Self-Assembled Monolayers of Dendritic Polyglycerol Derivatives on Gold That Resist the Adsorption of Proteins

Conrad Siegers,[a] Markus Biesalski,[c] and Rainer Haag*[a, b]

Dedicated to Professor Dr. Armin de Meijere on the occasion of his 65th birthday

Abstract: Highly protein-resistant, self-assembled monolayers (SAMs) of dendritic polyglycerols (PGs) on gold can easily be obtained by simple chemical modification of these readily available polymers with a surface-active disulfide linker group. Several disulfide-functionalized PGs were synthesized by *N*,*N*′-dicyclohexylcarbodiimide-mediated ester coupling of thioctic acid. Monolayers of the disulfide-functionalized PG derivatives spontaneously form on a semitransparent gold surface and effectively prevent the adsorption of proteins, as demonstrated by surface plasmon resonance (SPR) kinetic measurements. A structure–activity relationship relating the polymer architecture to its ability to effectuate protein resistance has been derived from results of different surface characterization techniques (SPR, attenuated total reflectance infrared (ATR-IR), and contact-angle measurements). Dendritic PGs combine the characteristic structural features of several highly protein-resistant surfaces: a highly flexible aliphatic polyether, hydrophilic surface groups, and a highly branched architecture. PG monolayers are as protein resistant as poly(ethylene glycol) (PEG) SAMs and are significantly better than dextran-coated surfaces, which are currently used as the background for SPR spectroscopy. Due to the higher thermal and oxidative stability of the bulk PG as compared to the PEG and the easy accessibility of these materials, dendritic polyglycerols are novel and promising candidates as surface coatings for biomedical applications.

Keywords: biocompatible materials · dendrimers · polymers · proteins · surface chemistry

Introduction

Nonspecific protein adsorption may occur at the solid–liquid interface when a surface is exposed to a biological environment containing proteins (for example, blood). An example of a circumstance where this phenomenon is unfavorable is protein adsorption to artificial surfaces, such as medical devices or biosensors.[1,2] Nonspecific protein adsorption in biosensors can impair their proper function. Moreover, complications initially arising from protein adsorption to surfaces of, for example, catheters,[3,4] implants,[1,5] or artificial organs[3,6] introduced into the human body may lead to irritations, chronic infections, or thrombosis.[7,8] Despite the large impact of this problem on the above-mentioned applications and extensive research efforts, the details of the mechanism of nonspecific protein adsorption are not yet completely understood. However, it is believed that parameters such as van der Waals interactions, electrostatic forces, and hydrogen bonding govern nonspecific adsorption.[1,8]

A common approach to avoiding problems arising from protein adsorption is coating a device used in biomedical applications with a layer of a material that prevents nonspecific interactions. Materials that have been used for this purpose include heparin,[9] dextran,[10] and poly(ethyloxazoline);[11,12] however, the most common and prominent example of a material used to render a surface inert to nonspecific protein adsorption is poly(ethylene glycol) (PEG), a linear, flexible, hydrophilic, and water-soluble polyether.[13]

[a] Dipl.-Chem. C. Siegers, Prof. Dr. R. Haag
Freiburger Materialforschungszentrum und
Institut für Makromolekulare Chemie
Universität Freiburg, Stefan-Meier-Strasse 21
79104 Freiburg (Germany)
E-mail: rainer.haag@uni-dortmund.de

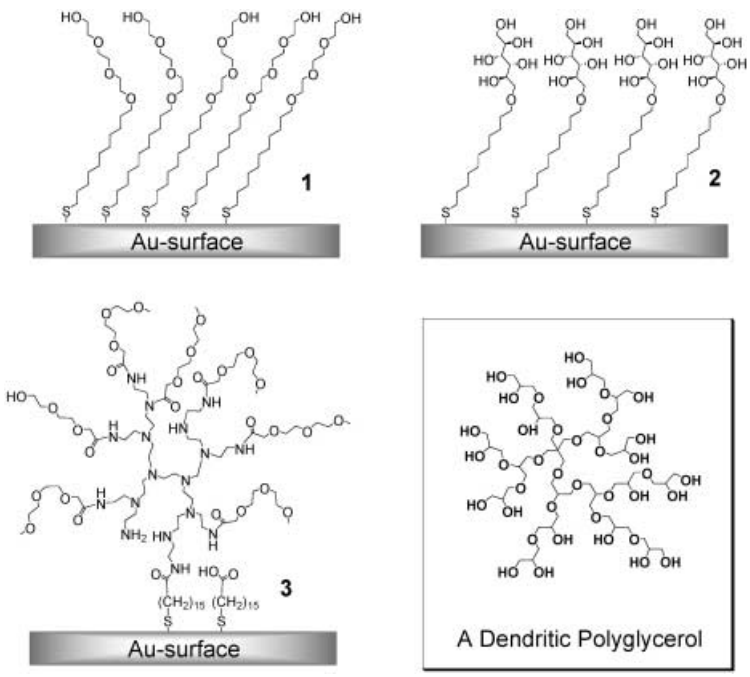
[b] Prof. Dr. R. Haag
Current address: Organische Polymerchemie
Universität Dortmund, Otto-Hahn-Strasse 6
44227 Dortmund (Germany)
Fax: (+49) 231-755-6148

[c] Dr. M. Biesalski
Institut für Mikrosystemtechnik (IMTEK)
Universität Freiburg, Georges-Köhler-Allee 103
79110 Freiburg (Germany)

Supporting Information for this article is available on the WWW under http://www.chemeurj.org or from the author.

