Protein Resistance of PEG-Functionalized Dendronized Surfaces: Effect of PEG Molecular Weight and Dendron Generation

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ABSTRACT: Dendronized surfaces were prepared by chemisorption of poly(ethylene glycol) monothiol (HS-PEG_{650}-OH) onto gold-coated silicon wafers followed by functionalization of the PEG terminal OH group with aliphatic polyester dendrons, generation 1−4, using divergent dendron growth. PEG monomethyl ether (PEG-OMe) chains of various molecular weight (MW) were covalently attached to the peripheral hydroxyl groups of the dendronized surfaces via EDC coupling and investigated for protein adsorption. Protein adsorption studies were carried out using fibrinogen (Fg) and lysozyme (Lys) as model proteins from phosphate buffered saline (PBS) (Fg, Lys) and plasma (Fg). In the first part of this study, the effect of functionalization of the peripheral hydroxyl groups with PEG-OMe oligomers (M_n = 350 Da) on protein adsorption was investigated. Results showed that adsorption of both Fg and Lys was reduced when dendronized surfaces were grafted with PEG-OMe oligomers. To investigate the effect of molecular weight on protein adsorption, PEG-OMe chains of greater length (750, 2000, and 5000 Da) were coupled to first generation dendronized surfaces (Au-G1(OH)). Results showed that protein adsorption decreased with increasing PEG-OMe MW up to 2000 Da. To further investigate the effect of dendron generation on protein resistance, dendronized surfaces of generation 1−4 were coupled with PEG_{2000}-acid. Subsequent protein studies showed a decrease in Fg and Lys adsorption with increasing dendron generation.

Introduction

Development of surfaces with antifouling properties is of significant interest in biomedical applications, where biocompatibility of materials continues to be the primary issue.[1−8] Nonspecific protein adsorption, known as the first event that follows implantation of biomaterials, is very important in initiating further events that determine host responses, such as blood coagulation, thrombus formation, and bacterial infection.[9,10] In the past two decades a number of studies investigating low-fouling surfaces have been reported in the literature; however, only few materials exhibit a significant reduction in nonspecific protein adsorption sufficient to prevent such adverse events as platelet adhesion.[6,9,11−15] A number of experimental studies showed that surfaces displaying poly(ethylene glycol) (PEG) are known for their resistance to protein adsorption and cell adhesion.[5−10] It is believed that the effectiveness of PEG for repelling proteins is related to its highly hydrated nature under physiological conditions and formation of a steric barrier by the PEG chains.[16−18] Several theories have been reported in the literature on the mechanism of protein resistance of PEG-functionalized surfaces, but a fundamental understanding of protein resistance at the molecular level has yet to be achieved.[19−26] From the various literature-reported protein-repelling surfaces modified with short or long segments of PEG, a number of criteria responsible for protein repulsion have been identified. Several of these reports attribute protein repulsion to the high dynamics of the PEG chains in water[27] and molecular conformation of the segments at the interface.[28,29] Other criteria for enhanced protein resistance were reported by Whitesides and co-workers and included high hydrophilicity, the presence of hydrogen-bond acceptors, and the absence of hydrogen-bond donors.[30] However, other studies have demonstrated that good protein resistance can be achieved with molecules comprising a large number of hydrogen-bond donors, such as hydroxyl groups and carboxylic acid groups.[31,32]

When analyzing the reported structural properties of protein-resistant surfaces as well as previous results by Haag and co-workers[33] and Zhu and co-workers,[34] it became apparent that the presence of highly flexible and hydrophilic groups, combined with a highly branched architecture, can lead to good protein resistance. On the basis of these findings, we have prepared a series of gold surfaces coated with hydrophilic aliphatic polyester dendrons, in an attempt to study their protein resistance properties.[35] By analogy to previous results reported on branched architectures,[36] the aim of our study was to covalently functionalize PEG-grafted surfaces with aliphatic polyester dendrons to improve the overall surface coverage and surface hydrophilicity, which would potentially lead to enhanced protein resistance based on previous observations.[33,34,36] As expected, the hydrophilicity of the resulting surfaces increased with increasing dendron generation as a result of the numerous hydroxyl groups at the periphery. However, contrary to our expectation, protein resistance was found to decrease when the surfaces were covalently functionalized with the hydrophilic dendrons.[35] It was postulated that several factors could be responsible for the increased protein adsorption to the dendronized surfaces, including increased surface area, the introduction of hydrogen-bond donor groups,[14,30] and a decrease in the mobility of the surface-grafted polymers as a result of inter- and intramolecular hydrogen bonding between the numerous hydroxyl groups at the dendron periphery. To circumvent these confounding phenomena, while maintaining surface hydrophilicity, we have chosen to covalently attach PEG-OMe chains of various molecular weight to the peripheral hydroxyl groups of first to fourth generation dendronized surfaces. Here we report the synthesis and characterization of the resulting surfaces, functionalized with PEG-OMe star polymers having 2, 4, 8, and 16 arms and PEG-OMe molecular weight of 350, 750, 2000, and 5000 Da. We present the results of protein adsorption studies to these surfaces using fibrinogen and lysozyme.

