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Inhibition of adhesion of yeasts and bacteria by poly(ethylene oxide)-brushes on glass in a parallel plate flow chamber

Astrid Roosjen,[1] Hans J. Kaper,[1] Henny C. van der Mei,[1] Willem Norde[1,2] and Henk J. Busscher[1]

[1]Department of Biomedical Engineering, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

[2]Laboratory of Physical Chemistry and Colloid Science, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, The Netherlands

Correspondence
Henk J. Busscher
h.j.busscher@med.rug.nl

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Poly(ethylene oxide) (PEO)-brushes are generally recognized as protein-repellent surfaces, and although a role in discouraging microbial adhesion has been established for some strains and species, no study exists on the effects of PEO-brushes on a large variety of bacterial and yeast strains. In this paper, a PEO-brush has been covalently attached to glass and silica by reaction in a polymer melt. Subsequently, the presence of a PEO-brush was demonstrated using contact angle measurements, X-ray photoelectron spectroscopy and ellipsometry. For five bacterial (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus salivarius*, *Escherichia coli* and *Pseudomonas aeruginosa*) and two yeast strains (*Candida albicans* and *Candida tropicalis*), adhesion to PEO-brushes was compared with adhesion to bare glass in a parallel plate flow chamber. The initial deposition rates of *Sta. epidermidis*, *Sta. aureus* and *Str. salivarius* to glass were relatively high, between 2400 and 2600 cm^{-2} s^{-1}, while *E. coli* and *P. aeruginosa* deposited much more slowly. The initial deposition rates of the yeasts to glass were 144 and 444 cm^{-2} s^{-1} for *C. albicans* GB 1/2 and *C. tropicalis* GB 9/9, respectively. Coating of the glass surface with a PEO-brush yielded more than 98% reduction in bacterial adhesion, although for the more hydrophobic *P. aeruginosa* a smaller reduction was observed. For both yeast species adhesion suppression was less effective than for the bacteria and here too the more hydrophobic *C. tropicalis* showed less reduction than the more hydrophilic *C. albicans*. The PEO-brush had a thickness of 22 nm in water, as inferred from ellipsometry. Assuming that on bare glass the adhered micro-organisms are positioned only a few nanometers away from the surface and that the brush keeps them at a distance of 22 nm, it is calculated that the brush yields a sevenfold attenuation of the Lifshitz–Van der Waals attraction to the surface between the micro-organisms and the surface. Decreased Lifshitz–van der Waals attraction may be responsible for the suppression of the microbial adhesion observed.

INTRODUCTION

Micro-organisms tend to adhere strongly to surfaces at the onset of the formation of a complex adhering microbial community, called a biofilm (Gottenbos *et al.*, 1999). 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, in-dwelling voice prostheses and vascular and urinary catheters (Gristina, 1987). While the mechanism of bacterial adhesion to the biomaterial surface has not been fully elucidated, interactions between biomaterial surfaces and bacteria have been reported to include non-specific and specific interactions, such as electrostatic and Lifshitz–Van der Waals forces, hydrophobic interactions and a variety of specific receptor–adhesin interactions (Hermansson, 1999; Busscher & Weerkamp, 1987).

Several surface modifications have been developed with the aim of discouraging microbial adhesion. For instance, hydrophobic silicone rubber could be made hydrophilic by repeated argon plasma treatments (Everaert *et al.*, 1998). It was found in *in vitro* experiments that microbial adhesion over a 4 h time span to hydrophilized silicone rubber was generally less than on original, hydrophobic silicone rubber. Also, positively and negatively charged surfaces have been investigated and adhesion of bacteria was found to be lower

Abbreviations: PEO, poly(ethylene oxide); XPS, X-ray photoelectron spectroscopy.

on negatively charged surfaces as compared to positively charged ones (Gottenbos *et al.*, 2001). However, although many of the approaches taken have been shown to yield statistically significant and scientifically interesting reductions in microbial adhesion, almost none of the approaches taken so far have shown clinically significant reductions, of more than 98 % (Tsibouklis *et al.*, 1999).

