Microbial dynamics in subgingival biofilms
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Summary
SUMMARY

Periodontal diseases are the most common presentation of oral pathology and affect the tooth supporting tissues, including the cementum, the periodontal ligament, the alveolar bone and the gingival lining. Severe forms of periodontitis affect 10-15% of the adult population and result in the loss of teeth when left untreated. The aetiology of periodontitis is complex and is the result of bacterial accumulations on the tooth surface, compromising host factors like gene-polymorphisms or medical disorders like diabetes and modified by lifestyle factors such as smoking. A model that describes how the different factors involved in periodontal pathogenesis interact is described in Chapter 1. Periodontitis starts with the accumulation of bacteria on the tooth surface where they form biofilms. To study the role of these biofilms in periodontitis, molecular techniques are developed and optimized for oral bacteria. In Chapter 2 Denaturing Gradient Gel Electrophoresis (DGGE) is introduced in oral microbiology. DGGE fingerprints of complex microbial communities are used to study shifts in the population after treatment and *Exiguobacterium aurantiacum* was identified in 13 out of 25 samples. It was possible to detect the most relevant periodontal pathogens in DGGE profiles to a level comparable with culturing and species-specific PCR (Chapter 3). These promising findings proved the applicability of DGGE in oral microbiology and made us expand the experimental setup to study the microbiological effects of scaling and root planing (SRP) in a randomized clinical trial in which full-mouth SRP (FM-SRP) and multiple-session SRP (MS-SRP) are compared. The microbiological effects of SRP are monitored by taking samples before treatment, immediately after, after 1, 2, 7, 14 and 90 days and are described in Chapter 4. DGGE analysis of the subgingival microbial population from preselected pockets revealed that SRP is limited in the elimination of bacteria but induces a transient change in the Actinobacteria and Firmicutes population structure, which returns to baseline population structures after three months. At baseline these populations are skewed with a few species present in almost every patient (*Streptococcus* sp., *A. israelii* and *A. odontolyticus*) and most species being present in a limited number of samples. After three months, additional species also become prevalent, including *Rothia dentocariosa*. In the Cytophaga-Flavobacteria-Bacteroides cluster (CFB) population, *Tannerella forsythia* and an unidentified band are present in almost every patient. After SRP, the composition of the CFB-cluster population does not
return to baseline values and not a single species is detected in more than 15 patients. Moreover, pockets ≤4 mm at three months have a CFB population that is significantly more different from baseline than in pockets >4 mm. There was no difference observed for Actinobacteria and Firmicutes and not when comparing FM-SRP with MS-SRP. This leads us to conclude that SRP only results in a change in the composition of bacterial populations. This change is temporarily for Actinobacteria and Firmicutes and may last for at least three months for the CFB population. Pocket depth after three months seems to be more important than treatment option in establishing a CFB population different from baseline. To be able to discuss these findings, a better understanding of the architecture of subgingival biofilms was needed. In Chapter 5 we provide the first in vivo visual localization of different species in subgingival plaque. With fluorescently labeled species-specific probes the most important subgingival bacteria are localized in the subgingival plaque on seven extracted teeth. The data shows convincingly the dominance of Actinomyces sp., T. forsythia, Fusobacterium nucleatum, Spirochaetes and Synergistetes in different layers in the subgingival plaque. This observation supports a previous hypothetical model of oral biofilms. Synergistes is a recently identified member of subgingival plaque with a possibly important role in host-pathogen interaction due to its localization in close proximity to immune cells. Moreover, Lactobacillus sp. are identified as the central cells of bacterial aggregates in subgingival plaque, whereas Streptococcus sp. and the yeast Candida albicans form corncob structures in supragingival plaque. Finally, periodontal pathogens colonize already formed biofilms and form micro-colonies therein. These in vivo observations on oral biofilms provide a clear vision on biofilm architecture and the spatial distribution of predominant species.

In Chapter 6 it is shown that FM-SRP and MS-SRP result in significant improvements in probing pocket depth (PPD), plaque index (PII), bleeding on probing (BoP) and reduction in the general detection frequency of 5 periodontal pathogens after three months, but without a significant difference between both treatments. Analysis of selected pockets in a test quadrant revealed that FM-SRP results in significant less recolonization of Treponema denticola compared to MS-SRP. An additional finding was that the detection frequencies of T. forsythia and T. denticola continued to decrease up to one week after treatment, without additional treatment. This observation lead us to suggest that SRP also induces an immune response. The elimination of bacteria might therefore be the concerted action of mechanical therapy and the induced immune system of the host. In Chapter 7 an
attempt was made to evaluate the microbiological and clinical experimental results and to provide a rationale for the mechanisms of mechanical periodontal therapy. It is concluded that clinical hypothesis testing without adhering to CONSORT-group statements is erroneous. These statements are formulated to improve the reporting of clinical trails. Moreover, “better” treatment options should be defined as less time consuming, less side effects or less expensive instead of aiming for more reduction in pocket depth. Based on our microbiological findings we hypothesize that mechanical periodontal treatment has preliminary a disturbing effect on the subgingival biofilm and a stimulating effect on the host immune response. To determine the exact sequences and mechanisms of biofilm formation and its interaction with the host, further experiments are needed, for which we present the molecular tools. These goals can be combined in a clinical trial that is designed to reduce the bacterial load, disrupt the subgingival biofilm and upregulate an immune response in a treatment modality with reduced disturbing side effects within a limited amount of time to further improve periodontal therapy.