 DOI: 10.1002/chem.200306073

Self-assembled monolayers (SAMs) presenting oligo(ethylene glycol) (OEG) groups (as in $HS(CH_2)_{11}(EG)_nOH$ ($n \geq 3$)) on a gold surface also prevent the adsorption of proteins, even if the number of EG units presented is as low as three (for example, **1**, where $n = 3$; Scheme 1).[14] PEG is widely employed to coat the surfaces of biomedical devices, but it nevertheless exhibits the disadvantage of thermal instability[15] and rapid autooxidation, either through O_2 in presence of transition metal ions or enzymatically in vivo, to yield aldehydes and acids.[16] These drawbacks of PEG are spurring interest in the development of alternative protein-resistant materials.



Scheme 1. Highly protein-resistant surfaces selected from the literature (**1**–**3**) and a dendritic polyglycerol that could be attached to a surface to give a novel protein-resistant material combining all the structural features of **1**–**3** (highly flexible aliphatic polyether, rich in hydrophilic groups, and highly branched architecture).

The mechanism of protein resistance of PEGylated surfaces is still subject to discussion. One of the many and diverse mechanisms presented in the literature was proposed by Nagaoka et al., who ascribed the suppression of protein adsorption to surfaces modified with short PEG segments directly to the high dynamics of such segments in water.[17] The authors reasoned that protein adsorption would greatly restrict the mobility of the chain segments, a restriction that is synonymous with a thermodynamically unfavorable decrease of entropy.[17,18] Grunze and co-workers correlated the protein-resistant properties of OEG chains to their molecular conformation at the interface. They showed that the conformation is different on gold and silver substrates due to different packing densities. On silver, the OEG segments form the all-*trans* conformation, whereas SAM formation on a gold substrate gives rise to a helical structure,[19] which is favored by solvation in water.[20,21] Hence, the presence of the OEG segments in the helical conformation was considered crucial for the occurrence of protein resistance.[19] The authors stated that the protein resistance of the helical OEG phase may not be a property of the molecule itself; instead, the helical phase may prevent a direct interaction between the surface and the protein by forming a stable solid–liquid interphase involving tightly bound water.[19,21]

The first systematic correlation between protein resistance and different chemical structures as surface coatings was performed by Whitesides and co-workers for a large number of low molecular weight compounds attached to gold surfaces through a thioundecanoic amide.[16] Based on this detailed study several criteria for the occurrence of good protein-resistant properties were formulated for small molecules.[22] The desired structural features of a protein-resistant material included a high hydrophilicity, the absence of hydrogen-bond donors, and the presence of hydrogen-bond acceptors. These criteria were demonstrated when substitution of acidic protons with methyl groups led to a reduction in protein adsorption.[22] In this respect, a few polymeric structures showing good protein-resistant properties were also investigated;[7] amongst them was a branched poly(ethylenimine), which was further derivatized with OEG groups. A monolayer of this branched PEG analogue (**3** in Scheme 1) exhibits good protein-resistant properties. This new polymer-grafted surface layer covers small heterogeneities in the underlying support and has a gel-like character (a characteristic that is associated with resistance to the adsorption of proteins[18]). However, in some cases, good protein-resistant properties were observed even for molecules with a large number of free hydrogen-bond donors (that is, OH groups). For example, Mrksich and co-workers prepared a monolayer presenting mannitol groups (**2** in Scheme 1) and demonstrated that this surface is inert to protein adsorption[23] even though other simple sugar derivatives do not resist the adsorption of proteins.[16] Several groups immobilized dextran, a biopolymer consisting of mainly 1,6-linked glucose units,[9] onto various surfaces and showed a substantial reduction in protein adsorption due to the coating procedure.[24–26] Polymers based on methylated sorbitol have also recently been shown to be highly protein resistant.[27]

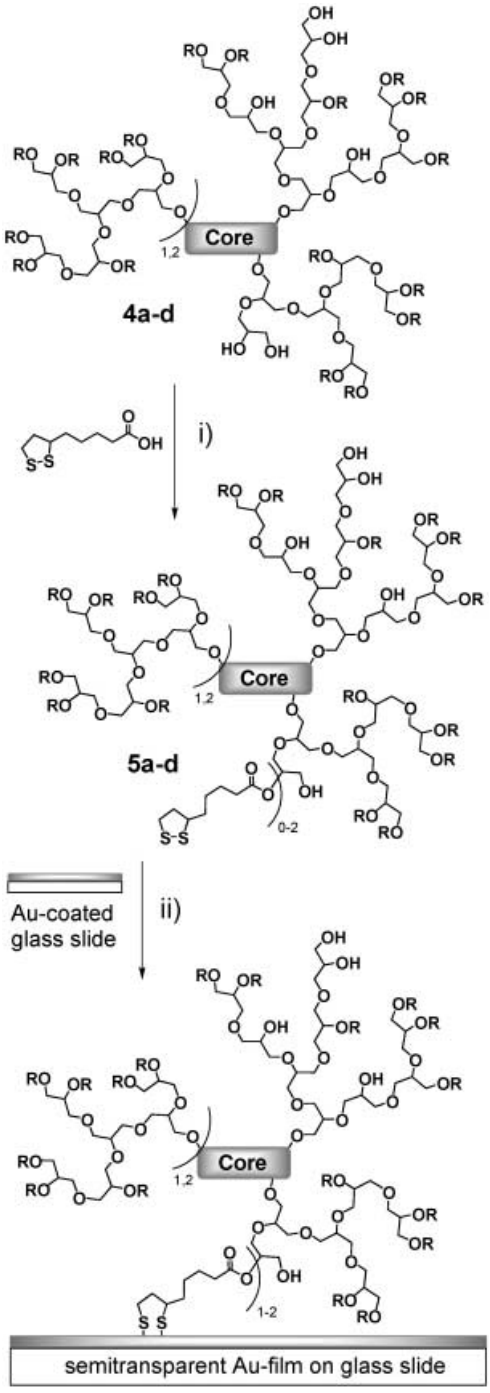
From a structural analysis of the protein-resistant surfaces with **1**–**3** (Scheme 1) and dextran, three structural features become apparent: the presence of highly flexible, aliphatic polyether and hydrophilic groups (that is, OH groups) and a highly branched architecture. Dendritic polyglycerols (PGs)[28–32] exhibit all the above-mentioned structural fea-

