Oral Biofilm as a Reservoir for Antimicrobials

Otten, Marieke Petronella Theodora

Chapter 6

General discussion

In this thesis, the hypothesis that the substantivity of antimicrobial oral health care products is enhanced by absorption and release of antimicrobial components in and from oral biofilm left behind after brushing was substantiated. In this chapter, we will consider the following biological aspects of the biofilm as a reservoir for antimicrobial agents:

- development and prevention of oral diseases via changes in oral ecology
- clinical relevance of the biofilm as a reservoir
- perspectives for the future.

Development and prevention of oral diseases via changes in oral ecology

In 1676 Antonie van Leeuwenhoek was the first to use a microscope to study dental plaque and he discovered the presence and existence of “animalcules”, later called bacteria. In the following years, the relationship between bacteria and diseases was observed and finally proven by Robert Koch, in his postulates, in 1890. Harald Löe and his coworkers were able to demonstrate that dental plaque is the primary etiologic factor in both gingivitis[1] and caries[2], in 1965 and 1970 respectively. These findings indicate that removal of the disease-causing bacteria will prevent the development of the disease. The most efficient way to remove disease-causing bacteria, incorporated in a biofilm, is complete removal of the total biofilm. This, however, is an impossibility, because 100% removal of the oral biofilm can never be achieved[3]. Apart from being impossible, it is probably undesirable since oral biofilm is a part of the resident microflora and normal ecology of the oral cavity[4]. Disturbance of this ecology can lead to the development of bacteria-related diseases, like caries and periodontal disease, but also to overgrowth of potentially pathogenic microorganisms like yeasts[4]. Modifying the composition of the oral biofilm might therefore have more clinical

impact than aiming at complete removal. Therefore, it is important to obtain a better insight of the composition of the oral microflora to understand the exact role of bacteria in disease and how to act upon the bacteria. Currently, it is known that the oral biofilm has an open architecture, with a variety in spatial distribution of dead and live bacteria[5]. Furthermore, the oral biofilm composition is site and subject specific[6]. Nowadays, more insight is gained in the composition of oral biofilms by the use of diagnostic tools, like DGGE[6], a technique described in chapter 5.

The inclusion of fluoride in toothpastes was a major factor in the decline of caries development[7;8]. This emphasizes the importance of adding chemo-therapeutical agents to oral hygiene products, like toothpastes and mouthrinses. Since bacteria are responsible for the development of oral diseases like caries and periodontal disease, controlling bacteria by adding agents, that reduce oral biofilm formation or its pathogenicity, to toothpastes and mouthrinses is a logical development. In chapter 2 it was studied whether known antimicrobial toothpastes and mouthrinses were capable of influencing oral biofilm development. To meet the *in vivo* situation, where saliva and water dilute the concentration of toothpaste and mouthrinse components, dilution series were made and studied. The outcomes of this study showed that dilution of the products did not result immediately in less growth inhibition, depending on the product and dilution studied. Nevertheless all products showed a significant decrease in growth inhibition at the highest dilution studied. In accordance with our findings, it was shown that rinsing with a beaker water after brushing and therewith diluting the toothpaste components, resulted in a higher level of caries[9;10].

Clinical relevance of the biofilm as a reservoir

Dental diseases like caries and periodontal disease are one of the most common bacterial infections in humans. The prevention of biofilm accumulation, and therewith gingival inflammation and caries, is an important part of preventive dentistry, and often done by recommending brushing twice per day with a toothpaste and possibly the supplementary use of a mouthrinse. The anti-plaque, anti-gingivitis and anti-caries effects of toothpastes and mouthrinses are therefore extensively promoted by their manufacturers. The anti-caries effect of toothpastes and mouthrinses is proven in multiple studies[7;11] and a comprehensive review on the anti-plaque and anti-gingivitis effects of several toothpaste and mouthrinse formulations was made by Gunsolley[12]. Despite the promising results of using toothpastes and mouthrinses in combination with mechanical cleaning, oral biofilm is always left behind[3]. Thus it can be concluded that chemical plaque-, gingivitis- and caries-control are challenging topics and subjects for discussion. From the literature it is already known that biofilms can be used as a reservoir for fluoride ions[13]. Therefore we hypothesized that biofilm left behind can also be used as a reservoir for antimicrobial toothpaste and mouthrinse components, and substantiated in chapters 2, 3, 4. In these chapters we demonstrated that biofilm can indeed act as a reservoir for oral antimicrobials, therewith contributing to prolonged action of the antimicrobial product.

