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Microorganisms in root canal infections: a review

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SUMMARY

A traditional concept is that apical periodontitis is the result of pathogenic effects of the microorganisms colonizing the root canal system and the response of the host defence system. The composition of the microflora of root canals differs in primary endodontic treatment and retreatment cases. Persistent disease in the periapical region after root canal treatment presents a more complex situation as it was thought earlier. Scientific evidence indicates that unsatisfactory outcome of cases in which treatment has followed the highest technical standards mainly is associated with microbial factors, comprising extraradicular and/or intraradicular infections.

Key words: apical periodontitis, biofilm, persistent disease, bacterial taxonomy.

INTRODUCTION

Apical periodontitis should be considered as an inflammatory reaction in the periapical tissues to the presence of bacteria within the root canal system [1, 2]. It is evident that an infected root canal system is a unique niche for the selective species of microorganisms [3]. The composition of microflora of root canals has been the focus of considerable research over the years. Results of studies clearly defined the microbial differences between primary endodontic treatment and retreatment [4]. Apical periodontitis persisting after root canal treatment presents a more complex etiological and therapeutic situation [5]. It appears that certain species of microorganisms, especially Gram-positive facultatives, which often have expanded representation in retreatment cases in comparison with primary endodontic treatment, possess greater resistance to antimicrobial agents used during endodontic treatment than anaerobes. This has shifted the focus of scientists to these microorganisms in recent years.

Another important factor which is started to be evident during the last years is that microbes in the root canals can grow not only as planctonic cells or in aggregates, co-aggregates, but they can also form biofilms consisting of a complex network of different microorganisms [6, 7]. Biofilm formation in root canals is probably initiated some time after the first invasion of the pulp chamber by planktonic oral microorganisms after some tissue breakdown [8]. Biofilms are composed of microcolonies of bacterial cells that are distributed in a matrix which consists of exopolysaccharides, proteins, salts and cell material in an aqueous solution. The matrix takes about 85% of the volume of a biofilm. Bacterial biofilms are reported to be the most common cause of persistent inflammation [9]. The morphology of root canal systems is complex and this favors growth of bacteria in the form of biofilms [7]. Studies have showed that biofilms protect microorganisms from adverse environmental changes and effects of biocides more than one thousand times in comparison with the same microorganisms in planktonic form [9, 10, 11]. Introduction of the biofilm concept to endodontic microbiology was the major step forward to the understanding of root canal infections, especially those of the persistent kind.

Now it is evident that the management of persistent apical periodontitis is more complex and less uniform regarding the choice of intracanal medicaments

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and sequence of their use than in the management of apical periodontitis affecting non-treated teeth [12].

The purpose of this paper is to rewiev the specificity of the root canal microflora and its impact on the success rate of endodontic treatment procedures.

ROLE OF MICROORGANISMS IN DEVELOPING OF APICAL PERIODONTITIS

In 1894 W. D. Miller was the first who published observations from the root canals with infected pulp space, but at that time he was unable to verify his findings [13]. Since that time bacteria was implicated in infections of endodontic origin. Further studies and development of anaerobic sampling techniques, demonstrated that the endodontic environment is selective and supports the growth of specific microorganisms [1, 2]. In 1982 Fabricius et al. showed the succession of strict anaerobes over facultative anaerobes with time in the root canal, which most likely occurred due to the changes in ecology of root canal system [14].

Over time the diversity of microorganisms became evident and their classification for identification of species became necessary. Bacterial taxonomy was based on their main characteristics (Table) [15]. Due to innovations in identification techniques, the taxonomy of bacteria associated with endodontic infection was changed.

Precise identification of microorganisms participating in the pathogenesis of apical periodontitis is important in order to understand the disease process and to provide effective antimicrobial treatment. For a long time culturing and serial dilution methods were considered as standard methods used in research. Due to development of new technologies, identification of pathogens now generally also involves microscopy, immunological assays and molecular methods. Molecular methods (for example polymerase-chain-reaction-based method (PCR) was thought to replace the need for microscopy, culturing and immunological assays due to their characteristics. They are easier, faster and more sensitive in comparison with standard methods. Using them it can be detected less than 10 bacteria per sample. However it is important to differentiate between culturing and molecular methods. The culturing as method measures viable bacterial cells as colony-forming units while molecular methods measure nucleotide sequences and viable microorganisms are not required. The PCR method allows amplification of very minute quantities of DNA to detectable levels. A disadvantage of molecular methods is that when using them it is impossible to get information about viability of microorganisms and they are not available for other tests or research studies. Cultivation discloses a wider spectrum of microorganisms in root canal system. By using this method is easier to detect contamination of the sample during laboratory manipulation, viability of microorganisms cells could be detected and these microorganisms could be used further with other tests and in research studies [16]. Due to implementation of culturing and molecular methods in laboratory analysis, results of studies showed that root canal infection is more complex than it was believed earlier and researchers were enable to identify previously unidentified and uncultivable microorganisms and to expand understanding about endodontic pathogens.

