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REVIEW ARTICLE

Endodontic Microflora- A Review

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ABSTRACT

It is essential that clinicians have a thorough understanding of the interplay between root canal microflora and the endodontic disease processes in order to develop an effective rationale for treatment. Newer information on endodontic micro flora improves our ability to effectively debride and disinfect the root canal system and manage infections of endodontic origin. New molecular based techniques have been introduced thus bringing about a massive change in procedures for microbial identification. This knowledge can be put into practical use resulting in improvements in the quality of treatment offered to our patients. This review focuses on the microflora in primary and root-filled canals of deciduous and permanent teeth.

Keywords: Endodontics, Root canal microflora, Primary infection, Root filled microflora

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INTRODUCTION

Endodontics has become an increasingly routine aspect of patient care in general dental practice. The study of microbes infecting root canals and periradicular tissues has greatly increased thereby increasing our knowledge and understanding of reasons for endodontic failures. Newer information on endodontic micro flora improves our ability to effectively debride and disinfect the root canal system and manage infections of endodontic origin. Molecular methods and other research techniques have helped detect and identify many previously unknown endodontic pathogens.

Primary infected root canals are untreated canals, into which the microorganisms have gained access to colonize the pulpal tissue (1). When failure of endodontic treatment due to persistence of bacterial infection in the root canal system occurs, it is called as secondary infection (2,3). Necrotic pulpal tissue investigated in primary root canal infections has commonly exhibited the presence of polymicrobial flora with an average of 4-7 intra-canal species, which are often Gram-negative anaerobic (4-9). Obligate anaerobic bacteria are found to be the dominant species in several studies in root canal infections, which comprise 90% of all bacterial species. However, facultative bacteria such as, Enterococci, are more likely to survive chemo-mechanical instrumentation and root canal medication due to their ability to survive with or without oxygen in the environment.

MILESTONES IN DISCOVERY OF ROOT CANAL MICROFLORA

- The Chinese believed that a white worm with a black head lived in the tooth and it caused abscesses. The worm theory, which was followed until the middle of 18th century, prevented the pursuit of a bacterial cause for pulpal disease (10).
- Van Leeuwenhoek investigated the root canal of a badly carious tooth and described the presence of microorganisms in the 17th century, However his findings were corroborated by W.D. Miller at the end of 19th century (11).
- In 1894, Miller conducted bacteriological investigation of root canal infection and published his findings. This was followed by systematic culturing of root canals and in the 1930's; microbiological techniques were used to determine the biological basis of endodontic methods (12).

- In the 1960's, with the development of anaerobic culturing, many unknown microorganisms were identified in the root canal and it came to be believed that endodontic microbiota were predominantly anaerobes (13,4).
- 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).

With the advent of modern molecular assays, there has been a manifold increase in the identification of new species and species which are difficult to culture.

MICROBIAL INVASION OF ROOT CANALS

The dentine-pulp complex is normally protected from the microbial invasion by the intact enamel and cementum. The invasion of bacteria occurs when there is a break in the integrity of the overlying enamel and dentine by caries, trauma, contamination of the pulp during dental treatment, seepage of saliva through cracks or inadequate coronal restorations (15). In cases of trauma not including pulpal exposure, the dentinal tubules serve as portal of entry. With the establishment of infection, the microbial flora changes from an initially predominant facultative gram-positive flora to a completely anaerobic gram-negative flora when the canals have been infected for three months or more (14).

The number of isolated species has been usually found to be usually low in the initial phase of a root canal infection. In cases where pulpal involvement occurs due to carious lesion, the bacterial species are similar to those found in the carious lesion. In cases where there is no apparent communication with the oral cavity and the bacteria penetrate through dentinal tubules, as in trauma cases without pulp exposure, there is no clear pattern of primary bacterial invaders (4,5). A correlation seems to exist between the size of the periapical lesion and the number of bacterial species and cells in the root canal. A higher density of bacteria has been observed in root canals of teeth with long-standing infections and large lesions than teeth with small lesions.

