Insights into the Bacterial and Fungal Ecology of Endodontic Infections

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SUMMARY: INSIGHTS INTO THE BACTERIAL AND FUNGAL ECOLOGY OF ENDODONTIC INFECTIONS

The research presented in this thesis aims to give further insight into the complexity of root canal infections and into their interactions with the host as well as the interactions within the infection itself. A better understanding of the aetiopathogenesis of apical periodontitis aids in preventing it and in developing successful treatment strategies.

The first study evaluated the bacterial ecology of primary root canal infections (**chapter 2a**). Twenty-three extracted teeth were processed by cryo-pulverization. Bacterial communities of apical and coronal root halves were profiled using tagged 454 pyrosequencing. Analysis revealed immense diversity of the infections and a different ecology of in apical and coronal roots. Infections in apical roots had a higher diversity and contained more species than coronal roots. Also, apical roots contained more Proteobacteria and fastidious obligate anaerobes, suggesting a distinct ecological niche in the apical region. This may explain the difficulty of antimicrobial treatment of the infection and stresses the need for new treatment strategies.

Some of the bacteria found in this study are capable of forming spores, which enables them to survive harsh environments. Thus far, no study has identified bacterial spores present in the root canal system. In a follow-up pilot-study, 15 root canal infections were examined for the actual presence of this treatment-resistant bacterial phenotype using paper point sampling (**chapter 2b**). Samples were incubated at 80°C to eliminate vegetative cells. Subsequently, spore germination was determined by anaerobic and aerobic cultivation. Examination by microscopy and by cultivation in chopped meat broth revealed no bacterial spores. Absence of spore-forming bacteria was confirmed by 16S rRNA sequencing of the 6 isolated bacilli, which identified *Propionibacterium acnes* and *Propionibacterium avidum*. Further research could use more sensitive techniques applied on a larger sample size in order to definitively dismiss the presence of bacterial spores within root canal infections.

The role of bacteria in causing apical periodontitis has been established, while the role of other microorganisms is studied less thoroughly and less consistently. In a systematic review and meta-analysis of the literature, the prevalence and diversity of fungi in root canal infections was studied (**chapter 3**). After screening of 1 041 titles and abstracts and full-text reading of 167 articles, 54 studies were included. The overall prevalence of fungi in root canal infections was 7.5% and *Candida albicans* was the most frequently isolated species. Subgroup analyses revealed no factor influencing the prevalence. Further *in vivo* studies using better standardized techniques should give a more detailed and accurate representation of the prevalence and nature of fungi in root canal infections.

The next study used Illumina sequencing of both the bacteriome and mycobiome to give insight into the bacteria and fungi present in root canal infections (**chapter 4**). Twenty-six extracted teeth were processed by cryo-pulverization. The previously found immense bacterial diversity was confirmed, although no differences were observed between apical and coronal root infections. Fungi were present in 57% of the teeth. *Candida* and *Malassezia* were the most prevalent fungi. Fungal diversity was lower than in the salivary microbiome. Moreover, fungal presence is accompanied by a distinct bacteriome. Root segments positive for fungi contained a more acidogenic bacteriome. Bacterial-fungal interaction may complicate the infection. Therefore, more research into the interaction between bacteria and fungi in root canal infections is necessary.

The fungal-bacterial interaction and its effect on the inflammatory response was further studied using an *in vitro* model (**chapter 5**). Immune cells were stimulated with *C. albicans* and *Enterococcus faecalis* biofilms, either untreated or treated with the irrigants sodium hypochlorite (NaOCl) or chlorhexidine. Only untreated *E. faecalis* biofilms stimulated Toll-like receptors (TLRs). No stimulation was observed when the bacteria were treated or when they were co-cultured with *C. albicans*. Furthermore, *C. albicans* failed to stimulate TLRs on its own. The interplay between the two microorganisms and the host resulted in decreased TLR stimulation, which may lead to a modification of the innate immune response. This interplay will have consequences for treatment efficacy.

Since root canal infections appear to be more diverse and complex than previously considered, effective treatment is essential. Because contemporary root canal treatment is unable to remove the full microbial community, a novel strategy using the vanadium chloroperoxidase (VCPO) enzyme was tested (**chapter 6**). VCPO uses available substrates to generate antimicrobial reaction products. The VCPO reaction products were used to treat 24-hour *E. faecalis* biofilms and were similarly effective in reducing the viability of four different strains of *E. faecalis*. Thus, VCPO may provide an added benefit to current endodontic treatment strategies, possibly as an antimicrobial dressing.

The enzyme was further optimized for the local pH of the root canal system and tested for its biocompatibility (**chapter 7**). Reaction products generated by the modified VCPO inactivated 24-hour biofilms of *E. faecalis* after 5 minutes and even more after 30 minutes. Additionally, cytotoxicity tests demonstrated preliminary biocompatibility. Therefore, an interappointment dressing containing VCPO could aid in improving current endodontic treatment through continuous and local generation of antimicrobials.

In conclusion of this thesis, the bacteriome and mycobiome of root canal infections were found to be complex and this has consequences for their interaction with the host. Future research should study the mechanisms underlying the functioning of the microbiome of root canal infections, and if and how the microbiome interacts with the host. If a detrimental effect on the host is evident, more effective strategies to eliminate the infection and preserve oral function and well-being should be developed, possibly using VCPO.