Characterization of poly(ethylene oxide) brushes on glass surfaces and adhesion of *Staphylococcus epidermidis* *

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**Abstract**—Poly(ethylene oxide) brushes have been covalently bound to glass surfaces and their presence was demonstrated by an increase in water contact angles from fully wettable on glass to advancing contact angles of 54°, with a hysteresis of 32°. In addition, electrophoretic mobilities of glass and brush-coated glass were determined using streaming potential measurements. The dependence of the electrophoretic mobilities on the ionic strength was analyzed in terms of a soft-layer model, yielding an electrophoretic softness and fixed charge density of the layer. Brush-coated glass could be distinguished from glass by a 2–3-fold decrease in fixed charge density, while both surfaces were about equally soft. Adhesion of *Staphylococcus epidermidis* HBH276 to glass in a parallel plate flow chamber was extremely high and after 4 h, $1.9 \times 10^6$ bacteria were adhering per cm$^2$. In contrast, the organisms did not adhere to brush-coated glass, with numbers below the detection limit, i.e. $0.1 \times 10^6$ per cm$^2$. These results attest to the great potential of polymer brushes in preventing bacterial adhesion to surfaces.

**Key words:** Polymer brushes; poly(ethylene oxide); electrophoretic mobility; *Staphylococcus epidermidis*; bacterial adhesion.

**INTRODUCTION**

Biomaterials are widely used in modern medicine or the production of artificial organs and in a variety of intra- and extra-corporeal prostheses. However, their application can give rise to biomaterial-centered infections (BCIs) that defy treatment.
with antibiotics. Therefore, the only effective remedy appears to be the removal of the infected device [1]. The development of biofilms causing BCIs occurs in several steps: firstly, a substratum surface is covered with a conditioning film of surface-active molecules, in particular proteins. Secondly, bacteria are transported towards the substratum and adhere. Initial adhesion is through long-range interactions, acting over distances up to a few tens of nanometers between the bacterium and the substratum, after which short-range interactions acting on a sub-nanometer scale become operative.

The long-range interactions in bacterial adhesion may be calculated by applying concepts from colloid and interface science [2]. Thus, the DLVO theory, originally formulated to describe the adhesion of inanimate colloidal particles, has been applied to bacterial adhesion with varying success. According to the DLVO theory, the overall bacterium–substratum interaction is governed by contributions from Lifshitz–Van der Waals forces and forces resulting from overlapping electrical double layers. The description of short-range interactions (e.g., hydrogen bonding, ion pairing, hydrophobic interaction) depends on detailed knowledge of the chemistry for each surface involved and such information is usually not available for biological surfaces.

Two strategies may be followed to reduce the risk of BCIs and to prevent or delay the adhesion of infectious bacteria to the substratum surface:

(i) modifying the surface with (charged) groups that make the surface less attractive for the bacteria [3]; and

(ii) introducing steric hindrance that keeps the bacteria at a distance from the surface where long-range attractive interaction forces are reduced to an ineffective magnitude [4, 5].

Steric hindrance may be achieved by decorating the surface with polymer molecules that are attached through an anchor to the surface, whereas the other part (the buoy) is moving freely in the surrounding medium. When the density of the polymer is high enough, the polymer molecules are forced to stretch out and the resulting layer is called a ‘molecular brush’ (see Fig. 1). The brush is essentially penetrable for solvent and low-molecular-weight ions, but depending on its packing density $\sigma$ and extension $L_0$, may prevent the deposition of larger components such as protein molecules and bacteria [6].

Figure 1. Schematic representation of polymer brush molecules. Upon increasing the grafting density, the polymer molecules are forced to stretch out.
So far, most of the research, both experimental and theoretical, on biomedical applications of molecular brushes has focused on the prevention of protein adsorption using poly(ethylene oxide) (PEO) as the polymer material. Work on bacterial adhesion includes the experiments by Park et al. [7], who grafted PEO molecules with different lengths and end-groups to a polyurethane surface, and Vacheethasanee and Marchant [5], who modified polyethylene and pyrolytic graphite substrata with a surfactant consisting of a poly(vinyl amine) backbone with grafted PEO and hexanal. In both experiments, surface modification with PEO molecules of sufficient length and grafting density was effective in preventing bacterial adhesion.

