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The use of polymer brush coatings to prevent microbial adhesion

Roosjen, Astrid

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Microbial adhesion to poly(ethylene oxide) brushes: Influence of polymer chain length and temperature

Glass surfaces were modified by end-grafting poly(ethylene oxide) (PEO) chains having a Mw of 526, 2000 or 9800 Da. Characterization using water contact angles, ellipsometry and X-ray photoelectron spectroscopy confirmed the presence of the PEO brushes on the surface with estimated lengths in water of 2.8, 7.5 and 23.7 nm, respectively. Adhesion of two bacterial (*Staphylococcus epidermidis* and *Pseudomonas aeruginosa*) and two yeast (*Candida albicans* and *Candida tropicalis*) strains, to these brushes was studied and compared to their adhesion to bare glass. For the bacterium *P. aeruginosa* and the yeast *C. tropicalis*, adhesion to the 2.8 nm brush was comparable to their adhesion on bare glass, whereas adhesion to the 7.5 and 23.7 nm brush was greatly reduced. For *S. epidermidis*, adhesion was only slightly higher to the 2.8 nm brush than to the longer brushes. Adhesion of the yeast *C. albicans* to the PEO brushes was lower than to glass, but no differences in adhesion were found between the three brush lengths. After passage of an air bubble, nearly all microorganisms adhering to a brush were removed, irrespective of brush length, whereas retention of the adhering organisms on glass was much higher. No significant differences were found in adhesion nor retention between experiments conducted at 20 or 37°C.

Introduction

Microorganisms tend to adhere to surfaces as the onset of the formation of a complex adhering microbial community, called a “biofilm” [1]. Biofilm formation on 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 [2]. Although the mechanism of microbial adhesion to a biomaterial surface has not been fully elucidated, interactions between biomaterial surfaces and microorganisms have been reported to include non-specific contributions from e.g. electrostatic, Lifshitz-Van der Waals and hydrophobic forces and a variety of specific receptor-adhesin interactions [3,4].

A way to prevent or reduce the adhesion of microorganisms as well as other particles (e.g. proteins) to surfaces is to graft poly(ethylene oxide) (PEO) chains on the surface. As the PEO chains are highly mobile [5] and attain extremely large exclusion volumes [6] they make the surface difficult to approach by incoming particles. Raising the temperature reduces the solubility of PEO in water [7]. The resistance for particle deposition at a PEO tethered surface is therefore expected to decrease. Several studies have shown this effect for proteins [8-10].

Most 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 [11-14]. 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. This has been confirmed by several researchers investigating adsorption of proteins [8,10,15,16]. However, in some studies [17,18], such an effect of PEO chain length has not been observed, while in other [10] very low adsorption was found even on layers of very short PEO molecules. This apparent controversy may be due to the fact that in the various studies different methods have been used to graft PEO molecules to a surface leading to very different chain densities and, hence, to very different conformations of the applied PEO layer.

As opposed to protein adsorption, microbial adhesion on PEO coatings has not been studied as a function of the thickness of the PEO layer and of temperature. However, due to size differences, the trends observed with microorganisms may deviate from those for

proteins, because a stronger Lifshitz-Van der Waals attraction to the surface is expected for the larger particles and microorganisms are less able to penetrate in between the brush chains.

The aim of the present study is to determine adhesion and retention of two bacterial (dimension about 1 μm) and two yeast (dimension about 3 μm) strains at surfaces grafted with PEO molecules of different molar mass (526, 2000 and 9800 Da). The grafting density of the three samples was taken such that the PEO layer assumes a brush conformation. The brushes are characterized by contact angle measurements, X-ray photoelectron spectroscopy (XPS) and ellipsometry. Microbial adhesion and retention measurements were done at 20 and 37°C under defined flow conditions.

Materials and methods

Materials. Microscope glass slides were obtained from Menzel-Gläser, Emergo, Landsmeer, The Netherlands. RBS 35, used for cleansing, was obtained from Omnilabo International BV, Breda, The Netherlands. Methanol, nitric acid 65%, chloroform, NaCl, K_2HPO_4 , KH_2PO_4 and M_w 526 methacryl-terminated poly(ethylene oxide) (PEO 526) were purchased from Merck. M_w 2000 and M_w 9800 methacryl-terminated poly(ethylene oxide) (PEO 2000 and PEO 9800) was purchased from Polymer Source Inc., Dorval, Quebec, Canada and used as received.

