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Microbiology of Endodontic Infection: A Reviews

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ABSTRACT

BACKGROUND

The association between the presence of microorganisms and endodontic pathology was first reported by Miller in 1984. Since then, understanding of biofilm formation, role of pathogens in different endodontic pathology, and their virulence factors became major concern to improve the outcome of endodontic therapy.

AIM

This review aims to discuss the microbiology involved in endodontic infections, its role in causing infection, several molecular techniques for the identification and underlying concepts.

CONCLUSION

The persistent microorganisms are the most difficult pathogens to irradiate through disinfection measures. However, thorough debridement and meticulous disinfection might reduce the number of pathogens to the threshold level which can cause intraradicular or extraradicular secondary infection.

CLINICAL SIGNIFICANCE

Advancement and evolution in the root canal therapy has led to the higher success rate. One key explanation is the full understanding of the microbiology involved in the endodontic pathology. It has become a basis for changing concept in endodontic therapy.

KEYWORDS

Biofilm; Culture; *E. fecalis*; Endodontic microflora

INTRODUCTION

Apical periodontitis is an inflammatory disease of microbial origin which arises due to complex interaction of bacteria with host defense.

Though the primary etiologic agent of apical periodontitis is bacteria, recently the role of fungi, virus and archaea is also well established for its progression and persistence [1].

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Pulpodentin complex is a sterile environment isolated from microbiota via enamel, dentin and cementum [2]. However, any breach in integrity due to caries, trauma, faulty restoration, microcracks, periodontal procedures establish a direct route of oral microbiome to the pulp [3]. Once the microbiome gain access to the pulp tissue, they form biofilm which helps them to sustain harsh conditions. The type of biofilm is mediated by the environmental conditions. The primary source of nutrients is necrotic pulp tissue, tissue fluids, salivary constituents and products from other bacteria. The endodontic treatment's ultimate goal is to either prevent apical periodontitis from developing or to provide suitable circumstances for periradicular tissue repair [4]. Such goal is achieved by thorough debridement, eradication of microbes and creating a fluid tight seal. This article highlights about the complex endodontic microbiome along with the infections caused.

HISTORY

Bacteria were first reported by Antony von Leeuwenhoek in 17th century named as “animalcules” [5]. In 1894, WD Miller found association of 3 forms of bacteria with apical periodontitis (cocci, bacilli and spirilla) [6]. In 1965, Kakehashi S examined histologic sections of exposed pulp and suggested that pulp necrosis with apical periodontitis is only seen in presence of germs whereas in germ free environment vital pulp and repair response was noticed [7]. Sundqvist G in 1976 applied advanced anaerobic culturing techniques to evaluate the bacteria present in root canal with necrotic pulp due to trauma and concluded that over 90% of the bacteria were anaerobic [8]. In 1981, Moller showed that there is absence of pathology in non-infected pulp [9]. Later PN Ramachandran Nair proposed the role of biofilm in endodontic infection [10].

MECHANISM OF MICROBIAL PATHOGEN-ECITY AND VIRULENCE FACTOR

Ability of microbes to cause disease is known as pathogenicity whereas degree of pathogenicity is called its virulence. There are various microbial products, structural cellular components or strategies (virulence factor) that contribute to pathogenicity. The majority of microbes employed in endodontic infection are opportunistic pathogens. Diffusion of bacterial products cause pulpal inflammation. On pulp exposure, surface is colonized by biofilm which results in severe inflammation ultimately resulting in necrosis. Microbes then move towards apical part of canal till the entire pulp is necrotic. At this stage pioneer microbes modify the environment and new species access the site resulting in shift of microbiota. Tissue destruction is a consequence of direct effect of exotoxins, enzymes, metabolites and indirectly via host immune reaction [3]. The various virulence factor are as follow:

- ***Lipopolysaccharide:*** It is an integral part of cell wall of gram-negative bacteria. This endotoxin is associated with pulpal pain, periapical inflammation, activation of complement and periapical bone destruction [11,12].
- ***Peptidoglycan:*** It is a major component if cell wall of gram-positive bacteria which is released on cell lysis. It reacts with innate immune system cause upregulation of proinflammatory and anti-inflammatory cytokines in T cells [13].
- ***Lipotechoic Acid:*** It is a part of cell wall of gram-positive bacteria with pathogenic properties similar to lipopolysaccharide. It is released during cell lysis which binds to target cell and interacts with circulating antibodies activating the complement pathway [14].
- ***Fimbriae:*** Long filamentous macromolecule seen in Gram negative bacteria helps it to attach to the surface [15].
- ***Capsule:*** Outermost covering of bacteria made of polysaccharides. It provides protection against desiccation, phagocytosis and hydrophobic toxic material [14].