tures; thus, they meet the requirements for protein resistance because they comprise 1) a hydrophilic repeating unit, 2) a unit that can hydrogen bond with water and is hence well soluble (swellable) in water, 3) an oligomer/polymer that is conformationally very flexible due to aliphatic ether bonds, and 4) a highly branched architecture. These similarities led us to hypothesize that a monolayer of dendritic polyglycerol derivatives that combine all of the above-mentioned properties should possess good protein-resistant properties.

Experimental design and synthetic approach: For the examination of this hypothesis we have prepared several suitable PG derivatives that form SAMs on gold films and we have used surface plasmon resonance (SPR) spectroscopy[3,16] as a reliable detection system for the quantitative evaluation of protein adsorption from an aqueous solution. The amount of adsorbed protein was determined by exposing each modified surface to a protein solution in the flow cell of an SPR spectrometer (see the Experimental Section). SPR spectroscopy can determine changes in the optical thickness ($n \times d$, where n = refractive index and d = absolute thickness) of a layer system near the interface of a semitransparent gold film by measuring changes in the resonance angle at which *p*-polarized light reflected from the glass/gold interface has a minimum intensity.[1,3,33,34]

For the investigation of protein adsorption we used fibrinogen,[9] a protein that is present in relatively large quantities (0.2–0.4%) in the blood and that is commonly used to evaluate biocompatibility of artificial surfaces.[7,14,16,22,35] This protein is relevant for nonspecific adsorption and biofilm formation because it has a high tendency to adsorb onto most surfaces.[11,16] For a structure–activity relationship of the protein-resistant properties resulting from these dendritic architectures attached to a gold surface, we have varied the following molecular parameters: 1) the molecular weight of the dendritic polymer, 2) the initiator unit at the core, and 3) the functionalization of the OH groups. These parameters were all tunable by the polymerization conditions or subsequent functionalization steps.[28–32,36] To compare the protein resistance of the PGs with already established materials used for surface coatings, we studied protein adsorption to PEG- and dextran-modified surfaces. Furthermore, the thermal stabilities (under air and nitrogen) of the bulk PG and PEG under air were characterized by thermogravimetric analysis.

The synthetic approach to obtain various PG derivatives suitable for the formation of PG SAMs consisted of the partial esterification of OH groups (approximately one linker group per dendritic polymer molecule) of PG substrates **4a–d** with thioctic acid (Scheme 2), a linker molecule bearing a carboxylic acid linked to a 1,2-dithiolane ring and known to form stable SAMs.[37–40] Four different functionalized dendritic polymers, **5a–d**, were obtained by DCC-mediated ester coupling (Table 1). The following PG derivatives were used as substrates for the partial esterification: a polyglycerol with $M_n = 5000\ g\,mol^{-1}$ and trimethylolpropane (TMP) as the initiator (PG_{5000}–TMP, **4a**), polyglycerols with pentaerythrite (PE) as the initiator and molecular weights of $M_n =$ 2500 and 5000 $g\,mol^{-1}$ (PG_{2500}–PE, **4b**, and PG_{5000}–PE, **4c**, respectively), and a partially methylated PG derivative with glycerol as the initiator and an estimated molecular weight of $M_n = 5000\ g\,mol^{-1}$ (MeO–PG, **4d**; 88% of the initially



Scheme 2. Synthesis of disulfide-functionalized polyglycerol derivatives by DCC-mediated coupling with thioctic acid and spontaneous formation of self-assembled monolayers of PG–Thioc derivatives **5a–d** on semitransparent gold films for SPR spectroscopy evaluation of their protein-resistant properties (**4a–c, 5a–c**: R = H; **4d**, **5d**: R = Me). The polymers used are discussed in the text. Reagents and conditions: i) DCC, DMAP, DMF, 0 °C; ii) self-assembled monolayer formation on 40 nm Au/2.5 nm Cr/glass support. DCC = *N*,*N*′-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, DMF = *N*,*N*-dimethylformamide, Thioc = thioctic acid.

Table 1. Disulfide-functionalized PG derivatives **5a–d** synthesized by DCC coupling with thioctic acid.