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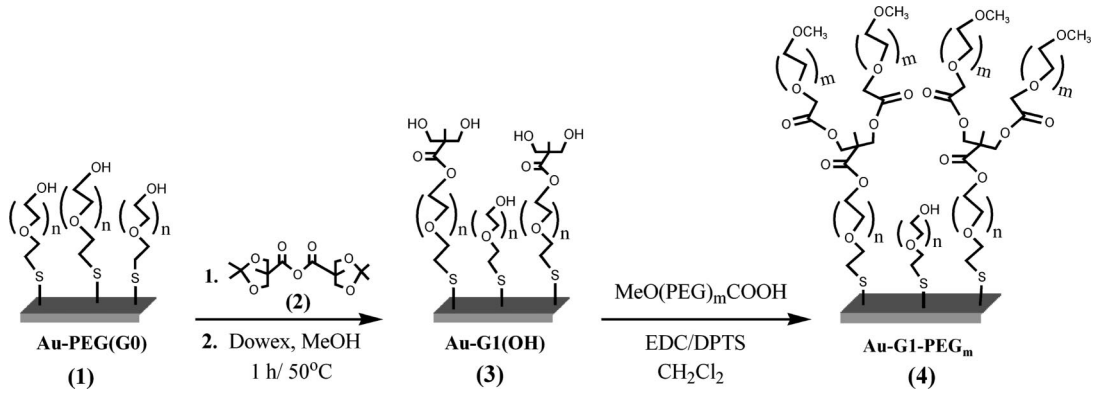
Scheme 1. EDC Coupling of PEG-acid to a G1(OH) Dendronized Surface



Experimental Section

General. DOWEX50W-X2 ion-exchange resin, 4-(dimethylamino)pyridine (DMAP, 99%), 2,2-bis(hydroxymethyl)propionic acid (bis-MPA, 98%), 2,2-dimethoxypropane (98%), *p*-toluenesulfonic acid monohydrate (TsOH, 98%), TEMPO free radical (98%), iodobenzene diacetate (98%), and poly(ethylene glycol) methyl ether (M_n = 350 and 750 Da) were purchased from Aldrich. 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC, 98+%) was purchased from Alfa Aesar. Poly(ethylene glycol) methyl ether (M_n = 2000 and 5000 Da) were purchased from Fluka. Thiol-terminated poly(ethylene glycol) (HS-PEG_{650}-OH) was purchased from Polymer Source, Inc. Dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), hexanes, methanol (MeOH), diethyl ether, and ethanol (EtOH) were purchased from Caledon. All reagents were used as received. Acetonide-protected anhydride of bisMPA (**2**)[37] and of 4-(dimethylamino)pyridine/*p*-toluenesulfonic acid (DPTS)[38] were prepared according to literature procedures.

Gold substrates prepared by electron beam evaporation of silicon wafers with an adhesive titanium tungstate (TiW) layer (300 Å) followed by a layer of gold (1000 Å) were purchased from Thin Film Technology, Buellton, CA, and diced into 1.0 × 1.0 cm^2 pieces. Prior to chemisorption, the surfaces were cleaned, using a literature procedure by immersing them in chromosulfuric acid (H_2SO_4 >92%, CrO_3 >1.3%) for 1 h to remove any organic contaminants, followed by ultrasonication in both Milli-Q water and ethanol for 10 min and extensive rinsing with Milli-Q water.[39] All reactions on surfaces were carried out by placing reaction flasks on a VWR S-500 orbital shaker at a medium speed setting, since no stirring was possible.

Characterization. NMR spectra were measured on Avance 600 MHz spectrometers. ^1H spectra were recorded at 600 MHz, and ^{13}C NMR spectra were recorded at 150 MHz in $CDCl_3$ or methanol-d_4. The nondeuterated solvent signal was used as the internal standard for both ^1H and ^{13}C spectra. Sessile-drop water contact angles were determined using water drops with a 1−2 μL volume. Advancing and receding angles were obtained using a Ramé-Hart NRL 100-00 goniometer (Mountain Lakes, NJ). Atomic force microscopy (AFM) analyses were carried out using a Digital Instruments NanoScope IIIa Multimode AFM equipped with a vertical engage "E" scanner with 15 μm full range scan. The images were recorded with standard tips in tapping mode at a scan rate of 0.5 Hz.

Proteins. Fibrinogen was purchased from Calbiochem (La Jolla, CA), dialyzed against Tris buffer, pH 7.4, aliquoted, and stored at −70 °C. The molecular weight and dimensions of fibrinogen are 3.4×10^5 Da and $450 \times 90 \times 90$ $Å^3$, respectively, and its isoelectric point is 5.5. Lysozyme was obtained from Calbiochem (La Jolla, CA) and used as received. The molecular weight and dimensions of lysozyme are 1.43×10^4 Da and $45 \times 30 \times 30$ $Å^3$, respectively, and its isoelectric point is 11.0.

General Procedures. *General Procedure for Surface Dendronization.* First generation aliphatic polyester dendrons (G1(Ac)) were covalently attached to the terminal hydroxyl groups of PEG_{650}-functionalized gold surfaces via an esterification reaction. In this reaction, excess acetonide anhydride (**2**) (0.1 g, 3.03×10^{-4} mol; excess/cm^2 of (**2**):PEG-OH = 5×10^4:1) was added along with a catalytic amount of DMAP (10 mg, 8.18×10^{-5} mol) in a mixture of CH_2Cl_2:pyridine (3:2 v/v, 5 mL). This step was followed by removal of the acetonide protecting groups to give the hydroxyl-terminated first generation dendrons. The deprotection step was carried out following a literature procedure, using the acidic resin DOWEX 50W-X2 in methanol at 50 °C for 1 h.[39] Subsequent esterification and deprotection reactions allowed surface dendronization up to the fourth generation as described previously.[35]

General Procedure for Oxidation of Poly(ethylene glycol) Monomethyl Ether (PEG-OMe)[40]. Poly(ethylene glycol) monomethyl ether (PEG-OMe) (M_n = 2000 Da) (3.01 g, 1.51×10^{-3} mol) was introduced to a round-bottom flask equipped with a

Table 1. Water Contact Angles of PEG-Grafted G1 Dendronized Surfaces: Effect of PEG Molecular Weight

surface	advancing contact angle (deg)	receding contact angle (deg)
bare Au	65 ± 3	40 ± 8
Au-PEG_{650}[a]	42 ± 4	36 ± 2
Au-G1(OH)	40 ± 2	22 ± 3
Au-G1-PEG_{350}	41 ± 2	20 ± 4
Au-G1-PEG_{750}	38 ± 3	19 ± 4
Au-G1-PEG_{2000}	30 ± 5	12 ± 2
Au-G1-PEG_{5000}	19 ± 2	12 ± 2

[a] 5 mM chemisorption solution of HS-PEG_{650}-OH in PBS at pH = 7.4.