- ***Extracellular Vesicle:*** Associated with gram negative bacteria, it is responsible for hemolysis, proteolysis and bacterial adhesion [16].
- ***Exotoxin:*** Released by living cells and target other microbes thus help in survival competence [17].
- ***Extracellular Protein:*** This group mainly consists of enzymes. It plays a major role in spread of infection. It included DNAse, hyaluronate lyase, chondroitin sulphatase [18].
- ***Short Chain Fatty Acids:*** Major by-product of fermentation process performed by obligate anaerobes. It includes butyric and propionic acid and contributes to infection process [19].
- ***Polyamines:*** Small polycationic molecule which contribute to clinical symptoms like pain and formation of sinus tract [20].
- ***Superoxide Anions:*** It is a biologically toxic, highly reactive free radicals produced by bacterial species as well as immune cells. This is responsible for erythrocytic lysis [21].

BIOFILM AND ITS MECHANISM

Biofilm is defined as assemblage of microbial cells that is irreversibly associated with a surface and enclosed in a matrix of primarily polysaccharide material [22]. The term biofilm was given by "Bill Costerton [10] whereas in endodontics it was introduced by P N Ramachandran Nair [23]. There are 4 stages in biofilm formation (Figure1). Initially microbes are irreversibly attached to the substratum which over a period of time excretes extra polysaccharide and start proliferating. Once the biofilm is mature, the inner surface liquefies resulting in dispersion of microbes. These biofilms can be interspecies or intraspecies. The various characteristic features along with mechanism is enlisted in Table 1.

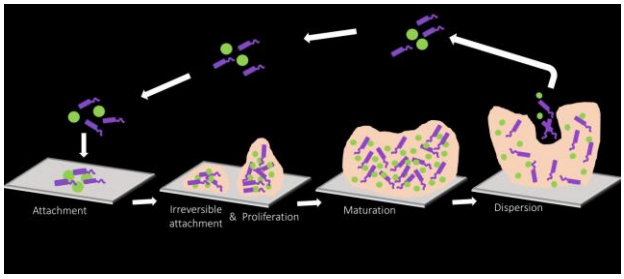

Figure 1: Steps of biofilm formation.

Composition: 97% Water+ Microbial cells+ Polysaccharide+DNA+RNA+Proteins+ Non cellular materials (Mineral crystal, clay, blood components etc depending on environment) [22]
Why microbes form biofilm: Defense, Nutrient rich area, Cooperative benefits, Default mode of growth
Special features (Attributable to resistance): Autopoiesis, Homeostasis, Synergism, Communality [24]
Nutrient influence: Type of nutrients determine initial conditioning film, matrix production, adherence of pioneer bacteria, competence and acceptance in environment
Quorum sensing: Cell to cell communication that enables bacteria to express energetically expensive processes as a collective only when impact is maximised. Gram negative bacteria releases acylated homoserine lactones (Autoinducers) and gram-positive releases oligopeptides. These autoinducers diffuse away when cell density is low and in biofilm due to high cell density is well detected [25].
Gene transfer: Conjugation, Transformation, Transduction [26].
Antibiotic resistance: Biofilm are 1000 times more resistant to antibiotic. Either antibiotics do not cross the membrane or just affects the superficial cells leading to growth and proliferation of persister bacteria [27].

BIOFILM IN ENDODONTICS

There are 4 types of biofilms in endodontics (Figure2).

