The use of polymer brush coatings to prevent microbial adhesion
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Stability and effectiveness against bacterial adhesion of PEO coatings in biological fluids

Poly(ethylene oxide) (PEO) coatings have been shown to reduce the adhesion of different microbial strains and species and thus have been suggested to be promising as coatings to prevent biomaterial centered infection of medical implants. Clinically, however, PEO coatings are not yet applied, as little is known about their stability and effectiveness in biological fluids. In this study, PEO coatings coupled to a glass substratum through silyl ether bonds were exposed for different time intervals to saliva, urine or phosphate buffered saline (PBS) as a reference at 37°C. After exposure, the effectiveness of the coatings against bacterial adhesion was assessed in a parallel plate flow chamber. The coatings appeared effective against Staphylococcus epidermidis adhesion for 24, 48 and 0.5 h in PBS, urine and saliva, respectively. Using XPS and contact angle measurements, the variations in effectiveness could be attributed to conditioning film formation. The overall short stability results from hydrolysis of the coupling of the PEO chains to the substratum.

Introduction

Biofilm formation on biomaterial implant surfaces and subsequent infectious complications are a frequent reason for failure of many biomedical devices, such as total hip arthroplasties, indwelling voice prostheses and vascular or urinary catheters [1]. The development of a biofilm is initiated by the formation of a conditioning film of adsorbed macromolecules, such as proteins, followed by adhesion of microorganisms, growth and anchoring through secretion of extracellular polymeric substances [2]. A poly(ethylene oxide) (PEO) coating is considered a promising method to prevent biomaterial centered infections (BCI), as it can form a barrier between the surface and approaching microorganisms and proteins [3]. For plasma proteins like albumin, γ-globulin and fibrinogen, PEO coatings are highly effective in the prevention of their adsorption [4]. Also for many bacterial strains involved in BCI, like Staphylococcus epidermidis, Staphylococcus aureus and Escherichia coli [5] PEO coatings generally reduce adhesion by more than 90% [6-8].

For PEO coatings, both grafting density and chain length are of critical importance for its effectiveness in preventing protein adsorption [9-12] and bacterial adhesion [13]. When the density is low penetration of the PEO coating can occur or parts of the bare substratum can be revealed [3,14]. Furthermore theories describing PEO layer-particle interaction predict stronger particle repellency for longer PEO chains, that is with increasing thickness of the PEO layer at the surface [15-18]. The reason is that a thicker PEO layer implies a larger separation between the surface and the incoming particles and hence a stronger attenuation of the long range Lifshitz-Van der Waals attraction.

PEO coatings have been demonstrated to be stable under sterilization conditions [19] or for an extended exposure to phosphate buffer saline (PBS) [20]. However, none of these studies examined the stability of PEO coatings after prolonged exposure to physiological fluids, such as saliva or urine. In fact, a clear and versatile method is lacking to determine the stability of a PEO coating. Sophisticated techniques, like ellipsometry, are sometimes used to provide information on the PEO coating, but these are not applicable on all biomaterials and do not directly indicate the effectiveness in reducing protein adsorption or bacterial adhesion.
Therefore, the aim of the present study is to determine the stability of the PEO coating and their effectiveness in reducing bacterial adhesion after prolonged exposure to urine and saliva, as compared with PBS. *Staphylococcus epidermidis* HBH 276 was used as a probe bacterium, as it is known to show relatively high adhesion on bare glass and negligible adhesion on a PEO coating [6]. PEO coatings, prior to and after exposure to body fluids were examined by contact angles and X-ray photoelectron spectroscopy.

**Materials and methods**

**Preparation of poly(ethylene oxide) coatings.** PEO with a molecular weight of 9.8 kD was applied on microscope glass slides using the reaction of surface silanol groups, with vinyl terminated polymers in a polymer melt, leading to covalent silyl ether bonds, as described by Maas *et al.* [21]. Briefly, glass slides (Menzel-Gläser, Emergo, Landsmeer, The Netherlands) were first sonicated in 2% RBS 35 (Omnilabo International BV, Breda, The Netherlands), followed by sonication in methanol and submersed in hot (95°C) nitric acid 65% (Merck, Darmstadt, Germany). Next, the slides were rinsed with demineralized water and dried. To graft the PEO chains on the slides, slides were covered with a 0.4 mM solution of methacryl-terminated 9.8 kD PEO (Polymer Source Inc., Dorval, Quebec, Canada) in chloroform. The solvent was evaporated in a stream of nitrogen, after which surfaces were annealed overnight in vacuum at 145°C. Prior to experiments, excess material was removed by washing with demineralized water. Previous research has shown that this method leads to high surface grafting densities (0.6 chains nm⁻²), where, in an aqueous environment, the PEO chains are in a brush conformation [6].

