Antibacterial measures for biofilm control

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Chapter 7

General discussion

Orthodontic treatment aims at improving function of the masticatory system, correcting dental irregularities and achieving facial harmony and esthetics, ideally without side effects caused by the accumulation of dental plaque or oral biofilm around the orthodontic appliances. In order to avoid biofilm formations during orthodontic treatment different antibacterial measures exist. This thesis describes an investigation of antibacterial measures for oral biofilm related infection, demonstrating that a combination of antibacterial measures would contribute greatly to the management of biofilm. These findings may be of considerable value to clinical practice and the society in general.

Motivating patients to maintain good oral hygiene by mechanical and chemical measures is daily practice for dental and orthodontic professionals. Removal of oral biofilm is important for prevention of dental diseases, but biofilm can never be thoroughly removed, especially not from fissures, buccal pits, interproximal areas, gingival margins and around orthodontic appliances. We adopted an *in vivo* model for such retention sites in the oral cavity using multi- strand wires. Two important findings are clinically relevant. First, a synergy between brushing mode and antibacterial-regimes exists, whereby the use of powered toothbrushes enhances the action of oral antimicrobials. The more 'fluffed-up', open state of the biofilm after powered brushing facilitates antimicrobial penetration. Second, by using different regimens of oral antimicrobials, an evident change in composition of oral biofilm can be achieved. Particularly the use of triclosan combined with an essential oil containing mouthrinse led to a distinct decrease in the prevalence of cariogenic bacteria, such as *Streptococcus mutans* (Loesche 1986) and *Lactobacilli* (Caufield et al. 2015), when compared to a solely Natrium Fluoride-containing toothpaste without antibacterial claims. This reduction in the presence of these cariogenic species might point to a shift in the composition of the adhering oral microbiome in a more healthy direction. After using the antimicrobial regime only one week, this change in composition of the biofilm was already clearly visible (see Chapter 3) and may become stronger after prolonged use of the oral antimicrobials. Especially because the oral microbiota is natural and provides benefits to the host (Marsh 2018), approaches to modify the microbiota to a more healthy and biological equilibrium is logical.

In oral self-care, mouthwash and toothpaste containing antimicrobials are commonly used to assist control of biofilm growth (James et al. 2017; Riley and

Lamont 2013; Marinho et al. 2003). However, extensive use of antimicrobials may result in development of resistant bacterial strains. Concerns about presence of antimicrobial resistant bacterial strains in the oral cavity arise, especially of *Staphyloccocus aureus* (Block and Furman 2002), commonly isolated from infections around implants (McCormack et al. 2015). In light of recent findings that bacteria in dental biofilm with reduced susceptibility to chlorhexidine, can also develop multidrug resistance (Saleem et al. 2016), investigation of mechanisms to develop antimicrobial resistance by oral bacteria may not be overlooked. Using surface enhanced fluorescence, nanoscopic bacterial cell wall changes after exposure of chlorhexidine were demonstrated to be dependent on the bacterial strain, indicating differential response and repair mechanism of *S. aureus* and *S. mutans*. Accordingly, *S. aureus* developed resistance toward chlorhexidine, while our *S. mutans* did not. These findings raise the question about the routine use of chlorhexidine in dentistry and its over-the-counter availability for the patient. Frequent use of chlorhexidine may induce resistance of *S. aureus*, making the treatment with antibiotics when necessary ineffective or even potentially unsafe for medically comprised patients. The precise mechanism behind these different survival strategies is worth exploring in further research. Using atomic force microscopy (AFM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) cytological evidence may be obtained to help explain the mode of action of bacterial resistance to chlorhexidine, eventually leading to recommendations for clinically safe and effective use of antimicrobials.

Although measures such as tooth brushing and the use of antimicrobials in toothpaste and mouthwashes are effective in maintaining a healthy equilibrium of the oral flora, these measures are dependent on patient compliance, which often declines during a long course of orthodontic treatment (Al-Jewair and Suri 2011). Alternatively, compliance independent pathways for interception of biofilm related problems can be very beneficial. In a survey for patients to identify relevant research topics, all respondents, including patients, parents of patients, orthodontists and paramedics scored highest for 'non-compliance' bacterial-killing adhesives with lasting killing effect. The outcome of this survey demonstrates that the opinion of end-users makes a valuable contribution, not only to establish a sound professional basic clinical relationship between doctors and patients, but also to get a better understanding of their needs. With increasing awareness of the importance of oral

health and recognition of academic research being part of a wider process in healthcare, the public's opinion is becoming a leading factor, influencing the policy makers in decisions on funding priorities and healthcare strategies. Therefore, obtaining public support through interactions between scientists and end-users is an important aspect for research in academia (Bouter 2010).