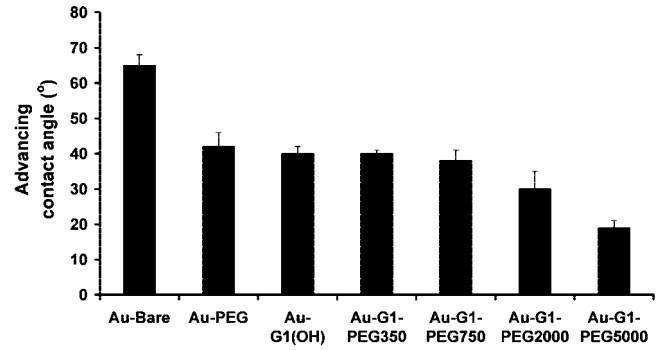


Figure 1. Advancing contact angles of Au, Au-PEG, Au-G1(OH), and Au-G1-$PEG_{350\text{-}5000}$ surfaces.

Table 2. Root-Mean-Square (rms) Roughness of PEG-Functionalized Dendronized Surfaces Obtained from AFM Data: Effect of PEG Molecular Weight

surface	rms (nm)	surface	rms (nm)
bare gold	1.26	Au-G1-PEG_{750}	4.97
Au-G1(OH)	3.27	Au-G1-PEG_{2000}	5.88
Au-G1-PEG_{350}	3.42	Au-G1-PEG_{5000}	6.14

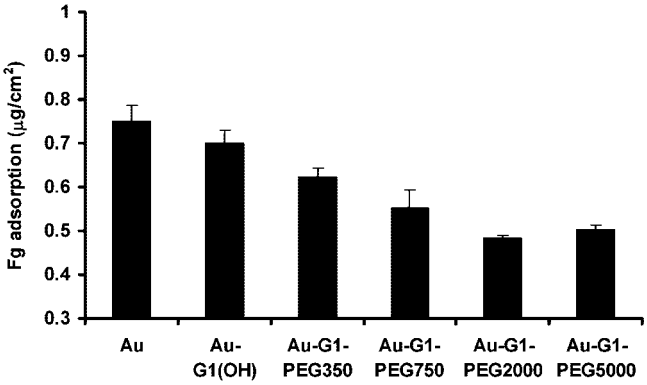


Figure 2. Fibrinogen adsorption from 1 mg/mL Fg−Fg−^{125}I in PBS-NaI on G1 dendronized surfaces functionalized with PEG chains of various molecular weight.

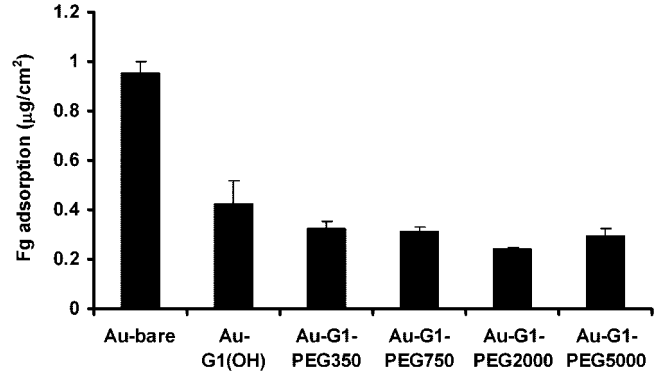


Figure 3. Fibrinogen adsorption from 1 mg/mL Fg−Fg−^{125}I in plasma on G1 dendronized surfaces functionalized with PEG chains of various molecular weight.

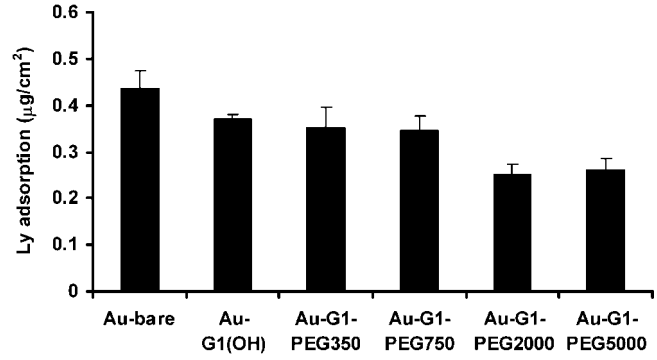


Figure 4. Lysozyme adsorption from 1 mg/mL Lys−Lys−^{125}I in PBS-NaI of G1 dendronized surfaces functionalized with PEG chains of various molecular weight.

magnetic stir bar as a solution in a 1:1 mixture of water/acetonitrile (5 mL). TEMPO free radical (0.047 g, 3.0×10^{-4} mol) was added to the solution, followed by iodobenzene diacetate (1.45 g, 4.5×10^{-3} mol). The reaction mixture was stirred at room temperature for 6 h (or overnight to ensure a complete reaction). The mixture was then concentrated to dryness in vacuo, and the residue was dissolved in a minimum amount of ethanol. The crude polymer was precipitated in cold diethyl ether and recovered by filtration through a glass fritted funnel. After drying in a vacuum oven overnight, PEG_{2000}-acid was obtained as a white powder (2.9 g, 97%). ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 3.36 (s, 3H, $-CH_3O$), 3.50−3.55 (m, 2H, $-CH_2OCH_3$), 3.55−3.69 (s-broad, 148H, $-CH_2CH_2O$), 3.69−3.72 (m, 2H, $-CH_2O$), 4.13 (s, 2H, $-CH_2CO_2H$). ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 59.1 ($-CH_3O$), 69.15 ($-CH_2OCH_3$), 71.37 ($CH_3O-CH_2CH_2O$), 71.58 ($-CH_2CH_2O$), 72.97 ($-CH_2CO_2H$), 174.01 ($-CO_2H$).