All slides used were only partly grafted with PEO 9.8 kD chains, which allowed studying the bare glass surface and a PEO coated surface in one and the same experiment.

**Collection of biological fluids and exposure of the PEO coatings.** From at least 10 healthy volunteers of both sexes, human whole saliva was collected into ice-chilled cups. Saliva was stimulated by the volunteers chewing Parafilm® (3M Company, Minneapolis, MN). After the saliva was pooled and centrifuged at 10,000 g for 5 min at 10°C, phenylmethylsulfonylfluoride (0.2 M, Merck) was added to a final concentration of 1 mM as
a protease inhibitor. The solution was again centrifuged, dialyzed overnight at 4°C against
demineralized water, and freeze-dried for storage. A solution of 1.5 mg ml⁻¹ of freeze-dried
stock in adhesion buffer (2 mM potassium phosphate, 50 mM potassium chloride, and 1 mM
calcium chloride, pH 6.8) will be denoted as saliva. Saliva was supplemented with 0.01%
natrium azide to prevent bacterial growth.

Urine was collected and pooled from six healthy male volunteers. After removal of
sediments such as epithelial cells, bacteria and debris by centrifugation (10,000 g, 10 min,
10°C), the urine was pooled, yielding a pH of 7.0. The pooled urine was sterilized through a
5.0 µm and a 0.45 µm pore size filter (Millipore) successively, divided into 30 ml portions
and stored at -20°C. Prior to use, urine was brought to room temperature and supplemented
with 0.05% natrium azide to prevent bacterial growth.

Glass slides with a partial PEO coating were placed for up to 48 h in saliva or up to
144 h in urine, both at 37°C. As a reference, glass slides with a partial PEO coating were
placed in PBS (10 mM potassium phosphate, 150 mM NaCl, pH 6.8) up to 144 h at 37°C. For
contact angle and XPS measurements, slides were washed for 30 min in demineralized water.
Prior to contact angle measurements, slides were dried for 40 min in ambient air.

Bacterial adhesion. *S. epidermidis* HBH 276 was first grown overnight at 37°C on an agar
plate from a frozen stock, which was kept at 4°C, never longer than two weeks. Several
colonies were used to inoculate 10 ml of tryptone soya broth (TSB, OXOID, Basingstoke,
UK). This preculture was incubated at 37°C in ambient air for 24 h and used to inoculate a
second culture of 200 ml that was grown for 16 h. The microorganisms from the second
culture were harvested by centrifugation for 5 min at 5000 g and washed twice with
demineralized water. Subsequently, the bacteria were sonicated on ice (10 s) and suspended in
200 ml PBS to a concentration of 3 × 10⁸ ml⁻¹.

Bacterial adhesion was studied in a parallel plate flow chamber, which has been
described in detail [22]. The flow chamber (175 × 17 × 0.75 mm) held a top plate made of
glass, while glass slides with a partial PEO coating, that were pre-exposed to PBS, saliva or
urine, constituted the bottom plate. Deposition was observed on this bottom plate with a
CCD-MX Ri camera (High Technology, Eindhoven, The Netherlands) mounted on a phase
contrast microscope (Olympus BH-2) equipped with a 40 × ultra long working distance objective (Olympus ULWD-CD Plan 40 PL). The camera was coupled to an image analyzer (TEA, Difa, Breda, The Netherlands). Each live image (512 × 512 pixels with 8 bit resolution) was obtained after summation of 15 consecutive images (time interval 1 s) in order to enhance the signal to noise ratio and to eliminate moving microorganisms from the analysis.

Prior to each experiment, all tubes and the flow chamber were filled with PBS, while care was taken to remove air bubbles from the system. Flasks, containing microbial suspension and buffer, were positioned at the same height with respect to the chamber to ensure that immediately after the flows were started, all fluids would circulate through the chamber at the desired flow rate of 0.013 ml s⁻¹, which yields a laminar flow at a wall shear rate of 8 s⁻¹. PBS was circulated through the system for 30 min followed by the bacterial suspension, both at room temperature (20°C). For each experiment, the number of bacteria adhering after 30 min was determined for both the bare and PEO coated glass after fluid exposure.

