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UNDERSTANDING THE ENDODONTIC MICROBIOME

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ABSTRACT

Apparently, various microbial flora has been reported in association with pulp, peri-apical and periodontal tissues. This discovery has led us to a new era of planning and treatment of the infection in the root canal system, inflammation in the periodontal region, and immunologic reaction caused by the by-products and enzymes released by these microbes in the host. In recent times, it is also found that there are a few species that are highly resistant to the host response and medicaments applied while treating the infection and can even be an etiology for the lesion after the root canal treatment. This article provides an overview of the endodontic microbiology and pathobiology and their pursuit in endodontic treatment strategy.

KEYWORDS: Endodontic microbiology, Root canal flora, Endodontic biofilm.

INTRODUCTION

The primary motive of an endodontic procedure is to conserve the tooth and regain its natural anatomical structure and function. Thorough knowledge and understanding of the response of the host and microbes led to a drastic increase in the success rate of such treatment.[1] Intrinsically, the dental root canal system is the chief site of interest for microbes leading to endodontic lesions and apical periodontitis, whilst many chemical and physical aspects have also been reported to cause periapical inflammation.[2] Focusing on scientific evidence, microorganisms are found to be the key element in advancing and externalizing periradicular inflammation.[2]

Accounting for the fact of wiping out the infection and microbial colonization, averting it from recurrence, and rehabilitating the healthy environment of the dental root canal system, endodontic treatments are entirely focused on a complete understanding of microbiota linked with various forms of infections and gaining Laurence worldwide.[5]

PATHOPHYSIOLOGY

Microbial colonisation is habitually found and coevolved in humans, in mucosal surfaces of the oral cavity, gastrointestinal system, urogenital tract, and skin surfaces, hence becoming passive against the host defense mechanism.[3] So when the host becomes immunocompromised or a satisfactory amount of pathogen invades the host, the disease develops. The organisms that causes the disease are called pathogens and their potential to cause the disease in the host is called pathogenicity.[4] These microorganisms are qualified to fuse, cleave, inhabit, live and reproduce themselves and simultaneously become obtrusive in host immune combating cells such as neutrophils, complements and antibodies cause damage to the tissue, directly generated by bacterial enzymes, metabolites, and endotoxins or indirectly could be begotten by host immune response itself which causes tissue damage driven through bacterial components like lipopolysaccharide (LPS), peptidoglycan (PG), lipoteichoic acid (LTA), fimbriae, outer membrane proteins, capsular components and extracellular vesicles.[4] The degree of anaerobiosis, pH level, the availability of exogenous and endogenous nutrients, as well as the surfaces accessible for adhesion like dentin, can all have an effect on the pathogenicity of bacteria. The amount of pathogenicity of microorganisms is referred to as virulence. Any medication leftovers and root-filling material in infected root-filled teeth are additional variables that might affect pathogenicity.[5]

VIRULENCE FACTOR

Many bacteria discovered in endodontic infections are commensals in the oral cavity that have entered the root canal pulp tissue during the caries process. The identification and characterization of several virulence factors that may play a role in endodontic infections are listed below.[6][8][9]

Lipopolysaccharide (LPS): Endotoxin is another name for LPS. LPS is a component of Gram-negative bacteria's

cell wall. When LPS is released, it has a variety of physiological impacts, including the activation of immunosurveillance systems in the pulp. These endotoxins are linked to pulpal discomfort, periapical inflammation, complement activation, and periapical bone degradation.

Prostaglandins (PG): PG is the major component of Gram-positive cell wall. PG is generated after cell lysis and can interact with the natural immune system as well as stimulate T cell overexpression of proinflammatory and anti-inflammatory cytokines. Through macrophages, PG may enhance an adaptive immune response. In the presence of LPS, the efficacy of PG is greatly increased.

Lipotechoic acid (LTA): LTA is a Gram-positive bacterium cell wall component made up of techoic acid and lipids. LTA and LPS have many pathogenic characteristics. LTA is produced after cell lysis and attaches to target cells, where it interacts with circulating antibodies, activating the complement cascade and causing damage.

Fimbriae: They are slender, filamentous polymers that may be discovered on the surface of many Gram-negative bacteria. Protein subunits are used to create thin hair-like projections (they are distinct from flagella). Fimbriae are important in surface adhesion and communication with other bacteria.