Microbial Colonization And Survival

There are several ecological factors that determine the survival of root canal microflora. The development of specific proportions of the anaerobic microflora is supported by the selective habitat of the endodontic environment. The most important factors driving this process are:

- Availability of nutrition
- Oxygen level
- Microbial symbiosis
- The local pH within the root canal

Nutrients may be derived from the oral cavity, degenerating connective tissue, dentinal tubule contents, or a serum-like fluid from periapical tissue. Establishment of microbial growth depends on the type and availability of nutrients. These factors in the root canal environment permit the growth of anaerobic bacteria capable of fermenting amino acids and peptides, whereas bacteria that primarily obtain energy by fermenting carbohydrates may be restricted by lack of available nutrients. The growth of anaerobic bacteria capable of fermenting amino acids and peptides are encouraged by endogenous proteins and glycoproteins that are the principal nutrients in the main body of the root canal system. Exogenous nutrients, such as fermentable carbohydrates, affect the microbial ecology of the coronal part of an exposed root canal by promoting growth of species that primarily obtain energy by carbohydrate fermentation. This is the likely reason why the flora is dominated by facultative anaerobic bacteria, such as Streptococci, in the coronal section of root canals exposed to the oral cavity, and anaerobic bacteria dominate in the apical section (6-9).

Oxygen and oxygen products play important role as ecological determinants in the development of specific proportions of the root canal microflora (7-9). The consumption of oxygen and production of carbon dioxide and hydrogen along with the development of a low reduction- oxidation potential by the pioneer species favor the growth of anaerobic bacteria. The succession of strict over facultative anaerobes with time is most likely due to changes in available nutrition, as well as a decrease in oxygen availability (6). Facultative anaerobic bacteria dominated by Streptococci grow well in anaerobiosis, however their prime energy source is carbohydrates.

Growth of mixed bacterial populations may depend on a food chain in which the metabolism of one species supplies essential nutrients for the growth of other members of the population (16-20). Black-pigmented anaerobic rods (Prevotella and Porphyromonas species) are examples of bacteria that have very specific nutritional requirements. They are dependent on vitamin K and hemin for growth. Other bacteria can produce Vitamin K (21). Hemin becomes available when haemoglobin is broken down, but some bacteria may also produce hemin. A wide range of nutritional interactions is recognized among oral bacteria and these may also influence the associations between bacteria in the root canal (22-24). After degradation of pulp tissue, a sustainable source of proteins develops because bacteria induce periapical inflammation that leads to an influx of a serum-like exudate into the canal. This fluid contains proteins and glycoproteins, and the bacteria that dominate this stage of the infection are likely to be those that either have a proteolytic capacity, or maintain a cooperative synergy with those that can utilize this substrate for bacterial metabolism. Initially, there may be no clear associations between bacteria, but strong positive associations develop among a restricted group of the oral flora

due to the type of nutrients in the environment (25). Thus, F. nucleatum is associated with P. micros, P. endodontalis and C. rectus. Strong positive associations exist between P. intermedia and P. micros and P. anaerobius. There is also a positive association between P. intermedia, and P. micros, P. anaerobius and the Eubacteria. In general, species of Eubacteria, Prevotella and Peptostreptococcus are positively associated with one another in endodontic samples. Properties that these bacteria have in common are that they ferment peptides and amino acids and are anaerobic, which indicates that the main source of nutrition in root canals is tissue remnants and a serum-like substrate (5).

The interrelationships between microbes in the disease process have been positively established by the studies of Fabricius *et al* on monkeys (6). In the studies of Fabricius *et al*, bacterial isolates from the root canal of a monkey were inoculated as a separate or combined strain into the root canals of other monkeys. The study revealed that the separate strains produced only a small lesion and mild periapical reaction in comparison to the combined strains. Similar experiments involving P.Oralis revealed that it did not survive as a single isolate. However, the presence of other bacteria seemed to favor its survival and dominance within the root canal. Enterococcus faecalis and Streptococcus milleri were also found to induce weak periapical reactions when inoculated as separate strains, although they could survive in the root canal as combined isolates. The synergistic mechanisms between the various endodontic pathogens involve interplay of various factors like:

- Providing nutrition
- Inhibition of phagocytosis (i.e. preventing opsonisation and inflammation, destruction of phagocyte)
- Secretion of growth factors and enzymes
- Decrease in the local oxygen concentration and
- Oxidation–reduction potential and local pH in the root canal.

These mechanisms facilitate the survival and pathogenesis of obligate and facultative anaerobes.