All values given in this chapter are the averages of experiments on three separately prepared glass slides with a partial PEO coating, and were carried out with separately grown bacteria.

**Contact angle measurements.** Advancing and receding water contact angles on bare and PEO coated glass after fluid exposure were measured at 20°C with a homemade contour monitor using the sessile drop technique. The syringe was placed in the water droplet (1-1.5 µl) after positioning it on the surface and the sample was carefully moved until the advancing angle was maximal. Then both the advancing and the receding contact angles were determined. On each sample, at least 3 droplets were placed at different positions and results of 3 separately prepared and exposed coatings were averaged.

**XPS measurements.** XPS was performed using a S-Probe spectrometer (Surface Science Instruments, Mountain View, CA, USA) equipped with an aluminum anode (10 kV, 22 mA) and a quartz monochromator. The direction of the photoelectron collection angle (θ) was 35°
with the surface of the sample and the electron flood gun was set at 10 eV. A survey scan was made with a $1000 \times 250 \mu m$ spot and a pass energy of 150 eV. Detailed scans of the C$_{1s}$ lines were obtained using a pass energy of 50 eV. Binding energies were determined by setting the binding energy of the C$_{1s}$ component due to the carbon-carbon bond at 284.8 eV. The experimental peaks were integrated after nonlinear background subtraction and the peaks were decomposed assuming a Gaussian/Lorentzian ratio of 85/15 by using the SSI PC software package. The elemental surface compositions were expressed in atomic %, setting % C + % O + % Si + % N to 100%.

The film thickness of the layer on top of glass was determined using an overlayer model, comparing the Si$_{2p}$ peak of glass with the C$_{1s}$ peak, as originating from the overlayer [23]. Accordingly, the thickness ($T$) is given by

$$T = \frac{\lambda_C}{\lambda_{Si}} \sin \theta \ln \frac{I_C I_{Si}}{I_{Si} \lambda_C}$$  \hspace{1cm} (1)

where $I_C$ and $I_{Si}$ are the C$_{1s}$ and Si$_{2p}$ intensities, $\lambda_C$ and $\lambda_{Si}$ are the mean free paths of the C$_{1s}$ and Si$_{2p}$ electron, taken to be 3.0 and 3.2, respectively. In order to determine the layer thickness of PEO and proteinaceous conditioning films, their contributions to the carbon C$_{1s}$ signal, were considered separately. In addition, a third contribution was necessary because of the presence of contamination. First, when appropriate, the protein contribution to the C$_{1s}$ signal was determined based on the protein N$_{1s}$ signal, using a C/N ratio of 3.9, as generally valid for proteins [24]. The PEO contribution to the signal was then determined by first decomposing the C$_{1s}$ peak and taking the component at 286.4 eV (C-O) as originating from the PEO molecule [25], while subtracting a contribution of C-O bonds due to proteins present. To this end, the ratio C-O/N was taken 1.46 [24]. The remaining C-O signal could be directly related to the C$_{1s}$ signal originating from PEO using the C/C-O ratio of 1.2 as obtained from measurements on the PEO surfaces prior to exposure. The remaining C$_{1s}$ signal was ascribed to external contaminations and layer thicknesses ($T_{Co}$), proteins ($T_{Pr}$) and PEO ($T_{PEO}$) were calculated from their respective contributions to the C$_{1s}$ signal. As in urine, proteins and urea both can contribute to the N$_{1s}$ signal, only the total layer thickness could be determined ($T_{Tot}$).

All measurements were performed on three separately prepared and exposed coatings.
Statistical analysis. To analyze deviations in the contact angles measurements or adhesion numbers, independent Student t-tests were performed with SPSS for Windows (SPSS Inc., Chicago, IL) using a significance level of 0.05.

Results

Effects of exposure to PBS

Bacterial adhesion. Figure 1 shows bacterial adhesion to glass and PEO coatings after exposure to PBS for different time intervals. Bacterial adhesion to glass for each exposure time was relatively constant between $620 \times 10^4$ and $940 \times 10^4$ cm$^{-2}$. The average adhesion on the PEO coating increased significantly between 0 and 4 h of exposure to PBS from $5.4 \times 10^4$ to $39 \times 10^4$ cm$^{-2}$, and subsequently remained relatively constant up to 24 h. After 42 h exposure to PBS, a sudden increase in staphylococcal adhesion to the PEO coating occurred, but here differences between separate experiments as well as on different positions of the same sample were large (Figure 2). Between 24 and 48 h exposure to PBS, adhesion to the PEO coating increased from $73 \times 10^4$ to $590 \times 10^4$ cm$^{-2}$ ($p<0.05$) and after 144 h adhesion to the PEO coating was comparable to glass.

