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Review Article

In search of endodontic pathogens

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Abstract
Success of root canal therapy depends on the complete eradication of microflora from the root canal system. A great deal of research is needed to identify and define the role of the pathogens which are involved in the pathogenesis of the periradicular diseases. This will help the endodontist to plan the best treatment by irradiation of pathogens which, in turn predict the outcome of the treatment. This article reviews the endodontic microflora, routes of microbial entry, methods to identify endodontic microbes and markers that permit the clinician to know when to conclude the treatment.

Key words: Endodontic flora, Anachoresis, Culture media, Polymerase Chain Reaction, Antibiotic sensitivity testing.

Most of the diseases of the dental pulp and periradicular tissues are due to microorganisms. Apical periodontitis is a sequel to endodontic infection and manifests itself as the host defence response to microbial challenge emanating from the root canal system. Successful endodontic therapy depends upon reduction or elimination of these microorganisms. Failures in endodontic therapy may be due to the persistence of infection. The question now is no longer whether the microbes are involved but specificity of microbial species. New endodontic pathogens are added to list because of expanding technology like molecular methods to help the endodontist to be more accurate in the treatment planning. Studying the flora will streamline the treatment planning.

Flora

The bacterial flora of the root canal has been studied over many years. A difference exists between the flora of infected root canals which have been open to the oral fluids for sometime and the flora isolated from freshly opened canals[1]. It is polymicrobial flora dominated by obligate anaerobic bacteria[2, 3]. Earlier studies, described a flora consisting predominantly of aerobic and facultative anaerobic micro organisms. The anaerobic micro organisms are currently the subject of extensive basic research as the main causative agent of endodontic infections. But these invaders are not found clinically in pure culture, because of their synergistic nature requiring specific nutrients for growth and the presence of other micro organisms to supply some of these products necessary for survival[4]. The wide variety of organisms found in root canals by different investigators can be partially related to the principal interests and culture techniques of these investigators. Any micro organism of oral cavity, upper respiratory tract or gastrointestinal tract can gain access to the root canal system. But many may not be able to cause infection in the new environment. On the other hand, endogenous nonpathogenic oral commensals may become invasive, destructive members of community of microbes producing toxins and enzymes that cause inflammation, tissue necrosis and infection.

Among aerobic micro organisms α haemolytic streptococci are the most commonly recovered organisms. *Streptococcus mitis* and *Streptococcus salivarus,* belonging to the viridans group, are the normal commensals of oral cavity and often present in the infected root canals.

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Streptococcus mitis has been cultured from heart valves after subacute bacterial endocarditis, therefore proving its pathogenicity in a site remote from the root canal. The cell wall components of the Streptococcal group give structural rigidity and also stimulate cytokine production which enhances destructive process in the tooth and bone [5]. Enterococci, are also commonly isolated, are very difficult to eliminate because of their resistance to many antimicrobial agents[6]. Gram negative rods like coliforms, pseudomonas, spirocheates have also been recovered along with Staphylococcus spp. and Neisseria spp[4].

The new improved, sophisticated anaerobic methods of culture and identification of micro organisms has enabled the growth of anaerobic organisms found in the root canal. 25% of isolated organisms are anaerobes. Several studies have reported anaerobic growth in teeth with necrotic pulps. The interpretation of the literature has become quite complicated due to the several taxonomic name changes for these organisms over the past several years. The predominant anaerobes causing endodontic infections belong to two major genera, Porphyromonas and Prevotella. These gram negative organisms elaborate enzymes like collagenase, hyaluronidase and have endotoxins in their cell walls. The clinical symptoms, inflammation and bone destruction are all a direct or indirect effect of the endotoxin and enzymes [5]. Some studies have demonstrated with increasing frequency the presence of actinomyces in the root canals. These are anaerobic/microaerophillic gram positive filamentous commensal bacteria. Usually surgical curettage is sufficient to remove this organism but sometimes persistent infections may require surgical re entry and aggressive oral antibiotic therapy for a month or longer.