Development of 'non-compliance' dental and orthodontic materials attracting less biofilms goes back for decades. One of the earliest examples of non-leaching organ silicon quaternary ammonium compounds (QAC) capable of killing microorganisms on contact was reported in the early 1970s (Isquith et al. 1972). Since then, many attempts have been made to develop effective antimicrobial adhesives to prevent biofilm. QAC is a promising antibacterial monomer for clinical applications (Makvandi et al. 2018). Yet, till date antibacterial materials are not commonly available for orthodontic patients. Most research on antibacterial composites were based on laboratory tests, where no unanimously accepted method exists in literature that can reliably evaluate the efficacy of bacterial contact-killing on these surfaces, making comparison between different studies meaningless. We showed that three out of five commonly used methods for evaluating contact-killing, including an established ASTM, are unsuitable. Methods found suitable are Petrifilm® and JIS Z 2801 (Japanese Industrial Standards), provided being used in combination with a zone-of-inhibition-assay to establish absence of antimicrobial leaching, which can potentially interfere with contact-killing. The modified JIS method is acceptable, but does not contain balanced amount of nutrients and should only be used with respect to a non-contact killing control. ASTM (E2149-13a) and bacterial spray methods are not reliable, the main reason being the lack of control over the applied bacterial challenge and actual contact of bacteria with the surface. The identification of suitable assays for evaluating bacterial contact-killing will greatly assist progress in this emerging field and may be valuable to the advancement of clinical downward translation. For future research, methods suitable for studying multi-species biofilm may extend our knowledge of the efficacies of contact-killing materials.

From engineering perspective manufacturing of 3D printable material with the unique feature of contact killing is promising for clinical applications. In orthodontics, 3D printing technology is currently used in aligner systems, where series of 3D printed models are made for producing aligners, in diagnostic set-ups, indirect bracket-bonding sets (Dawood et al. 2015) and removable retainers (van der

Meer et al. 2016). However, a relatively narrow range of biocompatible, 3D printable materials with a limited spectrum of physico-chemical properties still restricts the application of this disruptive technology (Umme Kalsoom et al 2016). Interest in printable, biologically 'smart' materials with extensive features such as bacterial killing and desirable mechanical strength is growing. The two strategies we presented in chapter 6-I demonstrated potent killing effect on bacteria associated with dental caries, without compromising the biological and mechanical properties of the materials required for their clinical performance, attributed to the incorporation of QAC in a resin matrix. For eventual clinical use, *in vivo* evidence about the prevention of caries or gingivitis due to the use of antibacterial composite is valuable, but not yet available (Pereira-Cenci et al. 2013). Future research into the long-term effect and *in vivo* effects of contact-killing materials is worth exploring.

A combination of different antibacterial measures decreases the risk for oral biofilm related infections in orthodontics and oral health in general. Oral biofilm control requires a well-maintained balance between efficacy and safety of the measure used. For optimal clinical application an anti-bacterial measure should be effective in killing pathogenic bacteria, disrupting biofilm matrix, and ideally aiming for a healthy equilibrium in the oral microbiome. In parallel, measures used should be save without detrimental effects in any part of the body.

Summarizing, in this thesis we explored pathways for modification of the composition of biofilm, mechanisms of antimicrobial resistance of oral bacteria and control of biofilm formation by 3D printable and contact-killing materials.

Based on the main findings from this thesis, a number of conclusions can be put forward:

1. Powered toothbrushing enhances the action of oral antimicrobials and results in significant reductions of oral biofilm and shifts in its compositions.
2. Different species of bacteria have different responses and repair mechanisms that may play a role in development of resistance toward chlorhexidine.
3. Among five commonly used methods, including an established ASTM, to evaluate the efficacy of contact killing, three are unsuitable. Methods found suitable (Petrifilm® and JIS Z 2801) should be used in combination with a zone-of-inhibition-assay to establish absence of antimicrobial leaching.
4. A biocompatible, 3D printable composite resin has been developed demonstrating potent bacterial killing upon contact with good clinical handling properties.

5. When considering societal impact of research, the opinion of end-users is valuable in selecting research topics and the role of (social) media cannot be denied in spreading scientific results.

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