Perspectives for the future

In this thesis, we described a new role for oral biofilm left behind after brushing as a reservoir for antimicrobial agents that may influence further biofilm development *in vitro* and *in vivo* upon their subsequent release from the biofilm.

For more clinically relevant parameters like caries decline, Cenci *et al.*[14] showed that using a fluoride toothpaste resulted in decreased caries progression. *In vitro*

research confirmed that fluoride, at low concentrations comparable to concentrations found in oral biofilm, can promote remineralization of enamel[15]. With respect to the current study, the effects of the antimicrobial agents absorbed in the biofilm on the surrounding oral surfaces *in vivo* in terms of clinical parameters needs further investigation. Another subject being worthwhile to study is to increase the penetration of antimicrobials in oral biofilm to enhance effects of absorption and subsequent release. For either fluoride[16] and chlorhexidine[17] it is known that both only have a superficial effect on an *in vivo* grown biofilm. The structure of the biofilm may restrict the penetration of an antimicrobial agent, i.e. by binding agents on the surface or outer layers[18], or the molecules are simply to big to penetrate in the dense biofilm. The role of combining mechanical oral biofilm removal, that may fluff up biofilm-left-behind, with chemical treatment of the biofilm could improve penetration of antimicrobials in the biofilm and is an exciting option that has not yet been explored. Sonic toothbrushes induce fluid shear effects[19;20] and yield the inclusion of air bubbles in the biofilm, exceeding the range of the bristles end. These processes result in non-contact removal of the biofilm, through fluid shear dynamics[19] or acoustic pressure[20]. Possibly, these fluid dynamics and acoustic forces can also contribute to the deeper penetration of therapeutic agents in or even through the biofilm, to increase the delivery of therapeutic agents at hard to reach places, therewith enhancing the efficacy of those agents. *In vitro* studies have already shown that fluoride penetration through *Streptococcus mutans* biofilms is increased after using a powered toothbrush compared to diffusion alone[21]. Ongoing research on the contribution of hydrodynamic fluid forces towards the delivery of therapeutic agents in biofilms, to enhance the efficacy of therapeutic agents in the prevention of dental diseases is needed to proof this concept.

Another approach to influence oral biofilms is to target the adsorbed salivary film on teeth to which the biofilm adheres. It was shown for instance, that amine

fluoride can adsorb to salivary conditioning films and influence initial bacterial adhesion and viability[22]. Recently, the structure and functional mechanism of the bacterial glucansucrase GH70 enzyme has been revealed[23]. The bacteria utilize the glucansucrase enzyme to produce polysaccharides that facilitate biofilm formation and enhances adhesion of bacteria to tooth surface from dietary sucrose. In the future, inhibitors of the glucansucrase enzyme might be developed, possibly resulting in anti-caries drugs or an improved penetration of antimicrobial or therapeutic agents in the biofilm.

General conclusion

There is *in vitro* and *in vivo* evidence that the oral biofilm can act as a reservoir for antimicrobial therapeutic agents, as described in this thesis for antimicrobial agents. Therewith, the biofilm as a reservoir contributes to the long-lasting activity of oral antimicrobials, derived from toothpastes and mouthrinses. This is important since the biofilm is mostly left behind on places that are both hard to reach, like interdental regions, pits and fissures, and most at risk for the development of diseases, like caries and gingival diseases.

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