Routes of micro organism entry [7]

The microbes reach the pulp

- By extension of the caries lesion into the dentine, and spread of bacteria to the pulp via the dentinal tubules
- By traumatic tooth fracture or pathological exposure due to tooth wear
- By traumatic exposure during dental treatment

But microbes have also been isolated from teeth with necrotic pulps and clinically intact crowns. Endodontic infections of such teeth are preceded by pulpal necrosis. Bacteria from gingival sulci or periodontal pockets may have reached these root canals via the pulpal blood supply during bacteraemia (anachoresis).

Sequelae[7]

The pulpal infections may spread to the adjacent tissues resulting in periapical and periodontal infections. Dentoalveolar infections are usually limited by anatomical barriers to the orofacial areas but complications such as mediastinal, intracranial or retropharyngeal abscess may occur. Therefore early diagnosis and treatment by the endodontist is imperative.

Microbiological examination

From centuries, microscopic examination and cultivation using artificial growth media have been the standard diagnostic tests in infectious diseases. As mentioned, dental infections are polymicrobial in origin. For the most part, previous studies utilized only aerobic cultivation methods which favoured organisms like streptococci, and ignored the anaerobic bacteria. Application of molecular genetic methods and immunological techniques to the analysis of the bacterial diversity in the oral cavity has revealed a still broader spectrum of bacteria than previously reported by cultivation approaches. These methods have made it possible to detect the uncultivable bacteria and also identify some fastidious bacterial species responsible for endodontic infections.

Sample collection

Proper sample collection is the key step in the microbiological diagnostic tests. Care should be taken to avoid any kind of contamination during the sample collection. Ask the patient to rinse the oral cavity. A microbial sample from a root canal is taken by first isolating the root with a rubber dam and then disinfecting it and the rubber dam with 5% or 10% iodine tincture. Drainage may be sampled with a charcoal-impregnated sterile paper point or aspirated with a 16- or 20-gauge needle. The paper point is kept in the root canal for 30 seconds to absorb the exudate. If the exudate is aspirated using a syringe, any aspirated air should be vented from the syringe. To sample a dry canal, a syringe is used to place pre reduced transport medium into the canal. A file is then used to scrape the canal walls to suspend microorganisms in the medium.

A sub mucosal swelling should be sampled by aspiration before an incision is made. After anaesthesia has been given, the mucosal surface is dried and disinfected and a 16- or 20-gauge needle is used to aspirate the exudate. If a sample cannot be aspirated, a specimen of purulent exudate is collected

on a swab after the incision has been made, taking care to prevent microbial salivary contamination.

The paper point or the aspirate should be taken immediately to the laboratory or injected into pre reduced transport medium like Stuart's or Moller's transport medium.

Microscopic examination

Microscopic examination of the specimen usually suggests any evidence of infection. Gram staining of samples obtained from the root canal is rapid, relatively simple to perform, and inexpensive. Gram stain reveals the morphology of the microbes (cocci/rods/yeasts) and the gram reaction (gram positive or gram negative). However, identification to genus or species level is impossible. In addition, several gram positive bacteria can stain negative and many gram negative bacteria may be extremely difficult to detect against the background created by remnants of disintegrating organic material from tissues. In addition, gram staining does not make it possible to select the proper antibiotics. [8]

Phase contrast microscopy and dark field microscopy do add to the usefulness of examination. The cell boundaries appear sharper in both phase contrast microscopy and dark field microscopy than in gram staining. The added advantage of dark field microscopy is in the detection of spirochetes in root canal samples, by their motility.[9]

But microscopy has limited sensitivity and specificity to detect microorganisms in clinical samples. Limited sensitivity is because a relatively large number of microbial cells are required before they are seen under microscopy (e.g. 10^4 bacterial cells/ml of fluid). Some microorganisms may even require special stains or procedures to become visible. Limited specificity is because of the inability to speciate microorganisms based on the pleomorphic morphology and staining patterns. Microscopy is just a supplement to culturing to obtain rapid tentative information about the infective flora.