Oxidation of Poly(ethylene glycol)$_{350}$ Monomethyl Ether (MeO-PEG$_{350}$-OH). The oxidation was carried out, as described in the general procedures, using PEG-OMe (M_n = 350 Da) (3.06 g, 8.57×10^{-3} mol), TEMPO free radical (0.268 g, 1.71×10^{-3} mol), and iodobenzene diacetate (8.28 g, 2.57×10^{-2} mol) in a 1:1 mixture of water/acetonitrile (15 mL). The crude polymer was precipitated three times in cold diethyl ether and filtered to isolate the desired product as a white powder (2.7 g, 90%). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 3.35 (s, 3H, $-CH_3O$), 3.53 (m, 2H, $-CH_2OCH_3$), 3.59−3.65 (s-broad, 22H, $-CH_2CH_2O$), 3.72 (m, 2H, $-CH_2O$), 4.13 (s, 2H, $-CH_2CO_2H$). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 59.1 ($-CH_3O$), 69.01 ($-CH_2OCH_3$), 70.62 ($-CH_2$-CH_2O), 71.28 ($CH_3O-CH_2CH_2O$), 72.03 ($-CH_2CO_2H$), 172.72 ($-CO_2H$).

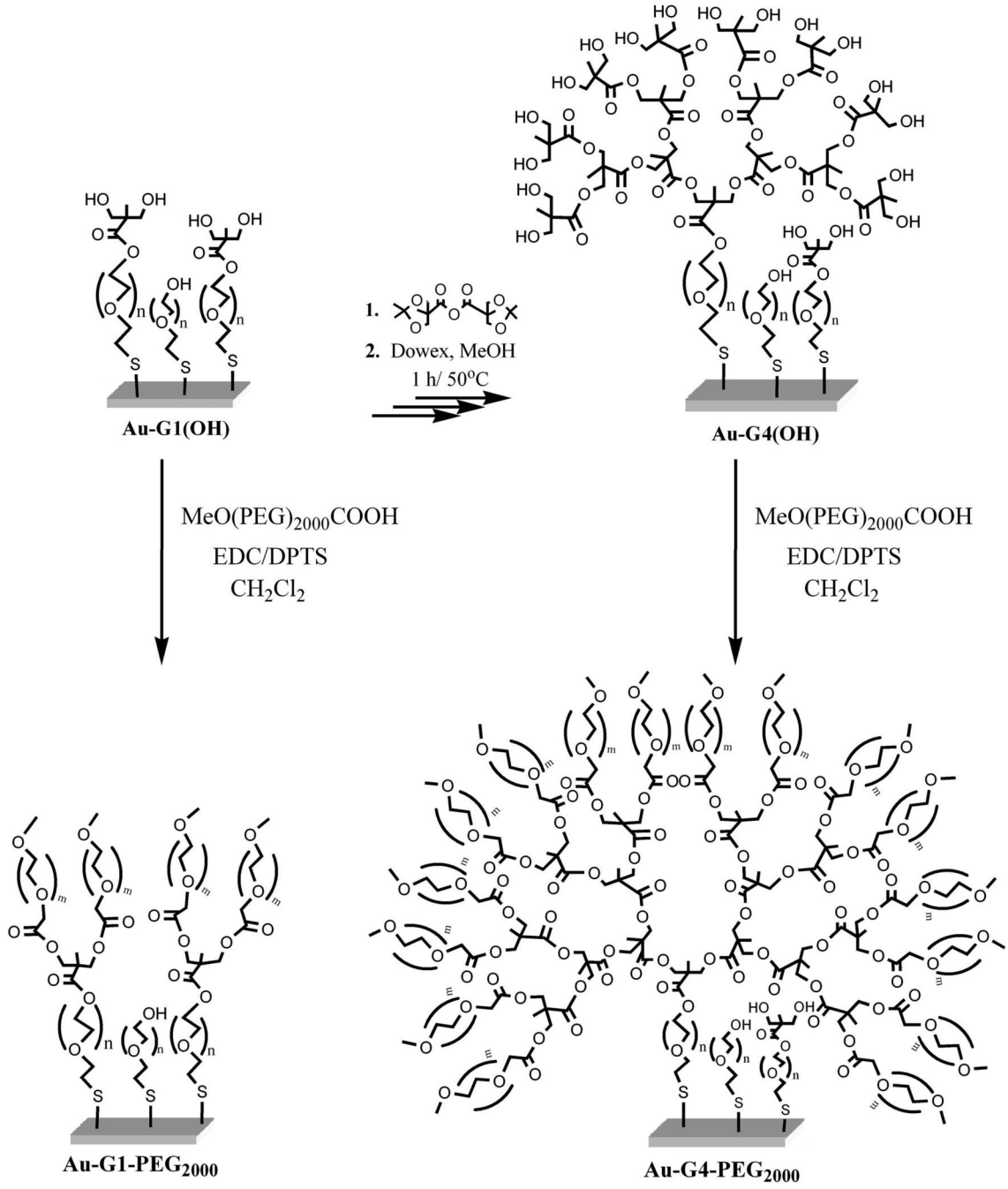
Oxidation of Poly(ethylene glycol)$_{750}$ Monomethyl Ether (MeO-PEG$_{750}$-OH). The oxidation was carried out, as described in the general procedures, using PEG-OMe (M_n = 750 Da) (3.02 g, 4.03×10^{-3} mol), TEMPO free radical (0.127 g, 8.13×10^{-4} mol), and iodobenzene diacetate (3.86 g, 1.19×10^{-2} mol) in a 1:1 mixture of water/acetonitrile (20 mL). The crude polymer was precipitated three times in cold ether and filtered through a fritted funnel to isolate the desired product as a white powder (2.84 g, 95%). ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 3.27 (s, 3H, $-CH_3O$), 3.48−3.52 (m, 2H, $-CH_2OCH_3$), 3.52−3.65 (s-broad, 72H, $-CH_2CH_2O$), 3.65−3.68 (m, 2H, $-CH_2O$), 4.08 (s, 2H, $-CH_2CO_2H$). ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 59.1 ($-CH_3O$), 69.26 ($-CH_2OCH_3$), 71.37 ($CH_3O-CH_2CH_2O$), 71.57 ($-CH_2CH_2O$), 72.98 ($-CH_2CO_2H$), 174.22 ($-CO_2H$).