Entry	Substrate		M_n [g mol^{-1}]	Initiator	R	Product		DF[a] [%]	N_{Thioc}[b]	Yield[c] [%]
1	PG_{5000}–TMP	**4a**	5000	TMP	H	PG_{2500}–Thioc	**5a**	1.7	1.1	34
2	PG_{2500}–PE	**4b**	2500	PE	H	PG_{2500}–Thioc	**5b**	2.2	0.7	22
3	PG_{5000}–PE	**4c**	5000	PE	H	PG_{5000}–Thioc	**5c**	0.6	0.5	82
4	MeO-PG[d]	**4d**	5000	glycerol	Me	MeO-PG–Thioc	**5d**	2.2	1.4	76

[a] *DF*: degree of functionalization = number of thioctic acid groups × (OH groups before functionalization)$^{-1}$ × 100. The uncertainty was calculated to be 7% (relative error). [b] N_{thioc} = average number of thioctic acid groups per PG molecule. [c] Yields of isolated product after extensive dialysis. [d] The degree of methylation was 88% (see text).

prevailing OH groups were converted into OMe groups).[36] The motivation for evaluating the methylated PG derivatives arose from the effect observed by Whitesides and co-workers[22] and Guan and co-workers[27] that protein resistance may be enhanced if acidic protons (such as OH groups) are substituted by methoxy groups, thereby eliminating the hydrogen-bond donors of a given structure.

By the described synthetic strategy (Scheme 2) it was possible to obtain different PG derivatives containing the appropriate disulfide linker group, which in contact with a gold surface forms two covalent bonds.[37,38,41,42] The number of linker groups per PG molecule is subject to a statistical distribution with an average of one group per PG molecule, as determined by ^{1}H NMR spectroscopy (Table 1).

SAM formation and characterization: To screen all the PG derivatives for their protein-resistant properties, self-assembled monolayers were formed on semitransparent gold substrates by immersing the surfaces into a methanol solution of the respective thiol or disulfide overnight (referred to as ex situ surface modification in the following description) and subsequent washing with organic solvent to remove the unreacted material (Scheme 2). The optimal time for complete SAM formation with the PG–Thioc derivatives **5a–d** was obtained from SPR kinetic studies, which consisted of monitoring the adsorption of different PG derivatives in the flow cell of the SPR spectrometer (referred to as in situ surface modification in the following description). A steady state in the sensogram of the adsorption of **5c** (Figure 1) was observed after 210 min, a result indicating complete SAM formation; this result was not observed if the PG derivative **4c** without the disulfide linker was used.[43]

For comparison of the different protein-adsorption experiments our SPR protocol was based on those found in the literature as follows:[3,16,22] 1) flowing PBS over the surface for 1.5 min, 2) replacing the buffer by flowing a solution of 20 mM sodium dodecylsulfate (SDS) in PBS over the surface for 3 min, 3) rinsing the surface of SDS by washing with PBS buffer for 10 min, 4) exposing the surface to protein by flowing a fibrinongen solution (1 mg mL^{-1} in PBS) over it for 30 min, and 5) finally rinsing the surface with PBS for an additional 10 min. The amount of adsorbed protein is proportional to the difference between the reflective units (*RU*) obtained from the SPR sensograms (see Figure 2 and the Supporting Information) after and before exposure to protein solution ($\Delta RU = RU_{\text{after exposure}} - RU_{\text{before exposure}}$). The amount of adsorbed fibrin-

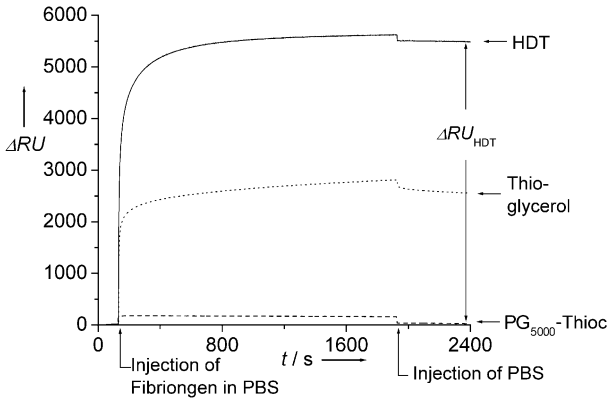


Figure 2. SPR Sensograms of fibrinogen adsorption to modified surfaces (SAMs consisting of hexadecanethiol (HDT, solid line), thioglycerol (dotted line), and PG_{5000}–Thioc, **5c** (dashed line)). The sensorgrams illustrate the adsorption of different amounts of protein onto different SAMs. The HDT SAM is the reference surface ($\Delta RU_{HDT} = 100\%$).

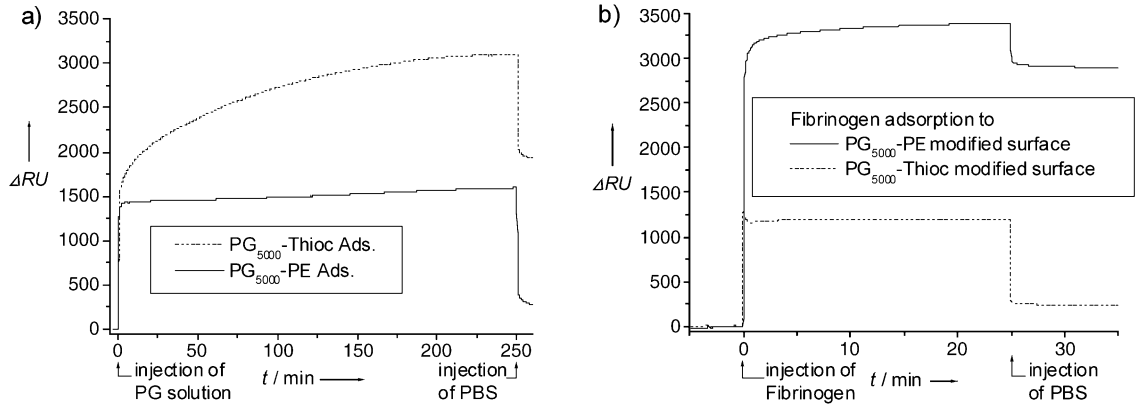


Figure 1. a) Sensogram of a polyglycerol (1 wt%) in phosphate-buffered saline (PBS; in-situ modification), before (PG_{5000}–PE, **4c**, solid line) and after (PG_{5000}–Thioc, **5c**, dashed line) partial esterification with thioctic acid, upon exposure to a gold surface. b) Sensogram of protein adsorption to the in situ modified gold surfaces with **4c** and **5c**.

ogen was determined relative to a hydrophobic reference surface, an HDT SAM, which is known to strongly adsorb proteins by nonspecific interactions.[16] Thus, the relative amount of adsorbed protein (%*PA*) was calculated from ΔRU values taken from sensograms according to the following equation: $\%PA = \Delta RU_{PG} \times (\Delta RU_{HDT})^{-1} \times 100$.

