Polymeric Thin Films That Resist the Adsorption of Proteins and the Adhesion of Bacteria

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This paper describes the design and preparation of thin polymeric films that resist the adsorption of proteins and the adhesion of bacteria to an extent comparable to, or better than, self-assembled monolayers (SAMs) that present tri(ethylene glycol) groups. These polymeric films were prepared by the reaction of a polyamine, for example, poly(ethylenimine), with a SAM that presented interchain carboxylic anhydride groups, and by the subsequent conversion of the amino groups of the polymer to amido groups on reaction with acyl chlorides. Polyamines functionalized with acetyl chloride produced films that resisted the adsorption of protein and the adhesion of bacteria to a useful extent. Functionalization of the polyamine with acyl chlorides that were derivatives of oligo(ethylene glycol) resulted in films that were 1−10 times more resistant than those obtained by acetylation. The removal of hydrogen bond donor groups from the surface of the polyamines upon acylation seems to be important for the generation of films that resist the attachment of proteins and bacteria.

Introduction

This paper describes a strategy for designing and synthesizing thin, polymeric films that are covalently grafted on the surface of self-assembled monolayers (SAMs) and that resist the adsorption of proteins and the adhesion of bacteria. These surface films were prepared using a three-step process (Figure 1): (i) A single-component SAM of mercaptohexadecanoic acid was converted to a SAM having terminal interchain carboxylic anhydride groups on the surface. (ii) The reaction of this SAM with polymeric amines generated a second surface composed of a thin, covalently grafted, polymer layer having multiple amino groups. (iii) The free primary and secondary amino groups of the immobilized polymers were acylated to introduce functional groups that resist the adsorption of proteins. The number of bacteria that adhered to the surface films that best resisted the adsorption of proteins was 10−100 times lower than that measured on commercially available medical grade poly(urethane) and on so-called "bare" gold.

We believe that this procedure illustrates a general strategy for generating surfaces that resist the adsorption of proteins and the adhesion of bacteria (for brevity, we call these surfaces "inert", meaning "inert to the attachment of macromolecules and microorganisms"). The procedure is experimentally straightforward; we believe that it can be adapted to the fabrication of surfaces for biomedical materials and devices.

Adsorption of Proteins to Surfaces. The interaction of proteins with surfaces has been studied actively to (i) find biocompatible materials for applications in sensors, assays, and biomaterials, and to (ii) understand the fundamental mechanisms of adsorption of proteins to surfaces. Although a number of classes of more-or-less biocompatible materials have been identified, the understanding of the mechanisms underlying biocompatibility and protein adsorption is not complete.[1,2]

The adsorption of protein to a surface is a complex problem; knowledge of both the kinetics of the adsorption and the structure of the layer of adsorbed protein is important in understanding this process.[3] Proteins often adsorb to surfaces irreversibly, and it is difficult to derive useful molecular information from kinetic models. Once adsorbed, many proteins undergo conformational and orientational changes. It has not been possible to determine the structures and orientations of the resulting adsorbed protein molecules in most cases.[3] Lateral interactions between neighboring adsorbed molecules can also influence the stability of the adsorbed layer.[4] These topics have been reviewed recently elsewhere.[3,5−7]

Bacterial Adhesion to Surfaces. Bacteria adhere to host cells and to artificial surfaces through both biospecific/

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selective interactions (carbohydrate−protein, protein−protein) and nonspecific interactions (hydrophobic or electrostatic).[8] The interaction of type I pili of *E. coli* with mannose groups is one example of specific interactions;[9] the interaction of so-called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) of *Staphylococcus aureus* with host plasma proteins adsorbed onto bone matrix and to bone implant biomaterials is a second.[8,10−13]

A bacterial infection localized on the surface of an in-dwelling device is usually the result of at least three processes: the adsorption of proteins and/or polysaccharides (presented in the host or secreted by the pathogen) to the surface of the device, the adhesion of bacteria to this surface, and the growth of the bacteria.[8] Formation of a carbohydrate gel layer covering the bacteria and hiding them from the immune system is often a fourth, important, process. In all cases, it appears that the presence of a layer of protein on a surface facilitates (or is required for) the adhesion of bacteria. Surfaces that resist the adsorption of proteins are, therefore, strong candidates as surfaces that resist the adhesion of bacteria.

Applications of Inert Polymeric Films. Inert surfaces are useful in the prevention of a wide variety of technological problems including (i) thrombosis caused by the adsorption of plasma proteins on in-dwelling medical devices such as intravenous catheters,[14−16] (ii) irritation caused by the adsorption of proteins and the adhesion of bacteria to external medical devices such as contact lenses, (iii) contamination of packaged food by microorganisms such as *Salmonella typhimurium* and *Staphylococcus aureus* that can lead to biofilm formation,[17] and (iv) "hard fouling", the adherence of barnacles and gorgonians to the hulls of ships.[18,19]

Surfaces that resist the adsorption of proteins may also inhibit the adhesion of bacteria; no material, however, completely inhibits the adhesion of bacteria. Any procedure that offers improvements over existing biomaterials would nonetheless be attractive to those designing biomedical devices.[20]

Strategies for the Design of Inert Surfaces. *Poly(ethylene glycol) (PEG).* PEG has been used extensively as a component in materials for biomedical applications:[21] when included as a comonomer in polymeric materials, or when grafted to a surface, it usually increases biocompatibility (as measured using several criteria). Often, a low surface density of PEG with high molecular weight (EG_n where $n \simeq 30$) is grafted on the surface of a material to improve its biocompatibility.[21,22]

Coatings made with PEG are widely used in biomedical devices to decrease the adsorption of proteins.[23,24] The usefulness of liposomes as drug delivery devices can be increased by grafting PEG to the lipid membrane;[25] these "stealth liposomes" circulate in the body for longer periods of time than liposomes without PEG. Since PEG is hydrophilic, it has also been used as an agent for enhancing solubility of proteins; PEG is incorporated in micellar structures to keep proteins in aqueous solutions of detergent.[21] Some therapeutic proteins functionalized with PEG have better pharmacokinetic profiles than the native forms.[21]

Although PEG is a very useful component of biomaterials, it is not clear if it is the *best* organic group of its type, that is, the most effective in reducing protein adsorption, bacterial adhesion, platelet activation, and other unwanted symptoms of bio-incompatibility.[14,15] It also has a specific technical deficiency. As a polyether, it has a tendency to autoxidize when exposed to O_2 and transition metals.[26−28] In vivo, the terminal hydroxyl group of PEG is oxidized to an aldehyde by alcohol dehydrogenase; the aldehyde group is oxidized further by aldehyde dehydrogenase.[29,30]

Other Surfaces that Resist the Adsorption of Proteins. Branched poly(ethylenimine) (BPEI) has been allowed to adsorb noncovalently to polystyrene to serve as the support onto which PEG or a polysaccharide could be immobilized.[31] Alternatively, PEG or a polysaccharide was allowed to react with BPEI prior to its noncovalent immobilization onto polystyrene. PEG has also been grafted onto poly(L-lysine) to generate polymers that adsorb noncovalently to metal oxide surfaces to reduce the adsorption of proteins.[32] Poly(urethane)s have been used extensively as materials for biomedical devices and, in particular, for catheters.[33,34] Other approaches have involved grafting phospholipids onto silicone rubbers or poly(urethane)-based materials,[35−37] or using metals such as platinum, titanium, or Nitinol.[38] Ratner et al. have provided an excellent review of the field of biomaterials.[38]

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SAMs That Resist the Adsorption of Proteins. Surfaces functionalized with a high density of short oligomers of $(EG)_n$ ($n = 3-6$) resist the adsorption of proteins to an extent comparable[39-42] to that found by others for surfaces functionalized with low densities of high MW PEG.[1,22] Model studies based on SAMs of alkanethiolates on gold have helped to clarify structure−property relationships relevant to the understanding and design of inert surfaces. We have used the combination of SAMs and surface plasmon resonance (SPR) to identify functional groups that, present as a terminal group on a SAM, made a surface inert.[43-45] In those studies, inert surfaces shared the following structural features; they were[44] (i) hydrophilic, (ii) overall electrically neutral,[46,47] and (iii) hydrogen bond acceptors, but (iv) *not* hydrogen bond donors. Conformational flexibility is a characteristic of many of these groups, but it does not seem to be required. Inert surfaces identified using this SAM-based screening protocol effectively prevented the adhesion of bacteria and mammalian cells.[43,46] These studies confirmed that there are a substantial number of different types of organic functional groups that can form the basis for SAMs that prevent the adsorption of proteins and the adhesion of bacteria and that grafting derivatives of ethylene glycol to a surface is not a unique strategy for generating inert surfaces.

