Modulating the Ecology and Phenotype of In Vitro Oral Biofilms.
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Summary

In the oral cavity, microbes are always present, mostly living in biofilms. A biofilm is a community of microbes, adhered to a surface and surrounded by an extracellular matrix. Commensal oral biofilms can be defined by the absence of pathology-related phenotypes. In contrast to pathological biofilms, commensal oral biofilms are rarely studied. In chapter 2, the effect of the initial inoculum and subsequent growth conditions on *in vitro* oral biofilms was studied. Biofilms were inoculated with saliva of ten individuals or with a pooled saliva sample of these individuals. The biofilms were grown anaerobically for up to three weeks in an artificial saliva medium. Serum was added to induce a gingivitis biofilm, sucrose was added to induce a cariogenic environment, and biofilms were also grown without any addition to the saliva growth medium. Pathology-related phenotypes of these biofilms (i.e., lactic acid accumulation and protease activity) were quantified and the community profile was determined using denaturing gradient gel electrophoresis (DGGE). Phenotype and DGGE profiles of biofilms inoculated with pooled saliva were similar to biofilms inoculated with the saliva of the individuals. This indicates that a pooled saliva sample can be used as inoculum to generate representative biofilms in this model system. DGGE analysis allowed differentiation of biofilms grown with sucrose, but not with serum. Lactate accumulation by biofilms was significantly increased by sucrose and protease activity was significantly increased by serum. Biofilms grown without serum or sucrose showed low activity for both phenotypes. Therefore, three clinically relevant *in vitro* biofilm models were developed and could be differentiated based on pathology-related phenotypes but not DGGE analysis.

In chapter 3, these phenotypes were also evaluated for biofilms where *Candida albicans* was introduced. *C. albicans* is an oral commensal fungus, which can be found in the oral cavity of 50-70% of healthy individuals. Its effect on oral ecology has mostly been studied using dual-species models, which disregards the complex nature of oral biofilms. The aim of this study was to culture *C. albicans* in a complex model to study its effect on oral biofilm ecology and phenotypes. Biofilms inoculated using pooled stimulated saliva with or without addition of *C. albicans*, were grown under anaerobic, aerobic or aerobic conditions with elevated CO_2-levels. *C. albicans* was only able to proliferate in biofilms grown under aerobic conditions. After two days, *C. albicans* did not induce differences in total biofilm formation, lactic acid accumulation (cariogenic phenotype) or protease activity (phenotype of periodontal diseases). The microbiome of young biofilms was determined five hours after inoculation using 16S rDNA sequencing. Facultative or strict anaerobic *Veillonella*, *Prevotella*, *Leptotrichia* and *Fusobacterium* species were significantly more abundant in biofilms with *C. albicans*. Biofilms without *C. albicans* contained more of the aerobic and facultative anaerobic species *Neisseria*, *Rothia* and *Streptococcus*. These results show that *C. albicans* generates a niche for anaerobic bacteria to proliferate within the boundaries of a non-pathogenic ecology. This confirms that *C. albicans* can be part of commensal oral

biofilms.

In chapter 4, maintenance of a non-pathogenic ecology by addition of the sugar alcohol erythritol was evaluated. This artificial sweetener is suggested to have caries-preventive properties, e.g., by reducing the abundance of the caries-related acedogenic bacteria. Since the effect of erythritol on gingivitis has rarely been studied, the aim of this study was to assess the effect of erythritol on the ecology and the gingivitis phenotype of oral microcosms. Biofilms were inoculated with stimulated saliva from twenty healthy donors, and grown in a gingivitis model in the continuous presence of 0% (control group), 5% and 10% erythritol. After nine days of growth, biofilm formation, protease activity (gingivitis phenotype) and microbial profile analyses were performed. Biofilm growth was significantly inhibited in the presence of erythritol and this effect was dose-dependent. Protease activity and the bacterial diversity of the biofilms were significantly lower when erythritol was present. Ecology analysis revealed that presence of erythritol induced a compositional shift from periodontitis- and gingivitis-related bacterial taxa towards early colonizers. This suggests that erythritol suppresses maturation of the biofilms towards an unhealthy composition. Furthermore, the gingivitis phenotype was suppressed and biofilm formation was reduced in the presence of erythritol. Consequently, erythritol likely contributes to maintaining a non-pathogenic oral biofilm *in vitro*.

Bacteria within oral biofilms communicate via quorum sensing (QS). QS regulates several phenotypic biofilm parameters, e.g., biofilm formation and the production of virulence factors. In chapter 5, we evaluated the effect of several QS modifiers on growth and on the cariogenic potential of microcosm oral biofilms. Biofilms were inoculated with pooled saliva and cultured in the presence of sucrose for two or four days. QS modifiers were continuously present. Biofilm formation and lactic acid accumulation were measured. Subsequently, the biofilm ecology was determined using 16S rDNA sequencing. Of the used QS modifiers only one completely abolished lactic acid accumulation by the biofilms without affecting biofilm growth; 3-oxo-N-(2-oxocyclohexyl)dodecanamide (3-Oxo-N). This compound was selected for further analyses. The active range of 3-Oxo-N was determined to be 10-100 μM. The homologous QS molecule did not counteract the reduction in lactic acid accumulation, suggesting a mechanism other than QS inhibition. Microbial ecology analyses showed a reduction in the relative abundance of streptococci in favour of the relative abundance of *Veillonella* species in the 3-Oxo-N exposed biofilms. The minimal inhibitory concentration of 3-Oxo-N for several streptococcal species varied between 8 and 32 μM. The compound 3-Oxo-N changes the ecological homeostasis of *in vitro* oral biofilms. It reduces its cariogenic potential by minimizing lactic acid accumulation. Therefore, 3-Oxo-N represents a promising compound in maintaining a commensal oral biofilm.