Protein-rejecting surfaces can be created by attaching poly-(ethylene oxide) (PEO) chains to surfaces (Harris, 1992). When PEO chains are attached to a surface and project into the surrounding medium, they form so-called mushroom structures at low grafting densities and brush structures at high grafting densities. Both structures are able to reduce protein adhesion by forming a steric barrier between the protein molecule and the surface. Notably in the case of brushes, incoming protein molecules have much difficulty in compressing or protruding into the heavily hydrated PEO chains. Therewith, a PEO-brush forms a steric barrier preventing close approach and thus attenuates the attractive Lifshitz–Van der Waals interaction between the protein and the surface. PEO-coatings have also been investigated for their ability to prevent bacterial adhesion (Park *et al.*, 1998; Razatos *et al.*, 2000; Ista *et al.*, 1996; Bridgett *et al.*, 1992). Varying results have been obtained, ranging from reductions in bacterial adhesion of 98 % (Razatos *et al.*, 2000; Ista *et al.* 1996), 90–98 % (Bridgett *et al.*, 1992) and between 0 and 90 % (Park *et al.*, 1998). These studies, however, have used only a small number of strains and the adhesion methodology employed involved dipping and slight rinsing, which may also cause detachment and accompanying low adhesion numbers (Gòmez-Suárez *et al.*, 2001). Hitherto, studies on polymer brushes have not included any yeast strains, impeding generalization.

The aim of the present study is to determine adhesion of five different bacterial and two different yeast strains to a PEO-brush, covalently attached to glass. Brushes are characterized by contact angle measurements, X-ray photoelectron spectroscopy (XPS) and ellipsometry. Adhesion results are discussed in terms of the attenuation of the Lifshitz–Van der Waals interaction energies caused by the presence of a brush and variations in size, shape and cell surface hydrophobicity of the micro-organisms used.

METHODS

PEO-brushes. Reaction of surface silanol groups such as on glass and silica, with vinyl-terminated polymers in a polymer melt was described by Maas *et al.* (2003).

Methacryl-terminated PEO with a molar mass of 9800 Da and a polydispersity index of $<1{\cdot}03$ was purchased from Polymer Source and used as received. Brushes were applied onto microscope glass slides (Menzel-Gläser) and used as substratum surfaces in bacterial adhesion, XPS and contact angle measurements, while brushes were applied on silica for ellipsometry, contact angle measurements and additional XPS experiments. Surfaces were first sonicated in 2 % RBS 35 detergent (Omnilabo International), 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 %; Merck) 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 covered with a solution of the methacryl-terminated PEO in chloroform (4 mg ml^{-1}). 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 and drying in a stream of nitrogen.

To reduce the biological variation in bacterial adhesion, glass surfaces used for adhesion studies were only partly grafted with PEO chains, which allowed the study of adhesion to a glass surface and a brush-coated surface in the same experimental run.

Characterization of PEO-brushes. Water contact angles on pristine glass, PEO-coated glass, pristine silica and PEO-coated silica were measured at 25 °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. These were obtained by keeping the syringe 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. On each sample, at least five droplets were placed at different positions and results of three separately prepared coatings were averaged.

The chemical compositions of pristine glass, PEO-coated glass, pristine silica and PEO-coated silica surfaces were determined by XPS using an S-Probe spectrometer (Surface Science Instruments). Three separate measurements were performed on each sample. The elemental surface compositions were expressed in atomic %, setting %C + %O + %Si to 100 %.

The film thickness of dry PEO layers on silica was determined using a null ellipsometer (Sentech SE-400) with a He–Ne laser light source ($\lambda = 632{\cdot}8$ nm) at an angle of incidence of 70°. The ellipsometry software from Sentech was used to calculate the film thickness on each of the three different spots measured per sample surface. The mean thickness of the PEO layer (D) was calculated by averaging the film thickness of ten separately prepared samples. Using the density of bulk PEO, $\rho = 1{\cdot}13$ g cm^{-3} for the density of the dry PEO film, the unit surface area per grafted molecule (A_m) was calculated according to the equation:

$$A_m = \frac{M_W}{\rho D N_A} \quad (1)$$

in which M_W is the molar mass, D the film thickness and N_A Avagadro's number. Based on the unit surface area per grafted molecule thus obtained, a monomer size, a, of 0·278 nm (Jeon *et al.*, 1991) and the number of monomers, $N = 220$, an estimation of the length of the PEO-brush in water (L) can be calculated using the Flory version of the Alexander model (Halperin, 1999):

$$L = aN\left(\frac{a^2}{A_m}\right)^{\frac{1}{3}} \quad (2)$$

This model assumes ideal chains that interpenetrate freely and exhibit no correlations and has been shown to give a fair prediction of PEO-brush length in water (Efremova *et al.*, 2001).

