Endodontic infections and apical periodontitis. An analysis of microbial factors prior, during and after endodontic treatment
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Chapter 8

SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

Bacteria play an essential role in the development and persistence of endodontic disease. The aim of treatment of endodontic infections is focused on elimination of bacteria from the root canal system and on prevention of reinfection. A lot of attention has been focused on improving measures to definitively eliminate bacteria after cleaning and shaping of the root canal and also to the presence and role of bacteria in the root dentinal tubules. The low numbers of microorganisms in the canal and in the dentinal tubules after cleaning and shaping have been considered responsible for persistence and development of periapical disease. To eliminate bacteria from the root canal system (root canal proper and root dentinal tubules) the use of intracanal dressings such as calcium hydroxide, chlorhexidine, iodine potassium-iodine (IKI) and volatile drugs such as camphorated p-monochlorophenol (CMCP) are advocated widely in endodontic therapy. In this thesis the presence of bacteria in the root canal and root dentinal tubules is discussed and studied, and clinical measures necessary for eradication are evaluated. In chapter 2, the fate and role of microorganisms present in root dentinal tubules is reviewed. The relevance and consequences of residual bacteria in the tubules of root dentine after cleaning and shaping of the root canal space is addressed. A second aim is to discuss the necessity of clinical interventions to eradicate those bacteria that are presumed to survive in the root dentine. It appeared that there is very little information on the presence of viable microorganisms in root dentinal tubules in necrotic infected pulps and the role they may play in lesions that are refractory to treatment. In-vitro research has shown that one important factor for microbial invasion of dentinal tubules is the availability of a nutrient source. Ingrowth or progress of bacteria is delayed or prevented by the presence of a smear layer and an intact root cementum and periodontal ligament.

The available clinical and experimental evidence supports the use of antibacterial dressings in cases where the root canal space remains temporarily unobturated after removal of necrotic and infected pulp tissue. There seems no evidence however that special measures should be taken to kill the bacteria in the dentinal tubules. In the vast majority of cases, those bacteria seem not to jeopardise successful outcome of root canal treatment. In chapter 3, a test model is described to quantify penetration of bacteria into dentinal tubules in-vitro. The model consisted of two compartments separated by a bovine dentine specimen with a thickness of 1.5 to 3.1mm.
The root cementum was removed from the root surface and the specimens were oriented in the model with the pulpal side facing the inoculated chamber of the test model. One compartment contained the test organism (Enterococcus faecalis or Actinomyces israelii) whereas the other was filled with sterile broth. The depth of bacterial penetration was measured in the dentine with or without a smear layer using both a histological and a quantitative recovering grinding technique after 6 weeks of exposure to the microorganisms. *E. faecalis* penetrated the dentine significantly deeper than *A. israelii*. After removal of the smear layer with EDTA, *E. faecalis* penetrated significantly deeper than in the dentine pre-treated with saline only or with a combination of saline and sodium hypochlorite. Microorganisms were found in 89% of the cultured specimens and in 80% of the specimens that were evaluated with light microscopy. Total penetration through the dentine specimen and infection of the broth in the test compartment of the model occurred in only two out of 72 specimens. With a longer exposure period, the number of specimens with total penetration could have been higher. The collection and immediate culturing of infected dentine dust and the counting of colony forming units (CFU) allowed an overview of the number of bacteria per sample. This technique was more sensitive than light microscopy.

In chapter 4, the presence of microorganisms in root dentinal tubules *in vivo* is evaluated. Two sets of teeth with apical periodontitis were collected at different geographic layers (Amsterdam and Glasgow) to study the identity and quantify the numbers of bacteria present in the root dentinal tubules. Teeth that could have been restored after endodontic treatment but were extracted on request of the patient were selected. Root dentine of 20 of these teeth was cultured from three locations between pulp and cementum (A, B and C). In addition, dentine from eight teeth was examined histologically.