Experimental Design and Motivation. Our goal in this work was to explore whether the structure−property relationships that characterized SAMs that resisted the adsorption of proteins could be extended to polymeric films grafted onto the surfaces of SAMs. These surfaces could serve as models for polymers, since the surfaces of polymers are often chemically and topographically heterogeneous and difficult subjects for study. We sought to develop a system that would have six characteristics: (i) it would support the synthesis of a range of structures, (ii) its grafted surface layer would have a structure that would enable it to cover small heterogeneities in the underlying support, (iii) it would serve as the basis for surfaces that are realistic models for biomedical devices, (iv) its grafted layers would have gel-like character (a characteristic that is associated with resistance to the adsorption of proteins),[1] (v) it would allow the formation of polymeric surface films that could incorporate functional groups known to make SAMs inert, and (vi) it would be compatible with aqueous buffers and with SPR measurements.

We grafted several types of polymeric amines onto a SAM that presented interchain carboxylic anhydride groups (Figure 1). These grafted polymeric amines presented a high density of amino groups; these groups could be acylated with functional groups associated with inert surfaces. We hypothesized that these surfaces would provide models for thin polymeric films and hoped that the thin grafted films (Figure 1) would cover heterogeneities in the surface of the SAM/gold. The use of a SAM on gold as the substrate onto which to graft the polyamine films made the system compatible with analysis of protein adsorption by SPR.

The polymeric thin films synthesized following the procedures described herein adsorbed <1% of the quantity of fibrinogen and lysozyme that adsorbed on an alkyl-terminated surface; these films also reduced the attachment of bacteria by 10^3 relative to the surface of medical grade polyurethane.

Results and Discussion

Formation of Covalently Attached Polyamine Films on SAMs. *Choice of Polymeric Amines.* Figure 1 summarizes the synthetic procedure used to graft films of polyamines to SAMs. Chart 1 shows the structures of the polymeric amines used. We chose to use polyamines for our studies for the following reasons: (i) Amino groups react in high yields with carboxylic anhydride groups or acyl chloride groups. They can therefore be grafted onto SAMs that present interchain carboxylic anhydride groups. (ii) Polyamines such as linear poly(ethylenimine) (LPEI) contain a very high density of amino groups; this high density allowed for the functionalization of polymer films with a correspondingly high density of groups intended to render the surfaces inert.

The wide variety of polyamines that are available commercially allowed us to evaluate the importance of several properties of the polymer on the ability of our thin films to resist the adsorption of proteins. (i) We investigated the effect of the molecular weight of the polymers on the properties of the films. (ii) Polyamines presenting $-NH_2$ and $-NHCH_3$ groups were useful to evaluate the importance of hydrogen bond donor groups (e.g., LPEI, PMVA, PAA, and PLYS, Chart 1). (iii) A comparison of BPEI to LPEI allowed us to survey the importance of cross-linking in the polymer film by comparing BPEI to LPEI.

Grafting poLymeric Amines to the Surface of SAMs. Previously, we characterized the formation of thin films of BPEI (750 kDa) attached to SAMs (in the fashion described in Figure 1) using polarized infrared external reflectance spectroscopy (PIERS) and X-ray photoelectron spectroscopy (XPS).[48-50] Throughout this paper, surfaces are labeled using the name of the polymer and that of the substituent; for example, a mixed SAM with a grafted LPEI layer (250 kDa) is SAM/$LPEI_{250}$, and one that has been further acylated with ClCOR is SAM/$LPEI_{250}$/COR (Figure 1).

Modification of Amino Groups with Acyl Chlorides. Reactions of the carboxylic anhydride terminated SAMs

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(45) This project was based on a screening protocol that activated SAMs of mercaptohexadecanoic acid by dehydration with trifluoroacetic anhydride (TFAA) to form reactive interchain carboxylic anhydride groups; these surfaces reacted readily with commercially available amines (H_2NR) that presented the functional groups of interest to generate surfaces that presented 1:1 mixtures of $-CONHR$ and CO_2H/CO_2^-.
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(48) Reaction of the polymer with the anhydride SAM generated covalent amide bonds and carboxylic acid groups.[49,50] The immobilized film of BPEI was ca. 3 nm thick (measured by AFM and ellipsometry); the advancing contact angle of water on this layer was $\theta_a^{H_2O} = 37°$; that of the SAM that presents interchain carboxylic anhydride groups was $\theta_a^{H_2O} = 72°$.[49,50] At this stage, the yield for the conversion of carboxylic anhydride groups to amides during the covalent attachment of PEI to the SAM has not been quantified, but we presume that it is high.
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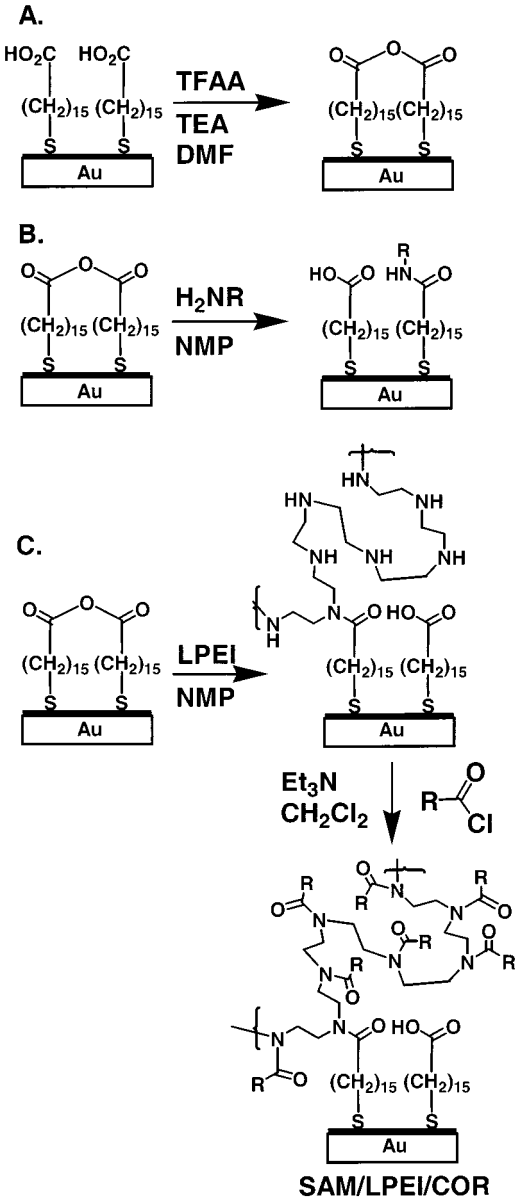
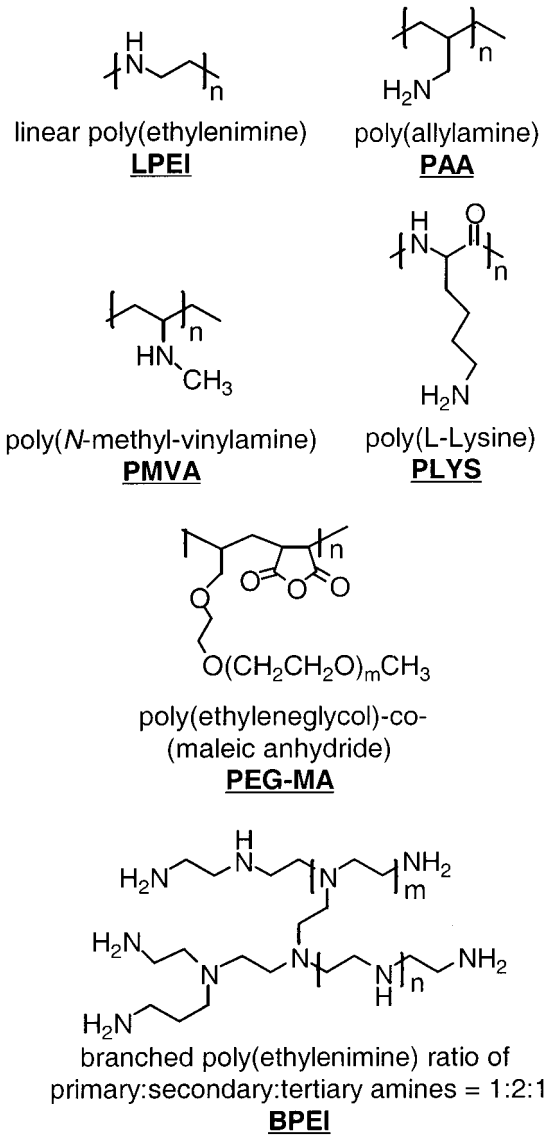