Contact angles. Figure 1 also shows the advancing water contact angles and contact angle hysteresis, i.e. the difference between the advancing and receding contact angle, on glass and PEO coatings after exposure to PBS for different time intervals. Advancing water contact angles on glass prior to and after 4 h exposure to PBS decreased somewhat from 70 degrees to 50 degrees (not statistically significant) and then remained around 50 degrees for longer exposure times. For the PEO coatings, there was a trend of decreasing advancing water contact angles with increasing exposure times to PBS. The contact angle hysteresis on both glass and the PEO coating was about 20 degrees for all exposure times, except for the PEO coating after 144 h exposure, where the average hysteresis was only 2 degrees.

XPS. Table 1 summarizes the elemental compositions as measured by XPS for glass and PEO coatings prior to and after exposure to PBS. Prior to exposure (0 h) glass shows a high oxygen
Figure 1. The number of S. epidermidis HBH 276 adhering after 30 min, advancing water contact angles and contact angle hysteresis after exposure of glass (white bars) and PEO coated glass (black bars) to PBS at 37 °C. Error bars represent the average standard deviation over three separate experiments.
Stability of PEO coatings

Figure 2. Micrographs of S. epidermidis HBH 276 adhering after 30 min in a parallel plate flow chamber on glass (left), PEO coated glass (middle) and PEO coated glass exposed to PBS at 37°C for 42 h (right) demonstrating surface heterogeneity of the coating after exposure to PBS. Micrograph was obtained by in situ phase-contrast microscopy.

and silicon content in proportions as expected for SiO₂. Furthermore, a small carbon content and contamination thickness of 0.6 nm on top of glass was detected, probably due to adsorption of carbonaceous contamination from the atmosphere [26,27]. PEO coating decreased the surface silicon concentration to 6.9% and the surface oxygen concentration to 33.6%, while the carbon concentration increased to 59.5%, yielding a PEO thickness of 3.7 nm. After both 4 and 144 h exposure of the PEO coating, the carbon signal decreased and the oxygen and silicon signals increased resulting in elemental surface compositions that resembled those of glass. Still 0.8 nm of the PEO coating was present after 4 h in PBS, and this decreased to 0.4 nm after 144 h of exposure.

Effects of exposure to saliva

Bacterial adhesion. Bacterial adhesion to glass and the PEO coatings after exposure to saliva for different time intervals is shown in Figure 3. Glass surfaces showed a significant decrease in bacterial adhesion after exposure to saliva. For PEO coated surfaces, exposure to saliva for 0.02 h to 0.5 h gave low staphylococcal adhesion ranging form 0.5 × 10⁴ to 3.3 × 10⁴ cm⁻². However, after 4 h exposure to saliva, bacterial adhesion to PEO coatings increased significantly to 260 × 10⁴ cm⁻² (p<0.05) and also after longer exposure times adhesion to the PEO coating was comparable to the one on glass.
Table 1. The percentage surface compositions carbon (C), oxygen (O), silicon (Si) and nitrogen (N), combined with PEO ($T_{\text{PEO}}$), protein ($T_{\text{Pr}}$) contamination ($T_{\text{Co}}$) and total ($T_{\text{tot}}$) thicknesses, prior to and after exposure to PBS. ± represents standard deviations over three separate experiments.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Exposure time (h)</th>
<th>% C</th>
<th>% O</th>
<th>% Si</th>
<th>% N</th>
<th>$T_{\text{PEO}}$ (nm)</th>
<th>$T_{\text{Pr}}$ (nm)</th>
<th>$T_{\text{Co}}$ (nm)</th>
<th>$T_{\text{tot}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>0</td>
<td>12.0 ± 1.0</td>
<td>56.9 ± 0.8</td>
<td>30.7 ± 0.2</td>
<td>n.f.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>PEO coating</td>
<td>0</td>
<td>59.5 ± 0.8</td>
<td>33.6 ± 0.3</td>
<td>6.9 ± 0.9</td>
<td>n.f.</td>
<td>3.7 ± 0.8</td>
<td>n.a.</td>
<td>0.0 ± 0.0</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
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<td>26.2 ± 5.1</td>
<td>49.5 ± 4.9</td>
<td>22.0 ± 1.1</td>
<td>n.f.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
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<tr>
<td>PEO coating</td>
<td>4</td>
<td>24.0 ± 7.2</td>
<td>52.3 ± 4.0</td>
<td>23.2 ± 3.4</td>
<td>n.f.</td>
<td>0.8 ± 0.6</td>
<td>n.a.</td>
<td>0.4 ± 0.2</td>
<td>1.2 ± 0.3</td>
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<tr>
<td>Glass</td>
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<td>32.3 ± 8.4</td>
<td>44.6 ± 5.8</td>
<td>20.4 ± 5.6</td>
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<td>n.a.</td>
<td>n.a.</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>PEO coating</td>
<td>144</td>
<td>26.2 ± 3.7</td>
<td>49.6 ± 2.3</td>
<td>21.5 ± 1.7</td>
<td>n.f.</td>
<td>0.4 ± 0.1</td>
<td>n.a.</td>
<td>1.2 ± 0.4</td>
<td>1.6 ± 0.5</td>
</tr>
</tbody>
</table>