Microbial strains and growth conditions. Five bacterial strains, *Staphylococcus epidermidis* HBH 276, *Staphylococcus aureus* ATCC 12600, *Streptococcus salivarius* GB 24/9, *Escherichia coli* O2K2 and *Pseudomonas aeruginosa* AK1 were used in this study, together with two yeast strains: *Candida albicans* GB 1/2 and *Candida tropicalis* GB 9/9. All strains were first grown overnight at 37 °C on an agar

plate from a frozen stock, which was kept at 4 °C, never longer than 2 weeks. Several colonies were used to inoculate 10 ml tryptone soya broth (TSB; Oxoid) for the staphylococci, Todd–Hewitt broth (THB; Oxoid) for *Str. salivarius*, nutrient broth (NB; Oxoid) for *P. aeruginosa* and brain heart infusion (BHI; Oxoid) for *E. coli*, *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 18 h. The micro-organisms from the second culture were harvested by centrifugation for 5 min at 9600 ***g*** for *Str. salivarius*, *C. albicans*, *C. tropicalis* and *P. aeruginosa* and 5 min at 5000 ***g*** for the other strains and washed twice with demineralized water. Subsequently, the bacteria were resuspended in 200 ml PBS solution (10 mM potassium phosphate, 150 mM NaCl, pH 6·8), for *Sta. epidermidis*, *Sta. aureus* and *P. aeruginosa* after sonication on ice (10 s), to a concentration of 3×10^8 ml^{-1}. Yeasts were resuspended in PBS to a concentration of 3×10^6 ml^{-1}. The hydrophobicities of the bacterial strains, as derived from measured water contact angles, have been published previously (Van der Mei *et al.*, 1998). The relative hydrophobicity of the yeast *C. tropicalis* GB 9/9 as compared to *C. albicans* GB 1/2 was also shown previously (Busscher *et al.*, 1997).

Parallel plate flow chamber and image analysis. The flow chamber ($175\times17\times0{\cdot}75$ mm) and image analysis system have been described previously (Busscher & Van der Mei, 1995). 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) mounted on a phase-contrast microscope (Olympus BH-2) equipped with a ×40 ultralong working distance objective (Olympus ULWD-CD Plan 40 PL) for experiments with bacteria and with a ×10 objective for experiments with yeasts. The camera was coupled to an image analyser (TEA; Difa). 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 micro-organisms from the analysis. An image covers a surface area of 0·0096 mm^2 at the magnification used for bacterial experiments and 0·18 mm^2 at the magnification employed in the experiments with yeasts.

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 10 s^{-1} (0·025 ml s^{-1}), which yields a laminar flow (Reynolds number 0·6). The microbial suspension was circulated through the system for 4 h and images were obtained alternately from the glass and from the PEO-coated part.

The initial increase in the number of adhering micro-organisms with time, was expressed in a so-called initial deposition rate j_0 (cm^{-2} s^{-1}), i.e. the number of adhering micro-organisms per unit area and time. The number of micro-organisms adhering after 4 h, $n_{4\ \mathrm{h}}$, was taken as an estimate of microbial adhesion in a more advanced state of the process.

All values given in this paper are the means of experiments on three separately prepared brush-coated surfaces and were carried out with separately grown micro-organisms. To analyse differences between bare glass and PEO-coated glass, independent *t*-tests were performed with SPSS for Windows (SPSS Inc) using a significance level of 0·05.