Figure 1. (A) Preparation of a SAM having interchain carboxylic anhydride groups by dehydration of terminal carboxylic acid groups. (B) Reaction of this activated SAM with a primary amine (RNH_2) to form a mixed SAM terminating in a 1:1 mixture of CONHR and CO_2^-/CO_2H groups. (C) Reaction of interchain anhydrides with linear poly(ethylenimine) (LPEI), followed by reaction of the surface-grafted LPEI with an acyl chloride (ClCOR. R = CH_3, $CH_2(OCH_2CH_2)_2OCH_3$), generates a thin, functionalized, polymeric gel on the surface of the SAM. Details of the procedures are in the Materials and Methods section.

Chart 1



with polyamines were carried out in aqueous buffer (PAA, PLYS), isopropyl alcohol (BPEI), or NMP (LPEI, PMVA); the resulting polyamine surfaces were allowed to react with low molecular weight acyl chlorides (0.3 mM) in dichloromethane.[51] We linked poly(ethylene glycol) (n = 30) derivatives to the surfaces of the grafted film by allowing the SAM/LPEI to react with poly(ethylene glycol)-*co*-poly(maleic anhydride) (PEG-MA). The residual anhydride groups of PEG-MA were hydrolyzed in the presence of buffer; the residual COOH groups can also be activated toward further reaction with amines with NHS/EDC. Alternatively, the residual anhydride groups can be allowed to react further with amino or diamino derivatives of ethylene glycol.

Surface Plasmon Resonance (SPR). We grafted polymers onto SAMs made on gold because these substrates are directly compatible with SPR. SPR is an optical technique that detects changes in refractive index at the interface between a thin film of gold and a solution in contact with this film.[52] The screening protocol was based on previous work:[40,43,44] surfaces were exposed to solutions of proteins for 3 min (Figure 2) followed by buffer for 10 min. As previously, two proteins were examined: fibrino-

(51) Yan et al. acylated a SAM/BPEI layer with $CH_3(CH_2)_{15}COCl$ and found that the resulting surface had $\theta_a^{H_2O} = 107°$; acylation with $CF_3(CF_2)_7COCl$ gave a surface with $\theta_a^{H_2O} = 118°$. These values of $\theta_a^{H_2O}$ are consistent with the formation of a hydrophobic layer with properties comparable to those of a SAM of hexadecanethiolate ($\theta_a^{H_2O} = 112°$).[49,50] The similarity of the values of the advancing contact angles of water on these surfaces suggests that there are few exposed polar functionalities on the acylated BPEI, but it does not provide any other information about the density of these groups.

(52) An SPR sensorgram is a graph of the change in response units as a function of time. For a detailed physical description of SPR, see: Raether, H. *Surface Plasma Oscillations and Their Applications*; Hass, G., Francombe, M., Hoffman, R., Eds.; Academic Press: New York, 1977; Vol. 9, pp 145−261. For a description of applications of SPR, see: Lofas, S.; Malmqvist, M.; Ronnberg, I.; Stenberg, E.; Liedberg, B.; Lundstrom, I. *Sens. Actuators, B* **1991**, *5*, 79−84. Mrksich, M.; Sigal, G. B.; Whitesides, G. M. *Langmuir* **1995**, *11*, 4383−4385.

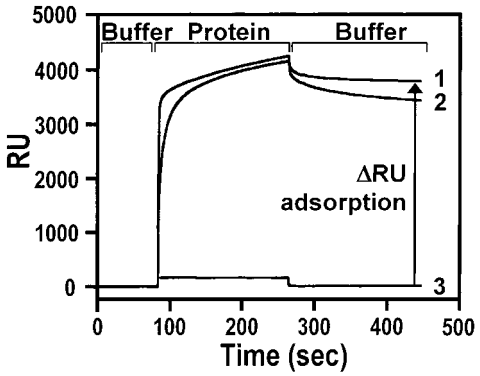


Figure 2. SPR sensorgrams illustrating the adsorption of fibrinogen to different SAMs. The surface of LPEI−$CO(CH_2)_{11}CH_3$ (25 kDa) adsorbs nearly a full monolayer of protein (curve 1). A mixed SAM that presents unfunctionalized LPEI also adsorbs almost a full monolayer of protein (curve 2). Acylation of the SAM/LPEI with $ClCOCH_2(OCH_2CH_2)_2OCH_3$ generates a surface that resists the adsorption of protein (curve 3). The amount of adsorbed protein is defined as the difference in signal between the end of the experiment and the beginning of the injection of protein; we illustrate that difference with an arrow labeled ΔRU adsorption. The percentages of adsorbed proteins were determined using eq 1.

gen and lysozyme.[40] The former adsorbs to hydrophobic surfaces, the latter to negatively charged ones.[53]

Adsorption of Proteins to SAMs That Present LPEI/COR, BPEI/COR, PAA/COR, PMVA/COR, and PLYS/COR. Both proteins adsorbed to a mixed SAM formed from the reaction of $CH_3(CH_2)_{10}NH_2$ with a SAM that presents anhydride groups;[40] an almost indistinguishable result was obtained with a SAM of hexadecanethiolate.[54] The actual quantity of protein that adsorbs to the alkyl terminated surface almost certainly represents a monolayer under the conditions of our experiment, but the structure, thickness, and index of refraction of this monolayer depend on the extents of adsorption and conformational change of the proteins on the surface.[55−57] We report the amount of protein adsorbed on other surfaces as a percentage of this value (eq 1) to simplify the relative comparison between the different surfaces. Here, RU = resonance unit (1 RU = 10^{-4}°) and ΔRU, illustrated in

$$\%\ ML_{protein} = \%\ \text{monolayer} = \frac{\Delta RU_{SAM/polymer/COR}}{\Delta RU_{\text{mixed SAM (/CON(CH}_2)_{10}CH_3)}} \times 100 \quad (1)$$

Figure 2, is the difference in response units between the end of the protein desorption (10 min after the completion of the protein injection) and before the beginning of the injection of the solution containing the protein. Generally, the percentage of adsorbed fibrinogen (% ML_{fib}) is higher than that of lysozyme (% ML_{lys}); fibrinogen is larger and it adsorbs to hydrophobic surfaces more tightly than does lysozyme.

