Endodontic Microbiology: Review of Literature

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Abstract
Since 1890, when Miller first observed microorganisms associated with pulp tissue, microorganisms have been implicated in infections of endodontic origin. Microbes seeking to establish in the root canal must leave the nutritionally rich and diverse environment of the oral cavity, breach enamel, invade dentine, overwhelm the immune response of the pulp and settle in the remaining necrotic tissue within the root canal. During that time they have to compete in a limited space with other microbes for the available nutrition. It is no accident that microbes berth in a particular environment there are ecological advantages for them to establish and flourish if conditions are favorable. This review will highlight the recent facts and controversies related to endodontics microbiology.

Introduction:
Microorganisms were observed in samples from teeth by Leeuwenhoek soon after he invented the microscope in 1684. Since Babylonian times, it was believed that a ‘tooth worm’ lived in the hollow portion of the tooth and caused decay. Leeuwenhoek challenged the ‘tooth worm’ theory of decay by identifying worm-infested cheese that he thought may be the source of disease (Cruse & Bellizzi 1980). Leeuwenhoek also described microorganisms that he scraped from teeth as ‘cavorting beasties’. However, it took over 200 years before his observation was confirmed and a cause and effect relationship was suggested by Miller (Henderson & Wilson 1998). Since
1890, when Miller first observed microorganisms associated with pulp tissue, microorganisms have been implicated in infections of endodontic origin.

Life is not easy for an endodontic pathogen. Microbes seeking to establish in the root canal must leave the nutritionally rich and diverse environment of the oral cavity, breach enamel, invade dentine, overwhelm the immune response of the pulp and settle in the remaining necrotic tissue within the root canal. During that time they have to compete in a limited space with other microbes for the available nutrition. It is no accident that microbes berth in a particular environment there are ecological advantages for them to establish and flourish if conditions are favorable. Through genetic exchange and mutation, microbes have developed specialized systems that facilitate their ability to find, compete and survive in these very specific environments (Sundqvist & Figdor 2003).

**Microbial invasion (routes of micro-organisms ingress):**

One of the primary functions of tooth enamel is to exclude these microorganisms from the underlying dentine–pulp complex. As long as the enamel and cementum layers are intact, the pulp and root canal are protected from invasion, but loss of these structures by caries, cracks or trauma opens an avenue for penetration of bacteria through the dentinal tubules (Sundqvist 1994, Siqueira & Janeiro 2002).

Dental caries is the most common cause of pulp injury. Most authors believe that acid-producing bacteria invade the dentinal tubules and demineralize the tubule walls. Thereafter proteolytic bacteria follow, acting on the organic matrix, which is denuded in the enlarged dentinal tubules. The bacteria in the front of the carious process are the first to reach the pulp. Gram-positive bacteria predominate among the advancing bacteria in a carious process. Some investigators have found exclusively lactobacilli whereas others also report streptococci (S. mitis, S. milleri; now termed S. oralis and S. anginosus), propionobacteria, Actinomyces, and some obligatory anaerobic gram-negative and gram positive nonsporulating rods. Most of the bacteria in the carious process are non-motile. Therefore bacterial penetration in the dentinal tubules is slow; the acids and other metabolites and toxic products produced by the bacteria diffuse faster. A reaction of the pulp occurs only a few hours after experimental application of bacterial products into dentin cavities (Samaranayake 2002).

In addition to caries, pathways for the entry of microorganisms into the pulp space include direct pulp exposure (e.g. trauma, and dental procedures), dentinal tubules, lateral/accessory/furcation canals, and anachoresis (Sundqvist 1994, Siqueira & Janeiro 2002).

**Intraradicular microbiology:**

Once the pulp is necrotic and the odontoblastic processes undergo autolysis, patent dentinal tubules (dead tracts) are traversed by microorganisms and infect the root canal system. When the pulpal tissue becomes necrotic, it loses its blood supply and the root canal system becomes a reservoir for microorganisms and their by-products. Because of the lack of circulation within the necrotic pulp, the root canal system
becomes a sanctuary for microorganisms from the immune system (Sundqvist 1994).

The root canal flora of teeth with clinically intact crowns, but having necrotic pulps and diseased periapices, is dominated (> 90%) by obligate anaerobes, usually belonging to the genera *Fusobacterium*, *Porphyromonas* (formerly *Bacteroides*), *Prevotella* (formerly *Bacteroides*), *Eubacterium*, and *Peptostreptococcus* (Sundqvist et al. 1989). In contrast, the microbial composition even in the apical third of the root canal of periapically affected teeth with pulp canals exposed to the oral cavity is not only different from root canal flora of teeth with intact crowns but also less dominated (< 70%) by strict anaerobes (Baumgartner & Falkler 1991).