Living conditions for endodontic pathogens are not easy and differ from that in caries lesions or periodontal pockets. In root canals microorganisms are restricted from the oral cavity. Changes in environment such as the type and availability of nutrients, oxygen tension and bacterial interactions influence the specificity of the root canal flora [17]. Microbiological analysis of the root canal flora in primary treatment cases of apical periodontitis is easier due to larger amount of bacterial cells and species in the root canal. Microbiological sampling and culturing from instrumented root canals or in retreatment cases is more difficult due to dramatic reduction of microbial cells after chemomechanical preparation or cells of surviving microorganisms which can be expected from 10 to 10^2 cells in a sample [18].

Current concepts suggest that the number of bacterial species in an infected root canals may vary from one to more than 12 and the number of bacterial cells from $<10^2$ to $>10^8$ per sample (4). Now it is evident that bacteria can inhabit not only the main root canal, but also enter the dentin tubules, apical canal ramifications, isthmuses and other morphological irregularities of the root. Number of studies has shown that invasion of bacteria into dentin tubules occurs in 60-90% of teeth with apical periodontitis [19, 20]. There are also suggestions that bacteria found in the dentin tubules are special and unique in comparison to the microflora of the oral cavity. Existing knowledge about the ability of different species to invade dentin shows that such species as gram-positive facultative *cocci*, *lactobacilli* and *actinomyces* are more often found as invaders among other bacteria species. Obviously the environment of the tubules restricts supply of nutrients to bacterial species making their life conditions less favourable [19, 20].

Number of studies has shown that the root canal microflora has a different nature when primary and retreatment cases are compared. These differences are mainly due to ecological changes which take place

in the root canal before and during treatment procedures. In 1998 two studies showed that the microbial flora associated with endodontic post-treatment disease is quite different from that found in the primary endodontic cases [21, 22]. The latter typically consists of a polymicrobial mix with approximately equal proportions of Gram-positive and Gram-negative species, dominated by obligate anaerobes capable of fermenting amino acids and peptides [14]. In canals of teeth with post-treatment disease less species of microorganisms are found and predominance of Gram-positive microorganisms is evident [21, 22].

ENDODONTIC PATHOGENS IN PRIMARY ENDODONTIC CASES

According to the results of studies primary root canal infection is a dynamic process and bacterial species dominating at different stages of this process differ. In experimental studies ter Steeg and van der Hoeven showed that the most important factors driving this process are: availability of nutrition, oxygen level (redox potential) and the local pH within the root canal [23]. Facultatively anaerobic bacteria often found in root canals in primary root canal infection grow well in anaerobiosis. However, their primary energy source is carbohydrates. Obviously that a decrease in availability of carbohydrates in the root canal occurs when there is no direct communication with the oral cavity. This fact limits growth opportunities for facultative anaerobes. Endogenous proteins and glycoproteins are the main nutrients in the root canal system of primary endodontic cases. The main source of proteins in the root canal is a process of degradation of the small volume of pulpal tissue and influx of serum like exudates from periapical tissues into the canal due to inflammatory process. Bacterial metabolism of the serum-like fluid also causes reduction of the redox potential and a rise in the pH within the root canal [24].

Table. Characteristics used for microbial classification [15].

Characteristic	Examples
Cellular morphology	Shape, gram stain, flagella, spores, size
Colonial appearance	Pigment, hemolysis, shape
Carbohydrate fermentation	Acid or gas production
Amino acid hydrolysis	Ammonia production
Fermentation products	Butyrate, lactate, acetate
Preformed enzymes	Glycosidases
Antigens	Monoclonal/polyclonal antibodies
Lipids	Menquinones, long chain fatty acid
Enzyme profile	Malate dehydrogenase
Peptidoglycan	Amino acid composition
DNA	Base composition, base sequence homology

The species commonly recovered by culture from root canals of teeth with apical periodontitis have been previously reviewed in detail [25]. Implication of new technologies in microbiological studies had impact on understanding of the etiology of primary endodontic infection. Application of molecular methods for microbial detection has added several additional species as typical of the microbial flora of the infected root canals.

Talking about the primary endodontic cases black-pigmented bacteria (BPB) are the species which have frequently been isolated. Due to their proteolytic activity these microorganisms are also implicated in apical abscess formation [26]. Generally most often *Prevotella* and *Porphyromonas* species are discussed when talking about participation of BPB in pathogenesis of primary endodontic pathology.