The smallest value of % ML we can detect reliably is 0.2%; this number corresponds to approximately 10 RU. The quantity of adsorbed protein measured at different positions on the same sample or on samples that were prepared by following the same protocol on different days varied by ca. 5% (relative error). We do not know the origin of these differences, but they are not significant for this work. SAMs on gold terminated with $(EG)_3OH$, $(EG)_3OCH_3$, $(EG)_6OH$, and $(EG)_6OCH_3$ groups adsorbed <0.2% of a monolayer of fibrinogen or lysozyme; *these surfaces represent the standards for resistance to adsorption of proteins to which we compare all other surfaces*; *the standard for* adsorption *of protein is the alkyl-terminated surface of a SAM of* $HS(CH_2)_{15}CH_3$ (Figure 2).[2,41,42]

Derivatives of $(EG)_n$. SAM/LPEI/$COCH_2(EG)_2OCH_3$ and SAM/PMVA/$COCH_2(EG)_2OCH_3$ adsorbed amounts of fibrinogen and lysozyme comparable to those adsorbed by single-component SAMs with $(EG)_3OH$ groups (Table 1, Figure 2). The low amounts of protein that adsorbed to thin films of LPEI (25 kDa), grafted on SAMs, that were functionalized with $CH_3O(EG)_nCH_2COCl$ were similar for $n \geq 2$ (Table 2); this result is consistent with our previous experience that high densities of short oligomers of ethylene glycol render surfaces inert.[41,42]

Grafting poly(ethylene glycol)-*co*-(maleic anhydride) (PEG-MA) to a film of LPEI (25 kDa) also generated a surface that resisted the adsorption of fibrinogen and lysozyme in a manner comparable to SAMs terminated in $(EG)_n$ (n = 3, 6) groups. We attribute this result to the presence of the *long* side chains of PEG ($n \sim 30$). Although the reaction of PEG-MA with LPEI generates a film containing (after hydrolysis) a number of carboxylic acid groups, it appears, from the small amounts of protein that adsorb to SAM/LPEI/PEG-MA (% $ML_{Lys} = 0.2$ and % $ML_{Fib} < 0.2$), that the long $(EG)_n$ chains screen these groups.

Acetyl Groups. The reaction of acetyl chloride with the surfaces covered with polyamines generated surfaces with a high density of acetamido groups (R_2NCOCH_3). The amount of protein that adsorbed to acetylated films of LPEI was lower than that adsorbed to unfunctionalized, grafted, polymer films (Table 1); this observation is consistent with previous studies indicating that SAMs that present secondary or primary amino groups adsorb more protein than structurally similar groups that present the same amino groups in the form of amides ($NCOCH_3$).[43,44] Acetylation of the grafted polyamines with acetic anhydride generated surfaces that adsorbed slightly larger amounts of fibrinogen than those acetylated with acetyl chloride.[58] Polymer films grafted with $-COCH_2(EG)_2OCH_3$ groups adsorbed 1−0.1 times the amount of protein that adsorbed on the acetylated films (Table 1).

Unfunctionalized Polymers. Proteins adsorbed to the unacylated thin films of the polyamines because they are positively charged (R_3N^+H); they also have a high density of hydrogen bond donor groups (Table 1). The surfaces of SAMs coated with polymeric amines adsorbed from 1 to 8 times more fibrinogen than lysozyme (Table 1). It is probable that the partially positively charged films repelled the positively charged lysozyme (pI = 11) and attracted the negatively charged fibrinogen (pI = 5.5).

Hydrophobic Groups. The amounts of proteins that adsorbed to polymeric films acylated with hydrophobic groups such as $CH_3(CH_2)_{10}COCl$ and $CF_3(CF_2)_3COCl$ suggest that the surfaces were largely hydrophobic in character (Table 2). These results are consistent with the report of Yan et al. that the advancing contact angles of

(53) Fibrinogen is a large (340 kDa) blood plasma protein that adsorbs strongly to hydrophobic surfaces. Lysozyme, a small protein (14 kDa) that is positively charged under our experimental conditions (z_p = 7.5; phosphate-buffered saline (PBS), pH 7.4), is used often in model studies of electrostatic adsorption.

(54) Mrksich, M.; Sigal, G. B.; Whitesides, G. M. *Langmuir* **1995**, *11*, 4383−4385.

(55) The limiting amount of protein that adsorbs to hydrophobic surfaces is influenced by the specific properties of the surfaces and of the solutions used (pH, ionic strength, protein concentration).[56,57]

(56) Hook, F.; Rodahl, M.; Brzezinski, P.; Kasemo, B. *Langmuir* **1998**, *14*, 729−734.

(57) Hook, F.; Rodahl, M.; Kasemo, B.; Brzezinski, P. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12271−12276.

(58) The reaction was carried out in pH 10 sodium carbonate or pH 9 sodium borate buffers with acetic anhydride (0.3 mM).

Table 1. The Percentages of Monolayers of Fibrinogen (% Fib) and Lysozyme (% Lys) That Adsorbed to Polymers (Scheme 1) Grafted on SAMs[a]

			% ML					
			unfunctionalized[b]		$ClCOCH_3$[c]		$ClCOCH_2(EG)_2OCH_3$[c]	
entry	polymer	MW (kDa)	Fib	Lys	Fib	Lys	Fib	Lys
1	LPEI	0.23	79	15[d]	2.8	0.4	< 0.2[h]	0.2
2	LPEI	0.42			5.9[e]	2.0[e]	0.4	< 0.2[h]
3	LPEI	25	87	19	1.9	3.3[e]	0.4	0.5[e]
4	LPEI	100			0.9	1.1	0.6	0.3[e]
5	LPEI	250			0.4	0.5	0.3	0.3
6	BPEI	0.8			1.5	0.5	1.0[e]	< 0.2[h]
7	BPEI	25	82	27	2.2	0.9	0.2	0.3
8	BPEI	750			1.1	0.3	0.3	< 0.2[h]
9	PMVA	~200	117[f]	18	0.5	0.3	0.3	0.4
10	PAA	70	99[g]	88	30[g]	7.2[g]	4.5[e]	2.3
11	PLYS	25	91[g]	29	71[g]	50[d]	0.7[e]	0.3

[a] The listed polymers (Chart 1) were reacted with a SAM that presented interchain carboxylic anhydride groups and functionalized further with the acyl chlorides listed in the first row of the table. "Unfunctionalized" in the first row refers to polymers that were not reacted with acyl chlorides. The surfaces were exposed to a solution of protein (1 mg/mL) for 3 min and then to buffer for 10 min. The reported values are percentages of the amount of protein that adsorbed to a mixed SAM formed by the reaction of an anhydride SAM with $CH_3(CH_2)_{10}COCl$. [b] The standard deviation (SDEV) in all measurements in this section was ≤5, unless specified otherwise. [c] The standard deviation in all measurements in this section was ≤0.3, unless specified otherwise. [d] SDEV ≤ 17. [e] SDEV ≤ 1. [f] Some surfaces allow the adsorption of more protein than the hydrophobic standard used here; this happens rarely. [g] SDEV ≤ 8. [h] We cannot measure less than 0.2%.