Preparation of poly(ethylene oxide) brushes. The PEO 526, 2000 and 9800 samples were applied on microscope glass slides using the reaction of surface silanol groups such as on glass and silica, with vinyl terminated polymers in a polymer melt as described by Maas *et al.* [19]. Previous research has shown that this leads to high surface grafting densities, where the PEO chains extend in a brush conformation in an aqueous medium [20]. The brushed surfaces were used as substratum surfaces in microbial adhesion, XPS and contact angle measurements, while furthermore brushes were applied on silica for ellipsometry. Surfaces were first sonicated in 2% RBS 35 detergent (Omnilabo International BV, Breda, The Netherlands), rinsed in demineralized water, sonicated in methanol and rinsed in demineralized water again, to remove oil contaminations and fingerprints. Next, possible metallic oxides on the surfaces were removed by submersing the slides in hot (95°C) nitric

acid 65% for 60 min. Finally, the surfaces 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 surfaces, surfaces were first covered with a 0.4 mM solution of the methacryl-terminated PEO molecules in chloroform. The solvent then 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 and drying in a stream of nitrogen.

In order to reduce experimental errors in microbial adhesion, only a part of the glass surfaces used for adhesion studies were grafted with PEO 526, 2000 or 9800 chains, which allowed studying adhesion to a glass surface and a brush-coated surface in one and the same experimental run.

Characterization of PEO brushes. Equilibrium water contact angles on pristine glass and glass coated with a PEO 526, 2000 or 9800 brush were measured at 20°C with a homemade contour monitor using the sessile drop technique. Surface homogeneity, prior to and after coating, was investigated by measuring advancing and receding water contact angles, obtained by keeping the syringe in the water droplet (1-1.5 μ l) after positioning it on the surface and by carefully moving the sample until the advancing angle was maximal. On each sample three droplets were placed at different positions and results of three separately prepared coatings were averaged.

The chemical compositions of pristine glass and glass coated with PEO 526, 2000 or 9800 were probed by XPS using an S-Probe spectrometer (Surface Science Instruments, Mountain View, CA, USA). Three separate measurements were performed on each sample. The elemental surface compositions were expressed in atomic %, setting % C + % O + % Si to 100%.

The vacuum-dried PEO layer thickness on glass of each sample was calculated using an overlayer model, comparing the Si_{2p} signal of glass with the C_{1s} signal originating from the overlayer [21]. Accordingly, the overlayer thickness (T_{XPS}) is given by:

$$T_{XPS} = \lambda \sin \theta \ln \frac{I_C \lambda_{Si}}{I_{Si} \lambda_C} \quad (1)$$

where I_C and I_{Si} are the C_{1s} and Si_{2p} intensities and λ_{Si} and λ_C are the mean free path of the Si_{2p} and C_{1s} electrons, taken to be 3.2 and 3.0 respectively.

For silica coated with air dried PEO layers, the film thickness was determined using an auto-nulling imaging ellipsometer (nanofilm EP3, nanofilm technologie GmbH, Gottingen, Germany) using a wavelength λ of 532 nm at an angle of incidence of 75°. The ellipsometry software provided by the manufacturer was used to calculate the film thickness on each of the three different spots measured per sample surface. The average thickness ($T_{\text{ellipsometry}}$) of the PEO layer was obtained from the film thicknesses of three separately prepared samples of each coating. Taking for the density of the dry PEO film that of bulk PEO [22], $\rho = 1.13 \text{ g cm}^{-3}$, the chain grafting density (D) was calculated according to

$$D = \frac{\rho T_{\text{XPS}} N_A}{M_w} \quad (2)$$

in which M_w is the molar mass of PEO and N_A Avogadro's number. The grafting density was compared to the the Flory radius (R_{Fl}) of the PEO chains in aqueous solution using

$$R_{Fl} = aN^{\frac{3}{5}} \quad (3)$$

with a monomer size a of 0.35 nm [23]. The degree of polymerization N indicates the amount of ethylene oxide (EO) units in each polymer chain, calculated by the ratio M_w/M_{EO} ($M_{EO} = 44 \text{ Da}$). If the chains are forced in a brush conformation, i.e. for $1/D < 2(R_{Fl})^2$, the chain grafting density (D) can be used to determine the theoretical length of the PEO brush in water (L) using the Flory version of the Alexander model [13,24],

$$L = aN(a^2D)^{\frac{1}{3}} \quad (4)$$

This scaling model assumes ideal chains that do not interact with each other and has been shown to give a fair approximation of the length of the brush (within 20%) [23,25].