Prevotella species such as *P. intermedia* and *P. nigrescens* were more often found in infected root canals. These two species have been cultured from 26–40% of root canals of teeth with apical periodontitis, although in one study they were detected in only 13% of infected root canals [27, 28]. It was shown that *P. nigrescens* is more common in endodontic infections than *P. intermedia* [27].

Example of sensitivity of methods used in identification of microorganism species in root canal is the detection of other BPB such as *Porphyromonas endodontalis* and *Porphyromonas gingivalis*. In culture studies they occur in frequencies lower than 10% [28]. In contrast, due to sensitivity of PCR method *P. endodontalis* and *P. gingivalis* were detected in 43% and 28% of samples from necrotic pulps respectively [27].

Without earlier mentioned BPB some species of microorganisms are strongly associated with primary endodontic cases. These are *Fusobacterium nucleatum*, *Veillonella parvula*, *Eubacterium* and other species. In root canal systems some of them are associated with other species which could be detected in the root canal. Numerous studies have shown the importance of a food chain in which the metabolism of one species found in the root canal supplies essential nutrients for the growth of other members of the population [29, 30]. One example of synergistic association between microbial species found in the root canal system could be strong association of *F. nucleatum* with *P. micros*, *P. endodontalis* and *Camylobacter rectus* [29, 30]. Strong positive associations were detected also between *Pr. intermedia* and *P. micros*, also between *P.*

anaerobius and the *Eubacteria* and *Peptostreptococcus anaerobius* [29].

In primary endodontic treatment cases, where direct communication with the oral cavity exists facultative anaerobic and aerobic microflora is dominating. Such teeth are characterized as more resistant to performed endodontic treatment procedures. E. Siren et al. has showed that root canals which were unsealed at some point during the treatment, harbored enteric bacteria more frequently than the canals with an adequate seal between the appointments [31]. Enteric bacteria were identified in 55% of cases when teeth were left open during the treatment, while in the group where only non-enteric bacteria were found 30% of the teeth had been open. Enteric bacteria were also more frequently isolated in cases with a high number of appointments before sampling. In the enteric bacteria group 35% of the samples were taken at the 10th visit or later [31]. *Enterococci* are more likely to survive chemomechanical instrumentation and root canal medication.

MICROFLORA OF ROOTFILLED TEETH

Generally it is accepted that persistence of disease in periapical tissues is most commonly associated with a difficulties or mishapes which occur during or after initial endodontic treatment. Inadequate aseptic control, poor access cavity design, missed canals, inadequate instrumentation and leaking temporary or permanent restorations are the main factors that may be critically important in post-treatment disease.

Based on numerous studies five main factors which may contribute to persistence of a periapical radiolucency after treatment have been detected [32, 33, 34]. These are:

1) intraradicular infection;
2) extraradicular infection;
3) foreign body reaction;
4) cysts;
5) healing via fibrous scar tissue.

It is generally believed that the major cause of post-treatment disease after root canal treatment is the persistence of microorganisms in the apical part of the root canal of rootfilled teeth. Some species of microorganisms found in such cases are capable to survive under harsh, nutrient-limited conditions of the rootfilled canal. Results of studies in which the microflora of teeth with persistence disease was studied showed a high prevalence of *enterococci* and *streptococci* followed by *lactobacilli, Actinomyces* species, *peptostreptococci*, *Candida*, *Eubacterium alactolyticus*, *Propionibacterium propionicum*, *Dialister pneumosintes* and *Filifactor alocis* [35].

Microbiological findings from filled root canals with persistent periapical disease have shown a high proportion of *enterococci*, ranging from 29% to 77% [36, 37, 38, 39]. Siren et al. showed larger number of *E. faecalis* in root canals of teeth which were left open between treatment sessions in order to give relief of symptoms [31]. Such findings showed that the idea of leaving root canals open in order to diminish symptoms is incorrect. Because it could change a simple case of primary endodontic infection into a more resistant type of infection which can withstand effect of intracanal medicaments. In untreared root canals *enterococci* constitute only around 5% or less of total microflora [40]. Such results raise the question of how and when *enterococci* invade the root canal system. It can be hypothesized that *E. faecalis* could be present in untreated canals, but in such low numbers that it is not recovered. Due to the changes in root canal environment this microorganism may grow to higher and recoverable proportions. Another explanation for the high prevalence of *E. faecalis* in root-filled canals associated with disease is that *E. faecalis* enters the canal in the process of treatment, during or between treatment procedures [41]. Numerous studies showed that *E. faecalis* has some special characteristics that allow them to survive in conditions that are commonly lethal for many other microorganisms. These properties include an ability to grow in high salt concentrations, a wide temperature range, tolerance a broad pH range, as well as persist in the presence of intracanal medicaments.