Table 2. The Percentages of Monolayers of Proteins That Adsorbed to SAM/LPEI/COR[a]

		% ML	
entry	ClCOR	Fib[b]	Lys[b]
1	unacylated LPEI	87	19
2	$ClCO(CH_2)_{10}CH_3$	93	76[c]
3	$ClCO(CF_2)_3CF_3$	91	69
4	$ClCOCH_2OCH_3$	0.3	<0.2[d]
5	$ClCOCH_2(OCH_2CH_2)_1OCH_3$	0.3	0.3
6	$ClCOCH_2(OCH_2CH_2)_2OCH_3$	0.4	0.5[e]
7	$ClCOCH_2(OCH_2CH_2)_3OCH_3$	0.2	0.2
8	$ClCOCH_2(OCH_2CH_2)_{5-7}OCH_3$	<0.2[d]	0.2
9	PEG-MA (14 kDa)[f]	<0.2[d]	0.2

[a] LPEI (25 kDa) was reacted with an anhydride SAM and then functionalized with the listed acyl chlorides (ClCOR). The determinations of the percentages of adsorbed proteins and the experimental protocol used to measure the adsorption of protein are described in the footnotes to Table 1 and in the text. [b] The standard deviation (SDEV) for entries 1−3 was ≤4. SDEV for entries 4−9 was <0.3, unless specified otherwise. [c] SDEV = 11. [d] We cannot measure less than 0.2%. [e] SDEV = 0.7. [f] The anhydride groups on the copolymer reacted with the free amines on LPEI.

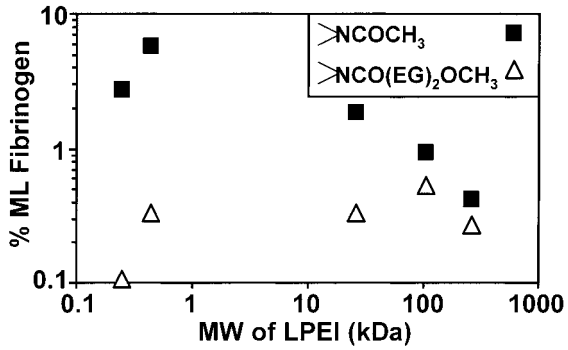


Figure 3. Dependence of the adsorption of fibrinogen on the molecular weight of LPEI used to form thin films. LPEI was grafted to a SAM terminated with anhydride groups (Figure 1) and it was then acylated with $ClCOCH_3$ (■) or $ClCOCH_2(OCH_2CH_2)_2OCH_3$ (△). Each point represents the average of two measurements performed on the same day. Values of % ML measured on different days varied by 0.5.

water on films of BPEI acylated with $CH_3(CH_2)_{15}COCl$ and $CF_3(CF_2)_7COCl$ are comparable to those measured on SAMs of hexadecanethiolate.[49−51,59]

Influence of the Structure of the Polymer on the Adsorption of Protein. *Molecular Weight*. The amount of protein that adsorbed to surfaces formed with LPEI/$COCH_3$ decreased with increasing molecular weight of the polymer (Figure 3); the results obtained with BPEI/$COCH_3$ are generally consistent with that trend. One of the possible explanations for this observation is that the larger polymers are likely to cover both surface heterogeneities and the CO_2^-/CO_2H groups of the SAM more effectively than their lower molecular weight variants. The amount of protein that adsorbed to surfaces coated with SAM/LPEI/$COCH_2(EG)_2OCH_3$ was independent of the molecular weight of the polymer; the amount that adsorbed was, however, so small that we would not have been able to identify slight trends.

Hydrogen Bond Donor Ability. The functionalized polymer films that best resisted the adsorption of protein were obtained by grafting thin films of polymers composed strictly of secondary amine groups (LPEI, PMVA) to the anhydride-terminated SAM and allowing these groups to react with acid chlorides ClCOR (R = CH_3, $CH_2(EG)_{n\geq 2}OCH_3$). On the basis of previous work, we believe that the elimination of hydrogen bond donor groups from the films formed by reaction of the amine groups with an acyl chloride may contribute to the resistance of these materials to the adsorption of protein.

The acetylation of films of LPEI, BPEI, and PMVA transformed hydrogen bond donor groups (RR′NH) into groups that were exclusively hydrogen bond acceptors ($R'RNCOCH_3$) and resulted in surfaces that were more effective in resisting the adsorption of proteins than the non-acetylated thin films. Acetylation of primary amino groups on films of PLYS and PAA generated secondary amido groups (HNCOR) that still had hydrogen bond donor groups; these surfaces still adsorbed proteins to a significant extent (Table 1).[43,44]

Although the amount of protein that adsorbed to polyamines acylated with acetyl chloride depended on the structure of the polymer, the amount that adsorbed on polyamines acylated with $ClCOCH_2(EG)_2OCH_3$ did not. The oligo(ethylene glycol) derivatives probably screened the hydrogen bond donor and charged groups more

(59) Yan, L.; Marzolin, C.; Terfort, A.; Whitesides, G. M. *Langmuir* **1997**, *13*, 6704−6712.

effectively than the acetyl amide groups, in part because of their larger size.

Adhesion of *Staphylococcus epidermidis* and *Staphylococcus aureus* to Mixed SAMs Terminating in Grafted Films of LPEI/COR. *Staphylococcus epidermidis* and *Staphylococcus aureus* cause 30−50% of the infections associated with in-dwelling devices. These strains adhere to the surfaces of host cells and artificial materials by a layer that contains both proteins and polysaccharides that are secreted by the bacteria; these molecules are recognized as bacterial adhesins.[10,11] We used an assay that determined the number of viable bacteria that adhered to the surface films after incubating the substrates in contact with a suspension of bacteria for 30 min followed by washing to remove unbound organisms. The adhering bacteria were removed from the surface of the substrate by sonication; the resulting suspension was diluted and plated onto agar plates followed by overnight incubation at 37 °C. The density of colony-forming units (cfu/mL) found in the solution obtained after sonication of the substrates was determined by counting the number of bacterial colonies on each plate; we assume that this number is monotonically related to the number of bacteria that adhered to each substrate. Sonication left some bacteria on the surfaces, especially on the bare gold control; thus, the control numbers are artificially low.

The smallest number of bacteria adhered to mixed SAMs presenting LPEI/$CO(EG)_2OCH_3$; we measured values that were $\sim 10^3$ lower than those obtained on bare gold and $\sim 10^2$ lower than those obtained on a SAM that presented $(EG)_3OCH_3$ groups (Figure 4).[60]

Adhesion of *E. coli* to Mixed SAMs Presenting LPEI−COR. The strain of *E. coli* used was isolated from a patient with urinary tract infections. An "imprint" assay measured the number of colony-forming units (cfu's) that adhered to test substrates. This assay was performed by contacting substrates covered with bacteria to agarose-coated plates, transferring bacteria from the gold substrates to agar plates, and counting cfu's after incubation (Figure 5). The number of bacteria that adhered to the bare gold surface, however, was too high to allow accurate counting, and the reported value represents a lower limit.

The number of attached, viable bacteria was lowest for SAMs functionalized with LPEI/$COCH_2(EG)_2OCH_3$ (Figure 5). Using LPEI/$COCH_3$ provided an improvement of 2 orders of magnitude over bare gold and 1 order of magnitude over commercially available PU. This coating performed as well as a SAM terminated with $(EG)_6OH$ groups (Figure 5) but not as well as SAM/LPEI/$COCH_2$-$(EG)_2OCH_3$.

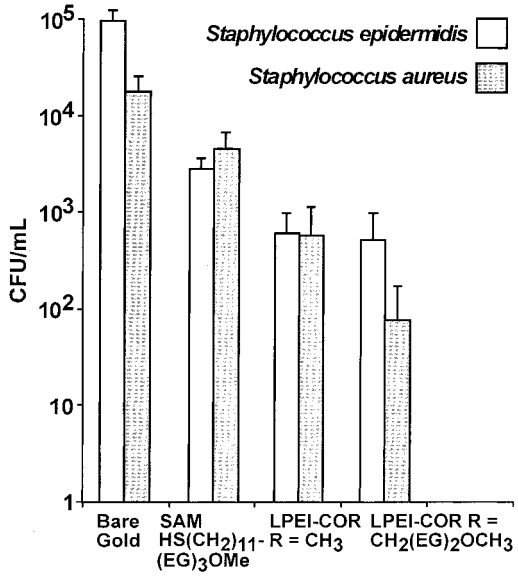


Figure 4. Adhesion of *Staphyloccus epidermidis* and *Staphylococcus aureus* to bare gold, a pure SAM of $HS(CH_2)_{11}EG_3OCH_3$, and mixed SAMs that present LPEI−$COCH_3$ and LPEI−$COCH_2(OCH_2CH_2)_2OCH_3$ (100 kDa). The quantity plotted on the *y* axis represents the number of bacteria (colony-forming units, cfu) that bound to each substrate. The error bars are the standard deviation of the measurements that were performed in triplicate. The experiments were performed on four separate days with similar results; for clarity, we only plot one set of results.

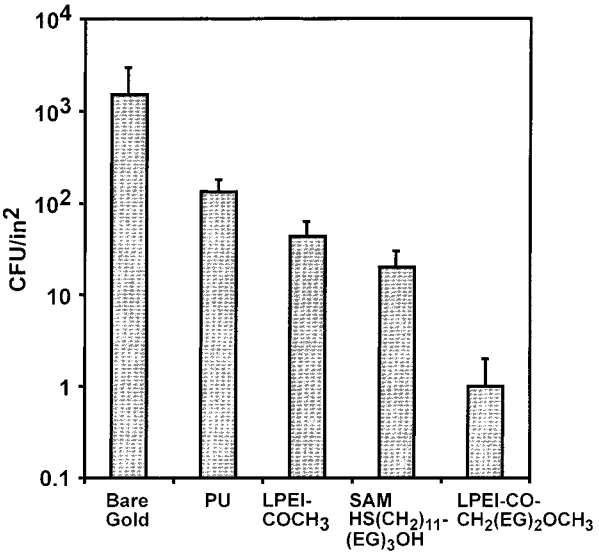


Figure 5. Adhesion of *E. coli* to bare gold, medical grade poly(urethane), a pure SAM of $HS(CH_2)_{11}EG_6OH$, and mixed SAMs that present LPEI−$COCH_3$ and LPEI−$COCH_2(OCH_2CH_2)_2$-OCH_3 (100 kDa). We plot the number of colony forming units per square inch. The value plotted for bare gold represents a lower limit; the number of bacteria that adhered was too high to be determined accurately. The error bars are the standard deviation of the measurements. The experiments were performed five times using four different batches of bacteria; the recorded values of cfu's/in^2 were averaged to generate the plots.

Conclusions

The most highly developed approaches to the rational engineering design of inert surfaces have relied on presenting low densities of high molecular weight poly(ethylene glycol) at surfaces. Here, a thin film of poly(ethylenimine) is grafted onto a SAM and then converted to an inert form by acylation with acyl chlorides (including those that allow incorporation of $(EG)_n$ groups). These studies demonstrate that some of the structural principles useful for designing SAMs that resist the adsorption of proteins[43,44] can be extended to thin polymer films grafted on the surface of SAMs. As in earlier studies,[43,44] the polymeric films that are successful in rendering a surface resistant to the adsorption of proteins are composed of

(60) It is possible that the polymeric coatings are better than SAMs at covering heterogeneities on the surfaces and that such defects have a more marked effect on the adhesion of bacteria than they do on the adsorption of proteins. This suggestion is strengthened by the observation that one hundred times more bacteria adhered to a SAM that presented $(EG)_3OCH_3$ groups than to a mixed SAM functionalized with LPEI/$COCH_2(EG)_2OCH_3$. Alkanethiols form a layer on gold that is one molecule thick; the presence of a defect such as a step in the surface would expose hydrophobic faces of the molecule that may cause protein adsorption and bacterial adhesion. Our polymeric coatings, on the other hand, cover rough features with a thick ($\leq$3 nm) amorphous layer and are, we presume, relatively insensitive to nanometer-scale imperfections or heterogeneities in the surfaces.

groups that are (i) hydrophilic and (ii) hydrogen bond acceptors but (iii) *not* hydrogen bond donors.

LPEI/$COCH_2(EG)_2OCH_3$ and LPEI/$COCH_3$ resist the adsorption of proteins from solution almost as effectively as single-component SAMs that present oligo(ethylene glycol) derivatives. Some of the most protein-resistant films (LPEI/$COCH_3$, PMVA/$COCH_3$) were obtained *without* using derivatives of ethylene glycol.

SAM/LPEI/$COCH_2(EG)_2OCH_3$ and SAM/LPEI/$COCH_3$ resisted the adhesion of bacteria in a manner comparable, or superior, to SAMs whose surface groups are $(EG)_nOR$ ($n = 3, 6$; R = H, CH_3). The number of bacteria that adhered to these films (relative to the control surfaces of SAMs terminating in $(EG)_nOR$ groups) depended on the strain of bacteria being examined. Although the mechanism of bacterial adhesion to surfaces depends on the strain, the results that we obtained with *E. coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* suggest that these films may show broad resistance to the attachment of bacteria.

The strategy described here has the advantages that the thin grafted films can be made from materials that are commercially available or from compounds that are simple to synthesize and that the thin films coat or cover heterogeneities on the surface of the substrate. The major disadvantage of this strategy is that it requires three synthetic steps that must occur *on* the surface of the material in its fabricated form: (i) chemical activation of surface functional groups, (ii) covalent immobilization of LPEI, BPEI, or PMVA, and (iii) acylation of the amino groups of the polymer with acyl chlorides. With this method, a device can be rendered inert only *after* its fabrication. Although inconvenient in some ways, the fact that the three separate chemical reactions are required, and that each can use a number of reagents with different structures, make the overall process a flexible one, and allow the introduction of different chemical functionalities at every step. This strategy is, thus, very well-suited for exploring structure−property relationships of thin polymer films that resist the adsorption of proteins and the adhesion of bacteria.

Materials and Methods

Materials. All chemicals were reagent grade unless stated otherwise. Fibrinogen (from bovine plasma, F8630), lysozyme (egg white, E.C. 3.2.1.17, L6876), and sodium dodecyl sulfate were purchased from Sigma (St. Louis, MO). Anhydrous toluene, anhydrous *N*-methyl-2-pyrrolidinone (NMP), 16-mercaptohexadecanoic acid, trifluoroacetic acid (TFA), trifluoroacetic anhydride (TFAA), acetyl chloride, methoxyacetyl chloride, lauroyl chloride, heptafluorobutyryl chloride, bromoacetic acid *tert*-butyl ester, triethylene glycol monomethyl ether, poly(ethylene glycol) monomethyl ether ($M_w = 350$), tetrabutylammonium bromide, 2-(2-methoxyethoxy)acetic acid, 2-[2-(2-methoxyethoxy)ethoxy]-acetic acid, 4-(dimethylamino)pyridine, linear poly(ethylenimine) (LPEI, pentaethylenehexamine $M_w = 232$), LPEI ($M_w \approx 423$), poly(2-ethyl-oxazoline) (PEOX, $M_w \approx 500\,000$ and $M_w \approx 200\,000$), poly(allylamine) (PAA, $M_w \approx 70\,000$), and poly(L-lysine) (PLYS, $M_w \approx 25\,000$) were purchased from Aldrich (Milwaukee, WI). Poly(*N*-methylvinylamine) (PMVA, $M_w \approx 25\,000$) and LPEI ($M_w \approx 25\,000$) were purchased from PolySciences, Inc. (Warrington, PA). Anhydrous *N*,*N*-dimethylformamide (DMF), triethylamine, acetic anhydride, and ethylene glycol were purchased from EM Science (Gibbstown, NJ). Absolute ethanol was purchased from Pharmcoproducts (Brookfield, CT). 2-(2-Methoxyethoxy)acetic acid chloride was synthesized as previously described.[61] Phosphate-buffered saline (PBS: 10 mM phosphate, 138 mM NaCl, and 2.7 mM KCl) was freshly prepared in distilled, deionized water and filtered through 0.22 μm filters prior to use. The ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$ on a Bruker spectrometer. Chemical shifts are reported in parts per million referenced with respect to $CHCl_3 = 7.26$ ppm.