n.f.: not found
n.a.: not applicable
Figure 3. The number of S. epidermidis HBH 276 adhering after 30 min, advancing water contact angles and contact angle hysteresis after exposure of glass (white bars) and PEO coated glass (black bars) to saliva at 37°C. Error bars represent the average standard deviation over three separate experiments.
Table 2. The percentage surface compositions carbon (C), oxygen (O), silicon (Si) and nitrogen (N), combined with PEO (T_{PEO}), protein (T_{Pr}) contamination (T_{Co}) and total (T_{tot}) thicknesses, prior to and after exposure to saliva. ± represents standard deviations over three separate experiments.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Exposure time (h)</th>
<th>% C</th>
<th>% O</th>
<th>% Si</th>
<th>% N</th>
<th>T_{PEO} (nm)</th>
<th>T_{Pr} (nm)</th>
<th>T_{Co} (nm)</th>
<th>T_{tot} (nm)</th>
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<td>Glass</td>
<td>0</td>
<td>12.0 ± 1.0</td>
<td>56.9 ± 0.8</td>
<td>30.7 ± 0.2</td>
<td>n.f.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
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<tr>
<td>PEO coating</td>
<td>0</td>
<td>59.5 ± 0.8</td>
<td>33.6 ± 0.3</td>
<td>6.9 ± 0.9</td>
<td>n.f.</td>
<td>3.7 ± 0.2</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
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<td>27.5 ± 6.2</td>
<td>10.5 ± 3.9</td>
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<td>0.1 ± 0.3</td>
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<tr>
<td>PEO coating</td>
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<td>53.9 ± 5.2</td>
<td>33.0 ± 2.6</td>
<td>10.7 ± 3.8</td>
<td>1.8 ± 3.1</td>
<td>2.1 ± 1.8</td>
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<td>144</td>
<td>62.0 ± 1.2</td>
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<td>8.4 ± 0.5</td>
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<td>4.1 ± 0.5</td>
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<tr>
<td>PEO coating</td>
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<td>29.8 ± 2.7</td>
<td>7.8 ± 1.9</td>
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<td>2.8 ± 0.6</td>
<td>0.4 ± 0.5</td>
<td>3.3 ± 1.5</td>
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</tbody>
</table>

n.f.: not found
n.a.: not applicable
Contact angles. Figure 3 shows the advancing water contact angles and contact angle hysteresis on glass and PEO coatings after exposure to saliva for different time intervals. Advancing water contact angles were relatively constant after saliva exposure, and amounted about 70 degrees for glass and 45 degrees for the PEO coating. Glass as well as PEO coatings were significantly more hydrophobic after 4 to 48 h exposure to saliva than after exposure to PBS for the same period (see Figures 1 and 3). Contact angle hysteresis on both glass and PEO coatings was about 25 degrees, which was significantly higher than after PBS exposure between 4 to 48 h.

XPS. XPS (Table 2) shows a high nitrogen amount on glass within 30 min after exposure to saliva, which is ascribed to an adsorbed protein layer of 2.9 nm. For the PEO coating after 30 min of exposure, 2.1 nm PEO was still present and only 0.5 nm protein thickness was found. After 48 h exposure to saliva, the PEO layer thickness decreased to only 0.1 nm. Furthermore, the surface composition of glass and PEO coatings were equal within the experimental errors, both with a relatively thick (3.6 and 2.8 nm, respectively) protein film.