Culture

The microbiota associated with different sites in the human body has been extensively and frequently scrutinized by studies using cultivation approaches. Making microorganisms grow under laboratory conditions presupposes some knowledge of their growth requirements. The type of culture medium used influences the results of root canal cultures. Appropriate medium, which specifically supports organisms commonly found in infected root canals, should be utilized. Various media have been used in endodontics. These include, brain heart infusion broth, with or without 0.1 percent agar, trypticase soy broth, with or without agar, thioglycollate broth, glucose ascites medium, cooked meat medium, and Moller's special medium. Sommer and his co-workers recommended glucose ascites medium for its wide range of growth potential. They stated that the agar allows for growth of aerobes and anaerobes, the ascitic fluid stimulates the growth of fastidious organisms, and the glucose allows better growth of acidogenic species. Filgueiras suggested meat broth with peptone and glucose for use in tropical climates, Leavitt and his co-workers advocated trypticase soy broth with 0.1 percent agar. The latter imparts varying levels of oxygen tension, allowing for anaerobic growth at the bottom, aerobic growth at the top, and microaerophillic growth in between. However, other investigators have claimed that at least two different media per canal are necessary to obtain maximum growth possibilities[4].

In spite of the precautions used in the preparation of the various media, studies show that the predominant flora obtained are gram-positive, and composed mainly of a relatively few species. These include: Streptococcus faecalis, Streptococcus mitis, Streptococcus haemolyticus, and miscellaneous streptococci. Even utilizing combinations of a few media, may fail to allow for growth of some species seen on smears. This could have resulted from either too few microbes in the inoculum, the antibacterial activity of the inflammatory response, or death of the microbes as a consequence of the metabolic product of the predominant streptococci.

The main advantages of cultivation approaches are related to their broad-range nature, which makes it possible to identify a great variety of microbial species in a sample, including those that are not being sought after. Engstrom and associates, in their investigation of the correlation of positive cultures with the prognosis for root canal treatment, reached the conclusion that persisting infection at the time of root filling is an important factor affecting adversely the prognosis in conservative root treatment [10]. Still, cultivation makes it possible to determine antimicrobial susceptibilities of the isolates and to study their physiology and pathogenicity. Also, cultures are recommended for use in immunocompromised patients.

However, cultivation-based identification approaches have several limitations: they are costly; they can take several days to weeks to identify some fastidious anaerobic bacteria (that can delay antimicrobial treatment); they have a very low sensitivity

(particularly for fastidious anaerobic bacteria); their specificity may be also low and is dependent on the experience of the microbiologist; they have strict dependence on the mode of sample transport; they are time-consuming and laborious.

Immunological methods

Immunological methods are based on the specificity of antigen-antibody reaction. It can detect micro-organisms directly or indirectly, the latter by detecting host immunoglobulins specific to the target micro-organism. The enzyme linked immunosorbant assay (ELISA) and the direct or indirect immunofluorescence tests are the most commonly used immunological methods for microbial identification. Advantages of immunological methods for identification of micro-organisms include: they take no more than a few hours to identify a microbial species; they can detect dead micro-organisms; they can be easily standardized and they have low cost. However, they have also important limitations as they can detect only target species and they have low sensitivity (about 10^4 cells), their specificity is variable and depends on types of antibodies and they can detect dead micro-organisms.

Molecular methods

The limitations of the culture techniques have led to underestimation of bacterial diversity within the root canal system. It is estimated that less than 50% of bacteria of oral cavity are cultivable [11]. It is imperative to identify the uncultivable species so that their contribution to the disease process can be assessed. The past decade has brought many advances in microbial molecular diagnostics, the most prolific being in DNA – DNA hybridization as well as in polymerase chain reaction (PCR) technology and its derivatives. The introduction of molecular methods into analyses of root canal samples has led to the identification of a number of fastidious organisms such as *Bacteroides forsythus* and *Treponema denticola,* which have not previously been described in endodontic infections[12].

