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The Microbial Maze: Navigating Bacterial Growth in Root Canals

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A B S T R A C T

The root canal system provides a unique, complex environment supporting microbial colonization and growth. The presence of necrotic pulp tissue and a nutrient-rich environment fosters the proliferation of bacteria, fungi, and other microorganisms. Polymicrobial biofilms within the canal further complicate disinfection and treatment, as they can resist standard antimicrobial approaches and host immune responses. This review explores the role of the root canal system as a biological host for microbial growth, and the factors influencing microbial colonization. Understanding these dynamics is essential for developing treatment protocols and outcomes in root canal therapy

Keywords: Biofilm, Endodontic microflora, Microbiology, Microbial growth, Root canal system

1. Introduction

The intricate architecture of the root canal system provides an ideal environment for the growth and survival of various microorganisms, particularly when the pulp tissue becomes necrotic due to infection, trauma, or decay. The presence of organic and inorganic materials in the necrotic pulp serves as a nutrient source for microbial populations, allowing them to thrive within the confined spaces of the canal system. Endodontic infections are predominantly polymicrobial, with a wide range of bacteria, fungi, and other microorganisms forming resilient biofilms. These biofilms are particularly challenging to eradicate due to their complex structure, which protects the microbes from antimicrobial agents and host immune responses. The root canal system's role as a host for microbial growth has significant implications for endodontic treatment. Despite advances in root canal therapy, complete elimination of microbial presence remains a challenge due to the anatomical complexity and the tenacity of biofilms.

PATHWAYS TO PULP:

Microflora a major deterrent in endodontic infection has many possible pathways to reach the dental pulp and periradicular tissues as shown in Figure 1. Main portals of pulp infection can be categorised as follows[1]:

- Caries
- Direct pulp exposure
- Invasion via dentinal tubules in the crown area or radicular dentine
- Periodontal and gingival diseases, lateral canals,
- Hematogenous pathway associated with bacteremia

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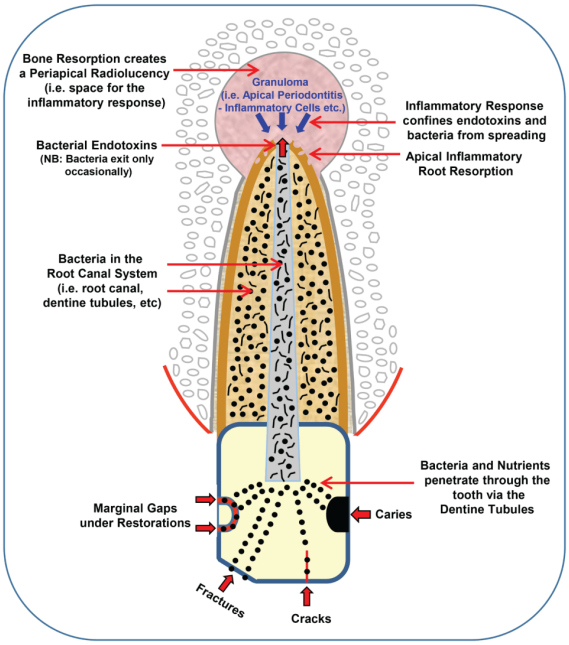

FIG. 1: Pathways For Bacteria to Reach Pulp And Periradicular Tissue.

Interaction between the host and microbes

The commensal microbiota present in the oral cavity co-evolve with its host and create an environment favourable for its survival. Invading microorganisms acquire their pathogenicity if the host defence mechanism is compromised. As a consequence, disease can develop and these disease-causing microorganisms are referred to as pathogens[2].They include bacteria, fungi, viruses, protozoa and higher parasites. These pathogens interfere with the innate and adaptive immune response and break host defense barrier. After discouraging host immunity, successful pathogens are able to adhere, colonize, survive, propagate, invade and evade host defense mechanisms such as neutrophils, complement and antibodies. In addition, pathogens can also initiate tissue destruction during inflammation either directly by enzymes and metabolites or indirectly by bacterial components.[3]

Fish in 1939 described the reaction of the periradicular tissues to bacterial products, noxious products of tissue necrosis, and antigenic agents from the root canal.[3]He established an experimental focus of infection in the guinea pigs by drilling openings in the jaw bone and packing it with wool fibers saturated with a broth culture of microorganisms.[4] Four well defined zones of reaction were found during the experiment as shown in Figure 2. [3,4,5]

1. Zone of infection or necrosis *(PMNLs)*
2. Zone of contamination *(Round cell inflitrate-lymphocytes)*
3. Zone of irritation *(Histiocytes and osteoclasts)*
4. Zone of stimulation *(Fibroblasts, capillary buds and osteoblasts).*