Capsules: A capsule is a coating of polysaccharides and other components found outside of the cell wall of bacteria. Capsules protect the bacterial cell resist decomposition, phagocytosis, microbial pathogens, and hydrophobic harmful compounds such as detergents. Bacteria and fungi use capsule development to prevent adaptive immunity and resist phagocyte ingestion.[7]

Extracellular vesicles: Gram-negative bacteria generate extracellular vesicles, which allow their products to be released into the extracellular space. Proteins and lipids are found in the contents and are engaged in a variety of activities such as hemagglutination, hemolysis, bacterial adhesion, and proteolytic activities. Extracellular vesicles allow bacteria to communicate with cells both prokaryotic and eukaryotic and can influence relationships between bacteria.

Exotoxins: Exotoxins are poisons generated by live cells that can cause excessive and abnormal T-cell activation. Bacterial toxins can also be used to kill other microorganisms; for example, bacteriocins, proteinaceous toxins generated by bacteria, are effective against bacteria.[7]

Extracellular proteins: Several of these extracellular matrix proteins are enzymes that bacteria make. These enzymes, which include proteases that neutralize immunoglobulins as well as cox-2, are produced after bacterial cell lysis and contribute to the spread of infection. Tissue degradation is aided by enzymes such as hyaluronate lyase, chondroitin sulphatase, beta-glucosidase, DNA polymerase, and acid phosphatase.[7]

Short-chain fatty acids: Butyric acid and propionic acid are significant leftovers of the transesterification reaction done by obligate anaerobes. These acids activate the inflammatory response and the production of pro-inflammatory cytokines, which aid in the course of infection.

Polyamines: Polyamines are tiny polycationic compounds such as putrescine, cadaverine, spermidine, and spermine that lead to subjective symptoms such as pain (particularly pounding pain) and sinus tract development. These polyamines work by regulating many ion channels.

Superoxide anions: Reactive oxygen species are extremely reactive free radicals that are physiologically hazardous. These are generated by a type of bacteria as well as immune system cells. They produce erythrocyte lysis and participate in interspecies interactions.

In endodontic infections, there is an unequivocal cause and association between bacterial pathogens and diagnostic signs and symptoms. Other strategies by which pathogens may control the infection process include the capacity of certain intracellular pathogens to eliminate the lethal strategies of phagocytic cells, allowing them to evade being destroyed by neutrophils and macrophages.[7] Furthermore, certain bacteria have the ability to genetically change their surface antigens, making it challenging for the inflammatory response to target these species. Understanding these virulence variables in depth aids in identifying treatment goals in endodontic infections.

TRAJECTORY OF MICROBIAL INCURSION

Bacteria may reach the pulp in a number of ways, therefore understanding them is essential for us to design our therapy. The following are the many methods that microorganisms take to reach the pulp.[8][9]

- Dentinal tubules: Microorganisms may enter the pulp via the dentinal tubules following a chronic wound or amidst dental procedures. When the dentin space between the border of the carious lesion and the pulp is 0.2 mm, bacteria gain entry to the pulp.
- Open cavity: Direct pulp exposure from a violent source, such as a coronal breakage, or from an iatrogenic source, such as medical interventions, undermines the protective wall that dental components establish and opens pulp to the contaminated oral environment.
- Periodontal membrane: Bacteria from the gingival crevice can enter the pulp chamber via the periodontium and either the apex or the peripheral channels. During dental prophylaxis, this route is opened up to microbes due to tooth luxation and, more importantly, the relocation of epithelial

infiltration to the formation of periodontal pockets.

- Blood stream: A normal day in the life of a healthy individual may include any number of events that contribute to transient bloodstream infections. Blood-borne bacteria would be attracted to the afflicted region following trauma or perhaps an intervention that created aggravation without accessing the tooth pulp. Endodontic infection can propagate through blood or lymph via this urge, which is known as anachoresis.
- Faulty restoration: Saliva can reach the periodontal area in less than 6 weeks in channels obturated with guttapercha and sealer, according to research. If the provisional seal is broken, the tooth architecture cracks before final restoration, or if the eventual repair is inadequate, pathogens can penetrate the pulpal tissue and cause sickness.

ENDODONTIC MICROBIOTA

The bacterial population of the root canal has been studied for years. In general, all oral cavity-dwelling bacteria have the capacity to infiltrate the pulp space both during and after pulp necrosis, contributing to canal infection, and enter the periapical tissues, resulting in periapical periodontitis.[9]

The endodontic microflora's bacterial profiles differ from person to person, indicating that apical periodontitis has a diverse aetiology with numerous bacterial combinations that may be involved in disease pathogenesis.