Preparation of SAMs. Gold films were prepared by e-beam evaporation as described previously and used within 3 weeks. SAMs were prepared by immersing the freshly e-beam evaporated gold substrates (24 × 50 mm glass cover slips) in a 2 mM solution of the appropriate alkanethiol in ethanol at room temperature overnight. These substrates were removed from the solution and rinsed with ethanol before being dried in a stream of nitrogen. Glass substrates that were used for SPR studies and for the measurement of the adhesion of *Staphylococcus aureus* and *Staphylococcus epidermidis* were coated with 1.5 nm of titanium and 38 nm of gold. The adhesion of *E. coli* was measured with glass substrates coated with 15 nm of Ti and 115 nm of gold; this was necessary to provide a transparent substrate. Recently, Biacore started selling glass substrates coated with bare gold for SPR studies.

Formation of Interchain Anhydrides.[59] SAMs were prepared by immersing gold substrates prepared as described above in a 2 mM solution of 16-mercaptohexadecanoic acid in a mixture of ethanol, water, and acetic acid (85:10:5 v/v) at room temperature overnight.[40] The substrates were removed from the solution, thoroughly rinsed with ethanol, and dried in a stream of nitrogen. The cleaned substrates were placed in a freshly prepared solution of 0.1 M trifluoroacetic anhydride (TFAA) and 0.2 M triethylamine in anhydrous DMF without stirring for 20 min at room temperature. The substrates were removed from the TFAA solution, rinsed with CH_2Cl_2, and dried in a stream of nitrogen.[59]

Grafting Polymers on SAMs and Functionalization.[50] SAMs with terminal interchain carboxylic anhydride groups were used immediately by covering them with the appropriate solution of polymeric amine for 20 min. Solutions of polymers were 0.5% (w/v); LPEI polymers and PMVA were dissolved in NMP, BPEI polymers were dissolved in isopropyl alcohol, and PAA and PLYS were dissolved in aqueous sodium phosphate buffer at pH = 10. We were careful not to contact the solution of polymer with the unfunctionalized side of the glass support. Contamination of the back of the substrate with polymer interfered with the optical interface of the SPR instrument. The substrates were then rinsed thoroughly with ethanol, dried in a stream of nitrogen, and immersed in a solution of RCH_2COCl (0.3 mM), triethylamine (0.3 mM), and 4-(dimethylamino)pyridine (0.01 mM) in CH_2Cl_2 at 35 °C for 1 h. The substrates were removed from the solution of acid chloride, rinsed thoroughly with ethanol, and dried in a stream of nitrogen. PEG-MA was grafted to surface-bound LPEI from an aqueous solution (1% w/w) for 30 min.

Surface Plasmon Resonance Spectroscopy. We used a Biacore 1000 SPR instrument. The substrates to be analyzed were mounted on a modified SPR cartridge as described previously.[54] The adsorption of proteins to SAMs was measured by (i) flowing a solution of sodium dodecyl sulfate (40 mM in PBS) over the surface of the SAM for 3 min followed by rinsing the surface with a solution of PBS buffer for 10 min or (ii) flowing PBS buffer for 2 min, then substituting the flow with a solution of protein (1 mg/mL in PBS) for 30 min, and finally injecting PBS buffer for an additional 10 min, Figure 2. The flow rate used for all experiments was 10 μL/min.

In Vitro Adhesion Model for *Staphylococcus epidermidis* and *Staphylococcus aureus*. Functionalized gold substrates on 18 mm^2 glass coverslips (and bare gold) were rinsed in 100% ethanol immediately before use and placed in sterile 100 × 15 mm polystyrene dishes (Fisher). An inoculum of either *Staphylococcus epidermidis* M187 or *Staphylococcus aureus* MN8M (100 μL of a 2.5×10^8 bacteria/mL suspension) was added to the Petri dish, and the sample was incubated at 37 °C for 30 min. The suspension was spread over the entire surface of the gold substrates with a sterile pipet tip. The gold-coated substrates were removed from the medium, washed five times in sterile PBS, and sonicated for 5 s in 10 mL of trypticase soy broth (TSB) containing 0.05% Tween. The resulting suspension was diluted (10-fold or 0-fold) before being placed on agar plates at 37 °C overnight. The number of colonies was counted to determine the density of colony-forming units (cfu's) in the suspension obtained from sonicating the gold-coated samples. Each sample surface

(61) Asakawa, M.; Brown, C. L.; Menzer, S.; Raymo, F. M.; Stoddart, J. F.; Williams, D. J. *J. Am. Chem. Soc.* **1997**, *119*, 2614−2627.

was tested in triplicate, with the bare gold surfaces done in quadruplicate; the experiment was repeated on three different days.

In Vitro Adhesion Model for *E. coli*. The particular strain of *E. coli* utilized was a clinical strain of *E. coli* (RB128, Type I pili) initially isolated from a patient suffering from urinary tract infections. Bacteria were grown in tryptic soy broth to mid-log growth phase, which corresponded to an optical density of approximately 0.3 at 650 nm. Bacteria were washed three times using phosphate-buffered saline (PBS) solution (pH = 7.4). During each washing step, 10 mL of PBS buffer was used to suspend bacteria in the test tube, followed by centrifugation at 3000 rpm for 10 min. The gold-coated substrates were placed vertically in a dish containing 200 mL of PBS buffer solution. The appropriate amount of the suspension of bacteria was added to the solution to adjust the bacterial concentration to 1×10^5 bacteria/mL. The dish containing the substrates and the suspension of bacteria was placed in a shaker with the water bath maintained at 37 °C. After 1 h, the substrates were removed from the suspension of bacteria and washed using PBS buffer solution (3×200 mL). After excess solution was allowed to drain off each test substrate, it was contacted to Petri dishes that had previously been covered with a 2−3 mm layer of tryptic soy agar gel (Gibco). The substrate remained in contact with the gel for 20−30 s and was then lifted from the gel using sterile tweezers. The number of colony-forming units (cfu's) was counted after incubating the gel overnight at 37 °C. The results are averages from five experiments using four different batches of *E. coli*. Polyurethane (PU, Tecoflex, Thermedics Inc.) was used as a control.

Syntheses. Preparation of LPEI. LPEI ($M_w \approx 250\,000$ and $M_w \approx 100\,000$) was prepared by the acid hydrolysis of PEOX ($M_w \approx 500\,000$ and $M_w \approx 200\,000$) with sulfuric acid followed by neutralization with NaOH and recrystallization from deionized water according to the procedure of Warakomski et al.[62−64] The ^{1}H NMR and ^{13}C NMR spectra both showed only one signal. This observation suggested that the LPEI was obtained from the complete hydrolysis of the PEOX.