Oxidation of Poly(ethylene glycol)$_{5000}$ Monomethyl Ether (MeO-PEG$_{5000}$-OH). The oxidation was carried out, as described in the general procedures, using PEG-OMe (M_n = 5000 Da) (3.08 g, 6.16×10^{-4} mol), TEMPO free radical (0.019 g, 1.22×10^{-4} mol), and iodobenzene diacetate (0.582 g, 1.81×10^{-3} mol) in a solution of a 1:1 mixture of water/acetonitrile (10 mL). The crude polymer was precipitated in cold ether and filtered to isolate the desired product as a white powder (2.94 g, 98%). ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 3.36 (s, 3H, $-CH_3O$), 3.53−3.55 (m, 2H, $-CH_2OCH_3$), 3.56−3.69 (s-broad, 432H, $-CH_2CH_2O$), 3.69−3.72 (m, 2H, $-CH_2O$), 4.12 (s, 2H, $-CH_2CO_2H$). ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 59.1 ($-CH_3O$), 69.26 ($-CH_2OCH_3$), 71.37 ($CH_3O-CH_2CH_2O$), 71.60 ($-CH_2CH_2O$), 72.98 ($-CH_2CO_2H$), 174.22 ($-CO_2H$).

General Procedure for EDC Coupling of PEG-acid to the Peripheral OH Groups of G1−G4 Dendronized Surfaces. First, the G1−G4 dendronized surfaces were synthesized using the divergent dendron growth, as described in detail in our previous work.[35] Then, flame-dried 15 mL vials were individually charged with Au-Gx(OH) (x = 1−4) surface in a solution of CH_2Cl_2:pyridine (3:2 v/v, 3 mL) under argon. Excess (50 mg) PEG-acid of specific MW (350, 750, 2000, and 5000 Da) along with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) (10 mg, 5.22×10^{-5} mol) and a catalytic amount of 4-(dimethylamino)pyridine/*p*-toluenesulfonic acid (DPTS) (2.5 mg, 8.50×10^{-5} mol) were added, and the vials were shaken for 72 h at room temperature using a shaker since stirring was not possible. The surfaces were then transferred to new individual vials and washed extensively with CH_2Cl_2 (~25 mL) to remove any unreacted EDC. This was followed by extensive rinsing with 1 M $NaHSO_4$ (~20 mL), 10% Na_2CO_3 (~20 mL), and brine (~10 mL) to remove any unreacted PEG-acid and DPTS. Finally, the surfaces were washed extensively with Milli-Q water (~20 mL) and dried with a stream of N_2 prior to analysis.

Protein Radiolabeling with ^{125}I. Fibrinogen (Fg) (Calbiochem, La Jolla, CA) was radiolabeled with Na^{125}I (ICN, Irvine, CA) using the iodine monochloride method.[41] The solution containing the ^{125}I-radiolabeled Fg was passed through a column packed with AG 1-X4 resin (Bio-Rad Laboratories, Inc.) in Tris Buffered Saline (TBS, pH 7.4) to remove unbound ^{125}I (held to <1% of total solution radioactivity). A free ^{125}I test was carried out on the ^{125}I-radiolabeled Fg using the trichloracetic acid (TCA) method to determine the

Scheme 2. EDC Coupling of PEG_{2000}-acid to G1−G4 Dendronized Surfaces



percent of unbound isotope following the iodination reaction. The same procedure was used to radiolabel lysozyme.

Protein Adsorption. Prior to protein adsorption experiments, gold-coated silicon wafers were equilibrated in PBS-NaI buffer overnight; "cold" NaI was added to the buffer to prevent uptake of unbound ^{125}I to the gold.[42] Protein adsorption experiments were performed in PBS-NaI buffer (pH 7.4) at a protein concentration of 1 mg/mL (10% labeled, 90% "cold"/PBS, and 2.5% labeled, 97.5% "cold"/plasma). The surfaces were placed in the wells of 24-well plates and incubated in a 1 mL solution of ^{125}I-labeled protein in PBS-NaI for 3 h at room temperature (22 °C). It was previously determined that no further adsorption occurred at times longer than 3 h.[17] The surfaces were rinsed three times (10 min each) with fresh PBS-NaI to remove any loosely bound protein. Surface radioactivity was determined, using a Perkin-Elmer WIZARD 3 in. 1480 automatic gamma counter. The percent reduction of adsorption on the PEO-functionalized and dendronized surfaces relative to the unmodified gold was determined.