Calculation of Lifshitz–Van der Waals interaction energies. The Lifshitz–Van der Waals interaction energy (U_{LW}), mediating microbial adhesion to surfaces, decays with the distance d between a sphere and a semi-infinite flat surface according to the equation:

$$U_{\mathrm{LW}} = -\frac{A}{6}\left[\frac{R}{d} + \frac{R}{d+2R} + \ln\left(\frac{R}{d+2R}\right)\right] \quad (3)$$

in which A is the Hamaker constant and R is the microbial radius (taken as 0·5 μm for bacteria and 2·5 μm for yeasts). Hamaker constants for micro-organisms interacting with glass surfaces are not well known and values ranging from $0{\cdot}56\times10^{-21}$ to $6{\cdot}9\times10^{-21}$ J have been reported (Busscher & Weerkamp, 1987; Rijnaarts *et al.*, 1995; Mafu *et al.*, 1991). Using these values, a range of possible Lifshitz–Van der Waals interaction energies can be calculated.

DLVO theory describes microbial adhesion as a balance between attractive Lifshitz–Van der Waals and repulsive or attractive electrostatic forces (Hermansson, 1999). Using this theory the micro-organisms can be calculated to be located in the so-called secondary interaction minimum, at approximately 3 nm from the surface under the current conditions (Bos *et al.*, 1999; Rutter, 1980).

RESULTS

Surface characterization

The water contact angle of a sessile drop of water on PEO-coated glass was $41\pm5^\circ$, whereas pristine glass showed complete wetting. Advancing and receding contact angles on PEO-coated glass were $48\pm5^\circ$ and $16\pm2^\circ$, respectively. Silica coated with PEO showed similar contact angles with a value of $36\pm5^\circ$ and advancing and receding contact angles were $41\pm6^\circ$ and $16\pm4^\circ$, respectively. Pristine silica also showed complete wetting. Table 1 summarizes the elemental surface compositions obtained by XPS for pristine glass, PEO-coated glass, pristine silica and PEO-coated silica. On glass surfaces, the PEO-coating decreased the surface silicon concentration from 30·7 to 6·9 % and surface oxygen concentration from 56·9 to 33·6 %. On the other hand, the carbon concentration increased from 12·0 to

Table 1. Percentage elemental surface composition of pristine glass and silica, as well as of PEO-coated surfaces, as determined by XPS

Data are presented ± SD over three separate measurements.

Element	Pristine glass	PEO-coated glass	Pristine silica	PEO-coated silica
C	12·0±1·0	59·5±0·8	19·1±2·2	60·4±2·8
O	56·9±0·8	33·6±0·3	52·2±2·2	32·7±1·4
Si	30·7±0·2	6·9±0·9	26·8±1·1	6·9±1·4

59·5 %. For silica coated with PEO, similar trends were observed: the surface concentrations of silicon and oxygen decreased, whereas the surface concentration of carbon increased. Furthermore, silica coated with PEO showed within error margins the same surface composition as glass coated with PEO. This justifies ellipsometry measurements on silica to be extrapolated to glass.

The thickness of the PEO layer on silica in ambient air was determined using ellipsometry and found to be $8{\cdot}3 \pm 2{\cdot}2$ nm. Using equation (1), an area per grafted molecule (A_M) of 1·7 nm^2 was found. This corresponds to a brush length in water of 22 nm, as calculated using equation (2).

Microbial adhesion

Fig. 1 shows *Sta. epidermidis* HBH 276 and *C. albicans* GB 1/2 adhering after 2 h on either side of the border between PEO-coated and bare glass as obtained in the parallel plate flow chamber. On the PEO-coated side, there are hardly any micro-organisms adhering, but the glass side is clearly covered by bacteria and yeasts.

The initial deposition rates (j_0) of adhering organisms are shown in Fig. 2, while numbers of adhering micro-organisms after 4 h ($n_{4\,h}$) are compiled in Fig. 3. The initial deposition rates (Fig. 2) of *Sta. epidermidis*, *Sta. aureus* and *Str. salivarius* to glass are relatively high, between 2400 and 2600 cm^{-2} s^{-1}, while *E. coli* and *P. aeruginosa* deposit much more slowly onto glass. The initial deposition rates of the yeasts on glass were 144 and 444 cm^{-2} s^{-1} for *C. albicans* GB 1/2 and *C. tropicalis* GB 9/9, respectively. Coating of the glass surface with a PEO-brush reduces the initial deposition rate by 95 % for *Str. salivarius* to even more than 98 % for *Sta. epidermidis*, *Sta. aureus* and *E. coli* ($P<0{\cdot}05$). For both *Sta. epidermidis* HBH 276 and *Sta. aureus* ATCC 12600 the values were as low as 14 cm^{-2} s^{-1}. Interestingly, deposition rates of the more hydrophobic *P. aeruginosa* AK1 are far less reduced in the presence of a brush than of the other strains. Also for yeasts, the more hydrophobic *C. tropicalis* GB 9/9 showed less reduction in deposition rates than the more hydrophilic *C. albicans* GB 1/2.