Microbial strains and growth conditions. Two bacterial strains, the hydrophilic *Staphylococcus epidermidis* HBH 276 and hydrophobic *Pseudomonas aeruginosa* AK1 [26] and two yeast strains, the hydrophilic *Candida albicans* GB 1/2 and hydrophobic *Candida tropicalis* GB 9/9 [27] were used in this study. *S. epidermidis* is a spherical bacterium and *P. aeruginosa* is rod-shaped, both are about 1 μm in size. Both yeast species have a spherical form and are about 3 μm in size. Microbial sizes were estimated from microscopic images.

All strains were 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, England) for *S. epidermidis*, nutrient broth (NB, OXOID) for *P. aeruginosa* and brain heart infusion (BHI, OXOID) for *C. albicans* and *C. tropicalis*. 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 5 min at 5000 g for *S. epidermidis* and 5 min at 9600 g for *P. aeruginosa*, *C. albicans* and *C. tropicalis* and washed twice with demineralized water. Subsequently, the bacteria were suspended in 200 mL PBS (10 mM potassium phosphate, 140 mM NaCl, pH 6.8), after sonication on ice (10 s), to a concentration of $3 \times 10^8 \text{ ml}^{-1}$. Yeast were suspended in PBS to a concentration of $3 \times 10^6 \text{ ml}^{-1}$.

Parallel plate flow chamber and image analysis. The flow chamber (175 × 17 × 0.75 mm) and image analysis system have previously been described [28]. The flow chamber was equipped with heat elements and kept at 37°C throughout the experiment when required. Images were taken from the bottom plate, which consisted of a partly PEO coated glass slide. The top plate of the chamber was made from glass. Deposition was observed with a CCD-MXRi 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) for experiments with bacteria and with a 10 × objective for experiments with yeast. 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. An image covers a surface area of 0.0096 mm² at the magnification used for bacterial experiments and 0.18 mm² at the magnification employed in the experiments with yeast.

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 shear rate of 15.7 s^{-1} (0.025 ml s^{-1}), which yields a laminar flow (Reynolds number 1.4). PBS was circulated through the system for 30 min followed by microbial suspension for 4 h at room temperature (20°C) or 37°C and images were obtained alternately from the glass and from the PEO coated part.

The initial increase in the number of adhering microorganisms with time, was expressed in a so-called initial deposition rate j_0 ($\text{cm}^{-2} \text{ s}^{-1}$), i.e. the number of adhering microorganisms per unit area and time. The number of microorganisms adhering after 4h, n_{4h} , was taken as an estimate of microbial adhesion in a more advanced stage of the process. Finally an air bubble was passed through the chamber, leading to a relatively high removal force (around $1 \times 10^{-7} \text{ N}$) [29], and the number of remaining microorganisms was determined.

All values given in this chapter are the averages of experiments on three separately prepared brush-coated surfaces, and were carried out with separately grown microorganisms. To analyze differences between experiments, independent t-tests were performed with SPSS for Windows (SPSS Inc., Chicago, IL) using a significance level of 0.05.

Calculation of interaction forces. The Lifshitz-Van der Waals force (F_{LW}) plays an important role in microbial adhesion to surfaces, and decays with the distance d between a sphere and a semi-infinite flat surface according to [30]

$$F_{LW} = \frac{AR}{6d^2} \quad (5)$$

in which A is the Hamaker constant, R is the microbial radius ($0.5 \mu\text{m}$ for bacteria and $1.5 \mu\text{m}$ for yeast). Hamaker constants for microorganisms interacting with glass surfaces are not well known and values ranging from 0.6×10^{-21} to $7.0 \times 10^{-21} \text{ J}$ have been reported [4,31,32]. Using the maximal Hamaker constant reported literature and the thickness of the brush L for d a maximal Lifshitz-Van der Waals force has been calculated for microbial interactions with glass with an adsorbed brush.

Results

Surface characterization. Results of water contact angle measurements on PEO coated glass are shown in Figure 1. Equilibrium contact angles were similar around 40 degrees for all chain lengths; the advancing contact angle however was significantly higher for the PEO 526 brush (59 degrees) as compared to the PEO 2000 or 9800 brushes (45 and 48 degrees). Also the receding angle of 37 degrees was relatively high for the M_w 526 brush and decreased to 24 degrees for the M_w 2000 brush ($p < 0.05$) and even further to 16 degrees for the PEO 9800 brush ($p < 0.05$). The hysteresis, i.e. the difference between the advancing and receding contact angles, was the highest for the PEO 9800 brush (32 degrees).