As the infection spreads, the root canal's nutritional and environmental conditions change. Anaerobic bacteria, of which only a small group is present in infected root canals, predominate in the root canal flora.

- Gram-positive organisms (75%) with most predominant being streptococci (28%), staphylococci (15%), corynebacteria (10–25%), yeasts (12%), and others.[9][10]
- Gram-negative bacteria (24%) include spirochetes (9–12%), Neisseriae (4%), Bacteroides (7%), fusobacteria (3%), pseudomonas (2%), coliform bacteria (1%), and others.[9][11]
- Using polymerase chain reaction (PCR) techniques, researchers have demonstrated that Tannerella forsythia is a prevalent member of the microbiota linked with endodontic infections, including abscesses.[12]
- It has also been determined that the gram-negative organism Fusobacterium nucleatum has five subspecies: fusiforme, nucleatum, polymorphum, vincentii, and animalis.[9][11]

Classification of Bacterial Genera Prevalent in Endodontic Infections[9]

Anaerobic gram-negative bacteria	Facultative gram-negative bacteria	Anaerobic gram-positive bacteria	Facultative gram-positive bacteria
Traponema Dialister Porphyromonas Tanerella Fusobacterium Prevotella Veillonella	Neisseria Capnocytophaga Haemophilus	Actinomyces Eubacterium Propionibacterium Peptostreptococcus	Enterococcus Streptococcus Lactobacillus

TYPES OF ENDODONTIC INFECTIONS

Endodontic infections are divided into intraradicular and extraradicular categories based on where they occur in relation to the root canal. Endodontic infections are polymicrobial in nature, with the microbiota of first infections being notably dominated by obligate anaerobic bacteria.

Intraradicular infections

The following are the endodontic microorganisms responsible for the primary intraradicular infections:

1)Among many of the black-stained Gram-negative anaerobic rods are Bacteroides melaninogenicus species. These bacteria have been classified into two genera: Prevotella, a saccharolytic species, and Porphyromonas, an asaccharolytic species.[9][11][14]

Prevotella species	Porphyromonas species
• Prevotella intermedia • Prevotella nigrescens • Prevotella tannerae • Prevotella multissacharivorax • Prevotella baroniae and • Prevotella denticola.	• Porphyromonas endodontalis • Porphyromonas gingivalis.

2)Tannerella forsythia, previously termed as Bacteroides forsythus or Tannerella forsythenis, became the first culprit discovered in an endodontic infection.[12]

3)Dialister species are asaccharolytic, symbionts anaerobic Gram negative coccobacilli that have been discovered frequently in endodontic infections.[11]

- Dialister pneumosintes and
- Dialister invisus.

4) Fusobacterium is also a common member of endodontic microbiota.

- Fusobacterium nucleatum
- Fusobacterium periodonticum[11]

5) Spirochetes are Gram-negative, spiral-shaped bacteria with periplasmic flagella that are extremely mobile. The genus Treponema contains all types of oral spirochetes.

- Treponema denticola
- Treponema sacranskii
- Treponema parvum
- Treponema maltophilum and
- Treponema lecithinolyticum.[11]

6)Endodontic microbiota has also been reported to include Gram-positive anaerobic rods.

- Pseudoramibacter alactolyticus
- Filifactor alocis
- Actinomyces spp.
- Propionibacterium propionicum
- Olsenella spp.
- Slackia exigua
- Mogibacterium timidum and
- Eubacterium spp.[10]

7)Gram-positive cocci seen in endodontic infections:

- Parvimonas micra (previously called Peptostreptococcus micros or Micromonas micros)
- Streptococcus spp. which include,

Streptococcus anginosus
Streptococcus mitisi
Streptococcus sanguinis

- Enterococcus faecalis.[10]

Fungi – particularly, Candida spp. (e.g.,) Candida albicans[13]

Archaea –These prokaryotes are a varied group that is apart from bacteria.

Although these microorganisms were formerly categorized as extremophiles, it has lately been shown that they can thrive in typical conditions, including the human body. Periodontal disease and persistent apical periodontitis have been linked to methanogenic bacteria.

Viruses: Viruses are particles that are made up of a genomic molecule (DNA or RNA) and even a polypeptide covering. In order to infect and reproduce the viral genome, these viruses must infect live host cells. As a result, they are unable to live in a dead root canal.