The numbers of organisms adhering to glass after 4 h ($n_{4\,h}$, Fig. 3) are relatively high for *Sta. epidermidis* and *Sta. aureus*, i.e. between 15×10^6 and 29×10^6 cm^{-2}. For *Str. salivarius* and *P. aeruginosa* the $n_{4\,h}$ values were lower and the lowest number of adhering bacteria was found for *E. coli* ($2{\cdot}3 \times 10^6$ cm^{-2}). For yeasts, the number of adhering micro-organisms after 4 h was lower than for the bacterial strains. Application of a PEO-coating to the glass surface reduced microbial adhesion after 4 h by more than 98 % ($P<0{\cdot}05$) for *Sta. epidermidis* ($4{\cdot}5 \times 10^4$ cm^{-2}), *Sta. aureus* (15×10^4 cm^{-2}) and *E. coli* ($3{\cdot}4 \times 10^4$ cm^{-2}). Also for *Str. salivarius* a large reduction in adhesion of 88 % after 4 h was found ($P<0{\cdot}05$). The adhesion of hydrophobic *P. aeruginosa* AK1 showed large differences after 4 h in separate experiments. In some experiments, reductions of more than 90 % were found, but in others adhesion of *P. aeruginosa* AK1 was similar to that on glass, leading to reductions that were not statistically significant ($P=0{\cdot}15$). The yeast strains *C. albicans* GB 1/2 and *C. tropicalis* GB 9/9 both showed a large decrease in adhesion after 4 h of 81 and 75 %, respectively ($P<0{\cdot}05$).