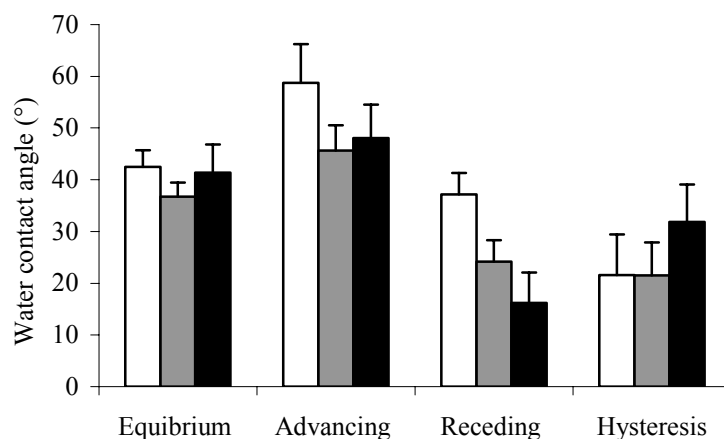


Figure 1. Equilibrium, advancing and receding water contact angles and contact angle hysteresis of the M_w 526 (white bars), 2000 (grey bars) and 9800 (black bars) PEO brushes. Error bars represent the average standard deviation over three separate experiments.

XPS results of pristine and PEO coated glass are compiled in Table 1. All PEO coatings showed an increase in surface carbon concentration and a decrease in surface oxygen and silicon concentration. These changes were larger for the longer PEO chains. Carbon present on the pristine glass is likely contamination due to adsorption of hydrocarbon components from the atmosphere [33,34].

Table 1. Percentage elemental surface composition of pristine glass and PEO coated surfaces, as determined by X-ray photoelectron spectroscopy. \pm denotes standard deviations over three separate measurements.

Element	Pristine glass	Coated glass		
		PEO 526 brush	PEO 2000 brush	PEO 9800 brush
% C	12.0 \pm 1.0	37.9 \pm 2.7	47.1 \pm 3.1	59.5 \pm 0.8
% O	57.2 \pm 0.8	43.3 \pm 2.2	40.5 \pm 1.0	33.6 \pm 0.3
% Si	30.8 \pm 0.2	18.8 \pm 1.0	12.4 \pm 1.9	6.9 \pm 0.9

The vacuum-dried PEO layer thickness as determined from XPS and ellipsometry (T_{XPS}), grafting density (D), Flory radius (R_{Fl}) and brush length (L) are shown in Table 2. The thicknesses as determined from XPS on glass, using Equation 1, were higher for the longer PEO chains and ranged from 1.8 nm for the PEO 526 brush to 3.3 nm for the PEO 9800 brush. The air dried PEO thickness on silica ($T_{ellipsometry}$) as determined from ellipsometry showed values comparable to those derived from XPS results on glass, but standard deviations were much larger. Hence XPS results were chosen to determine the grafting density (D , Equation 2). For the longer PEO chains lower grafting densities were found and for all PEO coatings it was observed that $1/D < 2(R_{Fl})^2$, confirming the PEO chains being in a brush conformation. Brush lengths in water (L , Equation 4) increase dramatically from 2.8 to 7.5 to even 23.7 nm, with increasing chain length.

Table 2. Dry PEO layer thicknesses as determined from XPS (T_{XPS}) and ellipsometry ($T_{ellipsometry}$) combined with the chain grafting density (D), Flory radius (R_{Fl}) and brush length in water (L) as extrapolated from XPS results. \pm , standard deviations over three separate measurements.

	PEO 526 brush	PEO 2000 brush	PEO 9800 brush
T_{XPS} (nm)	1.8 \pm 0.1	2.9 \pm 0.3	3.3 \pm 0.8
$T_{ellipsometry}$ (nm)	2.7 \pm 1.2	3.2 \pm 1.8	7.4 \pm 3.9
D (nm ⁻²)	2.3 \pm 0.1	1.0 \pm 0.1	0.2 \pm 0.1
R_{Fl} (nm)	1.5	3.5	9.0
L (nm)	2.8 \pm 0.1	7.5 \pm 0.3	23.7 \pm 2.1

Interaction forces. The maximal Lifshitz-Van der Waals forces (F_{LW}) between microorganisms and glass across the length of the brush (i.e. $d = L$), as calculated using Equation 5, are summarized in Table 3. In general, for a given brush, yeast cells experience an approximately three times larger attractive Lifshitz-Van der Waals than bacteria. Furthermore, the Lifshitz-Van der Waals force F_{LW} for the PEO 526 brush is on average 10 times higher as compared to the PEO 2000 brush and 72 times higher than the PEO 9800 brush.