All bacteria within the oral cavity share the same opportunities for invading the root canal space; however only a restricted group of species have been identified in infected root canals. The reason for the disproportionate ratio between potential and actual number of species is that the root canal is a unique environment where biological selection drives the type and course of infection. An anaerobic milieu, interactions between microbial factors and the availability of nutrition are principal elements that define the composition of the microbial flora (Sundqvist & Figdor 2003).

**Anaerobic milieu and microbial interactions:**
In 1894, WD Miller published his findings on the bacteriological investigation of pulps. He observed many different microorganisms in the infected pulp space and realized that some were uncultivable when compared with the full range observed by microscopy, and that the flora was different in the coronal, middle and apical parts of the canal system. Due to limitations of his sampling and cultivation technique, Miller was unable to verify this observation and it was not until 1982 that this could be shown by culturing. Differences in availability of nutrients and oxygen tension in the apical region compared with the main root canal are important reasons for the dominance of slow growing, obligately anaerobic bacteria in the apical region (Sundqvist & Figdor 2003, Sundqvist 1994, Wittgow Jr & Sabiston Jr 1975).

Studies on the dynamics of root canal infections have shown that the relative proportions of anaerobic microorganisms and bacterial cells increase with time and that the facultatively anaerobic bacteria are outnumbered when the canals have been infected for 3 months or more. When a combination of bacterial strains originally isolated from an infected root canal were inoculated in equal quantities into further canals in experimental infections, the original proportion of bacterial strains was reproduced and anaerobic bacteria dominated again. This illustrates that interactive mechanisms operate amongst these microorganisms, a concept further supported by the finding that when *Prevotella oralis* (formerly *Bacteroides oralis*) was inoculated on its own it was unable to survive, whereas when inoculated with other bacteria it survived and dominated the established flora. These experiments have shown that the endodontic milieu is a selective habitat that supports the
development of specific proportions of the anaerobic microflora (Sundqvist & Figdor 2003, Sundqvist 1994). Oxygen and oxygen products play an important role as ecological determinants in the development of specific proportions of the root canal microflora. The consumption of oxygen and production of carbon dioxide and hydrogen along with the development of a low reduction–oxidation potential by the early colonizers favor the growth of anaerobic bacteria (Loesche et al. 1983).

**Nutrition as an ecological driver:**
The type and availability of nutrients is important in establishing microbial growth. Nutrients may be derived from the oral cavity, degenerating connective tissue (Nair 2004), dentinal tubule contents, or a serum-like fluid from periapical tissue (Rocas et al. 2004). 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. This is the likely reason why the flora is dominated by facultatively 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. 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. Facultatively anaerobic bacteria grow well in anaerobiosis; however, their prime energy source is carbohydrates. A decrease in availability of carbohydrates in the root canal occurs when there is no direct communication with the oral cavity, which severely limits growth opportunities for facultative anaerobes (Sundqvist 1994).

The experiments of ter Steeg and van der Hoeven offer important clues about the likely dynamics of the root canal flora. Using serum as a substrate, they studied the succession of subgingival plaque organisms during enrichment growth. Three phases can be distinguished during growth (Sundqvist & Figdor 2003, Sundqvist 1994):

1. **Initially**, rapidly growing saccharolytic bacteria consume the low levels of carbohydrates in serum, leading to lactic and formic acid production.
2. **In a second phase**, proteins are hydrolyzed, some amino acid fermentation takes place, and there is digestion of remaining carbohydrates. Carbohydrates are split off from the serum glycoproteins. Growth during this phase are dominated by *Prevotella intermedia, Veillonella parvula, Fusobacterium nucleatum* and *Eubacterium species*.
3. **In a final phase**, there is progressive protein degradation. The predominant species during this phase are *Peptostreptococcus micros, F. nucleatum, and eubacteria*. The dominance of *P. micros* in cultures originating from subgingival microbiota, when grown in serum, has also been shown in another study. The ecological niche of *P. micros* may be related to its wide range of peptidase activities, making amino acids and peptides available from serum glycoproteins. These amino acids can be used by *P. micros*, but also by other bacteria that have little or no proteolytic activity in serum.
The black-pigmented anaerobic rods, *Prevotella intermedia/nigrescens*, *Porphyromonas gingivalis* and *Porphyromonas endodontalis*, are proficient in degrading serum proteins and make peptides and amino acids available for fermentation. The degradation of native proteins by *Prevotella and Porphyromonas* species enables the growth of bacteria that depend on the availability of peptides, such as *eubacteria, fusobacteria and peptostreptococci*, which produce peptidases but cannot hydrolyze intact proteins. This is also of importance for the capacity of root canal bacteria to induce periapical abscesses (Sundqvist & Figdor 2003, Jansen et al. 1996). Combinations of *P. micros* with *P. intermedia* or *P. endodontalis* have been implicated in the induction of periapical abscesses. Abscesses harboring a microflora that rapidly degrade serum proteins have been shown to be nearly three times larger than abscesses with a microflora that lack the capacity for breakdown of serum proteins (Siqueira et al. 2001).