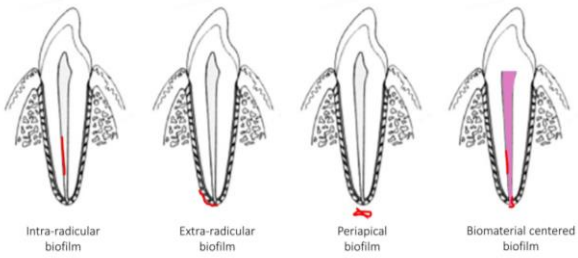

Figure 2: Types of endodontic biofilm.

- ***Intracanal:*** Present within canal on radicular dentin and usually associated with loose collection of rods, filaments and spirochetes.
- ***Extra Radicular:*** Present on root surface cementum and mostly associated with asymptomatic apical periodontitis and chronic periapical abscess. There are predominated by cocci and rods mostly.

- ***Periapical:*** Isolated biofilms independent of internal or external surface of tooth. *Actinomyces* and *Propionibacterium* are the most common bacteria related to this group.
- ***Biomaterial Centered:*** Also known as foreign body centered and is a result of opportunistic invasion. Commonly associated microbes include *E. fecalis, S. aureus, S. intermedius* on Gutta percha [24].

Bacteria colonizing the root canal system of teeth with primary or posttreatment infection are usually associated with sessile biofilm communities present in main canal. Lateral canal and isthmuses. Ricucci and Siquera JF in 2010 demonstrated the prevalence of biofilm and their association with diverse presentation of apical periodontitis. They suggested that almost 80% of root canal with primary or post treatment apical periodontits had intraradicular biofilm. The morphology of biofilm differs among individual. Lesion size and severity is directly proportional to the prevalence of biofilm. Also, these biofilms are commonly seen invading deep in dentinal tubules, and ramifications [28].

ENDODONTIC INFECTIONS

Oral cavity has is inhabited by wide range of microbial cells including virus, fungi, archaea, protozoa and bacteria. Around 10 million of it is bacteria out of which 50-60% remains unculturable. Endodontic infections are broadly classified into intraradicular and extraradicular. Intraradicular infections are further subclassified as primary, secondary and persistent. Clinically it is difficult to differentiate between secondary and persistant infections [29].

PRIMARY INTRARADICULAR INFECTIONS

This is responsible for primary apical periodontitis. The microbes involved are either the ones from earlier stages of pulp invasion that culminated the inflammation and further necrosis or latecomers that took advantage of environmental conditions in root canal after necrosis. The number of bacterial cells per canal varies from 10^3 to 10^8 and is directly proportional to the size of lesion. Though it is a mixed community infection, anaerobic bacteria prevail. The most prevalent species are gram negative bacteria namely *Fusobacterium, Dialister, Porphyromonas, Prevotella, Tannerella, Traponema, Campylobacter, Veillonella*. Gram-negative bacteria include *Parvimonas, Streptococcus, Propionibacterium, Actinomyces, Peptostreptococcus* and *Eubacterium*. The molecular studies revealed that 40%-66% unculturable phylotypes belongs to the group of *Dialister, Prevotella, Fusobacterium, Traponema, Eubacterium, Veillonella, Solobacterium* and *Olsenella*. However, various factors determine the microbial composition which covers the oxygen tension, Availability of nutrients, Bacterial interactions, temperature and pH. Early stages of infection are predominated by Saccharolytic bacteria which later on replaced by a saccharolytic microbe [3].

Apart from bacteria, even viruses like *Cytomegalovirus, Ebstein barr* virus are seen to be associated with primary apical periodontitis, The role of fungi and archaea in primary infection is questionable.

Secondary Infection

These are introduced during or in between treatment and are often responsible for endodontic flare up. Clinically, it is difficult to distinguish secondary and persistent infection. Usually, the microbes that resist the intracanal antimicrobial infections are the ones responsible for persistent infection, persistent symptoms, failure of endodontic treatment and interappointment exacerbations. The gram-negative bacterium involved are similar to the ones responsible for primary infection whereas gram positive facultative microbes include *Streptococcus, Propionibacterium, Actinomyces, Lactobacilli and E. fecalis.* Apart from these roles of Pseudomonas *aeruginosa, Candida albicans* and *Staphylococci* has also been well established in secondary infection [30].