Effects of exposure to urine

Bacterial adhesion. Bacterial adhesion to glass and the PEO coating after exposure to urine for different time intervals is shown in Figure 4. Adhesion to glass decreased significantly after exposure to urine for 4 h (p<0.05). An increase in staphylococcal adhesion to glass was found after exposure for longer time intervals, but also after 144 h the number of bacteria adhering was still significantly lower than prior to exposure. After exposure of the PEO coatings to urine for 4 to 48 h, average bacterial adhesion was $3.1 \times 10^4$ cm$^{-2}$, which was significantly lower than after exposure to PBS for the first 24 h. After exposure for 72 h, bacterial adhesion to the PEO coating increases to $160 \times 10^4$ cm$^{-2}$ (p<0.05) and after longer exposure times the adhesion became similar as to glass.

Contact angles. Figure 4 also shows the advancing water contact angles and contact angle hysteresis on glass and PEO coatings after exposure to urine for different time intervals. Advancing water contact angles on glass were 70 degrees on average for all exposure times to
urine, which was significantly higher than after PBS exposure. Also the advancing contact angles of the PEO coating after exposure to urine (55 degrees) were significantly higher as compared to after PBS exposure. The average hysteresis for both glass (31 degrees) and the PEO coating (31 degrees) was significantly higher than after PBS exposure.

**XPS.** The surface composition of glass after urine exposure for 4 h (Table 3) showed an increase in carbon and a decrease in the oxygen and silicon signal, while furthermore a nitrogen signal of 6.9% was measured. For the PEO coatings, a significantly smaller nitrogen signal appeared, indicative for the formation of a thin conditioning film composed of proteins and/or urea, as compared with that formed on glass. After 144 h the nitrogen signal decreases to 1.0% for glass and even below detection limits for the PEO coating.

**Table 3.** The percentage surface compositions carbon (C), oxygen (O), silicon (Si) and nitrogen (N), combined with total overlayer thicknesses ($T_{tot}$), prior to and after exposure to urine. ± represents standard deviations over three separate experiments.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Exposure time (h)</th>
<th>% C</th>
<th>% O</th>
<th>% Si</th>
<th>% N</th>
<th>$T_{tot}$(nm)</th>
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<td>Glass</td>
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<td>12.0 ± 1.0</td>
<td>56.9 ± 0.8</td>
<td>30.7 ± 0.2</td>
<td>n.f.</td>
<td>0.6 ± 0.1</td>
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<td>0</td>
<td>59.5 ± 0.8</td>
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<td>6.9 ± 0.9</td>
<td>n.f.</td>
<td>3.7 ± 0.2</td>
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<td>PEO coating</td>
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<td>1.7 ± 0.1</td>
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<td>n.f.</td>
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n.f.: not found
Figure 4. The number of S. epidermidis HBH 276 adhering after 30 min, advancing water contact angles and contact angle hysteresis after exposure of glass (white bars) and PEO coated glass (black bars) to urine at 37°C. Error bars represent the average standard deviation over three separate experiments.
Chapter 6

Discussion

Studies using model systems show the potential of PEO coatings to prevent biofilm formation, which in turn may reduce the risk of BCI [3]. However in vivo, little progress has been made. For instance, under clinical conditions blood proteins have been demonstrated to adsorb extensively to a PEO coated polymer, in contrast to in vitro results [28]. Also, in vivo research on PEO coatings in the oral cavity showed poor results [29], despite excellent in vitro reductions in salivary protein adsorption and oral bacterial adhesion to PEO coatings on glass and hydroxyapatite [29,30]. Possibly, the durability of the thin layer of grafted PEO chains in the oral cavity was not sufficient over the time scale used in vivo.