Using the culturing technique, bacteria were found in 77% of the dentine samples from set 1 (Amsterdam) and in 87.5% of the dentine samples from set 2 (Glasgow). At greater distance, in layer C, from the pulp bacteria were found in 62% (13 out of 21) of the dentine samples. Twenty-three percent (3 out of 13) of set 1 and 25% (2 out of 8) of set 2 contained more than 50,000 CFU/g of dentine in layer C. In layers closer to the pulp higher numbers of anaerobic bacteria and gram-positive rods were found as well as a larger number of bacterial species.

Identification revealed that the most dominant species were: *Prevotella intermedia, Prevotella prevotii, Prevotella buccae, Porphyromonas gingivalis, Porphyromonas asaccharolytica, Fusobacterium nucleatum, Fusobacterium necrophorum, Peptostreptococcus anaerobius, Peptostreptococcus micros, Actinomyces israelii, Actinomyces viscosus, Actinomyces naeslundii, Actinomyces odontolyticus, Streptococcus...*
coccus sanguis, Propionibacterium acnes, Bifidobacterium adolescentis, Lactobacilles acidophilus.

Histologic sections showed bacterial penetration in dentinal tubules in 5 of 8 teeth. In the other three teeth where the CFU/g recovered was <10,000, no histologic signs of tubule penetration was observed.

In more than half of the infected roots, viable bacteria were present in the deep dentine close to the cementum. In addition, anaerobic culturing of dentine appeared more sensitive than histology to detect these bacteria.

In chapter 5 the fate of microorganisms in the root canal of teeth with infected pulps and periapical bone lesions, after instrumentation and irrigation only or after the subsequent application of calcium hydroxide as a dressing, is described.

Endodontic samples were cultured and microorganisms were counted and identified in 43 teeth before (sample 1) and after (sample 2) instrumentation and irrigation during the first visit and before (sample 3) and after (sample 4) treatment during the second visit. In the first visit, teeth were instrumented and half of the teeth were filled with a thick slurry of calcium hydroxide in sterile saline, the other teeth were obturated with gutta-percha and AH-26 sealer. After 4 weeks the teeth with calcium hydroxide were accessed again and after removal of the calcium hydroxide and microbiological sampling, they were obturated with gutta-percha and AH-26 sealer.

The mean total colony forming units (CFU) counts of positive samples dropped significantly as a result of canal preparation during the first visit from 1.0x10^6 to 1.8x10^3 (between samples 1 and 2) but increased to 9.3x10^3 in the period of calcium hydroxide dressing between the two visits (sample 2 and 3). There was no difference in mean total CFU counts of positive samples between the end of the first (sample 2) and the end of the second visit (sample 4). The most frequently isolated species were Prevotella intermedia, Capnocytophaga spp., Actinomyces odontolyticus, Propionibacterium acnes and Peptostreptococcus micros.

Although a calcium hydroxide paste was placed in the prepared canals, the number of positive canals had increased in the period between visits. However, the number of microorganisms had only increased from 0.2 (sample 2) to 1% (sample 3) of the original number of CFU (sample 1). It is concluded that a slurry of calcium hydroxide in sterile saline limits but does not prevent regrowth of endodontic bacteria.

All cases described in chapter 5 were monitored. Evaluation of the healing of periapical lesions of teeth with positive and negative canal cultures at the time of obturation is described in chapter 6 together with the evaluation of periapical healing of teeth treated in one visit (without) or in two visits with an interappointment dressing of calcium hydroxide. Thirty-nine patients received endodontic treatment. In the first visit, teeth were instru-
mented and 18 of these teeth were filled (after biological sampling) with calcium hydroxide in sterile saline. The other 21 teeth were obturated with gutta-percha and AH-26 sealer after microbiological sampling. Four weeks later, the teeth with calcium hydroxide were accessed again and after microbiological sampling, they were obturated with gutta-percha and AH-26 sealer. Healing of periapical radiolucencies was recorded over a period up to 4\( \frac{1}{2} \) years.