Results and Discussion

Building on our results with dendronized surfaces, we set out to investigate the reason for increased protein adsorption on the previously reported surfaces. As stated in the Introduction, the possible reasons for increased fouling could be the presence of hydrogen-bond donors (OH groups) and a rigidifying effect of the underlying PEG layer due to inter- and intramolecular hydrogen bonding of the dendrons. Haag and co-workers have shown that methylation of the hydroxyl groups of surfaces functionalized with hyperbranched polyglycelrols slightly improves protein resistance.[33] We initially chose a similar approach using acetonide-protected G1 to G4 dendrons. However, protein adsorption increased likely due to increased hydrophobicity imparted by the methyl groups at the periphery of the protected dendrons (data given in the Supporting Information, Figure S3). Clearly, any attempt to eliminate hydrogen bonding must be

Table 3. Water Contact Angles (deg) of PEG_{2000}-Grafted G1−G4 Dendronized Surfaces: Effect of Dendron Generation

surface	advancing contact angle (deg)	receding contact angle (deg)
bare Au	65 ± 3	40 ± 8
Au-PEG_{650}[a]	42 ± 4	36 ± 2
Au-G1(OH)	40 ± 2	22 ± 3
Au-G1-PEG_{2000}	33 ± 2	12 ± 2
Au-G2-PEG_{2000}	30 ± 2	10 ± 2
Au-G3-PEG_{2000}	29 ± 2	9 ± 4
Au-G4-PEG_{2000}	27 ± 3	10 ± 3

[a] 5 mM chemisorption solution of HS-PEG_{650}-OH in PBS at pH = 7.4.

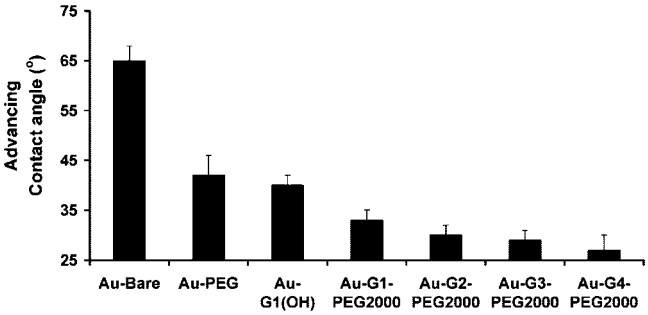


Figure 5. Advancing contact angles of Au, Au-PEG, Au-G1(OH), and Au-G1-PEG_{2000} to Au-G4-PEG_{2000} surfaces.

done in a way that maintains the initial surface hydrophilicity. Therefore, we chose to investigate the introduction of short PEG-OMe oligomers to first generation dendronized surfaces. These structures do not exhibit hydrogen-bond-donating groups but retain the hydrophilicity required.

Synthesis. The synthesis of dendronized surfaces was performed by divergent growth of aliphatic polyester dendrons as previously described.[35] The resulting hydroxyl-terminated dendrons were subsequently functionalized with PEG monomethyl ether (PEG-OMe) chains of different molecular weight using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) coupling with a PEG-acid derivative (Scheme 1). This was accomplished by first converting the terminal hydroxyl group of PEG-mono methyl ether (PEG-OMe) to a carboxylic acid group using the TEMPO-mediated oxidation reported by Masson et al.[40] The peripheral hydroxyl groups of the dendronized surfaces were subsequently reacted with excess PEG-acid of specific molecular weight in the presence of EDC and a catalytic amount of 4-(dimethylamino)pyridine/*p*-toluenesulfonic acid (DPTS). The coupling reaction was carried out for 72 h at room temperature.

Modification of Dendronized Surfaces with PEG Monomethyl Ether (PEG-OMe) Oligomers. A preliminary study was carried out by coupling PEG-acid oligomers (M_n = 350 Da) to first generation (G1) dendronized surfaces. The results from contact angles showed that the hydrophilicity of the original G1 dendronized surface was retained after coupling of the peripheral hydroxyl groups with PEG-acid oligomers (Table 1). More importantly, protein adsorption results showed that fibrinogen and lysozyme adsorption decreased as a result of this modification (Figures 2–4).

Encouraged by this result, we decided to investigate longer PEG-OMe chains (M_n = 750, 2000, and 5000 Da) to determine the effect of molecular weight on protein adsorption. Upon functionalization of the peripheral hydroxyl groups with PEG-acid chains of various MW, the effect of PEG-OMe length on surface hydrophilicity was measured by contact angle goniometry. The results, summarized in Table 1 and Figure 1, show that the advancing contact angles for the PEG-functionalized

Table 4. Root-Mean-Square (rms) Roughness of PEG-Functionalized Dendronized Surfaces Obtained from AFM Data: Effect of Dendron Generation

surface	rms (nm)
bare gold	1.26
Au-PEG_{650}(G0)	5.47
Au-G1-PEG_{2000}	5.88
Au-G2-PEG_{2000}	7.10
Au-G3-PEG_{2000}	8.93
Au-G4-PEG_{2000}	10.64

G1 dendronized surfaces ranged between 41° ± 2° and 19° ± 2°, and the receding angles ranged between 20° ± 4° and 12° ± 2° for PEG MWs of 350 to 5000 Da, respectively. These results reflect the hydrophilicity of the PEG-grafted G1 dendronized surfaces, which increased with increasing PEG-OMe chain length (Table 1, Figure 1). The data also indicate that the hydrophilic nature of the original G1 dendronized surfaces was retained and improved after coupling of the PEG-OMe chains, which was a requirement for the present study.

