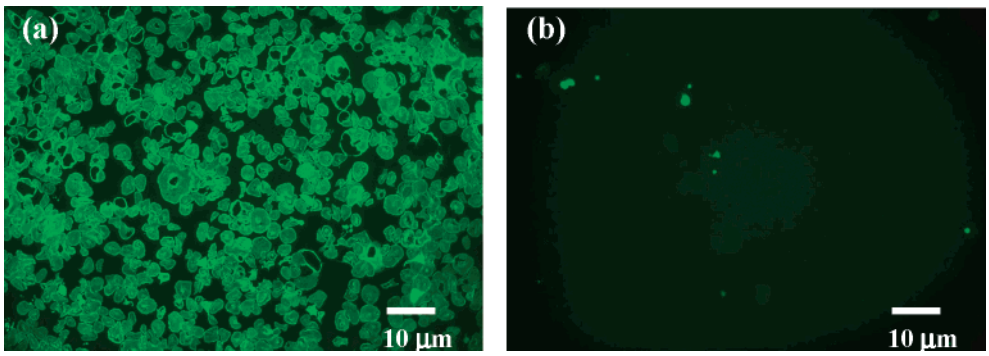


Figure 6. Platelet adhesion on (a) OTS-coated glass coverslip, and (b) dendritic surfactant polymers-coated OTS-glass coverslip.

and surfactant polymer surfaces by using platelet-rich plasma (PRP), and fluorescence microscopy was used to observe and evaluate the platelet adhesion.[26] Adherent platelets were stained with fluorescein isothiocyanate-tagged anti-CD41a (FITC-anti-CD41a), a monoclonal antibody that specially binds the integrin receptor GPIIb/IIIa on the activated platelet surface. From the analysis of the samples, significant differences were observed between the uncoated and surfactant-coated OTS surfaces, as shown in Figure 6. Many platelets and platelet aggregates were observed on the OTS surface (Figure 6a), indicating an activating surface. The PVAm-M4/Hex surface exhibited >90% less platelet adhesion (Figure 6b) than the OTS surface as determined from the relative fluorescence intensity. The adherent platelets tended to be smaller and less activated on the surfactant polymer surface than on the OTS-surface. These results indicate that the dendritic surfactant polymers can provide a sufficient depth of hydrated saccharide layer on the hydrophobic surface as a barrier to suppress platelet adhesion.

Conclusions

We present the first example of dendritic surfactant polymers as antifouling interface materials designed to reduce platelet adhesion. For the synthesis, acetal functionality was introduced conveniently and efficiently to the focal point of maltose dendron by conjugation of acetal-protected PAMAM dendron ($G = 2$) with maltonolactone. The dendritic surfactant polymers were synthesized via a two-step method by sequential addition of maltose dendron and hexanal to react with the amine groups on the PVAm backbone. The results show that the hexyl side chains facilitate adsorption of the surfactant polymers onto hydrophobic substrates, while the maltose dendron side chains provide a dense canopy of protective glycocalyx-like layer as an antifouling interface that suppresses platelet adhesion.

Acknowledgment. This work was supported by the National Institutes of Health (Grant EB002067), and we thank the facilities provided by the Center for Cardiovascular Biomaterials.

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BM050611P