In both treatment groups the size of the periapical lesions reduced significantly during the follow-up period. Complete radiographic healing was observed in 81% of the cases in the one-visit group and in 71% of the cases in the two-visit group. The probability of success increased continuously over time for both treatment groups. Seven out of eight cases (87.5%) that showed a positive root canal culture at the time of obturation healed. The number of colony forming units (CFU) in 6 out of 8 positive canals was <10\(^2\) CFU/ml.

Within the limitations of this study no significant differences in healing of periapical radiolucencies was observed between teeth that were treated in one visit (without) and two-visits with inclusion of calcium hydroxide for four weeks. The presence of a positive bacterial culture (CFU < 10\(^2\)) at the time of filling did not influence the outcome of treatment.

From the previous chapter it is clear that some bacteria survive clinical measures better than others do. Microorganisms that are found after treatment of the root canal are mainly strict anaerobic bacteria. An evaluation of specific combinations of bacteria in the root canal system is described in chapter 7. Eighty-one combinations of microorganisms were found and tested for a symbiotic relationship. Four of these combinations were considered to interact by stimulating each other’s presence. Prevotella intermedia with Prevotella oralis and Peptostreptococcus micros, P. micros with Actinomycyes odontolyticus -and Bifidobacterium spp. with Veillonella spp. The first three combinations consisted of microorganisms that were also found in high numbers in the root canal at all 4 stages of treatment (chapter 4) as well as in infected dentine (chapter 3).

In the root canal with a necrotic pulp, these strict anaerobic bacteria are mainly found in the apical third. Eradication may be difficult because of the anatomical conditions in this part of the root canal. The use of calcium hydroxide to eliminate these bacteria seems to be unpredictable.

Alternative dressings with a more vaporising action may have more effect in the apical part of the root canal. However, it should be kept in mind that many of these dressings have a toxic side effect on the host. In general it should be emphasised that eliminating bacteria from the root canal system is mainly dependent on the instrumentation and irrigation techniques used and the amount of infected dentine that is removed from the inner part of the root canal. The use of sodium hypochlorite in copious amounts for
irrigation is undoubtedly still the most predictable tool to reduce the number of microorganisms. The low numbers that can be reached this way seem not to interfere with periapical healing.

CONCLUSIONS

• Viable microorganisms are found in root dentinal tubules in high numbers.
• Instrumentation of the root canal system supported by irrigation with sodium hypochlorite results in a significant reduction of cultivable microorganisms.
• Inclusion of calcium hydroxide limits but does not prevent regrowth of endodontic bacteria and does not further reduce the number of microorganisms.
• Small numbers (< $10^2$ CFU/ml) of microorganisms left in the root canal system after cleaning, shaping and irrigating seem not to jeopardise the outcome of endodontic treatment.
• In the root canal bacteria may occur in certain clusters possibly resulting in pathogenic synergism

FUTURE DIRECTIONS

In the studies presented in this thesis microbial culturing of root dentine and the root canal content have been important tools to evaluate the extent and characteristics of endodontic infection. These techniques have limited sensitivity and are not able to detect low numbers of microorganisms resulting in false negative outcomes. This drawback can be bypassed by current polymerase-chain-reaction (PCR) techniques in which small amounts of bacterial DNA can be amplified and detected. The PCR is a highly sensitive and specific detection method for bacteria in biological samples. Especially quantitative PCR techniques will allow a more detailed insight in the microbial component of root canal infections and the influence of treatment modalities. Viability of microorganisms can only be established when RNA is demonstrated using PCR techniques. The shortcomings of culture and common PCR techniques can be overcome by the development of in-situ, intracellular fluorescent labels and probes that facilitate the rapid assessment of microbial physiology without cultivation to assess respiratory activity using 5-Cyano-2,3-ditolyltetrazolium (CTC) in endodontic biofilms.