Table 3. Expected separation distances between adhering microorganisms and glass together with maximal Lifshitz-Van der Waals forces.

Substratum	PEO 526 brush	PEO 2000 brush	PEO 9800 brush
d (nm)	2.8	7.5	23.7
F_{LW} (N) Bacteria	7.4×10^{-11}	1.0×10^{-11}	1.0×10^{-12}
F_{LW} (N) Yeast	2.2×10^{-10}	3.1×10^{-11}	3.1×10^{-12}

Microbial adhesion and retention at different temperatures. Adhesion and retention of the bacteria *S. epidermidis* HBH 276 and *P. aeruginosa* AK1 are compiled in Figure 2. For *S. epidermidis* both the initial deposition rate and number of bacterial adhering after 4 h are much lower for the PEO brushes of all molecular weights as compared to glass ($p < 0.05$), although adhesion on the PEO 526 brush tends to be higher than on the PEO 2000 and PEO 9800 brushes. However, for *P. aeruginosa* the initial deposition rates and number of bacteria adhering after 4 h are approximately the same for bare and PEO 526 coated glass, and a significant reduction was found only for the PEO 2000 and PEO 9800 brushes. Both bacteria hardly detached from the bare glass surface (around 1% for *S. epidermidis* and 20% for *P. aeruginosa*). For *S. epidermidis*, however on the PEO 526 brush, bacteria were much more loosely attached and detachment percentages were 39% at 20°C and 69% at 37°C. The high standard deviations found are likely due to the low number of bacteria initially present on the surface, which also impeded measurement of bacterial detachment for the PEO 2000 and 9800 brushes. Also for *P. aeruginosa*, a passing air bubble detached significantly more bacteria from the PEO coated surfaces (around 80%) than from glass. For both bacteria, no significant differences in adhesion and retention were found between 20 and 37°C.