In all cases the advancing static contact angle of a water drop was measured before the substrate was mounted in the flow cell of the SPR spectrometer.

For comparison with PEG SAMs that do not adsorb significant amounts of protein, we also studied the fibrinogen adsorption to a SAM of compound **1** (HS-$(CH_2)_{11}$-$(OCH_2CH_2)_3$-OH), which is known to be highly protein resistant,[16] and to the surface of a CM5 sensor chip (Biacore), which is a gold surface coated with partially carboxymethylated dextran.[44] The latter surface (Table 2, entry 10) is typically used as the background in SPR experiments.

Table 2. Protein adsorption and contact angles of surfaces modified with the dendritic polyglycerols **4a** and **5a–d** and reference surfaces.

Entry	SAM		Multiplicity of core unit	Surface active group	M_n [g mol^{-1}]	*PA* [%]	θ_{water}[a] [°]
1	HDT (reference)		–	SH	–	100.0 ± 4.6	97 ± 3
2	bare gold		–	–	–	45.5 ± 6.0	74 ± 5[b]
3	thioglycerol		–	SH	–	47.4 ± 4.2	20 ± 2
4	PG_{5000}–TMP	**4a**	3	none	5000	39.7 ± 6.3	40 ± 5
5	PG_{5000}–Thioc	**5a**	3	1,2-dithiolane	5000	3.7 ± 2.5	31 ± 2
6	MeO-PG–Thioc	**5d**	3	1,2-dithiolane	5000	1.6 ± 0.5	50 ± 3
7	PG_{2500}–Thioc	**5b**	4	1,2-dithiolane	2500	0.8 ± 0.1	20 ± 2
8	PG_{5000}–Thioc	**5c**	4	1,2-dithiolane	5000	0.8 ± 0.2	20 ± 3
9	HS-$(CH_2)_{11}EG_3OH$		–	SH	–	0.8 ± 0.3	34[c]
10	dextran[d]		–	–	–	2.7 ± 0.8	n.d.[e]

[a] Advancing static contact angle. [b] After rinsing with MeOH and drying. [c] Water contact angle reported in the literature: see ref. [48]. [d] CM5 research-grade sensor chip from Biacore, partially carboxymethylated dextran matrix (see text). [e] n.d. = not determined.

Results and Discussion

To inspect the quality of the SAM formation, the modified gold surfaces were initially characterized by kinetic SPR experiments (Figure 1). Adsorption experiments of PG_{5000}–Thioc **5c** and PG_{5000}–PE **4c** in PBS gave strong evidence for the formation of stable SAMs mediated by thioctic acid. The sensogram in Figure 1a shows a sudden initial increase in the ΔRU value in both cases, after which a slower increase persists over the exposure time for PG_{5000}–Thioc **5c** only; this value eventually reaches a constant level after 210 min. The initial increase can be attributed to the bulk effect resulting from substitution of running buffer with the PG solution within the flow cell of the SPR device. The continuous increase in the ΔRU value towards a constant level is typical for the formation of SAMs.[45,46] Another indication for the presence of immobilized PG at the surface is the fact, that, if the disulfide group is present, the ΔRU value does not drop back to 0 after rinsing the surface with buffer and SDS.

Further evidence for the chemisorbed versus physisorbed PG SAM (assuming its protein resistance) is given by the protein-adsorption experiments to the in situ modified surfaces (Figure 1b). Protein resistance can only be observed when the gold surface was exposed to the PG derivative **5c** bearing a thioctic acid moiety; strong fibrinogen adsorption is observed for the PG derivative **4c**. From the change in reflectivity caused by adsorption of PG_{5000}–Thioc **5c** onto semitransparent gold (in situ modification in MeOH), we have calculated the layer thickness after the adsorption to be 2 ± 1 nm with the assumption of a refractive index of n = 1.45 for the swollen PG layer in MeOH (see the Supporting Information). The error in the layer thickness is mainly due to the uncertainty in the value for the refractive index. This layer thickness is in good agreement with results obtained from atomic force microscopy measurements.[47]

For the structure–activity relationship, several PG SAMs have been evaluated for their fibrinogen adsorption and compared to reference surfaces (Table 2). Typical examples of sensograms of protein-adsorption experiments to surfaces modified ex situ in an organic solvent are shown in Figure 2. From the results presented in Figure 1, Figure 2, and Table 2 it becomes obvious that the PG derivatives bearing a surface-active 1,2-dithiolane linker, **5a–d**, form a highly protein-resistant surface layer (Table 2, entries 5–8), while physically adsorbed PG **4a** (Table 2, entry 4) shows only marginal improvements over the bare gold surface (Table 2, entry 2). The surfaces employed in the protein-adsorption experiments with **4a** and **5a** (Table 2, entries 4 and 5) were both modified with the same type of polymer (a polyglycerol with a trifunctional core group and identical molecular weight); however, only one surface was covalently modified with the linker-containing PG derivative **5a**. The significant difference in the relative adsorptions (%*PA*) of these two surfaces is consistent with the necessity of the sulfur-containing linker between the polyglycerol and the gold surface. If the polymer is merely bound by weak interactions (for example, physical adsorption), it cannot resist the adsorption of proteins effectively and so the protein-adsorption behavior to a surface immersed in a solution of PG_{5000}–TMP **4a** is similar to that of a bare gold surface. It is assumed that most of the physisorbed PG layer has been removed by the extensive washing steps prior to the protein-adsorption step, while the shift to a smaller contact angle as compared to bare gold is caused by the few remaining PG molecules (see also Figure 1).

