Summary

The placement of orthodontic appliances impedes the maintenance of proper oral hygiene in most orthodontic patients, provides oral bacteria with numerous surfaces to adhere, and changes the oral microflora. Bacterial adhesion and biofilm formation can cause a variety of serious problems during orthodontic treatment, such as enamel demineralization and soft tissue inflammation. Understanding bacterial adhesion forces to orthodontic materials is an important challenge in both clinical and fundamental orthodontics and allows for the development of preventive measures. Chapter 1 reviews biofilm formation and preventive measures in orthodontics. In the first part, the composition and mechanisms of orthodontic biofilm formation are addressed. Influential factors on biofilm formation are presented from the aspects of orthodontic materials, including brackets, adhesives, ligating devices, arch wires, and retainers. In the second part, possible clinical consequences of orthodontic biofilms and the most prevalent complications such as enamel white spot lesions and gingival inflammations are discussed. In the third part, treatment and preventive measures are described. Treatments of enamel demineralization and soft tissue inflammation are summarized. Recent progresses in both fundamental research and clinical practice are elaborated upon. Preventive measures toward orthodontic biofilm formation include mechanical removal, chemical control, and modification of orthodontic materials by incorporation of antimicrobial agents. The review ends with a discussion on the challenges and opportunities in orthodontic biofilm research for the future.

Clinical observations indicated that the bracket-adhesive-enamel junction is the most crucial and vulnerable site for bacterial adhesion. Bacterial adhesion forces determine the stability of biofilms formed at the junction site. In Chapter 2, the adhesion forces of nine oral bacterial strains, including both initial colonizers and cariogenic strains, to three materials constituting the bracket-adhesive-enamel junction are measured using atomic force microscopy (AFM), in the presence and absence of a salivary conditioning film. Physicochemical properties of these
materials are investigated to explore the relationship between bacterial adhesion forces and orthodontic materials. Our results showed that the roughest adhesives exerted the strongest adhesion forces, followed by stainless steel and enamel in the absence of a salivary conditioning film. The salivary conditioning film significantly reduced both the bacterial adhesion forces to all the materials, and also the differences in adhesion forces between the materials. In general, the initial colonizers (*Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus oralis* and *Actinomyces naeslundii*) adhere stronger than cariogenic bacterial strains (*Streptococcus sobrinus*, *Streptococcus mutans* and *Lactobacillus acidophilus*).

In Chapter 3 we investigated the nature of bacterial bond strengthening on saliva-coated enamel. Streptococcal adhesion forces increased from about -0.7 nN to -10.3 nN at 0 s to 120 s contact with saliva-coated enamel. Initial colonizers of saliva-coated enamel surfaces (*S. mitis*, *S. sanguinis*) have stronger adhesion forces than the more cariogenic strains (*S. sobrinus*, *S. mutans*). Poisson analyses decouple the adhesion forces into a hydrogen-bonding force and a non-specific force contribution, and indicate that the non-specific forces are repulsive, around +0.3 nN, and hydrogen-bonding forces are attractive, around -1.0 nN for initial and -0.8 nN for later colonizers. The slightly stronger attractive hydrogen-bonding forces of initial colonizers are considered to have resulted in the stronger adhesion forces at both 0 s and 120 s contact with saliva-coated enamel *in vitro*. The important transition from reversible to more irreversible adhesion of oral streptococci to saliva-coated enamel is thus attributed to the progressive involvement of hydrogen bonds.

Many studies on the nature of bacterial adhesion forces have been performed on non-conductive surfaces including glass, silicon, and enamel. However, no study has yet looked into the nature of bacterial adhesion forces on conductive surfaces such as stainless steel. Therefore, Chapter 4 investigated the nature of bacterial adhesion forces on stainless steel surfaces using Poisson analysis. Adhesion forces measured between stainless steel, both in the absence and
presence of an adsorbed salivary conditioning film, increased with increasing contact time between the streptococcal AFM probe and the surface. Concurrent with the increase in adhesion force, there was an increase in the number of minor force peaks in the retract force-distance curves. Poisson analyses of the adhesion forces indicated repulsive non-specific Lifshitz-Van der Waals forces for streptococci adhering to saliva-coated stainless steel, but interestingly and for the first time, attractive non-specific forces were revealed on stainless steel in the absence of a salivary conditioning film. We tentatively attribute this to attraction between the negatively charged streptococci and their positive image charges in the conducting material, which can not develop in a non-conducting material or in the presence of a non-conductive protein layer on the stainless steel surface.

Orthodontic adhesive usually possesses a rougher surface than enamel and bracket surfaces in clinical situations, protecting bacteria against oral removal forces. In Chapter 5, two most commonly used orthodontic composite resins were evaluated for the influence of their surface roughness on bacterial adhesion forces. The surface roughnesses of the composite surfaces were adjusted by grinding and polishing and amounted 20 nm, 150 nm, and 350 nm in the absence of a salivary conditioning film, and 17 nm, 80 nm, and 250 nm in the presence of a salivary conditioning film. The initial adhesion forces in absence of a salivary conditioning film were between -0.7 and -0.9 nN for the smoother composite resins and increased to between -1.0 and -2.0 nN for the roughest surfaces. In the presence of a conditioning film, rougher surfaces still exert stronger adhesion forces, irrespective of the type of composites or bacterial strains. In conclusion, streptococcal adhesion forces to orthodontic composite resins increase with increasing roughness of the composite surfaces. Adhesion forces of *S. mutans* were less affected by composite surface roughness than of *S. sanguinis*.

Chapter 6 is aimed to explore the incorporation of a non-bactericidal monomer (QAC, 3-(Methacryloylamino)propyl trimethylammonium chloride) in an orthodontic adhesive. The QAC-modified composite showed strong contact killing of different
oral streptococcal strains within 15 min, depending on the amount of QAC incorporated, with the minimal required concentration of QAC between 16% and 20% w/w. Contact killing reduced after coating the modified composite with a salivary conditioning film, although killing remained significant. No cytotoxic effect was observed in human skin fibroblast cells in contact with QAC-modified composites. Bond strength of the composite with enamel surfaces (12 ± 3 MPa) was negatively affected with increasing amount of QAC incorporated and reduced to 50% of the original value at 20% w/w QAC in the composite. These results suggest that QACs can be effectively incorporated in orthodontic composites to provide bactericidal activity without cytotoxicity. However, it needs to be established whether the loss of bond strength is clinically acceptable or whether further modifications are required to restore the bond strength.

In Chapter 7 the main results and conclusions of this thesis and their clinical implications are discussed, as well as possible new preventive measures based on the results of this thesis.