MICROFLORA OF PRIMARY ENDODONTIC CASES

Primary root canal infection is a dynamic process and bacterial species dominating at different stages of this process differ. Sundqvist found that primary root canal infections are polymicrobial, typically dominated by obligatory anaerobic bacteria (5). It has been recently shown that primary infection is characterised by a mixed consortium composed of 10 to 30 species per canal (18-20). The bacterial load varies from 103 to 108 cells per canal (26-28). The most frequently detected culturable species in primary infection belong to the Gram-negative genera Porphyromonas, Prevotella, Fusobacterium, Tannerella, Dialister, Campylobacter and Treponema. Gram-positive anaerobes from the genera Peptostreptococcus, Parvimonas, Eubacterium, Filifactor, Actinomyces, Propionibacterium and Pseudoramibacter, as well as facultative or microaerophilic Streptococci can also be commonly found in primary infection. Black-pigmented bacteria (BPB) are the species that have frequently been isolated. Due to their proteolytic activity these microorganisms are also implicated in apical abscess formation (4). They are closely associated with clinical symptoms like pain, tenderness on percussion and swelling. 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 (29-31). It was shown that P. nigrescens is more common in endodontic infections than P. intermedia (30). 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% (31). 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 (30).

The other predominantly isolated bacteria in the root canal, namely Fusobacterium nucleatum, comprise three subspecies – nucleatum, polymorphum and vincentii. The majority of the Streptococcus species included S. anginosus and S. mitis. Peptostreptococcus micros and P. anaerobius comprised of 1/3 of the samples in the study by Sundqvist, while 34 % of the root canals comprised of black-pigmented anaerobic rods, mainly Prevotella intermedia, and 6 % constituted P. loescheii and P. denticola. Porphyromonas endodontalis was more predominant than P. gingivalis among the black-pigmented asaccharolytic species. Eubacterium alactolyticum and E. lentum were isolated in 1/3 of the root canals. Actinomyces species comprised 15 % of the microflora and the dominant species was A. israelii (11%). Similar results were reported in other studies examining teeth with necrotic pulps. Actinomyces israelii has been found to be commonly associated with root canal failures. Gram-negative asaccaharolytic rods were identified as Fusobacterium and Bacteroides when butyric acid and acetic acid or succinic acid, respectively, was formed during fermentation (5).

Numerous other anaerobic species that have been isolated are Porphyromonas asaccaharolytica, Prevotella melaninogenica, P. bivia, P. oulora, Tissierella praeacuta, Bacteroides fragilis, Bifidobacterium adolescentis, Clostridium clostridiforme, Peptostreptococcus productus, P. parvulus, P. asaccharolyticus, P. magnus, Eubacterium tenue, E. combesi, E. saburreum, E. limosum,

E. aerofaciens, Fusobacterium varium, F. mortiferum, F. naviforme, Lactobacillus cellobiosus, L. casei subspecies rhamnosus, L. crispatus, L. fermentum, L. plantarum, and Mitsoukella dentalis . Enterobacter agglomerans, Staphylococcus epidermidis, S. aureus, and Bacillus, Acinetobacter, and Corynebacterium species and spirochetes have also been found in the canals (5).

PCR has enabled the identification of previously difficult to culture species like Dialister pneumosintes (66%) and Filifactor alocis in (46%) root canals of teeth with apical periodontitis. Thus the endodontic flora has predominantly gram-negative anaerobes but gram-positive facultatives are also seen. However, aerobic bacteria like Pseudomonas aeruginosa are found only when they enter the canal during the treatment (32). Enterococcus and streptococcus have been found in teeth with endodontic failure (32), whereas Actinomyces species predominate in teeth with persistent periapical lesions (33). Candida has been detected mostly in teeth with persistent apical periodontitis (34,35) while spirochetes are associated with endodontic abscesses (36). Propionibacterium propionicum are also related to persistent root canal infection (37).

In most cases, failure of root-canal treatment occurs when treatment procedures have not met a satisfactory standard for control and elimination of infection (38, 39). Persisting bacteria in root canals may be those originally present in the necrotic pulps that survive the biomechanical procedures, which may be located in missed canals or uninstrumented areas of the canals (40, 41). Conversely, bacteria may originate from the oral cavity, contaminate the root canal during treatment owing to inadequate aseptic control or invade the root canal via coronal leakage after root-canal treatment (42,43). Although it has been reported that non-microbial factors may be implicated in root-canal treatment failure, the literature suggests that persistent intraradicular or secondary infections are the major causes of the failure of root-canal treatment (29).