Several theoretical models exist to predict the interaction of a tethered layer with incoming particles as a function of its parameters, such as the grafting density and polymer chain length [8–11]. Jeon et al. [8, 9] assumed that the polymer chain is uniformly stretched with the end points located at the layer–solvent boundary. This corresponds to long and densely grafted polymer molecules. Szleifer [10] has pointed out that these assumptions do not hold in many practical situations. He has developed a model based on a self-consistent mean field approach. In this approach, interactions such as the intramolecular interactions between the monomer elements and the interactions between the brush polymer and the protein are taken into account. The model has been worked out for a PEO–lysozyme system. It was found that protein adsorption depends mainly on the grafting density, whereas the thickness of the grafted layer influences the kinetics of the adsorption process. It is doubtful whether this conclusion also holds for bacterial adhesion. Proteins are small enough to reach the substratum by diffusing through the layer. Bacteria, however, can adhere only by compressing the brush layer.

Halperin [11] adopted a simple model for the polymer molecule, which was described as a string of non-interacting monomer elements. According to this model, an incoming particle may penetrate the brush and adsorb in the (absolute) primary minimum at the substratum, or it may be trapped in the secondary minimum at the aqueous edge of the brush.

Various techniques, offering different degrees of control over the brush lengths versus densities, have been applied to attach the polymer molecules to a surface [12]. These techniques may be divided into non-covalent attachment, where the anchor has a high physico-chemical affinity for the substratum surface, and chemical grafting, where the anchor forms a chemical bond with surface groups. As the binding energy of a chemical bond exceeds that of a physical bond by approximately an order of magnitude, chemically grafted brushes are likely to be more robust. A technique that is often used for chemical grafting is the ‘grafting from’ method, where polymer molecules are grown from the substratum. This can be accomplished, for instance, by covering the surface with covalently linked initiators from which polymer chains grow in a solution containing the monomers, resulting in a polydisperse brush [13], or by using radio-frequency glow discharge plasma deposition to coat the surface with a thin, covalently bound polymer layer [14]. In the latter case, however, depending on the reaction conditions, the polymer mole-
Figure 2. Vinyl-terminated poly(ethylene oxide) covalently bound to a hydrated silica surface.

MATERIALS AND METHODS

Poly(ethylene oxide) (PEO) brushes

Methacryl-terminated poly(ethylene oxide) with a molar mass of 9800 Da, corresponding to approximately 250 monomer units, and a polydispersity index less than 1.03 was purchased from Polymer Source (Dorval, Quebec, Canada) and used as received. Microscope glass slides of size $76 \times 26 \times 1$ mm (Menzel-Gläser) were
used as a substratum surface. The slides were first sonicated in 2% RBS detergent (Omnilabo International, Breda, The Netherlands), rinsed in warm tap water and demineralized water, sonicated again in methanol, and rinsed in demineralized water, to remove oil contamination and fingerprints. Next, possible metallic oxides on the glass surface were removed by submersing the slides in hot (95°C) nitric acid (65%; Merck, Darmstadt, Germany) for 45 min. Finally, the slides were extensively rinsed with demineralized water and Millipore-Q water and dried in a heat box at 80°C for 5 h.

To graft the PEO chains on the glass surfaces, the slides were covered with a solution of the methacryl-terminated PEO in chloroform (4 mg/ml). The solvent was evaporated in a stream of nitrogen, after which the slides were annealed overnight in a vacuum at 145°C. Prior to experiments, excess material was removed by washing the slide with Millipore-Q water and the slides were dried in a stream of nitrogen.

Water contact angle measurements

Water contact angles were measured at room temperature with a home-made contour monitor using the sessile drop technique. Advancing and receding water contact angles were obtained by keeping the syringe needle in the water droplet (1–1.5 μl) after positioning it on the surface and by carefully moving the sample until the advancing angle was maximal. Contact angles with water droplets at rest will be referred to as equilibrium contact angles. On each sample, at least ten droplets were placed at different positions.

Streaming potential measurements

For a solid surface in contact with a liquid, streaming potentials $\Delta E_{str}$, arising from a forced flow of the liquid under the influence of a pressure $\Delta p$, depend on the electrokinetic potential $\zeta$ at the solid–liquid interface according to

$$\frac{\Delta E_{str}}{\Delta p} = \frac{\varepsilon \varepsilon_0}{\eta \kappa_{sp}} \zeta,$$

where $\varepsilon \varepsilon_0$ is the dielectric permittivity, $\eta$ is the viscosity and $\kappa_{sp}$ is the specific conductivity of the liquid. By comparison with the electrokinetic model of colloidal particles, the electrophoretic mobility $\mu$ for a flat surface is related to the electrokinetic potential $\zeta$ by [17]

