Oral Biofilm as a Reservoir for Antimicrobials
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Summary
Oral diseases like dental caries and periodontal disease are a major concern in both dentistry and public health, and have a causal relationship with oral biofilms. Either removing the biofilm or influencing the composition of the biofilm are nowadays the most accepted measures in controlling the biofilm, and therewith contribute to maintaining oral health. Unfortunately, no-one is capable to completely remove the biofilm with the common personal oral health care devices and products, like toothbrushes, interdental cleaning devices, toothpastes and mouthrinses. This thesis concerns the new idea that biofilm-left-behind after brushing can be used as a reservoir for antimicrobial components of toothpastes and mouthrinses to enhance the substantive or long-lasting antimicrobial effect of these products.

Chapter 1 gives an overview of oral biofilm (also called dental plaque) control measures by either mechanical removal, like toothbrushing and using interdental cleaning devices, and chemical influences. An important property of antimicrobial toothpastes and mouthrinses is that the antimicrobial components remain active in the oral cavity for a longer period than the average brushing or rinsing time by interaction with surfaces, a process called substantivity. The hypothesis that a biofilm can act as a reservoir for antimicrobials from toothpastes and mouthrinses, is first introduced in this chapter. An *in vitro* study regarding acute antimicrobial effects of four mouthrinses (Corsodyl®, Crest Pro Health®, Listerine®, and Meridol®) and six toothpastes (Crest Pro Health®, Crest Cavity Protection®, Oral B Pro Expert®, Colgate Total®, Zendium Classic® and Prodent Coolmint®) on an initial oral biofilm formation is discussed in Chapter 2. All products resulted in a decrease in biofilm development. Since rinsing with water and saliva dilutes mouthrinses and toothpastes *in vivo*, first dilution series of the products were studied. It was shown that dilution yielded loss of efficacy, or for some products even stimulated biofilm growth. A second aim of this chapter was to investigate the substantive action of antimicrobials in the toothpastes and mouthrinses through absorption in a biofilm. Both 4 h old and 24 h old biofilms were exposed to three
antimicrobial products, Corsodyl® mouthrinse, Crest Pro Health® mouthrinse and Crest Pro Health® toothpaste. Subsequently a new biofilm was grown on top of the exposed biofilm. Antimicrobial absorption in and release of antimicrobials in effective concentrations was demonstrated for all three selected antimicrobial products, meaning that antimicrobials remain bio-available for substantive action on new biofilms. These findings were confirmed by in vivo research, described in chapter 3 and 4. In chapter 3, volunteers used a control toothpaste, Prodent Coolmint®, for two weeks, followed by two weeks of additional use of an antimicrobial mouthrinse, Listerine®, Crest Pro Health® and Meridol®. Plaque and saliva were collected 6 h after the last brushing with the control toothpaste or 6 h after the last brushing with the additional use of an antimicrobial mouthrinse. The contribution of plaque and saliva towards substantivity was assessed by combining plaque obtained after mechanical cleaning with control toothpaste only with plaque and saliva obtained after additional use of an antimicrobial rinse. It was concluded that plaque, collected 6 h after rinsing with antimicrobial mouthrinses, contained a surplus of antimicrobial activity. The same protocol was followed in chapter 4, but instead of using antimicrobial rinses in addition to brushing, three antimicrobial toothpastes, Crest Pro Health®, Colgate Total® and Zendium Classic®, were used. In this study, plaque and saliva were collected both 6 h and 12 h after the last brushing, to gain more insight in the length of the substantive effects. Also in this chapter, it was concluded that plaque, either collected 6 h or 12 h after the last brushing, contributed to the prolonged activity of antimicrobials. Unfortunately, the substantive effects could not be found for all toothpastes studied, and in general the results were less obvious than in the mouthrinse study. In both the mouthrinse study, described in chapter 3, and the toothpaste study, described in chapter 4, no contribution of saliva towards the substantive action of antimicrobials could be found. Nevertheless, in both chapters we could clearly establish that the oral biofilm can act as a reservoir for oral antimicrobials, and therewith contribute to
their long-lasting antimicrobial activity in the oral cavity. **Chapter 5** describes the effects of the antimicrobial products studied in chapter 3 and 4 on the bacterial composition of the oral biofilm, using polymerase chain reaction - denaturing gel gradient electrophoresis (PCR-DGGE). All experimental plaques, collected after using an antimicrobial product were compared to control plaques, collected after using a control toothpaste without antimicrobial claims, with respect to their bacterial composition and differences were expressed as percentage similarity between both biofilms. All antimicrobial products influenced the bacterial composition as compared to biofilms obtained during the use of the control toothpaste, within the limitations of the small group of volunteers involved. Interestingly, the dissimilarity between experimental and control biofilms was largest, when the viability of the biofilms, mentioned in chapters 3 and 4, was lowest. In the general discussion, **chapter 6**, the outcomes of this thesis, describing the biofilm as a reservoir for antimicrobials, are placed in a broader spectrum. Future research might concentrate on combining powered toothbrushing, which induces fluid streams that may fluff up the biofilm and works beyond the bristles end, with chemical biofilm control measures, to enhance penetration of antimicrobials in the biofilm.

Summarizing, biofilm-left-behind after brushing can act as a reservoir for antimicrobials and therewith contribute to the substantive action of antimicrobial in oral health care products. It is important to note that the results of this thesis should not be taken as a pledge for incomplete brushing. However, since no-one is capable of complete removal of oral biofilm by brushing, regardless of the mode of brushing, this thesis presents a strong argument for the use of antimicrobial toothpastes and mouthrinses.