General discussion
Microbial adhesion onto the surface of medical implants, like hip prostheses and urinary catheters, followed by biofilm formation, often leads to infections on these implants. To prevent microbial adhesion, the surface of an implant may be modified. One modification strategy is to attach water soluble polymers, like poly(ethylene oxide) (PEO), to a surface. When PEO chains are attached to a flat surface they can exist in two different conformations, the so called “mushroom structure” at low chain grafting densities and “brush structure” at higher chain grafting densities, where the chains are forced to stretch in the medium (see Figure 1). Both structures are expected to create a barrier between the microorganism and the surface thereby reducing adhesion. Although in general a reduction in microbial adhesion by PEO coatings is reported in literature, the quantitative effects are difficult to interpret as the PEO coatings are often not well defined.

**Figure 1.** A schematic representation of a PEO coatings: mushroom conformation on the left hand side and brush conformation on the right hand side.

Influence of PEO coatings on microbial adhesion. It is expected that PEO coatings are generally anti-adhesive. We found very low adhesion for 7 medical strains and also, in experiments not reported in this thesis, for the marine bacterium *Vibrio alginolyticus* 138-2, the soil bacterium *Bacillus subtilis* 168 and the oral bacterium *Streptococcus mitis* BA. Several microbial strains used in this thesis are able to adhere to a PEO coating, these were however specifically selected for their adhesive nature to provide more scientific insight.

Interestingly, even for the ten low-adhesive bacteria, adhesion was never completely inhibited. Some bacteria were always found adhering on the surface, up to around $1 \times 10^4$ per cm$^2$ (around $1 \times 10^7$ on a bare surface). This may be explained by the fact that the oxygen atoms in PEO can act as a Lewis base allowing attractive interaction with Lewis acids on
biological particles. Furthermore, hydrophobic interactions with PEO may also occur, especially with smaller particles which more easily penetrate the PEO brush. Outside the bacterial cell wall is a layer composed of proteins, lipids, polysaccharides or polypeptides [1]. Certain components of these extracellular substances may penetrate and attach to the PEO chains leading to the remaining adhesion found.

Practical applicability. A coating of short durability as used in this thesis may have a positive effect in certain applications. When a biomaterial is placed in the body a so called “race for the surface” occurs [2]. Both host tissue and bacteria try to colonise a surface, and often bacteria already adhere per-operatively with an influence of course on the race for the surface. A PEO coating can prevent the adhesion of bacteria that contaminate the wound during operation. When after a short period, the native surface is revealed it can be colonised by the host tissue without competition. Especially in the case of hip prostheses a coating with short durability can be beneficial as ingrowth of bone to the implant is essential to secure the prosthesis in the body [3]. The coating procedure used in this thesis may also be used for hydroxide groups of medical stainless steel as used in hip prosthesis.

Of course other applications are under constant pressure of contaminating bacteria, like urinary catheters [4] and orthopedic pins and screws. For these applications this thesis has shown that PEO brushes can suppress adhesion of medically relevant microbial strains, indicating that development of a stable PEO coating is worth the effort.

Another important group of applications is those under strong conditioning film forming conditions. Oral applications and voice prosthesis can be covered with proteins from saliva and components from natural sea water can cover ships hulls. For these applications a coating needs to be developed that prevents both protein adsorption and bacterial adhesion. Another approach is to develop a semi-stable coating of one or multiple layers. This layer can be removed by wear or cleaning, providing a fresh layer without conditioning film or bacteria.

Suggestions for further research. The development of a stable coating is now of essential importance to advance application of PEO-brush coatings. This is not a simple task, because a facile coupling to a surface is usually also susceptible to cleavage. Furthermore the coating
has to be applied to real biomaterials like polyurethane, silicone rubber, polyethylene or fluoridated hydrocarbons (PTFE- and FEP Teflons). This will be more difficult than to glass, as used in this thesis, as for instance poly(ethylene) does not have reactive groups on its surface and silicone rubber is very heterogeneous. However, techniques are available in literature to graft molecules to different surfaces, which can be adapted to provide PEO coatings. Inspired by research reported in this thesis Irina Cringus in the polymer chemistry group of professor A. J. Schouten has produced a coating of water soluble poly(acryl amide) molecules. This coating have demonstrated low bacterial adhesion combined with high durability and will in the near future be applied to silicone rubber.

A PEO coating in the mushroom conformation may suppress microbial adhesion as well, making the development of a coating more simple. The differences in adhesion on a PEO coating in different configurations may be investigated by using model surfaces where the PEO chains are grafted with gradually increasing chain density [5]. A model surface can also be used to test if the few bacteria adhering to a PEO coated surface are able to act as a nucleus for biofilm formation under circumstances as found in the site of application.

Furthermore, when the exact mechanism for adhesion of different biological particles on a PEO coating is known, this may lead to new ideas on how to prevent adhesion. To obtain this knowledge polystyrene spheres with microbial dimensions and adjustable well defined surface properties could be employed [6]. As these spheres do not have proteins of polymeric substances on their surface they may show no adhesion to a PEO coating. Furthermore, characteristics of the bacteria can be systematically altered by using mutants of a strain, for instance, mutants that produce varying amounts of extracellular polymeric substances [7].

References