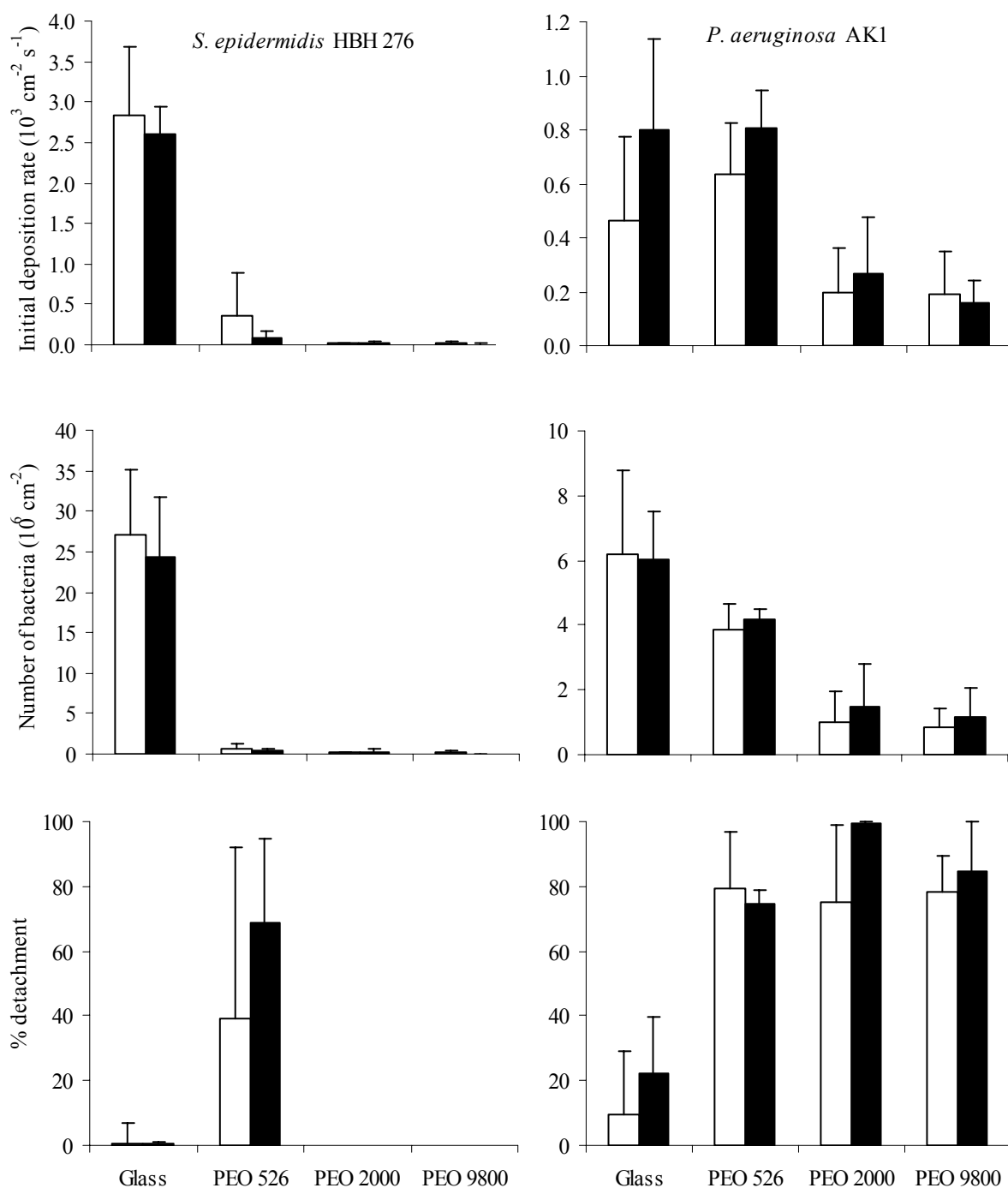


Figure 2. The initial deposition rates, number of bacteria adhering after 4 h and % detachment of *S. epidermidis* HBH 276 and *P. aeruginosa* AK1 at 20°C (white bars) and 37°C (black bars) on glass and glass coated with the PEO M_w 526, 2000 and 9800 brushes. For detachment of *S. epidermidis*, only data for glass and glass coated with PEO M_w 526 are shown. Error bars represent the average standard deviation over three separate experiments.

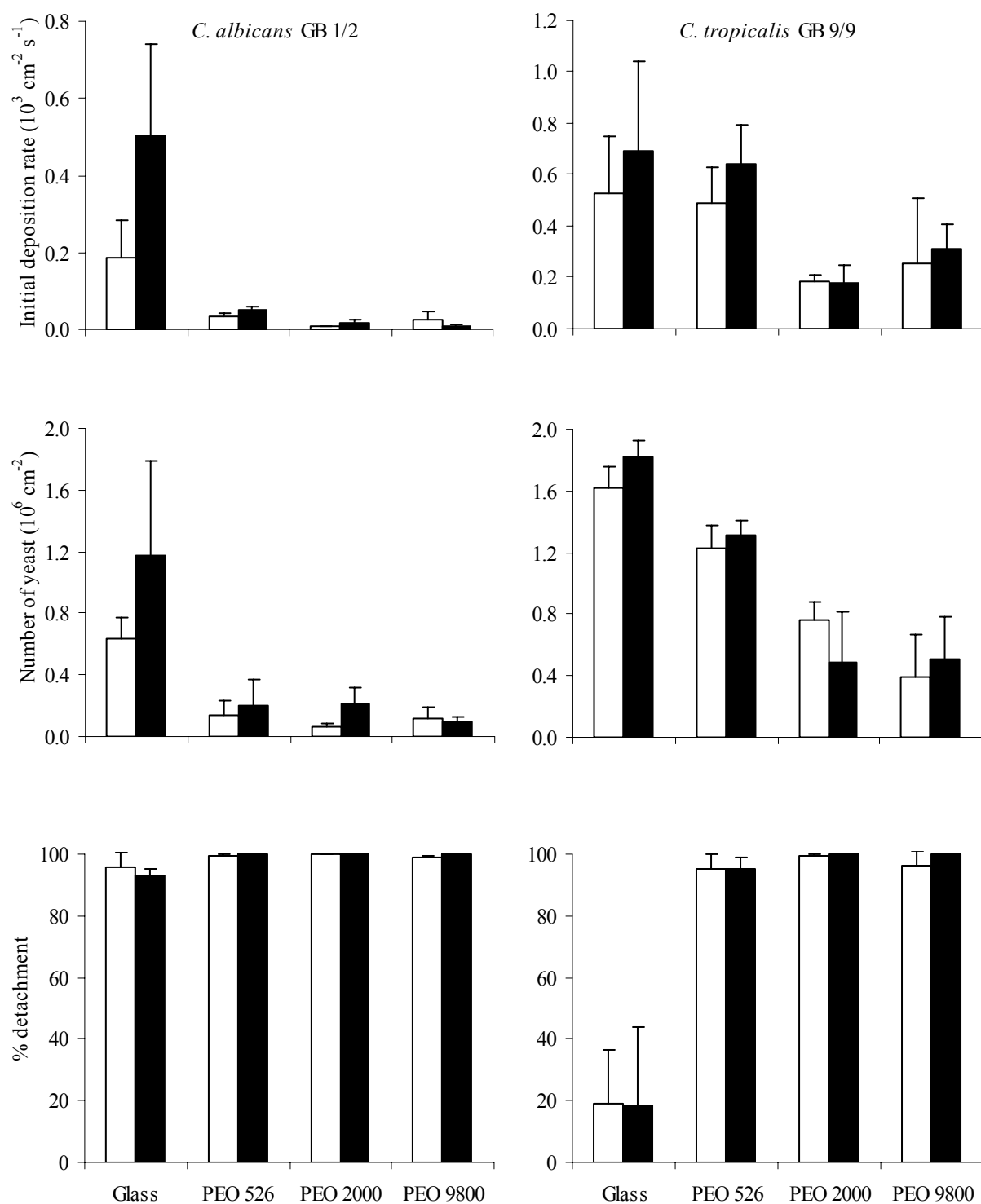


Figure 3. The initial deposition rates, number of bacteria adhering after 4 h and % detachment of *C. albicans* GB 1/2 and *C. tropicalis* GB 9/9 at 20°C (white bars) and 37°C (black bars) on glass and glass coated with the PEO M_w 526, 2000 and 9800 brushes. Error bars represent the average standard deviation over three separate experiments.

Adhesion and retention of the yeast strains *C. albicans* GB 1/2 and *C. tropicalis* GB 9/9 are collected in Figure 3. For *C. albicans*, a significantly higher initial deposition rate was found on glass at 37°C ($5.1 \times 10^2 \text{ cm}^2 \text{ s}^{-1}$) than at 20°C ($1.9 \times 10^2 \text{ cm}^2 \text{ s}^{-1}$). The initial deposition rate decreases significantly for all brush coated surfaces, but no significant differences were found between the three chain lengths. These trends for the initial deposition rate were also reflected in the numbers of *C. albicans* adhering after 4 h. For *C. tropicalis* different trends were found, since adhesion on the PEO 526 brush was comparable to adhesion on glass and only decreased significantly for the PEO 2000 and PEO 9800 brush. Yeast detached from the glass surface by 90% or more for *C. albicans*, but only around 20% for *C. tropicalis*. PEO brushes increased these percentages to close to 100% for both yeast strains. No significant differences in adhesion and retention on the brush coated surfaces were found between 20 and 37°C.

Discussion

PEO brushes have shown great potential in retarding or even preventing both protein adsorption [35] and microbial adhesion [20,36,37]. However, microbial adhesion, as opposed to protein adsorption, has never been systematically studied varying potentially critical factors, like temperature and brush length. The effects may be very different, as interaction forces involving larger particles are usually more extensive than arising from proteins. In our study, glass was coated with PEO brushes of three different lengths, and adhesion and retention of four different microbial strains was evaluated in a parallel plate flow chamber at 20 and 37°C.

PEO brushes were characterized by various surface sensitive techniques. The decrease in receding contact angle with increasing PEO chain length may be due to the extension of the polymer chain in the water droplet. The longer PEO chains extend further, thereby providing more interaction with the water droplet. As glass consists of 67% oxygen and 33% silicon and PEO of 33% oxygen and 67% carbon, the decrease in oxygen and silicon and increase in carbon as obtained by XPS are in agreement with a thicker dry PEO layer for the PEO brush with higher chain length. The larger values for the dry layer thickness of PEO as measured by ellipsometry as compared to those obtained from XPS may be due to the hygroscopic nature

of PEO. Water from the environment may be absorbed in the PEO layer during ellipsometry in ambient air, thereby irregularly increasing the thickness, whereas XPS is conducted in high vacuum. In view of the large standard deviation in the ellipsometry data, the XPS results were used for further calculations and the derived grafting density on glass was between 0.2 and 2.3 chains per nm². This density is high as compared to those of brushes of comparable molecular weight formed in solution, for which values between 0.01 and 0.2 nm² are usually reported [17,38,39]. This is most likely due to the higher concentration of polymers when in a polymer melt, leading to a higher probability of reaction with the surface. Lower grafting densities with increased chain length have been shown previously [40] and are likely due to steric inaccessibility of reaction sites.