This study directly assessed that in vitro our PEO coating is effective for only 24 h in PBS at 37°C, which is in contrast to another study, where ellipsometry indicated that under similar conditions a PEO coating remained stable for 24 days [20]. In both cases, degradation most probably does not occur at the alkyl or ether bonds of the PEO polymer itself, as these can only be degraded by aggressive chemicals [31], high temperatures [32] or specific bacteria [33]. The grafting of the PEO chain to the surface is, in general, the weak point of a PEO coating. For instance, the often used organosilane linkage is susceptible to hydrolytic cleavage [34]. In this study, the PEO chains are coupled by a Si-O-C linkage to the glass substratum, which may readily hydrolyze as well [21]. Furthermore, the PEO chains used here contain an ester group close to the anchoring point to glass, which is also sensitive to hydrolysis [35]. Thus it is anticipated that PEO chains are removed over time by reaction of water with one of the unstable bonds. For PEO coatings, the grafting density is of critical importance; when the density is too low penetration of the coating can occur or parts of the bare substratum are revealed, both leading to an increase in adsorption of proteins and adhesion of microorganisms [3,14]. This reasoning explains the large variability in adhesion after 42 h exposure to PBS, when the grafting density is thought to be at the transition point between effective and non-effective implying that small variations in density can lead to high variations in bacterial adhesion. Furthermore, the decreasing brush density in time may also explain the short durability of the coating as more bacteria can adhere to a less dense coating. This theory is supported by the decrease in advancing contact angles after exposure to PBS,
where the PEO chains are removed and the more hydrophilic glass becomes exposed. The very low hysteresis of the PEO coating after 144 h of exposure to PBS is probably due to complete removal of all PEO chains. The decrease PEO thickness with immersion time also supports removal of PEO chains.

In addition to stability, another important aspect of using coatings of any kind is their resilience against mechanical stress. Here, the edge of a glass objective was used to make a vertical scratch on the PEO coating using minimal force. Figure 5 clearly shows bacteria adhering in vertical alignment, showing that the scratch had damaged or removed the PEO coating to such a level that it is not longer effective. As PEO is much softer that glass [36,37] and PEO coatings are at most a few tens of nm thick scratching will probably readily occur, also with clinically used biomaterials.

![Micrograph of S. epidermidis HBH 276 adhering after 4 h in a parallel plate flow chamber on a fresh PEO coating scratched vertically by glass.](image)

**Figure 5.** Micrograph of S. epidermidis HBH 276 adhering after 4 h in a parallel plate flow chamber on a fresh PEO coating scratched vertically by glass.

Both saliva and urine exposure induced conditioning film formation as was indicated by the increase in advancing contact angles and contact angle hysteresis. XPS also indicated a protein layer on the PEO coating after exposure to saliva and a layer containing proteins and/or urea after exposure to urine. A salivary conditioning film decreased the effectiveness of a PEO coating on hydroxyapatite in reducing adhesion of the oral bacterium *Streptococcus mutans* [30,38]. Furthermore Efremova et al. [39] have shown that PEO may bind to mucins by hydrogen bonding, mucins are highly glucosylated proteins, which constitute about 15% of the total protein content in stimulated saliva [40]. It is therefore anticipated that salivary
proteins, especially mucins, adhere to and penetrate between the polymer chains, therewith covering the PEO coating and thus reduce its long term effectiveness. In human urine several factors are known that inhibit the adhesion of *E. coli* [41] and other uropathogens [42], by competitive inhibition of bacterial adhesins. The primary of these factors, Tamm Horsfall Protein, is thought to reduce overall bacterial adhesion by increasing the surface negative charge, thereby increasing electrostatic repulsion [43]. Thus supposedly, the combined effect of the remnant PEO coating and the anti adhesive effect of some urine components lead to the lower average adhesion and longer effectiveness of the PEO coating.

In future applications biomaterials containing a PEO coating remain in the body for months or years are subjected to mechanical stress and are exposed to several conditioning film forming fluids. The *in vitro* method as described here makes it possible to quickly assess the influence of these factors on the effectiveness of the PEO coating thereby making it possible to adjust research lines in an early stage. This, in turn, should lead to higher success rates *in vivo* a thus earlier practical use of such coatings on biomaterials.

**Conclusions**

A method is forwarded to assess the long term *in vitro* stability of a PEO coating under conditions of the formation of a conditioning film from e.g. saliva or urine. Although the present coating could only repress bacterial adhesion for at most 48 h and thus is not suitable for clinical applications, important prerequisites could be determined for *in vivo* use of a PEO coating. Firstly, the linkage to the surface has to be stable, and secondly protein content and concentration of the biological fluid in which the biomaterial is to be used has to be taken into account. Finally, biomaterials with a PEO coating should be handled very carefully to prevent scratching. Still, although an everlasting PEO coating for every application may be an utopia, a carefully developed and *in vitro* tested PEO coating could greatly reduce bacterial adhesion and, hence, the risk of BCI.
References