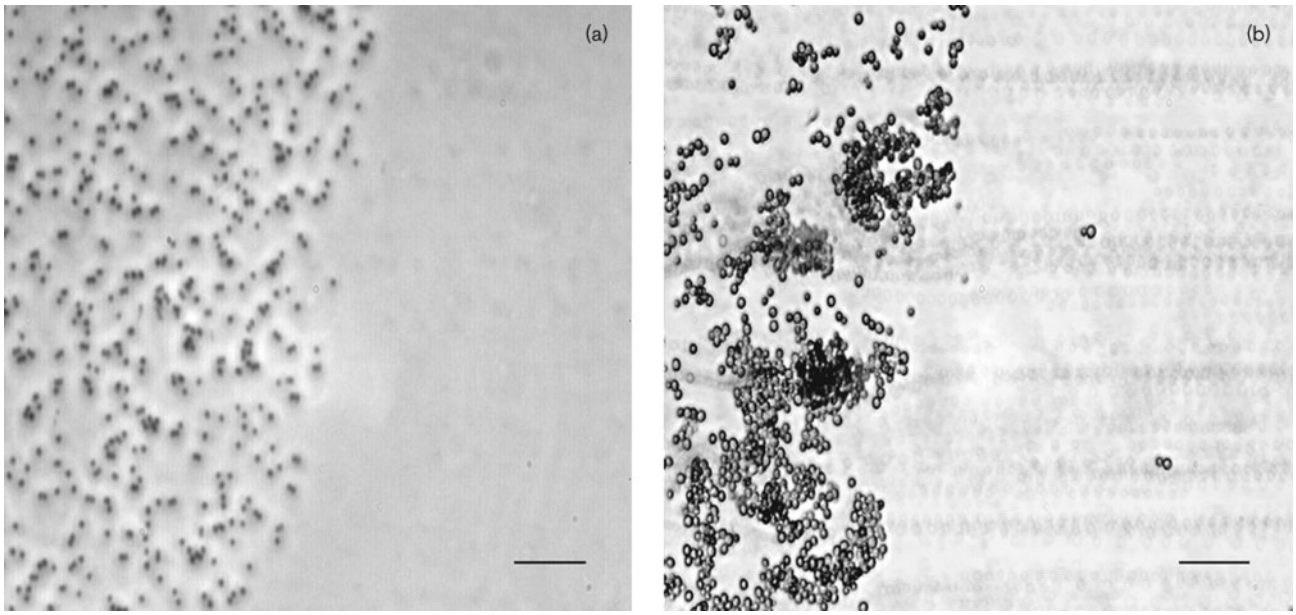

Fig. 1. Micrographs of *Sta. epidermidis* HBH 276 (a) and *C. albicans* GB 1/2 (b) adhering after 2 h in a parallel plate flow chamber around the border zone between glass (left-hand side) and PEO-coated glass (right-hand side). Micrographs were obtained by *in situ* phase-contrast microscopy. Bars, (a) 10 μm; (b) 40 μm.

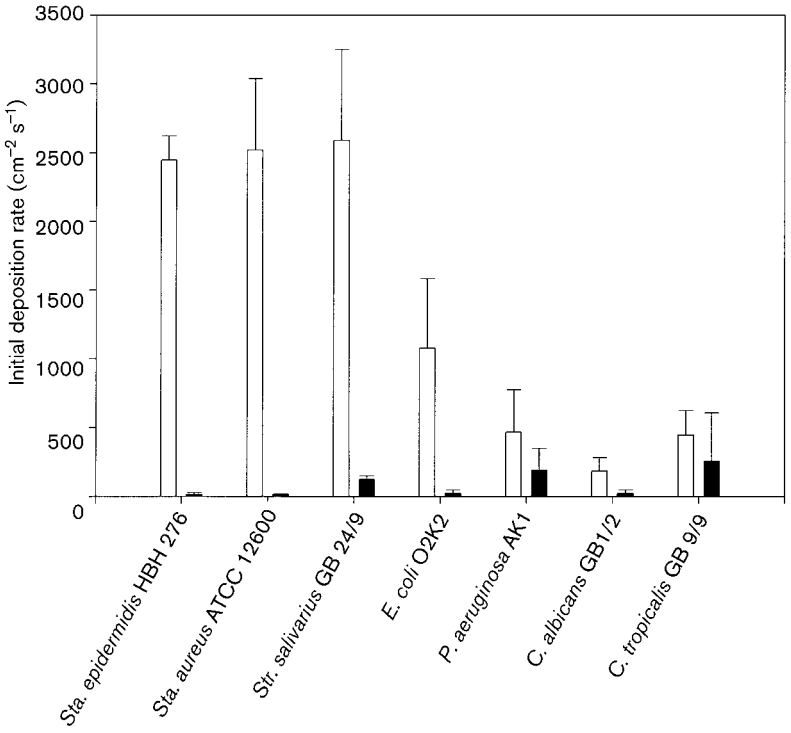

Fig. 2. The initial deposition rates of the various bacterial and yeast strains employed in this study on glass (white bars) and PEO-coated glass (black bars). Error bars represent the mean SD over three separate experiments.

Lifshitz–Van der Waals interaction energies

The extension of the PEO-brush in water, as derived from the grafting density using equation (2), amounts to 22 nm. This determines the distance at which the micro-organisms are kept away from the underlying surface. For the bacteria it then follows that, using Hamaker constants from literature and a radius of 0·5 μm, Lifshitz–Van der Waals interaction energies can vary from $2{\cdot}0 \times 10^{-21}$ to 25×10^{-21} J (0·5 to 6·3 kT) per bacterium as calculated using equation (3). As the Hamaker constant for each strain can be considered constant, it is calculated that for a given bacterium, the Lifshitz–Van der Waals interaction energy at the edge of the brush is about seven times lower than at 3 nm separation