PCR amplification of the bacterial 16S or 23S rRNA gene (r DNA) or other DNAs is more sensitive and more efficient than culturing and biochemical identification of endodontic flora[13, 14]. The genes contain some regions that are virtually identical in all representative of a given domain (conserved regions) and other regions that vary in sequence from one species to another (variable regions). Variable regions contain the most information about the genus and species of the bacterium, with unique signatures that allow specific identification. Thus 16S or 23S rRNA gene sequence data can be used widely to evaluate the members of diverse microbial communities including uncultivable micro-organisms and establish phylogenetic relationships among the organisms. Once the amplification is complete, sequences can be analyzed using various fingerprinting techniques, such as denaturing gradient gel electrophoresis (DDGE) and terminal restriction fragment length polymorphism (T-RFLP). In DDGE, multiple samples can be analyzed concurrently, making it possible to compare the structure of the microbial community of different samples and to follow changes in microbial populations over time, including after antimicrobial treatment [14]. T-RFLP helps to assess subtle genetic differences between microbial strains as well as provide insight into the structure and function of microbial communities. DNA – DNA hybridization, checkerboard DNA – DNA hybridization and DNA micro array analysis utilize oligonucleotide probes to detect bacteria in endodontic infections [13]. Quantitative results of micro-organisms in clinical samples can be obtained by using real- time PCR assays[15].

However, molecular methods are laborious and costly. These procedures can be affected by some factors, such as biases in homogenization procedures, preferential DNA amplification and differential DNA extraction. False positive results have the potential to occur because of PCR amplification of contaminant DNA. False negatives may occur on analysis of small volumes of sample or presence of specific inhibitors in the clinical samples which inhibit the PCR procedure or denature the DNA targets. False negative may be true with DNA – DNA hybridization techniques as the use of specific DNA probes limits the boundaries of the detection technique, as it assumes that these probes target the species of importance and do not account for any uncultivated bacteria or uncultivable biotypes of known species[13].

Antibiotic sensitivity testing

Debridement of the root canal system and drainage of the abscess stays the main tool in endodontic treatment. Once this is done follow-up on a daily basis should be done to see if any further treatment is indicated. Tools for the control of infections in modern dentistry are usually provided by antibiotics. Antibiotics are recommended only if there is any systemic illness or in patients with predisposing factors like rheumatic heart disease, or immunosuppression.

Ideally an antibiotic susceptibility test of the organism from the patient's infection is recommended before prescribing an antibiotic. But it takes days to culture, isolate and do an antibiotic

sensitivity testing. Root canal infections, including periapical abscesses are polymicrobial in nature. Most of them are sensitive to Pencillin V and this is regarded as the primary drug of choice. Amoxicillin has a broader spectrum and in combination with clavulanate can be used against beta lactamase producing organisms. Due to predominance of anaerobic micro organisms, Metronidazole can be given along with penicillin. Erythromycin / clarithromycin can be given to patients allergic to penicillin. Clindamycin can also be given for anaerobic infection. A study by Baumgartner and Xia indicated that the antibiotic susceptibility of 6 common types of bacteria associated with endodontic infections were 85% sensitive for Pencillin V, 91% for Amoxicillin, 100% for Amoxycillin+clavulanic acid, 96% for Clindamycin and 45% for Metronidazole[16].

These drugs may be used in high risk patients for bacteraemic infections as antibiotic prophylaxis before invasive dental procedures.

Conclusion

According to Grossman 'what comes out is more important than what goes in'. Eradiation of microbial agents is one of the important phases of root canal therapy. For the complete irradiation identifying the organism is mandatory. Even though there is no evidence of bacteraemia from root canal infection, because of risk in medically compromised individuals, prophylactic measures should be taken. Prescription of antibiotics during endodontic therapy may be very rare but because of emergence of bacterial resistance and identification of new species of microbes, antibiotics have to be carefully selected.

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