Antibacterial measures for biofilm control

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Chapter 1

General introduction and aim of this thesis

Orthodontic treatment and risks

Orthodontic treatment aims at improving function of the masticatory system, correcting the position irregularities of teeth and achieving better facial esthetics. However, orthodontic treatment also bears a potential oral health risk that can subsequently compromise oral function and dental esthetics. The main adverse effect is the accumulation of dental plaque or oral biofilm around the orthodontic appliances (Travess et al. 2004). Oral biofilm develops naturally on tooth surfaces and it is highly associated with caries and periodontal diseases (Marsh and Bradshaw 1995). Mechanical removal of biofilm by routine oral care is severely hampered by the presence of orthodontic fixed appliances, such as brackets, bands, tads and other auxiliaries due to the increasing crevices and niches introduced in the mouth. Composite bonding resins are prone to bacterial adhesion at the vulnerable bracket-adhesive enamel junction, especially since polymerization shrinkage may yield a gap at the contact interface into which bacteria can easily infiltrate. Temporary devices, such as mini-implants also create retention sites for oral biofilms. In these protecting niches, biofilm is difficult to remove mechanically and can grow undisturbed.

Also, removable clear appliances, such as positioners and aligners, gaining increasing clinical popularities, showed no advantage in terms of oral hygiene control compared with fixed appliances (Chibber et al. 2018). Aligners typically cover entire tooth surfaces and 1 to 2 mm of the gingiva. This extensive oral surface coverage has been shown to limit the flow of saliva, negating saliva's natural cleansing, buffering, and remineralizing properties (Addy et al. 1982). Moreover, the nature cleansing activities of the lips, cheeks, and tongue are interrupted, allowing undisturbed biofilm growth under the appliance (Moshiri et al. 2013). In short, a variety of additional surfaces introduced by orthodontic devices provides a favorable environment for microorganisms to grow in a biofilm mode and survive from mechanical removal.

Problems related to oral biofilm

Biofilm on oral hard and soft tissues can cause enamel demineralization and gingival inflammation (Marsh and Nyvad 2003). Demineralization of enamel, which in its mildest form yields white spot lesions indicative of subsurface decalcification, occurs

in 23-97% of the orthodontic treated patients (Ren et al. 2014). Decalcification can lead to caries and cavities, until restorative treatment is necessary.

Biofilm formed below the gingival margin can lead to inflammation of the gingiva, and in an extreme case periodontitis and tooth loss. Biofilm-related inflammation of soft tissues surrounding temporary devices, such as mini-screws, can cause inflammatory reactions similar to peri-implantitis, especially when it is related to biofilm formed on transgingival parts of the devices. These inflammations are associated with a 30% increase in failure rate of temporary anchorage devices (Miyawaki et al. 2003).

Daily oral care

Manual or powered brushing are still by far the most effective measure for oral hygiene maintenance in orthodontic patients. Manual toothbrushes with a special head design for orthodontics, v-shaped, or triple-headed, are more efficient than brushes with a conventional planar bristle field (Rafe et al. 2006). Powered toothbrushes reduce biofilm and gingivitis more than manual tooth brushing in the short and long term (Yaacob et al. 2014). Powered toothbrushes also promote gingival health more effectively than manual toothbrushes in orthodontic patients (Al Makhmari et al. 2017). In *in vitro* settings powered toothbrushes demonstrated noncontact removal of oral biofilm (Schmidt et al. 2013) up to brushing distances of 6 mm. Mechanisms of hydrodynamic action, passing air-liquid interfaces, and acoustic energy transfer are contributing to this beneficial impact (Busscher et al. 2010). Therefore powered toothbrushes are beneficial for patients with orthodontic appliances with additional crevices and niches that are difficult to reach by manual brushes (Sharma et al. 2015). It has been demonstrated *in vitro* that the structure of biofilm changes after powered brushing in favor of antimicrobials penetration to kill bacteria to a greater depth (He et al. 2014).

