The use of polymer brush coatings to prevent microbial adhesion
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Summary
Biofilm formation on biomaterials implants and subsequent infectious complications are frequently a reason for failure of many biomedical devices, such as total hip arthroplasties, vascular catheters and urinary catheters. 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, where after they grow and anchor through secretion of extracellular polymeric substances. Adhesion of microorganisms is influenced by the physico-chemical properties of a biomaterials surface, with or without conditioning film. Three surface modification techniques aimed at preventing biofilm buildup are reviewed in Chapter 1. Firstly, positively charged materials stimulate bacterial adhesion, but prevent growth of some strains of adhering bacteria. Secondly, the use of low surface free energy materials does not always reduce in vitro adhesion of bacteria, but has been found beneficial in in vivo applications where fluctuating shear forces prevail, like on intra-oral devices and urinary catheters. Finally, polymer brushes have shown a high reduction in in vitro adhesion of some strains and species. However, for clinical application, the problem of unstable coupling of polymer brushes to the substratum still has to be resolved.

The primary aim of this thesis is to investigate the mechanism by which poly(ethylene oxide) (PEO) brushes reduce the deposition of microorganisms on surfaces. Knowledge of that mechanism may provide directions for the application of PEO brushes to suppress biomaterials related infections.

In Chapter 2, a polymer brush was made by covalently attaching PEO chains to glass and silica by reaction in a polymer melt. PEO brushes are generally recognized as protein repellent surfaces, but no study exists on the effects of PEO brushes for a large variety of bacterial and yeast strains. 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 S. epidermidis, S. aureus and S. salivarius to glass were relatively high between 2400 and 2600 cm$^{-2}$s$^{-1}$, while E. coli and P. aeruginosa deposited much slower. The initial deposition rates of the yeasts to glass are 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 *P. aeruginosa* a smaller reduction was observed. For both yeast species adhesion suppression was less effective than for the bacteria, which may be explained by the fact that yeasts are considerably larger than bacteria, thus experiencing a stronger Lifshitz-Van der Waals attraction at a given distance from the surface. The PEO brush had a thickness of 22 nm in water, as inferred from ellipsometry. It is assumed that on bare glass the adhered microorganisms are positioned in the secondary interaction minimum only a few nanometers away from the surface and that the brush keeps them at a distance of 22 nm. At this higher separation distance it is calculated that the Lifshitz-Van der Waals attraction to the surface is sevenfold attenuated as compared to the bare glass. Decreased Lifshitz-Van der Waals attraction is considered to be the main reason for the suppression of the microbial adhesion observed.

The factors determining whether a bacterial strain does or does not adhere to a PEO brush coating, were investigated in Chapter 3. Bacterial adhesion was assessed in a parallel plate flow chamber. The bacterial adhesion results indicate that a distinction could be made between three adhesive and three non-adhesive strains of *P. aeruginosa*, while bacterial motility and zeta potential were comparable for all six strains. However, water contact angles indicated that the adhesive strains were much more hydrophobic than the non-adhesive strains. Furthermore, only adhesive strains produced surface active extracellular substances that may be engaged in attractive interactions with the PEO chains. AFM showed that the adhesion energy, measured from the retract curves of a bacteria coated cantilever from a brush coating, was significantly more negative for adhesive strains than for non-adhesive strains (p < 0.001). Through surface thermodynamic and extended-DLVO analyses these stronger adhesion energies could be attributed to acid-base interactions. However, the energies of adhesion of all strains with a brush coating were small when compared with their energies of adhesion with a glass surface. Accordingly, even the adhesive *P. aeruginosa* strains could be easily removed from a PEO brush coating by the passage of a liquid-air interface.
In **Chapter 4**, the effects of temperature and PEO chain length on microbial adhesion to PEO brushes were determined. Glass surfaces were modified by end-grafting PEO chains having a molecular weight 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 (*S. epidermidis* and *P. aeruginosa*) and two yeast (*C. albicans* and *C. 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 slightly higher to the 2.8 nm brush than to the longer brushes. This higher adhesion on shorter brushes is in accordance with stronger Lifshitz-Van der Waals attraction between the surface and the microorganism at shorter separation distances. 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. This indicates that both Lifshitz-Van der Waals attraction with the surface and direct interaction between the PEO chains and the microorganisms is relatively weak. No significant differences were found in adhesion or retention between experiments conducted at 20 or 37°C.

Microbial adhesion to surfaces can be interaction and/or mass transport controlled and often occurs despite high wall shear rates acting on the adhering microorganisms. In **Chapter 5** we compare the wall shear rates needed to prevent microbial adhesion to bare glass and PEO brush coated glass in a parallel plate flow chamber. Initial microbial deposition rates were determined for different wall shear rates between 4 and 1600 s\(^{-1}\) on the top and bottom plates of the flow chamber and expressed as deposition efficiencies \(\alpha_{SL}\), based on the Smoluchowski-Levich approach, neglecting sedimentation. Inclusion of a theoretical contribution of sedimentation to the mass transport reduced microbial deposition efficiencies from much larger than unity to realistic values between zero and unity. Experimentally, the contribution of sedimentation to the mass transport was eliminated by averaging the
deposition rates found for the top (negative contribution of sedimentation) and bottom (positive contribution of sedimentation) plates of the flow chamber, which also yielded realistic deposition efficiencies. Deposition efficiencies for *P. aeruginosa* D1, *E. coli* O2K2 and *C. tropicalis* GB 9/9 decreased with increasing wall shear rates and were lower for PEO brush coated glass than for bare glass. Characteristic shear rates reducing adhesion were around $10 \text{ s}^{-1}$ and $1.0 \text{ s}^{-1}$ for the bacteria on glass and the PEO brush and 36 and $3.4 \text{ s}^{-1}$ for the yeast strain on glass and the PEO brush, respectively.

Despite their high clinical potential PEO coatings are not applied, as little is known about their stability and effectiveness in biological fluids. In **Chapter 6**, PEO coatings coupled to a glass substratum through silyl ether bonds were exposed for different time intervals to saliva, urine and phosphate buffered saline (PBS) as a reference at $37^\circ\text{C}$. After exposure, the effectiveness of the coatings against bacterial adhesion was assessed in a parallel plate flow chamber. The coatings appeared effective against *S. epidermidis* adhesion after 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, while the overall short stability results from hydrolysis of the coupling of the PEO chains to the substratum.

**Chapter 7**, the general discussion, starts with considering the influence of PEO coatings on microbial adhesion. Next, for different applications the use of these coatings is discussed.