AFM Analysis. The topographies of the G1 dendronized surfaces prior to and after grafting with PEG-acid chains of various MW were examined by AFM. The results from the 3D AFM height images showed the presence of a large number of small bumps on the PEG-functionalized G1 dendronized surfaces. These observed features were absent in the control bare gold surface and Au-G1(OH) surface (Figure S1). Surface roughness was calculated, and the results, presented in Table 2, show an increase in surface roughness when G1 dendronized surfaces were coupled with PEG-acid chains, with values ranging between 3.42 nm for the Au-G1-PEG_{350} and 6.14 nm for Au-G1-PEG_{5000} surfaces. This increase in surface roughness is consistent with our previous results comparing the roughness of bare Au to PEG-functionalized and dendronized surfaces. Surface roughness increased with increasing surface functionalization, likely due to the formation of polymer-rich domains composed of PEG chains and dendrons on the gold.[18,43]

Protein Adsorption Studies. *Fibrinogen Adsorption.* The effect of surface functionalization with first-generation dendrons that were peripherally modified with PEG-OMe of various lengths on protein adsorption was investigated. In our previous study, it was found that protein adsorption increased upon surface dendronization with aliphatic polyester dendrons of generation 1−4 when compared to the control PEG-grafted surfaces.[35] To investigate the reason behind these results, we performed similar protein studies, but with G1 dendronized surfaces that were functionalized with PEG-OMe of different chain length. Initial studies using fibrinogen (Fg) were carried out from both phosphate buffered saline (PBS) and plasma (Figures 2 and 3, respectively). All protein experiments were repeated twice using triplicate samples at every functionalization

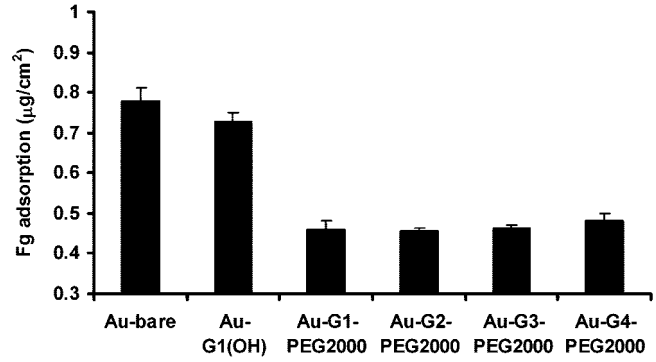


Figure 6. Fibrinogen adsorption from 1 mg/mL Fg−Fg−^{125}I in PBS-NaI of PEG_{2000}-grafted G1−G4 dendronized surfaces.

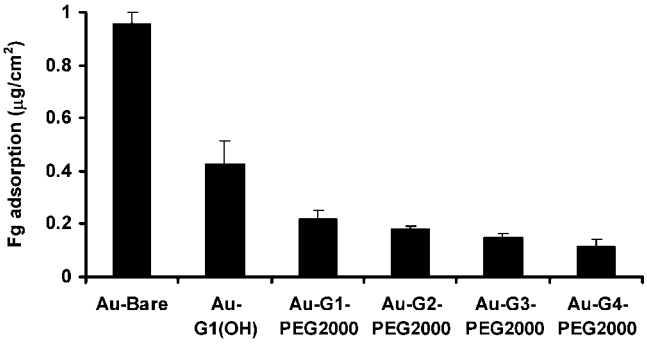


Figure 7. Fibrinogen adsorption from 1 mg/mL Fg−Fg−^{125}I in plasma of PEG_{2000}-grafted G1−G4 dendronized surfaces.

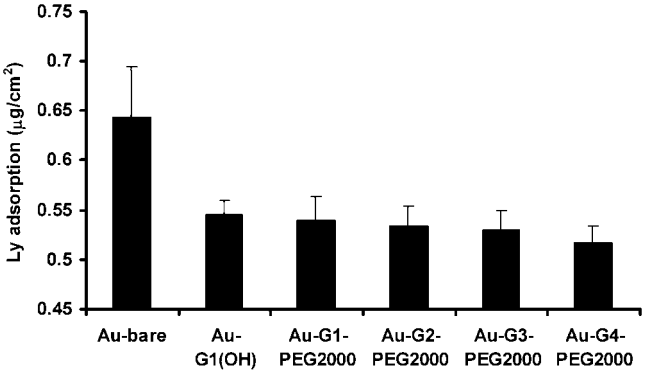


Figure 8. Lysozyme adsorption from PBS-NaI of PEG_{2000}-grafted G1−G4 dendronized surfaces.

step in order to confirm the observed trend of Fg adsorption onto the various surfaces. From these experiments, in contrast to OH-terminated dendronized surfaces, it was found that protein adsorption decreased when G1 dendronized surfaces were modified with PEG-OMe oligomers. This decrease in protein adsorption was even more significant when longer PEG-OMe chains were grafted to the dendronized surfaces, reaching an optimum decrease at MW of 2000 Da (Figures 2 and 3). Interestingly, when the PEG-OMe MW was further increased to 5000 Da, protein repulsion did not improve. Although we cannot provide a definite explanation for this latter result, some contributing factors might include the difficulty for the system to achieve optimum chain density and mobility with longer PEG chains, an observation previously reported by Brash and coworkers.[44]

Lysozyme Adsorption. To determine whether the degree of adsorption is influenced by the nature of the protein, we chose to also carry out these studies with lysozyme (Lys), which is a much smaller protein relative to fibrinogen. Additionally, under our experimental conditions (PBS, pH 7.4), lysozyme is positively charged while fibrinogen is negatively charged. Hence, it would be expected that these two proteins would respond to the PEG-functionalized dendronized surfaces in different ways. In our studies electrostatic interactions should not influence protein adsorption as the dendronized and PEG-functionalized surfaces are neutral under our conditions. Therefore, differences in size and surface functionality between the two proteins are expected to be dominant factors influencing adsorption. Moreover, the use of Lys allowed us to make quantitative comparisons with the results of Fg and Lys onto dendronized surfaces that we reported previously.[35] Similarly to fibrinogen, all protein studies were repeated twice using triplicate samples at every functionalization step to ensure reproducibility of the results. From these experiments, it was again found that functionalization of the G1 dendronized surfaces with PEG-OMe chains enhanced protein resistance, but the improved resistance was only significant when PEG_{2000} and PEG_{5000} chains were grafted (Figure 4). Again, it was found that increasing PEG-OMe MW from 2000 to 5000 Da did not improve protein resistance, indicating a limit to the effective MW for protein resistance. Overall, these results show that grafting PEG-OMe to dendronized surfaces improves protein repulsion irrespective of the nature of the protein, which is in agreement with previous studies.[15,35,44] The requirement for higher PEG molecular weights (relative to results with fibrinogen) before appreciable decrease in adsorption was observed is likely a result of lysozyme's greater ability to diffuse through small openings within the PEG coating to reach and adsorb to the Au surface.

Effect of Dendron Generation on Protein Adsorption. From the results obtained in the first part of this study, in which the effect of PEG-OMe MW on protein adsorption was investigated, it was found that surfaces functionalized with PEG_{2000}-OMe exhibited greatest protein resistance for both fibrinogen and lysozyme. On the basis of these results, PEG_{2000}-OMe was subsequently coupled to higher generation dendrons to produce PEG star-polymers having 2, 4, 8, and 16 arms. The synthesis of PEG star polymers on the Au surfaces was carried out by esterification of the terminal hydroxyl groups of surface bound G1 to G4 dendrons with PEG_{2000}-acid (Scheme 2).

Initial characterization of the surface bound PEG star polymers was accomplished by contact angle measurements. The results, summarized in Table 3 and Figure 5, show that the advancing contact angles for the PEG_{2000}-functionalized G1−G4 dendronized surfaces ranged between 33 ± 2° and 27 ± 3°, and the receding angles ranged between 12 ± 2° and 10 ± 3° for G1-PEG_{2000} to G4-PEG_{2000}, respectively. These results indicated that the hydrophilic character of the PEG star-functionalized surfaces was slightly superior to that of just the dendron-grafted surfaces at each generation (Table 3, Figure 5).[35]

AFM Analysis. The surface topographies of the functionalized gold surfaces prior to and after grafting of PEG_{2000}-acid to the G1−G4 dendronized surfaces were also examined by AFM. The 3D height images of the PEG_{2000}-functionalized dendronized surfaces showed the presence of a large number of small peaks and valleys, features that are absent on the control surface (Au-bare) and which became more significant with increasing dendron generation (Figure S2). The presence of these features resulted in a significant increase in surface roughness of the PEG_{2000}-grafted dendronized surfaces, which ranged between 5.88 nm for the Au-G1-PEG_{2000} and 10.64 nm for the Au-G4-PEG_{2000} (Table 4).

Fibrinogen Adsorption. Fibrinogen adsorption from PBS-NaI buffer decreased significantly on the PEG_{2000}-grafted dendronized surfaces compared to the control bare Au surface and the original Au-G1(OH) surface (Figure 6). However, no significant difference was noted in the Fg adsorption from PBS on the various generation dendronized surfaces. In contrast, when the surfaces were incubated in a solution of Fg in plasma, a more significant decrease in protein adsorption was observed with increasing arm number of the star polymer (Figure 7). When comparing these results to the ones obtained with the G1−G4 dendronized surfaces,[35] it is clear that introducing PEG-OMe chains to the periphery of the dendronized surfaces results in a significant decrease in protein adsorption. By coupling PEG-OMe chains to the peripheral hydroxyl groups, the hydrogen bonding between the dendrons is eliminated, allowing the new system to exhibit greater chain mobility. Functionalization of the dendronized surfaces with PEG-OMe chains also eliminates the presence of hydrogen-bond donor groups, which may be

an additional important contributing factor to the observed decrease in protein adsorption.

Lysozyme Adsorption. Lysozyme adsorption from PBS-NaI onto PEG_{2000}-functionalized G1−G4 dendronzied surfaces followed a similar trend to the one observed for Fg adsorption from PBS. Protein adsorption on the PEG_{2000}-grafted dendronized surfaces was significantly lower compared to the control bare Au surface. However, no major difference was noted when the number of the PEG-OMe arms was increased with increasing dendron generation (Figure 8). The similarity of these results to the ones observed with fibrinogen is another indication that protein adsorption onto PEG-functionalized dendronized surfaces follows the same trend regardless of protein size.

Conclusions

Modification of dendronized surfaces with PEG-OMe chains was carried out via EDC coupling of the peripheral hydroxyl groups to the carboxyl end group of PEG-acid. In the first part of this study, the effect of functionalization of G1 dendronized surfaces with PEG-OMe oligomers on protein adsorption was investigated. Results from fibrinogen and lysozyme adsorption studies showed that protein adsorption onto the PEG-OMe grafted surfaces was lower than for the original dendronized surfaces. To further investigate the effect of PEG-OMe molecular weight on protein resistance, PEG-acid chains with molecular weight ranging between 350 and 5000 Da were used. Protein studies comparing the original G1 dendronized surfaces to the PEG-functionalized dendronized surfaces showed that protein adsorption gradually decreased with increasing MW of PEG-OMe chains. Results also showed that increasing PEG-OMe MW beyond 2000 Da did not result in better protein resistance. In the second part of this study, the effect of dendron generation on protein adsorption was investigated using PEG_{2000}-acid. Protein studies showed a decrease in protein adsorption with increasing dendron generation regardless of protein size. The present results, when compared to our previous study,[35] showed that protein resistance was recovered when the dendronized surfaces were functionalized with PEG-OMe chains and both hydrogen bonding between the dendrons and the presence of hydrogen-bond donor groups were eliminated from the system. These results support previous studies indicating that chain mobility and dynamics in water may be critical to protein repulsion.[44,45] Additionally, this work shows that, when a surface incorporates a large number of hydrogen-bond donor groups that have a restricted motion at the water interface, it becomes more accessible to proteins and results in greater protein adsorption.

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Supporting Information Available: AFM data showing surface morphology for the dendronized surfaces as well as fibrinogen adsorption data from PBS buffer. This material is available free of charge via the Internet at http://pubs.acs.org.

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