Besides mechanical methods of oral hygiene, chemical products for oral care such as toothpastes, mouthrinses and varnishes containing antimicrobial agents assist in the control of oral biofilm. A variety of antimicrobials in toothpastes, mouthrinses and varnishes, are available and contain agents like chlorhexidine, quaternary ammonium compounds, triclosan, essential oils, metal salts and fluoride. Formulations with chlorhexidine, triclosan, and fluoride have demonstrated

significant antibiofilm efficacy *in vivo* (James et al. 2017; Riley and Lamont 2013; Marinho et al. 2003). Fluoride is most commonly used and is applicable in many different formulations and acts as a buffer to neutralize acids produced by bacteria and suppresses their growth (Khoroushi and Kachuie 2017). However, the benefits of fluoride mainly confine to the inhibition of demineralization (Busscher et al. 2010). Mouthrinses with chlorhexidine are considered the gold standard in dentistry with respect to antibacterial effects (Varoni et al. 2012). Chlorhexidine exhibits broad spectrum activity against both Gram-positive and Gram-negative bacteria, yeast, dermatophytes and lipophilic viruses (Beyth et al. 2003; Denton 1991) and is considered effective in helping reduce oral biofilm and gingivitis (James et al. 2017). Patients using chlorhexidine during orthodontic treatment have significantly less white spot lesions (Okada et al. 2016). Synergistic effects of different antibacterial chemicals have been shown in *in vivo* studies. A combination of different chemicals, such as an amine fluoride/stannous fluoride-containing toothpaste and mouthrinse with chlorhexidine showed improved cariostatic effects in an orthodontics-induced caries model compared with conventional fluoride formulation (Øgaard et al. 2001; Øgaard et al. 2017).

Antibiotic resistance

With wide application of antimicrobials worldwide, the development of antibiotic resistance has been a major concern in public health (Van de Belt et al. 1999; Neut et al. 2003; Howard et al. 2003). Oral antibiotics with non-selective antibacterial effect may be very effective, but resistance has emerged in clinical isolates resistant to multiple drugs, including chlorhexidine, such as in methicilin-resistant *Staphylococcus aureus* (Block and Furman 2002) and other oral strains (Saleem et al. 2016). Uncontrolled use of oral health products containing antimicrobial agents may stimulate development of multidrug resistant strains that can retain in oral biofilms left behind after brushing as 100% biofilm removal can never be achieved (Busscher et al. 2010). These resistant strains can act as a source for dissemination and pose a life threatening infection in a host with compromised immunological conditions (Davies 1994). Cell wall deformation plays an important role in understanding the bacterial susceptibility to antimicrobials and probably the development of resistance. An increase in deformation of the bacterial cell wall is accompanied by an increase in

the surface area of the lipid membrane, making it more susceptible for antimicrobials to penetrate. Reliable measurements of nanoscopic cell wall deformation as a result of bacterial adhesion to surfaces can be defined by exploiting surface enhanced fluorescence (Li et al. 2014; Carniello et al. 2018) and is highly important in the understanding bacterial responses to antimicrobials and recommendations for clinical use or the development of alternatives for current antimicrobials.

Prevention and control of oral biofilm

Strategies to prevent and control oral biofilm formation have been extensively studied in clinical research. Antibiofilm activity may be achieved by different mechanisms of action: by preventing bacterial adhesion, by limiting bacterial growth, by disrupting an already established biofilm or by altering the composition and/or pathogenicity of the biofilm (Sanz et al. 2013). One strategy of particular interest in dentistry is modifying dental materials with antimicrobial properties, by mechanisms based either on releasing antimicrobial particles from the material or modifying the material surface with 'contact-killing' features. For orthodontics and dentistry in general, prolonged antimicrobial action is desired, therefore materials that can kill bacteria upon contact are of great clinical relevance.

Polymers containing covalently bonded antimicrobial moieties, such as immobilized quaternary ammonium compounds, possess the unique feature of bacterial 'contact-killing' (Tiller et al. 2001; Imazato 2003). Adhering bacteria are killed upon contact by severe membrane disruption through extremely strong electrostatic attraction (Asri et al. 2014). Bacterial killing upon adhesion to cationic quaternary ammonium surfaces has been shown in many *in vitro* studies. *In vivo* efficacy, however, has only been shown in animal studies (Gottenbos et al. 2003; Imazato et al. 2004; Schaer et al. 2012). Another limitation is that there exists no ubiquitously accepted method to evaluate the efficacy of bacterial contact-killing on these surfaces. Nevertheless, bacterial contact-killing materials with long lasting actions are promising as a non-antibiotic based way to prevent biofilm formation. For clinical applications, it would be even better to incorporate the contact-killing property in a material with other unique features, e.g. 3D printability and mechanical versatility.

Aim of the thesis

The aim of this thesis is to investigate measures for oral biofilm control related to oral biofilm infections.

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