MICROFLORA FROM ROOT FILLED TEETH

Recent studies using advanced microbiological techniques for anaerobic species (34,35) have revealed the composition of root-canal microbiota after failed treatment differs from that normally found in untreated teeth. There is some diversity in species isolated from root filled teeth with persistent periapical disease, but there is a consensus amongst most studies that there is a high prevalence of Enterococci and Streptococci. (34,35, 44,45) Other species found in higher proportions in individual studies are lactobacilli, Actinomyces species and Peptostreptococci and Pseudoramibacter alactolyticus, Propionibacterium propionicum, Dialister pneumosintes, and Filifactor alocis (34, 46-55) and Candida albicans (27, 34, 35, 45, 56-59) As-yet-uncultivated phylotypes correspond to 55% of the taxa detected in treated canals (55).

Siren *et al*. showed larger number of E. faecalis in root canals of teeth that were left open between treatment sessions in order to give relief of symptoms (47). 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 that can withstand effect of intracanal medicaments. Enterococcus faecalis was the most commonly isolated species from root canals of teeth with failed endodontic treatment (30, 38). 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 of a broad pH range, as well as persist in the presence of intracanal medicaments. E.faecalis has special capacities as endopathogens: to invade dentinal tubules and adhere to dentin surface (48). Number of studies showed another extremely important characteristic 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 (48). 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 (48) Culture-dependent and culture-independent methods have revealed that E. faecalis is the species most frequently found in root-canal-treated teeth, with prevalence values reaching up to 90% of the cases (34, 35, 49-53). Root-canal-treated teeth are about nine times more likely to harbor E. faecalis than cases of primary infection. This suggests that the bleak environmental conditions within filled root canals do not prevent its survival. Taken together, all these properties help explain the significantly high prevalence of E. faecalis in root canal-treated teeth.

Fungi are only occasionally found in primary infection, but Candida species have been detected in root-canal-treated teeth in up to 18% of the cases (34, 35, 50, 51 56). Fungi gain access to root canals via contamination during endodontic therapy or they overgrow after inefficient intracanal antimicrobial procedures that cause an imbalance in the primary endodontic microbiota. Candida albicans is by far the most commonly detected fungal species in rootcanal- treated teeth. This species has several properties that can be involved in persistence following treatment, including ability to colonise and invade (57-59) and resistance to calcium hydroxide (60,61).

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 extraradicular endodontic infections. 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. Propionibacterium propionicum, a facultative anaerobic organism 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 (62).

MICROFLORA OF DECIDUOUS TEETH

Hu *et al*. (63) isolated 240 strains of bacteria from 22 infected deciduous root canals. Among 240 strains, 200 strains were obligate anaerobes, belonging to genera Peptostreptococcus, Bacteroides, Veillonella, Eubacterium, Propionibacterium, Actinomyces and Fusobacterium. Bacteroides & Fusobacterium especially P. gingivalis and F. nucleatum probably were related to acute periapical inflammation & Veillonella parvula from chronic periapical inflammation of deciduous teeth. Siqueira *et al* (64) examined the patterns of microbial colonization in primary root canal infections. The root canal microbiota consisted of cocci and/ or rods, often forming mixed communities. Different forms of rods could be found such as filaments, straight rods, curved rods and coccobacilli. Spiral bacterial cells were occasionally observed as single cells or as small clusters between other bacterial forms. Cocci were the predominant bacteria. Sato *et al*. (65) also reported a greater percentage of anaerobic than aerobic bacteria in primary teeth with necrotic pulp and periapical lesions indicated for extraction. Gram-negative aerobic rods, frequently found in periodontal pockets, were present in only 3 cases (15%). These outcomes are in agreement with those of Cohen *et al*. (66), who found these pathogens in 17% of primary teeth with necrotic pulp.

CONCLUSION

Our knowledge and understanding of the microbes infecting root canals and periradicular tissues has greatly increased. This information improves our ability to effectively debride and disinfect the root canal system and manage infections of endodontic origin. Success of any endodontic treatment is dependent on the effective elimination or maximal reduction of the involved microbiota. Modern day advances in laboratory procedures for identification of endodontic microflora, new therapeutic techniques and a clear understanding of the initiation and progression of the disease process will definitely take us a step closer to the goal of complete microbial elimination for successful root canal treatment.

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