E. faecalis has some virulence factors which are already identified and could be the reason for survival of this microorganism in a very harsh environment of the root canal system: secreted factors, adhesins, surface structures such as capsular polysaccharide and antibiotic resistance determinant. *E. faecalis* has special capacities as endopathogen: to invade dentinal tubules and adhere to dentin surface [42]. Number of studies showed another extremely important caharacteristic of this microorganism: capacity to withstand a wide pH range up to around 11.5 of intracanal medicaments such as calcium hydroxide which is generally a highly potent antimicrobial dressing [42]. Recently the mechanism of alkaline tolerance of this microorganism was shown and it was associated with a functioning cell-wall-associated proton pump, which drives protons into the cell in order to acidify the cytoplasm [42]. It was believed that *E. faecalis* is the microorganism which can withstand high pH of intracanal dressings like calcium hydroxide and play a critical role for its involvement in persistent infection in endodontic retreatment cases.

A study from North America showed that in cases where calcium hydroxide was not used frequently as intracanal medicament, *E. faecalis* was found in similarly high proportions in comparison with cases where this medicament was used frequently [43]. Earlier popular explanation that calcium hydroxide was the reason for such high proportion of *E. faecalis* found in retreatment cases and even in monocultures was not supported. Another characteristic of *enterococci* is an ability to survive even in the environment of low nutrient supply. This property was explored in a series of long-term starvation assays. There is a lot of information concerning availability and composition of nutrients in the apical region. Studies have shown that it might be serum-derivered fluid from periapical tissues which can sustain the microbial flora [44]. It was shown that cells of *E. faecalis* is capable of recovery upon addition of serum from periapical tissues [44].

Another microorganism which has been periodically identified in teeth with persistent post-treatment apical periodontitis is *Candida albicans* [37, 38, 45]. It is obvious that yeasts are rare inhabitants of untreated root canals, unless these canals have been open to the oral cavity. Some characteristics of yeasts are common with *enterococci*. One of them is that both these microorganisms can survive as a monoinfection and even invade dentinal tubules [45]. Studies have shown that sodium hypochlorite one of the most popular intracanal medicament, is a potent killing agent for *Candida* species while to the antimicrobial action of calcium hydroxide they are resistant. Both microorganisms *candida* and *enterococci* share several properties necessary to establish and survive in the harsh environment of the rootfilled canal. These properties include resistance to various antimicrobial agents, an ability to grow in monoinfections and survival in limitation of nutrients supply.

Actinomyces species belong to the primary colonizers of clean tooth surfaces and are relatively frequent isolates in endodontic infections. *Actinomyces* is also a well known pathogen found in therapy-resistant retreatment cases. The fimbriae on the cell surface of these microorganisms are important for its virulence and its establishment in extra-radicular endodontic infections (apical actinomycosis). This can be due to the possibility of this microorganism to migrate from periapical tissues to the root canal system. But the question how this microorganism invades periapical tissues is still controversial. It may be associated with the incorrect root canal debridement procedures or lack of asepsis during endodontic treatment procedures.

On species level *A. israelii* and *A. meyerii* are microorganisms which are more frequently found in treatment resistant cases and involved in periapical actinomycosis [33]. Recently a new species *A. radicidentis* has been identified in pure cultures from root canals of teeth with persistent periapical lesion [46]. *Propionibacterium propionicum* is a facultative anaerobic organism formerly known as *Arachnia propionica*. This bacterium is a normal resident of the oral cavity and has been repeatedly found in persisting intraradicular and extra-radicular endodontic infections that do not respond to conventional endodontic treatment. Although its pathogenic capacity still remains unclear, it seems that *P. propionicum* shares similar invasive characteristics as *actinomyces* [33].

CONCLUSIONS

It is evident that in primary endodontic cases root canal environment provides better nutritional supply rich with peptides and amino acids for bacterial inhabitants of root canal system. This favor growth of anaerobic proteolytic species. Whilst in the well-filled root canal most or all of the necrotic pulp tissue remnants are eliminated. In such conditions microbes experiences a static environment and starvation, until a serum-like fluid transudate from the periapical tissue will reach the regions of root canal invaded by microorganisms cells. Retreatment procedures are less predictable than treatment of primary endodontic cases. This is due to the specific more resistant species of microorganisms which can survive not only during antimicrobial treatment, but also rootfilling procedure. It is essential that further information is needed to get a thorough knowledge about residual post-treatment root infection and post-treatment apical periodontitis in order to improve quality of root canal debridement procedures.

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