Biomaterials associated infection (BAI) is one of the main causes of implant failure in an era in which the number of patients requiring biomaterials implant surgery is steadily increasing. Treatment of infected implants frequently includes long-term antibiotic use, however, often without success, so that finally the implant has to be removed. Devices implanted in revision surgery are even at a greater risk of infection likely due to the persistence of bacteria in adjacent tissue near the implant site. BAI begins with the initial adhesion of infectious organisms that subsequently grow to form a biofilm. Bacterial adhesion to surfaces is influenced by physicochemical properties of the surface and several attempts have been made to develop non-adhesive coatings, such as polymer brush-coatings, in order to prevent bacterial adhesion and subsequent infection. Brush-coatings are currently the most promising non-adhesive coatings that yield significant reductions, exceeding 90%, in microbial adhesion. Despite such impressive results no unambiguous judgment can be made yet whether polymer brush-coatings are capable of preventing implant associated infections.

**Chapter 1** reviews the main challenges that have to be dealt with before a polymer brush-coating can be practically used against BAI. The main problem is that the fate of the few bacteria adhering to a brush-coating is not known. Furthermore reports on *in vivo* evaluation of polymer brush-coatings are inadequate for drawing a conclusion. Therefore the main aim of this thesis is to investigate the fate of the few bacteria that adhere to a polymer brush-coating and to evaluate the effects of a brush-coating on bacterial colonization of biomaterial surfaces *in vivo.*

Tri-block copolymers of polyethylene oxide (PEO) and polypropylene oxide (PPO), i.e. \(\text{PEO}_n\cdot\text{PPO}_m\cdot\text{PEO}_n\), better known as Pluronic, can adsorb to surfaces either in a pancake or a brush-like conformation. The brush-like conformation is advantageous in numerous applications, since it constitutes a surface, repellent to indwelling particles,
such as proteins and microorganisms. The conformation of an adsorbed Pluronic layer depends on the hydrophobicity of the substratum surface, but the hydrophobicity threshold above which a brush-like conformation is adopted is unknown. In chapter 2 the conformation of Pluronic F-127 (PEO$_{99}$-PPO$_{65}$-PEO$_{99}$) adsorbed on surfaces with different hydrophobicities was investigated using a quartz crystal microbalance with dissipation. Adsorption in a brush-like conformation occurred on surfaces with a water contact angle above 80°, as inferred from the thickness, viscosity and elasticity of the adsorbed layer.

We used Pluronic F-127 to make a PEO brush-coating on medical-grade silicone rubber which has a water contact angle of 112°. This coating was very easily made by a dip-coating process and was used in our experiments for studying the adhesion of three bacterial strains: Staphylococcus aureus ATCC 12600, Staphylococcus epidermidis HBH 276 and Pseudomonas aeruginosa #3.

In order to have a measure of the interaction force between adhering bacteria and the substratum surface we developed a procedure which determines shear forces at the balance between bacterial attachment and detachment under flow. This protocol can be applied to determine adhesion forces in weakly adhering systems, e.g. polymer brush-coatings, not attainable with conventionally developed methods as shown in chapter 3. The adhesion strength of staphylococci was greatly decreased by the presence of a PEO-coating, while the adhesion strength of P. aeruginosa #3 was hardly affected.

Polymer brush-coatings, have so far only been investigated with respect to reducing initial bacterial adhesion, but never with respect to effects on kinetics of bacterial growth. In chapter 4, we compared adhesion and 20 h growth of three bacterial strains on pristine and brush-coated silicone rubber in a parallel plate flow chamber. Brush-coatings prevented adhesion of staphylococci to below $5 \times 10^5$ cm$^{-2}$ after 30 min, which is a 10-fold reduction compared to pristine silicone rubber. Biofilms grew on both brush-coated and pristine silicone rubber, while the viability of biofilms on brush-coatings was higher than on pristine silicone rubber. However, biofilms on brush-
coatings developed more slowly and detached almost fully by high fluid shear. In addition, we observed that the biofilm structure had altered from a contiguous layer on pristine silicone rubber to scattered microcolonies on a polymer brush-coating. Brush-coatings remained non-adhesive after *S. epidermidis* biofilm formation and subsequent removal. Adhesion, growth and detachment of *P. aeruginosa* were not significantly different on brush-coatings as compared with pristine silicone rubber, although here too the viability of biofilms on brush-coatings was higher.

The delay in the development of biofilms on brush-coatings and the formation of scattered colonies which, compared to a contiguous biofilm may be better penetrable to nutrients, made us speculate that the biofilm formation on a brush-coating is more susceptible to antibiotics. In chapter 5 we have investigated the growth of *S. aureus* biofilms, a common cause of BAI, on pristine and polymer brush-coated silicone rubber in the absence and presence of three concentrations of gentamicin (0.5, 5 and 50 µg ml⁻¹). Biofilms grew on silicone rubber and maintained their viability in the presence of gentamicin, regardless of its concentrations. On polymer brush-coatings, however, the presence of gentamicin in the growth medium suppressed biofilm formation and decreased the number of viable bacteria. Biofilm growth on brush-coatings was almost completely prevented in the presence of 50 µg ml⁻¹ gentamicin. We concluded that biofilms on polymer brush-coatings remain susceptible to antibiotics, which prevents biofilm formation.

Performance of brush-coatings against bacterial adhesion and growth is superb according to our *in vitro* studies, but the virtue of these polymer brush-coatings *in vivo* had to be investigated. The possible benefit of polymer brush-coated versus pristine silicone rubber in revision surgery was determined in chapter 6, using a murine model. BAI was induced in 26 mice by subcutaneous implantation of silicone rubber disks with a biofilm of *Staphylococcus aureus* Xen29. During development of BAI, half of the mice received treatment with rifampicin/vancomycin. After 5 days, the infected disks were removed from all mice, and either a polymer brush-coated or
pristine silicone rubber disk was re-implanted. Revision disks were explanted after 5 days and the number of cfu’s cultured from the disks and surrounding tissue was determined. None of the polymer brush-coated disks after antibiotic treatment appeared colonized by staphylococci, whereas 83% of the pristine silicone rubber disks were re-infected. Polymer brush-coated disks also showed reduced colonization rates in the absence of antibiotic treatment as compared with pristine silicone rubber disks. Tissue surrounding the disks was culture positive in all cases. Concluding, we state that polymer brush-coatings are less prone to re-infection than pristine silicone rubber when used in revision surgery, i.e. when implanted in a subcutaneous pocket infected by a staphylococcal BAI. Antibiotic pre-treatment during the development of BAI hardly had any effect in preventing colonization of pristine silicone rubber while it seemed to increase the effectiveness of brush-coating.

In chapter 7, various subjects from our findings in chapters 2 to 7 are discussed. Finally the main conclusions of the project are presented and some suggestions are given for future research.