Extraradicular infections

Intraradicular bacteria frequently contain themselves in the root canal due to the protective barrier. Pathogens can cross this defensive boundary and create an extraradicular infection in certain situations. This can result in an abrupt apical abscess with exudate infiltration in the periapical tissue. Extraradicular infections can occur in the presence or absence of an intraradicular infection. Anaerobic bacteria dominate the microorganisms present. The leading microbiota found are[14]:

- Actinomyces spp.
- Propionibacterium propionicum
- Treponema spp.
- Porphyromonas endodontalis
- Porphyromonas gingivalis
- Treponema forsythia
- Prevotella spp. and
- Fusobacterium nucleatum

Following root canal therapy and after intracanal disinfection techniques, bacteria still exist. After biomechanical preparation, some microorganisms that are susceptible to antimicrobial therapy can persist in the root canal.

Gram-negative anaerobic bacteria	Gram-positive bacteria
• Fusobacterium nucleatum • Prevotella spp. and • Campylobacter rectus.	• Streptococci (Streptococcus mitis, Streptococcus gordonii, Streptococcus anginosus, Streptococcus oralis) • Lactobacilli (Lactobacillus paracasei and Lactobacillus acidophilus) • Staphylococci • E. faecalis • Olsenella uli • Parvimonas micra • Pseudoramibacter alactolyticus • Propionibacterium spp. • Actinomyces spp. • Bifidobacterium spp. and • Eubacterium spp.

E. faecalis and yeast, particularly Candida albicans, have been regularly recognised as the species most commonly isolated from pulp chambers undergoing retreatment in situations of failure endodontically treated teeth and canals with chronic infections. Yeasts, most often Candida albicans, are occasionally discovered in trace levels. E. faecalis are facultative anaerobes and gram-positive cocci. They are common intestinal organisms that can be found in the oropharynx and gingival commissure. When present in tiny quantities, this bacteria is readily eradicated; but, when present in large amounts, it is challenging to eradicate. E. faecalis has numerous distinguishing characteristics that make it an extraordinary survival in the root canal. These microbes are capable of the following:

- Live and persist in a poor nutrient environment
- Form biofilms in medicated canals
- Invade and metabolize fluids within the dentinal tubules and adhere to collagen
- Convert into a viable but non-cultivable state
- Acquire antibiotic resistance
- 'Survive in low pH, high salinity, and high-temperature conditions
- Continue to be starved for extended periods of time while consuming tissue fluid that comes from the periodontal ligament.

BACTERIAL BIOFILMS

A biofilm is a type of microbial development in which dynamic communities of interacting sessile cells are enmeshed in an extracellular polymeric matrix and irreversibly bound to a solid substrate as well as to one another.[2][9][15]

Kishen et al asserts that a biofilm goes through four distinct stages as it develops, namely:

- Formation of a conditioning layer
- Planktonic bacterial cell attachment
- Detachment (Seeding dispersal)
- Bacterial growth and Biofilm expansion

Endodontic bacterial biofilms can be categorized as

- Intracanal biofilms
- Extraradicular biofilms
- Periapical biofilms
- Biomaterial-centered infections

Intracanal microbial biofilms: They are microbial biofilms that have developed on the root canal dentin of an infected endodontic tooth.[9]

Extraradicular microbial biofilms: They are also known as root surface biofilms because they develop on the root (cementum) surface of endodontically diseased teeth very next to the root apex.[9][16]

Periapical microbial biofilms: They are isolated biofilms found in the periapical region of endodontically infected teeth. Periapical biofilms may or may not be dependent on the root canal. These microbes are able to get past the host's defence mechanisms, flourish in the inflamed periapical tissue, and then cause an infection to spread to the periapical region.[9][17]

Biomaterial-centered infection: When bacteria attach to a surface made of artificial biomaterials and form biofilm structures, this is known as biomaterial-centered infection. The presence of biomaterials in close proximity to the host immune system can increase the susceptibility to biofilm. In endodontics, biomaterial-centered biofilms form on root canal obturating materials.[9][18]

CONCLUSION

The progression of the periapical disease is directly related to the microorganisms present in the pulpal tissue. Recent genomic approaches have enhanced the knowledge of taxonomic diversity. Such knowledge led to more biofilm-directed treatment strategies to eliminate the infection. However, the area of their complex interaction, their coexistence in the root canal, survival capacity, and interaction with each other still remain less known.

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