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. 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 (Jansen & van der Hoeven 1997). Vitamin K can be produced by other bacteria. Hemin becomes available when hemoglobin is broken down, but some bacteria may also produce hemin. Another example is *Campylobacter rectus* which can stimulate the growth of *Porphyromonas species* by producing a growth factor related to hemin. *C. rectus* itself derives a source of energy from the co-inhabiting microbial species. It is strictly dependent on a respiratory mechanism in which only formate and hydrogen can serve as electron donors and fumarate, nitrate, or oxygen as electron acceptors. This makes this organism dependent on bacteria producing formate or hydrogen. A wide range of nutritional interactions is recognized among oral bacteria and these may also influence the associations between bacteria in the root canal (Sundqvist & Figdor 2003).

Because the nutritional supply governs the dynamics of the microbial flora, it means that the bacteria present in the root canal will depend on the stage of the infection. 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 (Lana et al. 2001). 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 (Sundqvist 1994).
**Extraradicular microbiology:**

The development of periradicular lesions creates a barrier with in the body to prevent further spread of microorganisms. Bone tissue is resorbed and substituted by a granulation tissue containing defense elements, such as cells (phagocytes) and molecules (antibodies and complement molecules). A dense wall composed of polymorphonuclear leucocytes, or less frequently an epithelial plug, is usually present at the apical foramen, blocking the egress of microorganisms into the periradicular tissues. Very few endodontopathogens can advance through such barriers. However, microbial products can diffuse through these defence barriers and are able to induce or perpetuate periradicular pathosis (Vigil et al. 1997).

Ever since Miller demonstrated the presence of bacteria in diseased dental pulp, microorganisms have been suspected to play a causative role in periapical periodontis. As a result, voluminous literature exists to collaborate or disprove the presence of bacteria in apical periodontic lesion (Vigil et al. 1997).

Over years there has been conflicting reports regarding the presence and role of microorganisms in periapical lesions. Many histobacterialogic studies have been conducted on periapical lesions removed in toto, with complete connective tissue encapsulation and firm attachment. Stewart in 1947 and later supported by Hedman in 1951 proposed that bacteria were present in periapical lesions (Stewart 1947). Hedman’s study which was accomplished prior to present techniques for culturing anaerobic bacteria, bacteria were reported in 68% of 82 periapical lesions (Hedman 1951). Winkler et al using a modified gram stain for periapical tissue, were able to demonstrate the presence of bacteria in 87% or 13 out of 15 cases examined under a light microscope (Winkler et al. 1972).

Many investigators have challenged the concept of bacteria in periapical region. In a good number of studies, it was not possible to demonstrate the presence of bacteria in periapical lesions. Based on classic histology (Harndt 1926), many investigators therefore believe that ‘solid granuloma’ may not harbor infectious agents within the inflamed periapical tissue, but that micro-organisms are consistently present in the periapical tissue of cases with clinical signs of exacerbation, abscesses, and draining sinuses (Nair 2004). Quoting Kronfeld, Grossman said that “a tooth with a granuloma may have infected root canal, but a sterile periapical tissue in gram stained sections through infected pulpless teeth in situ that were examined; bacteria in abundance were always found within the root canal but granulation tissue and cysts attached to the apices of teeth were often free from microorganisms” and that “granuloma is not an area in which bacteria live, but in which they are destroyed” (Kronfeld 1974). Shindell reported 60 out of 63 specimens were negative for periapical region of endodontically treated tooth (Shindell 1961). In a histopathological and histobacteralogic study of 35 periapical endodontic surgical specimens, it was observed that although bacteria were identified in five specimens; in only one case were the bacteria located in the disintegrating tissue of root canal and periapical tissue (Laaangeland et al. 1977). Many recent investigations using electron microscopy supports granulomas being bacteria-free (Oguntebi et al. 1982).
One of the challenging aspects for determining bacteriological status in periapical tissue is microbial contamination of periapical samples. Microbial contamination of periapical samples is sometimes viewed as happening from the oral cavity and other extraneous sources. Many researchers believe that even if such ‘extraneous contaminations’ are avoided, contamination of periapical tissue samples with microbes from the infected root canal remains a problem. This is because microorganisms generally live at the apical foramen of teeth affected in both primary and post-treatment apical periodontitis. Here, microbes can be easily dislodged during surgery and the sampling procedures. Tissue samples contaminated with intraradicular microbes may be therefore reported as positive for the presence of an extraradicular infection (Nair 2004).