$$\mu = \frac{\varepsilon \varepsilon_0}{\eta} \zeta.$$

Combining equations (1) and (2) yields the connection between $\mu$ and $\Delta E_{str}$:

$$\mu = \kappa_{sp} \frac{\Delta E_{str}}{\Delta p}.$$
The pressure dependence of the streaming potentials was measured employing a parallel plate flow chamber [18]. The walls of the flow chamber were brush-coated microscope slides (26 × 76 mm) separated by a 0.2 mm Teflon gasket, while two rectangular platinum electrodes (5.0 × 25.0 mm) were located at both ends of the parallel plate flow chamber. Streaming potentials were measured in KCl solutions with ionic strengths of 5, 10, 15, 25, 50, 75 and 100 mM at ten different pressures ranging from 50 to 200 hPa. Each pressure was applied for 10 s in both directions.

Ohshima et al. [19] have proposed a model for the electrophoretic mobility of particles with fixed surface charges distributed across an ion-penetrable, porous layer. It unites the theory of electrophoresis of coiled structures of highly solvated polyelectrolytes with that of rigid spheres where the surface charge is located in an infinitesimal thin layer at the surface. In the Ohshima model, the ion-penetrable layer is characterized by its fixed charge density $\rho$ and a parameter $1/\lambda$, referred to as the electrophoretic ‘softness’ of the ion-penetrable layer, which depends on the frictional force exerted on the water when it flows through the ion-penetrable layer. For planar surfaces, under the conditions that (a) the charge densities are relatively low, (b) $1/\lambda$ is less than the thickness over which the liquid flow penetrates the soft surface layer and (c) the Debye length, $\kappa^{-1}$, is less than the thickness of the ion-penetrable layer (all being fair assumptions for PEO-grafted surfaces and to a somewhat lesser extent also for glass having a porous, jelly surface in aqueous media with a wide range of ionic strengths), the electrophoretic mobility as a function of the reciprocal Debye length $\kappa$ is approximated by

$$\mu = \frac{\rho}{\eta \lambda^2} \left[ 1 + \left( \frac{\lambda}{\kappa} \right)^2 \frac{1 + \lambda/2 \kappa}{1 + \lambda/\kappa} \right]. \quad (4)$$

For symmetrical electrolytes, $\kappa$ is related to the ionic strength as

$$\kappa^2 = \frac{2F^2 z_i^2 c_i}{\varepsilon \varepsilon_0 RT}, \quad (5)$$

where $F$ is the Faraday constant, $T$ is the absolute temperature, $R$ is the gas constant, $z_i$ is the valency and $c_i$ is the concentration of ion $i$.

The most salient feature of equation (4) is the fact that in contrast to the rigid surface model, the electrophoretic mobility does not approach zero as the electrolyte concentration increases. A least-squares fit of electrophoretic mobilities measured as a function of the ionic strength to equation (4) allows the evaluation of the softness of the polymer layer and the space charge density in the soft part of the layer. This is based on the assumption that the values for $1/\lambda$ and $\rho$ are invariant with ionic strength. All electrophoretic mobilities and softness values reported are the mean values of three different measurements with separately prepared brushes.

**Bacterial strain and adhesion experiments**

*Staphylococcus epidermidis* HBH276 was cultured in tryptone soya broth (OXOID, Basingstoke, UK). First, a strain was streaked from a frozen stock and grown
overnight at 37°C on a blood agar plate. A colony was used to inoculate 5 ml of growth medium, which was incubated at 37°C in ambient air for 24 h and used to inoculate a second culture in 150 ml of growth medium that was grown for 17 h. The bacteria from the second culture were harvested by centrifugation (5 min, 5000 g) and washed twice with Millipore-Q water. Subsequently, the bacteria were resuspended in PBS (pH 6.8). The suspension was sonicated on ice (10 s) to disrupt aggregates. The concentration of bacteria was determined using a Bürker-Türk counting chamber and adjusted to $3 \times 10^8$ bacteria/ml.

Adhesion experiments were carried out using a flow chamber and an image analysis system. Figure 3 shows an exploded view of the flow chamber used. The Teflon gasket between the upper and the lower part of the flow chamber determines the dimensions of the flow channel ($175 \times 17 \times 0.75$ mm). The top and bottom collector plates have the dimensions of a common microscope slide: $76 \times 26 \times 1$ mm. The top slide is made out of glass. The bottom slide is covered with the surface under study and microbial adhesion can be directly observed using a phase-contrast microscope equipped with a 40× ultra-long working distance objective. A pulse free flow (0.0325 ml/s) was created by hydrostatic pressure and the suspension was recirculated using a peristaltic pump. By means of a valve system, the flasks containing buffer or bacterial suspension can be connected with the flow chamber.

