Host-microbe interactions in reconstructed human gingiva in vitro

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Publication date
2020
Document Version
Other version
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Other

Chapter 7

Summary

Bacteria are always present in the mouth. The interactions between these bacteria and the host constantly influence the oral health. Like the commensal bacteria benefiting the gut and skin, oral commensal bacteria may also play an important role in our physiology and contribute to the oral health. Many studies have been done to investigate the influence of bacteria on the oral health and diseases, however, they were mainly focused on the oral pathogens which are involved in the development of common oral diseases, such as the formation of cavities (caries) and inflammation of the periodontal tissue (periodontitis). Little is known about the beneficial influence of commensal bacteria on oral health. The aim of this thesis was to gain more insight into the influence of the commensal bacteria in the oral cavity, in particular, on a 3D organotypic human gingiva model. Using this model, we investigated the interactions between gingiva and the commensal bacteria, and the combination of commensal bacteria and metals used in dental medical devices. The gingiva model consists of a multi-layered epithelium on top of an underlying connective tissue – a hydrogel populated with gingival fibroblasts. The gingiva model has notable physiological similarities with the native gingiva. In addition, representative multi-species oral biofilms grown from human saliva were used to mimic the *in vivo* microbial environment. The gingiva model was exposed to different conditions to answer the following questions:

1) Do the commensal biofilms beneficially influence the gingiva model?
In **chapter 2** the influence of the multi-species microcosm biofilm on the gingiva model was investigated. Without being exposed to any microbial biofilms, the gingiva model has already been shown to have morphological and functional features mimicking the native gingiva biological barrier. The epithelium continues to regenerate as it proliferates and differentiates from the basal cell layer towards the surface, contributing to the efficient clean-up of harmful invaders and the repair of damaged tissue. Furthermore, a basal secretion of functional cytokines is maintained by both the keratinocytes in the epithelium and the fibroblasts from the underlying connective tissue. But what happens to the gingiva model when it comes into contact with the multi-species microcosm biofilm? This was tested by exposing the gingiva model to a multi-species commensal biofilm for 1, 2, 4 and 7 days. After the biofilm exposure, a clear change in the epithelium was observed where the representative barrier characteristics were further improved. The epithelium became thicker, with increased proliferation and differentiation from the basal cell layer, upregulated secretion of cytokines and increased transcription

/ expression of antimicrobial peptides. This strongly indicated that in the native gingiva, exposure to a commensal microcosm biofilm provides a better defense against trauma and pathogenic and / or toxic agents. Currently, there are only a few *in vitro* models showing how the oral commensal bacteria influence the gingiva. Additionally, the exposure time in these models were set no longer than 48 hours in order to prevent contamination caused by unwanted overgrowth of bacteria during culture in a nutrient rich culture medium. In our study, the biofilm was exposed to the surface of the gingiva model (not via the culture medium) over a longer period (7 days). Since antibiotics penicillin / streptomycin were only present in the culture medium below the connective tissue of the gingiva model, the antibiotics had minimum effect on the biofilm which was applied to the surface of the gingival epithelium. Although the viability of the biofilm decreased significantly over 7 days, a clear beneficial influence was observed on the gingiva model. Nevertheless, to further look into the dynamic host-microbe interactions between the gingiva and the oral biofilm, it is necessary to develop an even more viable biofilm and a more stable co-culture model in the future.

2) Do pathogenic biofilms influence the gingiva model in a different way from the commensal biofilm?
To determine if the pathogenic biofilms and the commensal biofilm differentially influence the gingiva model, in **chapter 3**, we investigated the transcription of a group of genes involved in the Toll-Like Receptor (TLR) signaling pathway after different biofilm exposures. The gingiva model was exposed to three types of multi-species biofilms representing the commensal (healthy), gingivitis and cariogenic (pathogenic) biofilm exposures. With the commensal biofilm, many TLR signaling related genes were upregulated in the gingiva model. This response was much more announced than when the gingiva model was exposed to the pathogenic biofilms. This shows that the TLR signaling plays an important role in the gingiva in response to different oral biofilms. The TLR signaling may be activated by the commensal biofilm to initiate a complete host defense, which is necessary to prevent possible damage in the oral cavity. In contrast, the pathogenic biofilms showed less potential in activating such a defensive response.

3) Are the *in vitro* oral biofilms influenced by the host environment as well? For example, do different oral substrates, or does different nutrient availability, play a role in influencing the oral biofilms *in vitro*?

In **chapter 4**, the influence of the host environment on the oral biofilm was investigated. In the oral cavity, the host-microbe interactions are dynamic, and the environment contributes greatly to the maintenance and regulation of such interactions. Therefore, the influence of two host environmental factors, the nutrients and substrates, on the properties of the salivary biofilms was investigated over time (1, 3 and 5 days). The different host environments were mimicked by using two culture media: Brain Heart Infusion (BHI) and Thompson, and three oral substrates: gingiva (the gingiva model), teeth (hydroxyapatite discs, HAP) and dental implants (titanium discs, TI). In general, the compositions of the in vitro oral biofilms were comparable to the saliva inoculum within 5 days. On the biotic gingiva model, BHI and TP resulted in very similar biofilm compositions developing. This is in contrast to the biofilms which were grown on the two abiotic substrates, HAP and TI. These biofilms showed different compositions depending on the culture medium. This indicated that the combination of both culture media and oral substrates influenced the oral biofilms, thus providing information about how the biofilms are affected by the host in the oral cavity.

4) There are also other substances that are occasionally present in the oral cavity, such as metals from dental materials. Do the commensal bacteria also play a role in the reaction of the gingiva against these metals, for example nickel and titanium? In **chapter 5**, the influence of a commensal bacteria, *Streptococcus mitis*, was investigated on a gingiva and a skin model which were exposed at the same time to nickel or titanium. Cytokine secretion was increased after the exposure to *S. mitis* in the skin model but not in the gingiva model. In addition, the co-exposure of nickel and *S. mitis* caused an even higher cytokine secretion in the skin model. This was in contrast to titanium, which remained inert in both models. Taken together, this shows that the gingiva model and the skin model respond differently to the presence of the commensal bacterium *S. mitis*. They also respond differently to the co-exposure of *S. mitis* with nickel or with titanium. These observations suggest that commensal bacteria such as *S. mitis* may play an important role in the skin and the oral cavity when they are in contact with common metals such as nickel, thus potentially influencing the development of an allergic reaction in the skin or the oral cavity. Furthermore, looking into the transcription of the TLRs indicated that TLR1 and TLR4 may participate in this, each with their own function. This was the first time that living commensal bacteria had been co-exposed with potentially sensitizing metals in an organotypic skin / gingiva model. The clear difference

between nickel and titanium in the skin model shows how important the influence of the living commensal bacteria can be.