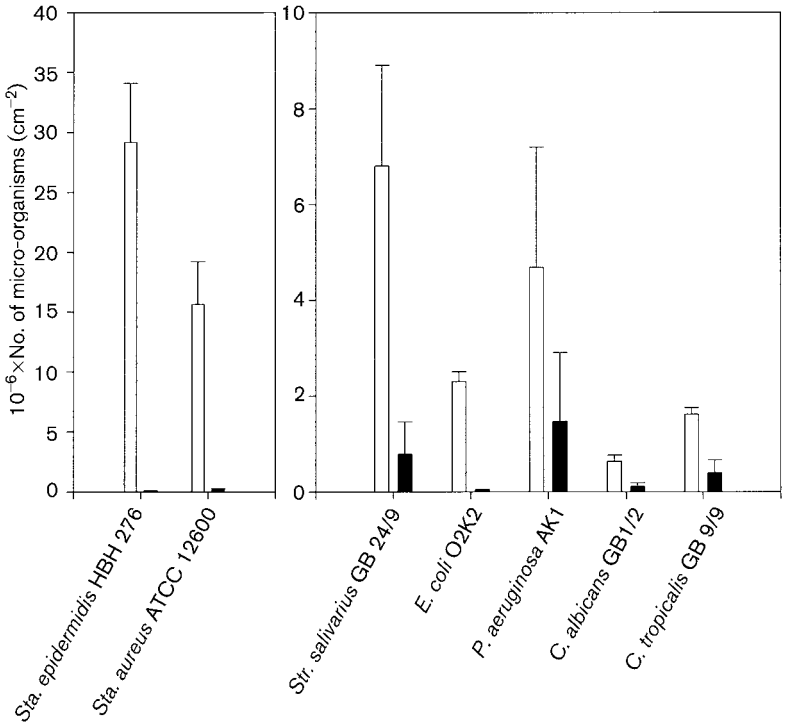

Fig. 3. The number of micro-organisms adhering after 4 h on glass (white bars) and PEO-coated glass (black bars). Error bars represent the mean SD over three separate experiments.

from the bare surface. A similar reduction by a factor of seven can be calculated for the yeasts, possessing a Lifshitz–Van der Waals interaction energy on the brush coating ranging from $1{\cdot}04 \times 10^{-20}$ to $12{\cdot}8 \times 10^{-20}$ J (2·6 to 32 kT).

DISCUSSION

In this study, glass surfaces were coated with PEO in a polymer melt reaction and the adhesion of several microbial strains, including two yeast strains, to the resulting PEO-brushes in aqueous environment was evaluated in a parallel plate flow chamber. PEO-brushes were characterized by various surface-sensitive techniques. Water contact angles indicated that pristine glass and silica surfaces were more hydrophilic than the PEO-coated surfaces, as in other studies (Park *et al.*, 1998; Lee & Laibinis, 1998). The high contact angle hysteresis ($>25^{\circ}$) found for both PEO-coated surfaces suggests penetration of water in the PEO layer and mobility of the PEO chains (Andrade *et al.*, 1985). XPS measurements indicated the presence of carbon on the pristine surfaces, likely to be due to adsorption of hydrocarbon components from the atmosphere (Seah *et al.*, 1994; Kasemo & Lausmaa, 1988). For both glass and silica an increase in carbon and a decrease in silicon and oxygen concentration was found after coating the surfaces with PEO. As glass and silica have a composition of 33 % silicon and 67 % oxygen and PEO is composed of 67 % carbon and 33 % oxygen, these changes are consistent with PEO being present on both surfaces. The mean separation between two polymer chains (1·3 nm) was calculated from the ellipsometric thickness. This small separation forces the PEO chains to stretch out from the surface into the solution, thus forming the so-called PEO-brush.

The mechanisms by which a PEO-brush discourages microbial adhesion are not fully elucidated.

One explanation is that PEO molecules in an aqueous environment are highly mobile and attain extremely large exclusion volumes. When a particle enters, the brush will be somewhat compressed leading to a repulsive osmotic force. Moreover, freedom of movement of the polymer chains will be reduced and this leads to an unfavourable decrease in conformational entropy. Halperin (1999) described a brush as being composed of simple chains forming a steric barrier between the surface and an approaching particle. Interactions between the particle and the brush-coated surface were thought to include three contributants: (a) short-range surface contact, (b) Lifshitz–Van der Waals attraction between the particle and the surface and (c) repulsive osmotic interaction between the particle and the brush. For a dense brush, large proteins can only adhere to the surface by adsorption in the secondary interaction minimum at the outer periphery of the brush as the protein is too large to interpenetrate between the polymer chains. Because bacteria and yeasts are larger than proteins, we assumed secondary adsorption for the micro-organisms involved in this study and calculated the Lifshitz–Van der Waals attraction at the edge of the brush.