The influence of obturation techniques on bacteria that remain in the canal and root dentinal tubules after cleaning and shaping is of great interest and should be investigated further. Their fate and possible influence
on healing is still not completely understood. In this thesis, the healing rate has been evaluated by clinical symptoms and radiographic evaluation. It should be realised however that radiographic signs of inflammation can only be seen when the cortical bone is affected. Lesions that are restricted to the spongious bone will not be visible on radiographs taken in a conventional way. It would be interesting to conduct a prospective clinical trial where more sensitive radiographic techniques, like computertomography, can be used. These techniques may elucidate small differences between treatment modalities. Smaller numbers of patients might than be needed for statistical evaluation which will make a prospective clinical trial more feasible.
Summary, conclusions and future directions

SAMENVATTING, CONCLUSIES EN AANBEVELINGEN

Bacteriën speelden een cruciale rol in het ontstaan van endodontische aandoeningen. Het deel van de wortelkanaalbehandeling is de verwijdering van deze bacteriële organismen en het voorkomen van herinfec tie van het wortelkanaal.

De bijzondere bacteriële colonie achterblijven in het wortelkanaal en in het worteldentine- en reiniging en vermijden worden vermeld als de belangrijkste middelen voor middenkoppeling. Om ook deze bacteriën uit het wortelkanaal te verwijderen moet het gebruik van wortelkanaal zoals calciumhydroxide, chloortetracycline, KI en een sterk desinfecterende, als CHCP, aangeraden worden.

In de praktijk wordt de aanwezigheid van bacteriën in het wortelkanaal en de enzymatische beschermen en bestuiven. De klinische handleidingen die gericht zijn op het verwijderen van deze micro-organismen worden hierin gegeven.

In deelsteek 2 wordt een overzicht gegeven van bacteriën in de loop van het worteldentine. De relevante van bacteriën die hier nuttig zijn voor de reiniging en de verrijging van het wortelkanaal wordt hier besproken. Bevorderlijk wordt aangemerkt voor een modermachtige bacteriën in worteldentine.

Volgens het experimenteel onderzoek is duidelijk geworden dat de aanwezigheid van bacteriën belangrijk is voor de invasie van worteldentine. De algehele huid van een ammonium-worteldentine en een parodontale ligament versnel voldoende het doordringen van bacteriën.

De klinische en experimentele onderzoeksgroepen rechtvaardigen het gebruik van een desinfecterende die gevallen waarbij het wortelkanaal na de reiniging en de verrijging, in de loop van de invasie, vergedelijk wordt getest. Aanvullende handleidingen die enkel gecentring zijn op het verwijderen van bacteriën uit het worteldentine lijken niet gecentreerd. In verband met de invasie van bacteriën lijk de aanwezigheid van kleine aanvallen bacteriën een succesvolle behandeling niet in de weg te staan.

In deelsteek 3 is een unifilm model ontwikkeld om de invasie van twee verschillende bacteriën in worteldentine te bepalen. Het model bestaat uit twee kamers die van elkaar gescheiden zijn door een schijf funderdenterine (waarvan het wortelkanaal is verwijderd) met een dikte van 1.3-3.1 mm. De pulpaale kant is in contact met de grootste kamer. Deze kamer bevat een testorganisme, Proteus vulgaris of Actinomyces israelii, de andere kamer bevat S. aureus. Na de proefperiode van 24 weken werd de penetratiedeep van het
on healing is still not completely understood. Hence, in this thesis the healing rate has been evaluated by clinical symptoms and radiographic evaluation. It should be realized, however, that radiographic signs of inflammation can only be seen when the cortical bone is affected. Lesions that are restricted to the cancellous bone will not be visible on radiographs taken in a conventional way. It would be interesting to apply prospective clinical trials where more sensitive radiographic techniques, like computed tomography, can be used. These techniques may elucidate small differences between treatment modalities. Smaller numbers of patients might then be needed for statistical evaluation which will make a prospective clinical trial more feasible.