E. fecalis as Persister

It is a gram-positive facultative anaerobe which is an opportunistic invader and seen in more than 90% of secondary or persistent cases. It is present almost 9 times more in failed root canal treatment than primary infection. This microbe is most commonly associated with asymptomatic cases and less common in acute condition. Survival and virulence factor of *E. fecalis* are:

- Endures prolonged period of nutritional deprivation
- Binds to dentin to a depth of more than 1000 micrometer.
- Alters host response.
- Suppress action of lymphocytes.
- Grow in extremely alkaline environment.
- Utilizes serum as nutrition source.
- Forms biofilm.
- Resists intracanal medicament (Calcium hydroxide for over 10 days in biofilm).
- ***Virulence factor:*** Gelatinase, Hemolysin, Extracellular superoxide aggregation substance, Cytolysin, Lipotechoic acid, Pheromones.
- Resists bile salts, heavy metals, ethanol and azide
- Can grow in 10°C - 45°C and survive 60°C for 50 minutes [31].

E. fecalis can survive calcium hydroxide due to its ability to passively maintain pH homeostasis using proton pump. Though it can be inactivated or killed at 11.5 pH, it is very unlikely that such a pH is maintain in dentinal tubules due to its buffering action [32].

Candida albicans and Persistent Infection

Seen in almost 3%-18% of the infected canal. It has the ability to bind to both dentin as well as prosthesis. Mechanism of pathogenicity includes production of B-glucan, affect macrophage activation, blocks function of lymphocytes, production of proteinase, activate complement system and adaptability to variety of environmental conditions, adhesion to variety of surfaces (collagen), production of hydrolytic enzyme, polymorphism and ability to form biofilm [33].

Extraradicular Infection

The most common form is acute apical abscess characterized by purulent inflammation in periradicular disease. This type of infection is either an extension of intraradicular infectious process or sequel of apical extrusion of debris during instrumentation. It can be dependent or independent of intraradicular infection. Independent extraradicular infection is mainly caused by *Actinomyces* and *Propionibacterium* because of their ability to form cohesive colonies and resistance to phagocytosis [34].

Methods of Microbial Identification

Culture: Process of propagating microorganisms in laboratory by providing them with the required nutrients and proper physicochemical conditions including temperature, moisture, atmosphere, salt concentration and pH. Endodontic samples are collected and transported to laboratory in a viability preserving non-supportive anaerobic medium. Sample collection is done after disinfecting the tooth via syringe/needle, paper point. This technique allows quantification of cultivable microbes and is widely available. However, over 55% of the oral microflora is non-culturable. Also, it is nearly impossible to collect samples from the accessory canal and adherend biofilms. Culturing is mainly done in cases of persistent infection to grow and isolate microbial flora for antibiotic resistance, to access bacteriologic status of canal before obturation and determine effectiveness of debridement procedure [35].

Polymerase Chain Reaction

It is the cloning of specific or targeted parts of a DNA sequence to generate thousands to millions of copies of DNA of interest. The primary components include DNA template, DNA polymerase, Primers and nucleotides. The first step is denaturation (96°C) resulting in separation of

the two strands of DNA, followed by annealing at 55°C so that primers anneal the template and lastly extension at 72°C where DNA polymerase adds nucleotides onto the ends of annealed primers. Developed by Kary Mullis in 1980, this molecular technique detects both cultivable and non-cultivable species and is much more sensitive as well as specific. However, these are expensive, laborious and most assays detects 1 or few different species at a time [36].

16srRNA gene sequencing: This is the most widely used technique nowadays. It helps in identification of specific bacterial strain.16srRNA is universally distributed among bacteria. It is long enough to be highly informative and short enough to be easily sequenced. For fungi and eukaryotes 18srRNA is used for identification [37].

DNA-DNA Hybridization

It utilizes DNA probes labelled with enzyme or radioactive isotope that binds to their complementary nucleic acid sequence. It can be used for large scale detection however there are chances of cross reaction [38].

CONCLUSION

Endodontic infection can arise following pulpal exposure or post treatment. The necrotic pulp, constant communication with the periapical as well as oral tissue makes the root canal ideal for microbial habitat. However, most of these microbes form a 3D complex structure in the form of biofilm. A better understanding of these complex is required for their complete eradication owing to success of endodontic treatment.

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