Theoretical models describing the mechanism of particle repellency of PEO brushes describe PEO chains as neutral, non-interacting polymers which resist compression or penetration by particles through osmotic repulsion [11-13]. Attraction of the particle at the outer edge of a PEO brushed surface is usually attributed to long range Lifshitz-Van der Waals forces. This is in agreement with the increased adhesion of most microorganisms on the shortest PEO brush, which has also been found for several proteins. The essential role of attractive Lifshitz-Van der Waals forces and their reduction by a brush, is furthermore collaborated by the more extensive adhesion of the larger yeast cells to the PEO 9800 brush as compared with adhesion of the smaller *S. epidermidis* and other bacteria previously investigated [20].

Also hydrogen-bonding, Lifshitz-Van der Waals and hydrophobic interactions may occur between a particle and the PEO brushed surface [41]. This can lead to attractive interaction as found between several proteins and PEO [23,42-45]. On all PEO brushes, *P. aeruginosa* adhered in larger numbers than *S. epidermidis* although similar Lifshitz-Van der Waals attraction is expected for both bacteria. It should be noted however that this expectation was based on assumed identical Hamaker constants for both organisms. The same applies for the large adhesion on PEO brushes of the yeast *C. tropicalis* as compared to *C. albicans*. Both *P. aeruginosa* and *C. tropicalis* have a more hydrophobic character than the two other strains [20], which seems to indicate a specific contribution from hydrophobic

interaction between these microorganisms and the PEO coating as was e.g. also found between the protein lysozyme and PEO [44].

Almost all microorganisms could be removed from the brushed surface by an air bubble, irrespective of the brush length, indicating that Lifshitz-Van der Waals interactions with the surface (between 1.0×10^{-12} and 2.2×10^{-10} N) and direct interactions with PEO are weaker than the force exerted by an air bubble (around 1×10^{-7} N) [29]. Reversible adsorption of proteins on a PEO brush has also been described, in line with the present observations for larger biological particles [46,47]. Most microbial adhesion studies on PEO coatings [48-53] include some kind of washing step, during which an unknown number of particles may be removed and results of such studies may not be unambiguously interpreted as indicative of microbial adhesion [54]. Low numbers of adhering microorganisms in studies involving washing steps may either point to a reduction in adhesiveness of the substratum surface or to a lower retention of adhering organisms, i.e. easier detachment.

Most microbial adhesion results as obtained here follow theoretical expectations and are in line with results found for proteins. The absence of a temperature effect on microbial adhesion is intriguing, however, since adsorption of several proteins to brushed surfaces increased with increasing temperature [8-10]. Temperature effects on protein adsorption to brushes may be explained in terms of interaction between the protein and PEO segments in the brush [23,41]. The invariance with temperature of microbial interaction with the brushed surface, as found in our study, suggests that the microbial cells cannot penetrate the brush.

Conclusions

Application of PEO brushes at surfaces reduces the adhesion of both bacteria (*S. epidermidis*, *P. aeruginosa*) and yeast (*C. albicans*, *C. tropicalis*). As a rule, both the initial deposition rate and the number of adhering bacteria after a prolonged period of time decrease with increasing length of the brush. Furthermore, the reduction tends to be less for the larger yeast cells as compared to the smaller bacteria. These results suggest that Lifshitz-Van der Waals interactions play a crucial role in the residual adhesion of the microorganisms at the outer edge of the brush. However, additional factors are not unimportant. For instance, the more hydrophobic organisms (*P. aeruginosa* and *C. tropicalis*) adhered in larger numbers to the

PEO chains than the hydrophilic ones (*S. epidermidis* and *C. albicans*). Adhering bacteria and yeast can be easily removed from the brush by a passing air bubble, whereas such a removal is much more difficult from the bare glass surface, indicating a relatively weak adhesion at the brush. Finally, changing the temperature from 20°C to 37°C does not lead to a significantly different adhesion pattern for the four microbial strains investigated.

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