The Lifshitz–Van der Waals attraction for bacteria at the edge of the brush, i.e. at a distance of 22 nm from the supporting surface, was calculated to be in the region of 0·5–6·3 kT. This is a sevenfold attenuation as compared to bare glass. These low Lifshitz–Van der Waals attractions could account for the almost complete lack of adhesion as observed for most bacterial strains. The large decrease in Lifshitz–Van der Waals attraction is in accordance with the more than 98 % reduction in adhesion on the brush as compared to bare glass, depending on the strain used (Fig. 3). This is a larger reduction than usually achieved *in vitro* with changing the hydrophobicity or other surface modifications. Park *et al.* (1998) showed that reduced Lifshitz–Van der Waals attraction by a PEO-coating with a molar mass of 1000 was not accompanied by a reduction in adhesion of *Sta. epidermidis* ATCC 12228. However, PEO with a molar mass of 3500 Da rendered the surface resistant to staphylococcal adhesion. Another study showed that surfaces with PEO chains with a molecular mass of 4500 and 20 000 Da blocked the adhesion of *E. coli* D 21 (Razatos *et al.*, 2000). Surprisingly, it has also been found that a self-assembled monolayer of PEO with only six PEO units is able to prevent the adhesion of *Sta. epidermidis* ATCC 14990 and *Deleya marina* ATCC 25374 (Ista *et al.*, 1996). Bridgett *et al.* (1992) showed that surfactants with PEO chains as short as three PEO units were able to reduce the adhesion of three *Sta. epidermidis* strains by about 97 %. This observation suggests that even with short PEO chains entropy repulsion outweighs attractive Lifshitz–Van der Waals interaction.

In contrast to the above-mentioned literature reports, focussing only on a small number of strains, we investigated the effects of a PEO-brush on adhesion of seven very different microbial strains: yeasts and bacteria, Gram-positive and Gram-negative bacteria, rod-shaped and spherical bacteria. Both spherical, Gram-positive bacterial strains (*Sta. epidermidis* HBH 276, *Sta. aureus* ATCC 12600, *Str. salivarius* GB 24/9) and a rod-shaped Gram-negative bacterial strain (*E. coli* O2K2) hardly showed any adhesion to the brush. This seems to indicate that form of the bacteria and composition of the cell membrane are not of a major influence on adhesion to the brush. It is difficult to explain why the brush exerts a much smaller reduction on the adhesion of the more hydrophobic organisms, *P. aeruginosa* AK1 and *C. tropicalis* GB 9/9. However, it has been discussed that PEO chains are not just simple non-interacting chains, but they may be engaged in attractive interactions with other components (Morra, 2000). For instance, hydrophobic proteins have been suggested to be able to adsorb to PEO-brushes through their hydrophobic moieties (Furness *et al.*, 1998), which was confirmed by the work of Sheth *et al.* (2000), demonstrating attraction of PEO to non-polar surfaces. It has furthermore been shown that PEO can exist in a protein-repulsive, polar, helical or random structure, and a protein-attractive, apolar, all-*trans* structure (Harder

et al., 1998; Currie *et al.*, 2003). The work of Efremova *et al.* (2001) showed that changing circumstances, such as temperature or compressive load, can change PEO from a protein-repellent to a protein-attractive state. Conclusively, one can imagine that the hydrophobic surface of a micro-organism can induce this attractive state, thus explaining the smaller reductions in adhesion.

A 70 % reduction in adhesion after 4 h due to the presence of the brush was found for both yeast strains, in accordance with the sevenfold reduction in Lifshitz–Van der Waals interaction. This decrease is less than for most bacterial strains, which can be explained by the fact that yeasts are considerably larger than bacteria and thus experience a stronger Lifshitz–Van der Waals attraction at a given separation from the surface.

In summary, it has been demonstrated that bacterial adhesion to PEO-brushes in a parallel plate flow chamber is greatly decreased with respect to adhesion to glass, except for hydrophobic bacteria. This decrease was thought to be largely caused by an attenuation in Lifshitz–Van der Waals attractive energies. Similarly, adhesion of yeasts was also decreased by the presence of a brush, but not to the same extent as observed for bacteria. This was ascribed to the fact that, owing to their larger dimensions, yeast cells experience a greater Lifshitz–Van der Waals attraction.

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