Procedure 1: 2-[2-(2-Methoxyethoxy)ethoxy]acetic Acid Chloride (1) ($CH_3(OCH_2CH_2)_2CH_2OCH_2COCl$). Oxalyl chloride (100 g, 0.78 mol) was added dropwise over 1 h to a solution of 2-[2-(2-methoxyethoxy)ethoxy]acetic acid (90 mL, 0.59 mol) and pyridine (0.1 mL, 1 mmol) in anhydrous toluene (250 mL), and the resulting solution was stirred at ambient temperature for 48 h. The reaction solution was concentrated in vacuo to afford $CH_3(OCH_2CH_2)_2CH_2OCH_2COCl$ as a slightly yellow oil (120 g, 96%). This compound was used without any further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.18 (s, 3H), 3.35 (m, 2H), 3.42 (m, 2H), 3.48 (m, 2H), 3.58 (m, 2H), 4.34 (s, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 58.36, 69.96, 70.21, 70.69, 71.27, 76.07, 171.54. HRMS-CI: *m/z* 214.0837 ($[M + NH_4]^+$, calcd for $C_7H_{13}O_4ClNH_4$ 214.0846).

Procedure 2: {2-[2-(2-Methoxyethoxy)ethoxy]ethoxy}acetic Acid *tert*-Butyl Ester (2a) ($CH_3(OCH_2CH_2)_3CH_2OCH_2COOC(CH_3)_3$). Bromoacetic acid *tert*-butyl ester (20 mL, 0.14 mol) was added over a 10-min period to a rapidly stirred mixture of triethylene glycol monomethyl ether (20 mL, 0.13 mol), tetrabutylammonium bromide (20 g, 0.062 mol), toluene (100 mL), and KOH (50% w/w, 100 mL), and the resulting heterogeneous reaction mixture was stirred at 0 °C for 10 min. The reaction mixture was warmed to ambient temperature and stirred for 1 h. Water (100 mL) was added, and the organic layer was separated. The organic layer was washed with saturated aqueous NH_4Cl (2×100 mL), saturated aqueous $NaHCO_3$ (100 mL), and brine (100 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo, and the residue was loaded onto a silica gel gravity column (200 g) and eluted with ethyl acetate to afford $CH_3(OCH_2CH_2)_3CH_2OCH_2CO_2C(CH_3)_3$ as a colorless oil (420 g, 12%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.46 (s, 9H), 3.37 (s, 3H), 3.54 (m, 2H), 3.63−3.72 (m, 10H), 4.01 (s, 2H). HRMS−FAB: *m/z* 301.1620 ($[M + Na]^+$, calcd for $C_{13}H_{26}O_6Na$ 301.1628).

{2-[2-(2-Methoxyethoxy)ethoxy]ethoxy}acetic acid (2b) ($CH_3(OCH_2CH_2)_3CH_2OCH_2COOH$) was prepared according to procedure 3. TFA (6 mL) was added to a solution of {2-[2-(2-methoxyethoxy)ethoxy]ethoxy}acetic acid *tert*-butyl ester (4.2 g, 15 mmol) in CH_2Cl_2 (24 mL), and the resulting solution was stirred for 3 h at ambient temperature. The solution was concentrated in vacuo to afford $CH_3(OCH_2CH_2)_3CH_2OCH_2CO_2H$ (3.3 g, 99%) as a slightly yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.45 (s, 3H), 3.69 (m, 10H), 3.76 (m, 2H), 4.22 (s, 2H). HRMS−FAB: *m/z* 245.1013 ($[M + Na]^+$, calcd for $C_9H_{18}O_6Na$ 245.1001).

{2-[2-(2-Methoxyethoxy)ethoxy]ethoxy}acetic acid chloride (2c) ($CH_3(OCH_2CH_2)_3CH_2OCH_2COCl$) was prepared according to procedure 1: oxalyl chloride (3.6 mL, 0.041 mol), {2-[2-(2-methoxyethoxy)ethoxy]ethoxy}acetic acid (3.3 g, 0.15 mol), pyridine (0.1 mL, 1 mmol), and anhydrous toluene (10 mL) to give $CH_3(OCH_2CH_2)_3CH_2OCH_2COCl$ as yellow oil (3.5 g, 99%). This compound was used without any further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.35 (s, 3H), 3.53 (m, 2H), 3.60−3.66 (m, 8H), 3.75 (m, 2H), 4.48 (s, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 58.98, 70.48, 70.58, 70.70, 71.25, 71.87, 76.61, 172.03. HRMS-CI(NH_3), MS (CI, NH_3) *m/z* (rel intensity) 239 (($[M - Cl + NH_2 + NH_4]^+$, 100) calcd for $C_9H_{19}NO_5NH_4$ 239).

Poly(ethylene glycol) monomethyl ether acetic acid *tert*-butyl ester (3a) ($CH_3(OCH_2CH_2)_{5-7}CH_2OCH_2COOC(CH_3)_3$) was prepared according to procedure 2: bromoacetic acid *tert*-butyl ester (20 mL, 0.14 mol), poly(ethylene glycol) monomethyl ether (20 mL, 0.13 mol, $M_n = 350$), tetrabutylammonium bromide (20 g, 0.062 mol), toluene (100 mL), and KOH (50% w/w, 100 mL) to give $CH_3(OCH_2CH_2)_nCH_2OCH_2CO_2C(CH_3)_3$ ($n = 5-7$) as a colorless oil (10 g, 40%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.46 (s, 9H), 3.33 (s, 3H), 3.53 (m, 2H), 3.64 (m, 20H), 3.58 (m, 2H), 4.00 (s, 2H). MS (FAB) *m/z* (rel intensity) 389 (($[M + Na, (n = 5)]^+$, 40), 433 ($[M + Na, (n = 6)]^+$, 100), 477 ($[M + Na, (n = 7)]^+$, 70), calcd for $C_{19}H_{38}O_9Na$ 433).

Poly(ethylene glycol) monomethyl ether acetic acid (3b) ($CH_3(OCH_2CH_2)_{5-7}CH_2OCH_2COOH$) was prepared according to procedure 3: TFA (10 mL), $CH_3(OCH_2CH_2)_nCH_2OCH_2CO_2C(CH_3)_3$ ($n = 5-7$) (10 g, 24 mmol), and CH_2Cl_2 (30 mL) to give $CH_3(OCH_2CH_2)_nCH_2OCH_2CO_2H$ (8.8 g, 99%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.43 (s, 3H), 3.66 (m, 22H), 3.75 (m, 2H), 4.20 (s, 2H). MS (FAB) *m/z* (rel intensity) 333 (($[M + Na, (n = 5)]^+$, 50), 377 ($[M + Na, (n = 6)]^+$, 100), 421 ($[M + Na, (n = 7)]^+$, 60), calcd for $C_{15}H_{30}O_9Na$ 377).

Poly(ethylene glycol) monomethyl ether acetic acid chloride (3c) ($CH_3(OCH_2CH_2)_{5-7}CH_2OCH_2COCl$) was prepared according to procedure 1: oxalyl chloride (3.6 mL, 0.041 mol), $CH_3(OCH_2CH_2)_nCH_2OCH_2CO_2H$ ($n = 5-7$) (8.8 g, 0.24 mol), pyridine (0.1 mL, 1 mmol), and anhydrous toluene (20 mL) to give $CH_3(OCH_2CH_2)_nCH_2OCH_2COCl$ as a yellow oil (9.0 g, 99%). This compound was used without any further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.35 (s, 3H), 3.53 (m, 2H), 3.61−3.66 (m, 20H), 3.75 (m, 2H), 4.48 (s, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 58.98, 70.47, 70.53, 70.56, 70.59, 70.70, 71.27, 71.89, 76.61, 172.03. MS (CI, NH_3) *m/z* (rel intensity) 283 (($[M - Cl + NH_2 + NH_4]^+$, 70, ($n = 4$)), 387 ($[M - Cl + NH_2 + NH_4]^+$, 100, ($n = 5$)), 371 ($[M - Cl + NH_2 + NH_4]^+$, 50, ($n = 6$)), 415 ($[M - Cl + NH_2 + NH_4]^+$, 25 ($n = 7$)), calcd for $C_{15}H_{31}NO_8NH_4$ 371).

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