Prior to an experiment, all air bubbles were removed from the tubing and the flow chamber and the system was perfused for 60 min with buffer. Subsequently, flow was switched to the bacterial suspension. During deposition, images of the bottom plate were recorded using a 512 × 512 pixel CCD camera and the bacteria present on the surface were counted by dedicated image analysis software [20].

On the one hand, the lower detection limit of the system is determined by the number of CCD pixels per bacterial cell that are necessary for the automatic
counting of the bacteria, and on the other hand, by the statistical error in the counting of the bacteria that one is willing to accept. For the current system, a statistical error of 10% results in a lower detection limit of approximately \(0.1 \times 10^6\) bacteria per \(\text{cm}^2\).

The initial adhesion experiments were carried out for 4 h, with bacteria suspended in PBS. The initial increase in the number of adhering bacteria was linearly extrapolated to \(t = 0\) to obtain the initial bacterial deposition rate \(j_0\), which represents the number of bacteria transported by convection and diffusion towards the substratum surface that subsequently have been able to adhere.

**RESULTS AND DISCUSSION**

*Physico-chemical characterization of the polymer brush*

Cleaned glass, i.e. the glass surface prior to application of the polymer, was fully wettable with water, while after application of the polymer, the surface was more hydrophobic (see Table 1). The equilibrium water contact angle increased to 43° and the advancing and receding water contact angles were 54° and 22°, respectively. This contact angle hysteresis either suggests [21] partial coverage of the glass surface by the brush, or attests to the mobility of the polymer chains in the brush. The advancing angle and the hysteresis are somewhat higher than the values measured by Park et al. [7], who reported advancing and receding angles of 44.6° and 30.2° on a polyurethene surface covered PEO with a molecular mass of 1000 Da. Harder et al. [22] found advancing water contact angles of 30–35° for oligo(ethylene oxide). Our PEO-coated surfaces are probably more hydrophobic than coatings made of smaller molecules, because small molecules possess a relatively higher fraction of hydroxyl to carbon groups, creating a more hydrophilic coating.

<table>
<thead>
<tr>
<th>Water contact angles</th>
<th>Electrophoretic properties</th>
<th>Bacterial adhesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\theta_{w,\text{eq}}) (degrees)</td>
<td>(\rho) (10^6 \text{ C m}^{-3})</td>
<td>(j_0) (cm^{-2} s^{-1})</td>
</tr>
<tr>
<td>(\theta_{w,\text{adv}}) (degrees)</td>
<td>(\lambda^{-1}) (nm)</td>
<td>(n_{4h}) (10^6 cm^{-2})</td>
</tr>
<tr>
<td>(\theta_{w,\text{rec}}) (degrees)</td>
<td></td>
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</tr>
<tr>
<td>Glass Wetting 43 ± 3</td>
<td>−1.5 ± 0.3</td>
<td>1860 ± 120</td>
</tr>
<tr>
<td>Wetting 54 ± 4</td>
<td>3.3 ± 0.3</td>
<td>19.0 ± 3</td>
</tr>
<tr>
<td>Wetting 22 ± 4</td>
<td>−0.6 ± 0.03</td>
<td>bd</td>
</tr>
<tr>
<td>Brush</td>
<td>3.6 ± 0.1</td>
<td>bd</td>
</tr>
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\(\pm\) indicates the SD over three separately prepared substratum surfaces, while in the case of bacterial adhesion, also three separately grown cultures were employed. bd, below detection.
Electrophoretic mobilities

Figure 4 shows the electrophoretic mobilities of clean and brush-covered glass as a function of the ionic strength. Both surfaces have a finite electrophoretic softness, as can be inferred from the fact that both curves tend to a non-zero electrophoretic mobility at elevated ionic strength. The electrophoretic softness of glass was calculated to be 3.3 nm and that of the brush 3.6 nm (Table 1). The results indicate that glass has a water- and ion-penetrable surface, which is in line with earlier measurements reporting thicknesses of ion-penetrable layers on glass of about 0.7 nm [23], 1.9 nm [24] and 4 nm [25]. Surprisingly, the softness of the brush hardly differs from that of the glass surface, which may point to strong immobilization of water in the polymer matrix of the brush. The soft outer region of the brush has a much lower charge density ($\rho_{\text{brush}} = (-0.6 \pm 0.03) \times 10^6 \text{ C/m}^3$) than in the soft glass layer ($\rho_{\text{glass}} = (-1.5 \pm 0.3) \times 10^6 \text{ C/m}^3$). This result is expected because PEO is essentially uncharged.