In general, there is a consensus of opinion among most of the researchers that chronic periapical lesion may harbor microorganisms if it is infected or it is an abscessed lesion (Cohen & Burns 2002). The periapical abscess is dominated (> 90%) by obligate anaerobes, usually belonging to the genera Bacteroides group, Peptostreptococcus spp., Peptococcus spp., and Fusobacterium spp (Goumas et al. 1997; Lewis et al. 1988; Siqueira Jr. et al. 2001). Lewis et al in their study on dentoalveolar abscess reported that anaerobic gram-negative bacilli are major pathogens in acute dentoalveolar abscesses (Lewis et al. 1988). Among these organisms there is high prevalence of P. endodontalis and P. Gingivalis(Siqueira Jr. et al. 2001) Studies have shown that suggests that P. endodontalis and P. gingivalis play an important role in the pathogenesis of periradicular diseases (Dymock et al. 1996).

Resurgence of concept of extraradicular microbiology:

In recent years there is resurgence of the idea of extraradicular microbes in apical periodontitis lesions with implied, controversial suggestion that extraradicular infection is the cause of many failed endodontic treatment (Vigil et al. 1997). Tronstad et al investigated the presence of periapical microbial flora of eight cases which had not healed with non surgical endodontic treatment. In all eight cases bacterial growth was evident. Three samples from each case were cultured and Tronstad et al. stated that their study clearly showed that anaerobic bacteria are able to survive in periapical tissue (Tronstrad et al. 1987). Haapasalo et al treated a case in which non surgical endodontics, calcium hydroxide, systemic erythromycin, and finally, a regimen of systemic metronidazole failed to resolve the draining fistula associated with a maxillary lateral incisor. Following periapical surgery, the lesion resolved the lesion. A mixed infection of anaerobic and facultative anaerobic microorganism was cultured from the root canal and the periapical lesion (Haapasalo et al. 1987). Presence of several species of bacteria have been reported at extraradicular sites of lesions described as “symptomatic periapical inflammatory lesion......refractory to endodontic treatment,” with the declaration that “.....these findings clearly end the erra of sterile periapical granuloma”( Cohen & Burns 2002). Iwu et al studied 16 periapical granuloma that were collected “during normal periapical curettage, apexectomy, or retrograde filling”. It was seen that most of the organism cultured were Veillonella species, Streptococcus milleri, Streptococcus sanguis, Actinomycetes
naeclundii, Propionibacterium acnes and Bacteroides species (Iwu & Wallace 1990). In majority of the recent studies on bacteriological and histological evaluation of periapical lesions, commonly isolated organisms were Staphylococcus epidermidis, Fusobacterium species, Propionibacterium acnes, Peptostreptococcus micococcus and Bacteriodes gracilis. Among these organisms, gram-negative bacteria like Propionibacterium acnes and Bacteriodes gracilis were the most commonly isolated ones (Wayman et al. 1992; Kiryu et al. 1994; Abou-Rass & Bogen 1998).

Black-pigmented bacteria in periapical lesions:
Black pigmented anaerobic gram negative bacteria are part of normal microbiota at various sites of human body and are often isolated from mixed infection sites. They belong to the family Bacteroides and are included in the genera Prevotella and Porphyromonas (Kiryu et al.1994; Trowbridge & Stevens 1992).

In majority of studies on bacteriological studies on periapical lesions, it has been observed that Porphyromonas and Prevotella had a higher prevalence in periradicular lesions associated with pain, purulent abscess and teeth refractory to conventional root canal treatment (Kiryu et al. 1994; Trowbridge & Stevens 1992; Bogen & Slots 1999; Yamasaki et al. 1998). Moreover, some black pigmented anaerobic rods are major pathogen in destructive periodontal diseases. It has been therefore proposed that these organisms may play an important role in the pathogenesis of periradicular diseases(Bogen & Slots 1999).