It is noteworthy that a gold surface modified with thioglycerol (Table 2, entry 3) under identical conditions does not show notable protein resistance, even though identical contact angles were measured for this surface and the PG–Thioc derivatives **5b** and **5c** (Table 2, entries 7 and 8). Thioglycerol can be considered as the smallest PG unit since it contains the terminal hydrophilic 1,2-diol group. Since a

SAM-stabilizing, unbranched alkyl chain between the thiol group and the functional group to be exposed at the interface is not present in this compound, poor monolayer formation has to be expected. In analogy to thioglycerol, SAMs with very short ethylene glycol chains (HS-$(CH_2)_{11}$-$(OCH_2CH_2)_n$-OH, $n=1$ or 2) also show poor protein resistance.[48] These results are consistent with the small-molecule thioglycerol, which possesses only a single 1,2-diol unit and has low molecular chain dynamics, lacking a substantial entropy loss on protein adsorption; this in turn leads to poor protein resistance. On the other hand, PGs show good protein resistance, due to their high molecular weight, flexibility, and dynamics. The contact angles are identical for thioglycerol and the best protein-resistant surfaces (those modified with **5b** and **5c**; $\theta=20°$) but the protein-resistant properties differ strongly. This reconfirms the fact that the hydrophilicity of a surface alone is not a sufficient feature to effectuate protein resistance.

By comparison of the relative adsorptions (%*PA*) for the PG–Thioc derivatives **5a–d** (Table 2, entries 5–8) only slight changes of the already very low protein adsorption were observed. However, these can be discussed in terms of the polymer architecture. The more globular PG_{5000}–Thioc **5c** with a tetrafunctional core unit is more inert to protein adsorption than PG_{5000}–Thioc **5a** with a trifunctional core unit ($\%PA_{\mathbf{5c}} < \%PA_{\mathbf{5a}}$, Table 2, entries 5 and 8). This behavior is consistent with a correlation between the higher polymer segment density for PG_{5000}–Thioc **5d** that arises from the tetrafunctional pentaerythrite core (as opposed to the trifunctional trimethylolpropane) and better protein resistance. On the other hand, no pronounced molecular-weight dependence could be detected in this structure–activity correlation for the polymers **5b** and **5c**; both SAMs show excellent protein-resistant properties. In order to confirm the protein resistance of these PG derivatives by direct detection methods as opposed to the indirect SPR measurements, attenuated total reflectance infrared (ATR-IR) spectra of the hydrophobic HDT surface and the PG SAM of **5c** were recorded (Figure 3). The characteristic amide bands of fibrinogen at 1650 and 1550 cm^{-1} (Figure 3a) are also visible on the hydrophobic surface after incubation in the fibrinogen solution (Figure 3b). This is not the case after incubation of the PG_{5000}–Thioc SAM (Figure 3c). This comparison underlines the results obtained from our SPR data.

With respect to the criteria formulated by Whitesides and co-workers regarding the molecular structure of protein-resistant materials,[16,22] it is surprising that the hydrophilic PG–Thioc derivatives **5a–c** with a very high number of hydrogen-bond donors (13.5 $mmol\,g^{-1}$ OH groups) show such excellent protein-resistant properties. Thus, in analogy to the mannitol SAMs prepared by Mrksich and co-workers[23] and to the dextran surfaces,[24,25] these PG derivatives are yet another exception to the correlation between protein resistance and the absence of hydrogen-bond donors.[16,22] Our incentive for the synthesis of the compound MeO-PG–Thioc **5d** in which the majority of the acidic hydrogen atoms were substituted by a CH_3 group was to verify whether these criteria also apply to PG derivatives. Surfaces modified with **5d** did not reveal significantly different protein resistance to the best hydrophilic PG–Thioc derivatives ($\%PA_{\text{PG–Thioc}} \approx \%PA_{\text{MeO-PG–Thioc}}$). However, a small improvement can be observed over a comparable trifunctional core PG derivative with free OH groups (Table 2, entries 5 and 6). This result is surprising with regard to the hydrophobicity of the methylated species **5d**. It is not soluble in MeOH and has a much higher contact angle. Nevertheless, this observation is in good agreement with the hypothesis referring to the absence of hydrogen-bond donors.[22]

A comparative protein-adsorption experiment to a dextran matrix showed that the immobilized PG SAMs of **5b** and **5c** are significantly more protein resistant than commercially available dextran-based sensor-chip surfaces that are typically used for SPR background measurements (Table 2, entry 10). For direct comparison we have also studied the protein adsorption to a SAM composed of a PEGylated alkanethiol HS-$(CH_2)_{11}$-$(OCH_2CH_2)_3$-OH, for which $\%PA_{\text{PEG}}$ values of 0.3–1% have been reported in the literature.[16,49] Our reproduced value for relative adsorption (%*PA*) to the above-mentioned SAM was $\%PA_{\text{PEG}} = 0.8 \pm 0.3\%$ (Table 2, entry 9). The slight differences between the experimental values may be ascribed to either different exposure times or the surface roughness of different gold substrates. Nevertheless, the present results show that the PGs effectuates inertness to protein adsorption as effectively as PEG derivatives under the applied experimental conditions.

In addition, the higher thermal and oxidative stability of the bulk PG as opposed to the PEG was determined by thermogravimetric analysis (TGA, Figure 4) to compare the long-term stability of the respective coating. The onset of thermal decomposition of PG_{5000} under nitrogen is 395°C (under air 225°C) and exceeds that of PEG_{20000} by 115°C (30°C under air).[15] This observation is extremely important for the application of PG derivatives as pro-

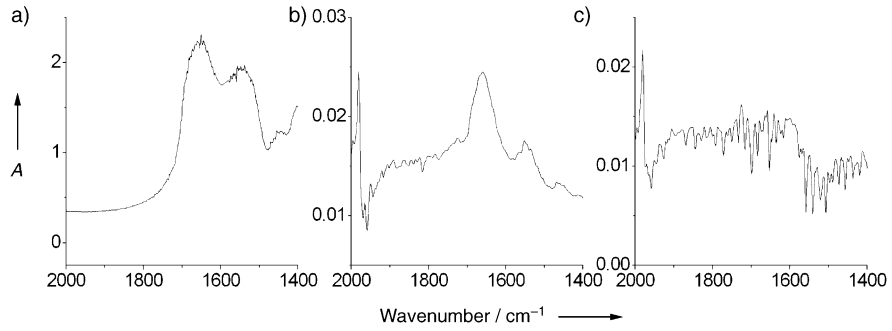


Figure 3. FT-IR spectra of a) fibrinogen in bulk, as well as b) a hydrophobic HDT SAM and c) the PG_{5000}–Thioc SAM after incubation in a fibrinogen solution and rinsing. The spectra in b) and c) were measured at the surface by using the ATR technique.

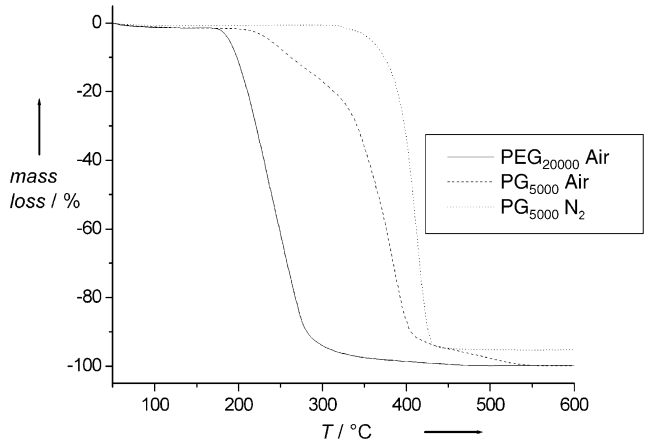


Figure 4. TGA diagram of PG with a molecular weight of 5000 g mol^{-1} under air and N_2 atmosphere and PEG with a molecular weight of 20000 g mol^{-1} under air. From this diagram, the following temperatures for the onset of thermal decompositions were extracted: PG_{5000} under N_2: 395 °C; PG_{5000} under air: 226 °C; PEG_{20000} under air: 195 °C.

tein-resistant coating material for biomedical devices, where heat is typically used for sterilization.

Conclusions

In summary, we have presented a new, biocompatible coating material, which suppresses the nonspecific adsorption of proteins. The synthesis of the PG–thioctic acid derivatives **5** is straightforward and the self-assembled monolayers form spontaneously on gold surfaces. A structure–activity relationship of the polymer architecture forming the self-assembled monolayers has been revealed by SPR and ATR-IR measurements, with detection of the adsorption of fibrinogen as well as contact-angle measurements of water droplets. The capability of these dendritic polyglycerol derivatives to resist the adsorption of proteins is compatible with the dynamic surface-layer mechanism proposed by Nagaoka et al.[17] Dendritic PGs combine the characteristic structural features of highly protein-resistant surfaces: a highly flexible aliphatic polyether, hydrophilic surface groups, and a highly branched architecture. While PG monolayers are as protein resistant as PEG SAMs, they are significantly more active than dextran-coated surfaces, which are currently used for SPR background measurements. Due to the higher thermal and oxidative stability of the bulk PG as compared to PEG and the easy accessibility of these materials, dendritic polyglycerols are promising candidates as surface coatings for biomedical applications. In addition, the multiple free OH groups of dendritic polyglycerols can be further functionalized with ligands for specific protein interactions with avoidance of nonspecific interactions at the same time. With consideration of practical applications, further research in our group is focused on dendritic polyglycerol derivatives with alternative reactive linker groups for the coating of commodity material surfaces (such as glass, plastics, and ceramics).

Experimental Section

The controlled syntheses of hyperbranched polyglycerols have been reported[28–32] and precise molecular weights of the polymers (controlled by the monomer/initiator ratio) with narrow polydispersities (<2) can be obtained. The unique, dendritic architecture of the hyperbranched PG shown in Scheme 1 has a typical degree of branching of 60 %, as determined by inverse-gated ^{13}C NMR spectroscopy.

Synthesis of PG–Thioc 5b: In a Schlenk flask PG_{2500}–PE (M_n = 2500 g mol^{-1}, 3.92 g, 1.57 mmol, 1 equiv), thioctic acid (0.324 g, 1.57 mmol, 1 equiv), and a catalytic amount of DMAP (10 mg, 0.08 mmol, 0.05 equiv) were dissolved in anhydrous DMF (25 mL) under an argon atmosphere. After the mixture was cooled to 0 °C, DCC (0.356 g, 1.73 mmol, 1.1 equiv) was added in DMF (1.4 mL) and the solution was stirred for 18 h at room temperature. The reaction mixture was filtered, the solvent was evaporated, and the residue was purified by extensive dialysis in MeOH. Evaporation of the solvent afforded the desired polymer **5b** (0.9 g, 22 %): ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 1.48 (m, 2H; H-Thioc), 1.65 (m, 4H; H-Thioc), 1.91 (m, 1H; H-Thioc), 2.45 (m, 3H; H-Thioc), 3.16 (m, 2H; H-Thioc), 3.40–4.25 (m, PG backbone and H-Thioc), 4.69 (m, OH), 4.89 (m, OH) ppm; ^{13}C NMR (75.4 MHz, CD_3OD, 25 °C): δ = 25.8 (C-Thioc), 29.9 (C-Thioc), 35.0 (C-Thioc), 35.7 (C-Thioc), 39.6 (C-Thioc), 41.6 (C-Thioc), 47.0 (central C atom of initiator), 57.8 (C-Thioc), 62.7, 63.0, 64.4, 66.9, 70.7, 71.0, 71.7, 72.1, 72.3, 72.5, 72.8, 74.0, 79.8, 80.1, 81.4, 81.6 (PG backbone), 176.0 (C-Thioc) ppm; IR (KBr) $\tilde{\nu}$ = 3370 (s), 2920 (m), 2880 (m), 1750 (w), 1650 (w), 1460 (w), 1350 (w), 1250 (w), 1120 (s), 1080 (s) cm^{-1}.

Preparation of SAMs: Gold substrates for SPR spectroscopy were prepared from glass coverslips (VWR International, no. 1, 20 × 20 mm^2) that were cut in half (resulting in glass chips of 10 × 20 mm^2), cleaned by immersing into deionized water and isopropanol in an ultrasonic bath, dried in a stream of nitrogen, and subsequently subjected to electron-beam evaporation of Cr (2.5 nm) followed by Au (40 nm). Preparation of SAMs from sulfur-functionalized polymers consisted of immersing the gold-coated glass coverslip in a PG solution (1 % w/w) in MeOH (for PG–Thioc) or THF (MeO-PG–Thioc) overnight, rinsing the surface in a stream of the respective solvent for 30 s, and drying it in a stream of nitrogen. SAMs from HDT, HS-$(CH_2)_{11}$-$(OCH_2CH_2)_3$-OH, and thioglycerol were prepared analogously by using 1 mM solutions in EtOH and MeOH, respectively.

SPR measurements: The measurements were carried out on a Biacore 1000 (Upgrade) spectrometer. The modified and dried, semitransparent gold substrates were mounted on an SPR cartridge with double-sided tape. The running buffer for the SPR spectrometer was PBS (10 mM PO_4^{3-}, 138 mM NaCl, 3 mM KCl, pH 7.4). All solutions used for SPR measurements were freshly prepared and filtered through a 0.22-μ filter prior to use. Fibrinogen and SDS solutions were used at concentrations of 1 mg mL^{-1} and 20 mM in PBS, respectively. The standard procedure for the recording of sensograms was 1) flowing PBS buffer over the surface for 1.5 min, 2) replacing the buffer by flowing SDS solution for 3 min, 3) rinsing the surface of SDS by flowing PBS buffer over it for 10 min, 4) exposing the surface to protein by flowing fibrinongen solution over it for 30 min, and 5) finally rinsing the surface with PBS for an additional 10 min. An experimental value for $RU_{\text{before exposure}}$ was acquired by averaging the $RU(t)$ values within the time interval 180–90 s prior to the fibrinogen injection. An experimental value for $RU_{\text{after exposure}}$ was acquired by averaging the $RU(t)$ values within the time interval 420–510 s after completion of the fibrinogen injection. The ΔRU values of four protein-adsorption measurements for the same modified surface were used to calculate the standard deviation, which was further used to estimate the error in relative adsorption (%PA) by using the mean square method.

Contact angles: The advancing static contact angle of a water drop (MilliQ) was measured prior to the SPR measurement by using a OC20 apparatus from Data Physics.

IR measurements: IR spectra from surfaces were carried out with an ATR-IR machine (IFS88) from Bruker.

Thermogravimetric analysis: To compare the onset of thermal decomposition for PG and PEG, a sample of PG with a molecular weight of 5000 g mol^{-1} and a PEG sample with a molecular weight of 20000 g mol^{-1}

were subjected to thermogravimetric analysis under air or nitrogen by using an STA 409 apparatus from Netzsch.

Acknowledgements

The authors thank H. Türk for the synthesis of unfunctionalized polyglycerol, A. Fauler for the preparation of semitransparent gold substrates, and Prof. R. Mülhaupt for his generous support. We thank Prof. K. Aktories, Dr. J. Kuhlmann, Prof. J. Rühe, Priv.-Doz. Dr. G. Schmidt, M. Vogelsgesang, and M. Wyszogrodzka for their support concerning SPR measurements. We thank the Prochimia Corporation (Gdansk, Poland) for the genereous gift of the PEGylated alkanethiol. C.S. is indebted to the Rehau-AG for a research award. M.B. and R.H. are grateful to the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie for financial support.

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Received: December 12, 2003

Published online: April 26, 2004