The thickness $L_0$ of the brush layer can be estimated using the equation [11]

$$L_0 \approx a N \left( \sigma a^2 \right)^{1/3},$$  \hspace{1cm} (6)

where $a$ is the length of a monomer unit and $N$ is the number of monomer units.

The applicability of this equation to approximate the thickness of PEO brushes has recently been demonstrated by Efremova et al. [26]. As we used the same grafting

![Figure 4](image_url)

**Figure 4.** Electrophoretic mobilities of glass and brush-coated glass as a function of the ionic strength of a KCl solution. The lines indicate the best fit to equation (4), i.e. the Ohshima soft-layer model.
technique as and comparable materials to Maas et al. [16], we assume a similar grafting density. Adopting the grafting density that Maas et al. found for \( M_w = 20000 \) (i.e. \( \sigma^{-1} = 0.33 \text{ nm}^{-2} \)) and inserting \( N = 250 \) and \( a = 0.278 \text{ nm} \) [27], \( L_0 \) is calculated to be 21.6 nm. Hence, every polymer molecule in the brush occupies a volume of \( L_0/\sigma \) equal to 65 nm\(^3\). The molar volume of an EO segment is equal to 38.9 cm\(^3\) [28]. Therefore, the volume taken by the monomer segments of the PEO molecule is 16 nm\(^3\), corresponding to approximately 25\% of the total brush volume. This fraction is high enough to ensure that virtually every water molecule in the brush layer is located in the vicinity of a polymer molecule. Since ethylene oxide is known for its strong hydration, it is thus plausible that the water is strongly immobilized in the soft brush layer.

**Bacterial adhesion**

In Fig. 5, a representative example of the results of a bacterial adhesion experiment is given. From these plots one can determine the numbers of *S. epidermidis* adhering after 4 h, as well as the initial deposition rates \( j_0 \), which are calculated by linear regression of adhesion numbers during the first 30 min of deposition. Table 1 summarizes the results of the bacterial adhesion experiments quantitatively. The numbers of *S. epidermidis* adhering after 4 h to pristine glass are several orders of magnitude higher than to the brush-coated glass. In fact, the brush effectively decreases bacterial adhesion to below the detection limit (0.1 \( \times \) 10\(^6\) per cm\(^2\)) for direct counting in the parallel plate flow chamber. The results appear to be consistent with other experiments on bacterial adhesion to ‘brush-like’

![Graph](image)

**Figure 5.** Example of the deposition kinetics of *S. epidermidis* HBH276 to glass with (circles) and without (triangles) a PEO brush.
layers, although the experiments differ in surface modification techniques and/or experimental methods to assess the efficacy of the surface layer, making a fair comparison difficult. Hendricks et al. [14] tested a plasma-deposited PEO coating on polyetherurethane under flowing conditions and reported reductions of over 99% in the initial adhesion of *Pseudomonas aeruginosa* with respect to a control, as well as over 90% reductions after 18 h of growth. Park et al. [7] also reported reductions of 90% in the adhesion of *S. epidermidis* on a polyurethane surface with PEG-3.4 kDa modification after allowing the bacteria to grow for 24 h. Surprisingly, a PEG-1 kDa-modified substrata performed significantly worse for *S. epidermidis*.

Finally, in order to illustrate the effectiveness of the brush layer in reducing bacterial adhesion, Fig. 6 shows a micrograph of a partly brush-coated glass slide after 4 h of exposure to a bacterial suspension in the parallel plate flow chamber. Clearly, far less bacteria have adhered to the brush-covered surface than to the bare hydrophilic glass.

**CONCLUSIONS**

Based on this study, the following conclusions can be drawn:

(1) polymer brushes applied on a glass surface can be distinguished from untreated glass by an increased hydrophobicity and a decreased fixed charge density, as derived from an electrokinetic soft-layer model;
poly(ethylene oxide) brushes on a glass substratum strongly discourage the adhesion of an *S. epidermidis* strain. Hence, it can be anticipated that polymer brushes will constitute effective non-adhesive coatings for the control of BCIs.

REFERENCES