Enterococcus Faecalis in periapical lesions:
Enterococcus Faecalis is a normal human commensals adapted to the nutrient-enriched, oxygen-depleted, ecologically complex environments of the oral cavity, gastrointestinal tract, and vaginal vault. It is a nonspore-forming, fermentative, facultatively anaerobic, Gram-positive coccus.

E. faecalis is significantly more associated with asymptomatic cases of primary endodontic infections than with symptomatic ones. Furthermore, E. faecalis was much more likely to be found in cases of failed endodontic therapy than in primary infections (Pinheiro et al. 2003; Rocas et al. 2004).

Periapical actinomycosis:
Actinomycosis is a chronic, granulomatous, infectious disease in man and animals caused by the genera Actinomyces and Propionibacterium (that normally colonize the mouth, colon, and vagina). They are non-acid, fast, non-motile, Gram-positive organisms revealing characteristic branching filaments that end in clubs or hyphae. The intertwining filamentous colonies are often called "sulphur granules" because of their appearance as yellow specks in exudate. Four clinical forms of actinomycosis account for most of these human infections: the cervicofacial, thoracic, abdominopelvic, and cerebral forms. Cervicofacial actinomycosis presents as a chronic, slowly evolving induration in the mandibular preauricular region, often accompanied by fistular tracts to the skin that discharge typical sulfur granules.
Invasion of the microorganism is usually a result of the disruption of the mucosal barrier after trauma or dental manipulation (Nair 2004).

The endodontic infections of *actinomyces* are a sequel to caries and are caused by *Actinomyces israelii* and *Propionibacterium propionicum*, commensals of the oral cavity. Because of the ability of the actinomycotic organisms to establish extraradicularly, they can perpetuate the inflammation at the periapex, even after orthograde root canal treatment. Therefore, periapical actinomycosis is important in endodontics. *A. israelii* and *P. propionicum* are consistently isolated and characterized from the periapical tissue of teeth which did not respond to proper conventional endodontic treatment (Hirshberg et al. 2003). A strain of *A. israelii*, isolated from a case of failed endodontic treatment and grown in pure culture, was inoculated into subcutaneously implanted tissue chambers in experimental animals. Typical actinomycotic colonies were formed within the experimental host tissue. This would implicate *A. israelii* as a potential etiological factor of failed endodontic treatment. The properties that enable these bacteria to establish in the periapical tissues are not fully understood, but appear to involve their ability to build cohesive colonies that enable them to escape the host defense system (Nair 2004). Periapical actinomycosis is thought to be rare. Nevertheless, it is assumed to be more frequent than is commonly believed. There are only limited data on the frequency of periapical actinomycosis among periapical lesions or on the correlation between periapical and cervicofacial actinomycosis (Hirshberg et al. 2003).

**Viruses in periapical lesions:**
Most recently, a series of publications appeared in various journals from one research group that reported the presence of certain viruses in inflamed periapical tissues and suggested an ‘etiopathogenic relationship’ to apical periodontitis. The reported viruses are present in almost all humans in latent form from previous primary infections (Nair 2004).

One of these studies has investigated the occurrence of herpes viruses in periapical granulomas. cDNA identification of genes transcribed late during the infectious cycle of herpes viruses was used to indicate an active herpes virus infection (Sabeti et al. 2003). It was proposed that herpes viruses may cause periapical pathosis as a direct result of virus infection and replication or as a result of virally induced damage to the host defense (Slots et al. 2003).

A strong association of human cytomegalovirus and Epstein- Barr virus with the acute exacerbation of periapical lesions has been reported (Sabeti, Simon & Slots 2003). Periapical lesions harboring dual cytomegalovirus–Epstein-Barr virus infection tended to exhibit elevated occurrence of anaerobic bacteria, be symptomatic, and show large size radiographic bone destruction. Cytomegalovirus and Epstein-Barr virus, in cooperation with specific bacterial species, have also been associated with various types of advanced marginal periodontitis and several non oral infectious diseases (Sabeti et al 2003; Sabeti, Simon & Slots 2003). (Figure 1)
Fungi in periapical lesion:
Fungi have also isolated in periapical lesions but in far rear instances. Among fungi, *Candida* is most frequently found, although other fungi have been cultivated. It has been detected in therapy resistant periapical lesions (Takahashi 1998).

Conclusion:
Microbes seeking to establish in the root canal must leave the nutritionally rich and diverse environment of the oral cavity, breach enamel, invade dentine, overwhelm the immune response of the pulp and settle in the remaining necrotic tissue within the root canal. During that time they have to compete in a limited space with other microbes for the available nutrition.

References:


