Bacterial adhesion is the main cause of implant failure, despite the existence of numerous preventive strategies and use of antibiotics. Procedures regarding the sterile environment in the operating theatre have been optimized to a near maximum and due to the fading discovery of new and better antibiotics combined with the rise of antibiotic resistant bacterial strains, there are decreasing options for treatment [1-4]. Surface coating of materials has been applied as a biomaterial surface modification to prevent the adhesion of bacteria, the first step in biofilm formation (see fig. 1). In Chapter 1, we provide an overview of the current developments in antimicrobial surface coatings. The method of choice has long been to achieve anti-adhesive surfaces, for which polymer-brush coatings have been used extensively. By forming a highly hydrated layer of extended polymer chains, polymer-brushes represent a steric barrier that prevents bacteria from adhering to the surface. However, even the best performing polymer-brush surface is not able to completely prevent all bacteria from adhering, and bacteria that do manage to adhere can grow into a full biofilm when given the opportunity [5]. Inclusion of some sort of anti-bacterial substance is therefore necessary to achieve enhanced results regarding bacterial adhesion and biofilm formation. In addition, in applications where tissue incorporation is required, surface coatings need to allow tissue cells to adhere and integrate the implant into the body, which in turn offers the best protection against further bacterial contamination. In this thesis we describe a number of anti-bacterial surface coatings, while also trying to enhance the current state of knowledge about the mechanisms by which bacteria adhere, since this can offer valuable information for the design of new strategies to prevent bacterial adhesion and biofilm formation.

**Micro-patterned surfaces**

Polyethylene glycol (PEG)-based surface coatings have long dominated the field of anti-adhesive surface modifications [7]. As an excellent repellant to both proteins and bacterial cells, it has been a promising candidate to serve as a coating for biomedical implants and devices to withstand bacterial adhesion and biofilm formation. However, as a repulsive surface coating, PEG polymer-brushes also hinder tissue cells to accommodate the surface and thereby prevent tissue integration in applications where it might be desirable [8]. In Chapter 2, we used micro-patterned surfaces of PEG-hydrogels to create surfaces with a repulsive nature towards bacteria, by taking advantage of its anti-adhesive character, while at the same time by introducing unmodified patches, exposing cell-adhesive surfaces, we create anchor points for mammalian cells to bypass the anti-adhesiveness of the coating. Whereas many previous studies have used cell recognition peptide-sequences, i.e. RGD, to stimulate cell adhesion on PEG modified surfaces [9,10], we showed that combining PEG hydrogel patches with the appropriate amount of bare surface can effectively prevent bacterial adhesion, while mammalian cells are still able to adhere and proliferate. An inter-gel spacing of 1 µm showed to be the optimum spacing which could sufficiently reduce bacterial adhesion and allow osteoblasts to adhere to and spread on the surface. This inter-gel spacing, of the same order of magnitude as bacteria, prevents bacteria from adhering while osteoblasts use their focal contacts and focal adhesions, which can extend hundreds of nanometers, to reach the adhesive spaces in between the hydrogels.
FIGURE 1 Schematic representation of the different steps in biofilm formation consisting of initial attachment, early growth phase, biofilm maturation, and dispersal of the biofilm [6]. Copyright by the Montana State University Center for Biofilm Engineering.

DNase I containing surface modification

eDNA has been pointed out as an important component of extrapolymeric substances (EPS) and playing an important role in adhesion of bacteria and the subsequent formation and maintenance of biofilm. Besides DNase I, several other enzymes already have been studied as possible surface coatings to prevent bacterial adhesion, including lysozyme and dispersin B [11,12]. In Chapter 3, we have shown a proof of principle of a functional DNase I coating, effective in preventing bacterial adhesion and biofilm formation, by using dopamine as an intermediate for surface attachment of DNase I. By presenting DNase I at the material-bacteria interface, bacterial adhesion could be prevented and biofilm formation after 14 h was drastically reduced as well. Dopamine is generally considered as a chemically stable attachment method, with studies reporting only a slight loss of surface attached compounds after exposure to phosphate buffered saline (PBS) for time periods between 7 and 30 days [13,14]. However, as an implant is inserted into the body, it is exposed to other harsh conditions that can be detrimental to any ‘soft’ surface coating. The handling of the implant by the surgeon is one of these, but nevertheless mechanical testing of surface coatings is seldom reported. The presence of DNase I and maintaining its activity is another challenge in the surface coating process. Therefore, in Chapter 4, we used poly-(lactic-co-glycolic)-acid (PLGA) as a ‘hard’ polymer surface coating that could withstand mechanical stress, and incorporated DNase I in a sugar
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glass, inulin, to protect the enzyme and retain its activity during the coating process. As a degradable polymer approved by the U.S. Food and Drug Administration, PLGA released the inulin-packaged DNase I for an extended time period and coating of a titanium surface reduced the number of adhering bacteria up to 99% and lowered the biomass of biofilms formed in 20 h significantly. More importantly, the PLGA coating proved to be able to withstand a number of storage and handling conditions that mimicked the environment to which an implant could be exposed in the operating theatre.

**Lateral forces in bacterial adhesion**

As the first step of biofilm formation, initial adhesion of bacteria is a main focus in the prevention of biomaterial associated infections. However, despite the increasing number of potential strategies developed to fight bacterial adhesion, the exact mechanisms remain poorly understood [15,16]. Major advances have been made by the use of cell probe microscopy in which bacterial adhesion forces of single bacteria to surfaces have been studied, and even the forces between specific cell-wall appendages and surfaces can be measured this way [17–19]. However, all these studies have in common that the direction of the forces between the bacteria and the surface are measured perpendicularly to the surface. In most real-life situations, and in many studies on bacterial adhesion, bacteria approach a surface from a flowing carrier liquid [20–22], which means the forces arising between these bacteria and the surface are actually laterally oriented. In this perspective, we studied the lateral forces arising between *Staphylococcus epidermidis* ATCC 35983 and several polymer brush coatings when moving a bacterium along the surface using lateral force microscopy (Chapter 5). Using two types of PEG molecules in different concentrations, we created brushes with different softness and could conclude that not only the friction forces between polymer-brush modified surfaces and bacteria are highly correlated to the number of adhering bacteria, but also the softness of a polymer-brush plays a n important role in the success of resisting bacterial adhesion. On soft polymer-brushes bond-maturation is much more extensive compared to rigid brushes, from which bacteria easily desorb.

In addition to the lateral forces between bacteria and polymer-brushes, in Chapter 6 we studied the influence of specific interactions between bacteria and surfaces on the lateral forces that arise when bacteria move along the surface. To this extend we used *Streptococcus mutans* with antigen I/II on its outer surface together with an isogenic mutant strain, not possessing this particular adhesin. The structure of antigen I/II has been revealed into detail in the last decade and it has been demonstrated that both its distal part as well as the cell-wall anchoring domain are able to interact with salivary agglutinins (SAGs) present in salivary conditioning films (SCFs) [23,24]. Comparison of both the normally-oriented and the lateral adhesion forces between these two strains and glass surfaces coated with SCFs suggests that the specific interactions between antigen I/II and SAG are directional depended and are better capable to resist lateral forces than forces oriented normally to the surface. This suggest that *S. mutans* has adapted to the highly dynamic environment of the oral cavity, where mastication, tongue movement and presence of fluids all create a high amount of shear forces. The nature of these high affinity binding site between
bacterial ligands and salivary receptors suggests that similar mechanisms could also hold true for other bacterial strains capable of forming bonds using specific interactions.

CONCLUSIONS
Antibiotic-independent strategies to prevent infection of medical devices and implants are a crucial factor for the success of future medicine. Problematic in the design of surface modifications for these applications is the wide variety of existing devices and implants, of which each comes with its own requirements regarding stability, tissue integration and duration of use. Our results suggest that surface patterning of anti-adhesive patches can aid in the tissue integration component of implants, while still offering a high degree of resistance against bacterial adhesion. Another approach to prevent bacterial adhesion is by applying natural components, like enzymes, that have specific activities to disturb bacteria in their adhesion and ability to form biofilms. Using biodegradable polymers makes it possible to create robust surface coatings that release these substances and keep the implant free of bacteria until tissue integration is achieved. However, as discussed in the final Chapter 7, these surface modification strategies will not be universally suitable for all applications, and therefore it is important to keep studying the mechanisms by which bacteria colonize surfaces, since this remains poorly understood. We made a start in unravelling the nature of lateral, or friction, forces between bacteria and surfaces whereas before only the normal adhesion forces have been directly measured. A better understanding of the role of lateral forces can help in future engineering of new surface modifications to resist bacterial adhesion.

REFERENCES
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