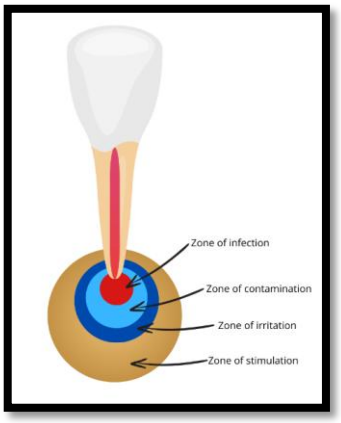

Fig.2: Fish's zones of reaction

In infected root canal system, bacteria and bacterial products are released from dentinal tubules and cause inflammation reactions in the pulp. A dynamic encounter between microbial factors and host defense at the interface between infected radicular pulp and periodontal ligament results in local inflammation, resorption of hard tissues and destruction of other periapical tissues. The equilibrium at the periapex, in favor of or against the host defense, determines the histological picture of the lesions. Once the foreign antigens released from bacteria enter the pulp, pulpal antigen presenting cells provide necessary signals to activate T lymphocytes. Initiation of immunological responses occur in the pulp with the help of immunocompetent cells in the form of helper/inducer T cells, cytotoxic (suppressor T cells, macrophages, class II antigen expressing cells).[3] Tissue damage during inflammation occurs more rapidly in the pulp due to its hard tissue encasement.[3] During infection, neutrophils not only fight the micro-organisms but also release leukotriene and prostaglandins. The former (LTB4) attract more neutrophils and macrophages into the area, and the latter activate osteoclasts resulting in bone resorption.[6]

In shallow carious lesion, induction of type I cytokines in pulp tissue and CD8 (+) T cells activation suggest major influence on initial pulp lesion pulpal pathology.[3] The process of advancing caries and pulpitis involves irreversible tissue damage, healing and repair with both specific and non-specific inflammatory reactions. Quantitative real-time PCR has revealed that gram-negative bacteria dominate the microbiota of advanced carious lesions and pulpitis.[3]

Modes on colonization within the root canal system

Microorganisms colonizing in the root canal space may either be free-floating as single cells (planktonic form) or attached to each other or on to the root canal walls or both. Bacterial cells can wander in a suitable environment, drifting for nutrition, reproduction and survival. This state is referred to as a planktonic existence.[7] Organisms present in a planktonic state require a liquid phase. Biofilm is a mode of microbial growth where dynamic communities of interacting sessile cells are irreversibly attached to a solid substratum, as well as each other, and are embedded in a self-made matrix of extracellular polymeric substances (EPS).[3] A microbial biofilm is considered a community with autopoiesis, homeostasis, synergy and communality.[5] Biofilm is composed of several morphotypes which grow in multilayers or as aggregates on the dentin walls of the root canal.[5]Endodontic bacterial biofilm can be categorized as intracanal biofilms, extraradicular biofilms, periapical biofilms, and biomaterial-centered infections.[3, 8]

A. Intracanal microbial biofilms

Intracanal microbial biofilms are formed on the root canal dentine of an endodontically infected tooth. This biofilm exist both as loose collection and biofilm structures. It mainly comprises cocci, rods, and filamentous bacteria, dead bacterial cells, and pockets of viable bacterial cells.[3] Most resistant bacteria, *E. faecalis*forms biofilm on root canal dentin surface (Figure 3) develop in distinct stages[3]which can be categorised as:

1. *E.faecalis* cells adhere and form microcolonies on the root canal dentine surface.
2. Dissolution of the mineral content from the dentine substrate is induced which results in localized increase in the calcium and phosphate ions for mineralization of biofilm.
3. Maturation of biofilm occurs with the formation of carbonated-apatite structure and the coaggregation interactions between *E.faecalis* and other microbes like *F. nucleatum*.[3]

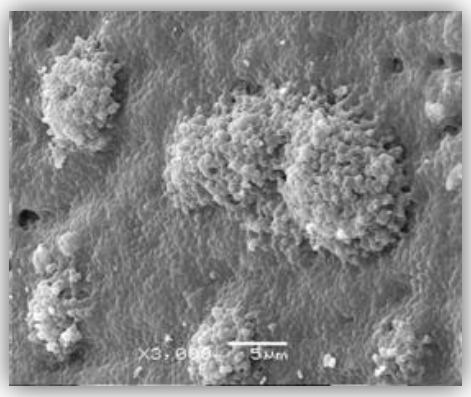

Fig. 3: Scanning electron microscopyview of Root dentinal surface completely covered by *E. faecalis* biofilm.

B. Extraradicular microbial biofilms

Extraradicular microbial biofilms are formed on the root cementum surface. These root surface biofilms are polymicrobial in nature and dominated by cocci and short rods, with cocci attached to the tooth substrate in the filamentous and fibrillar forms as shown in Figure 4.[1] A smooth, structure-less biofilm structure consists of extracellular matrix material with embedded bacterial cells at the root apex.[3]

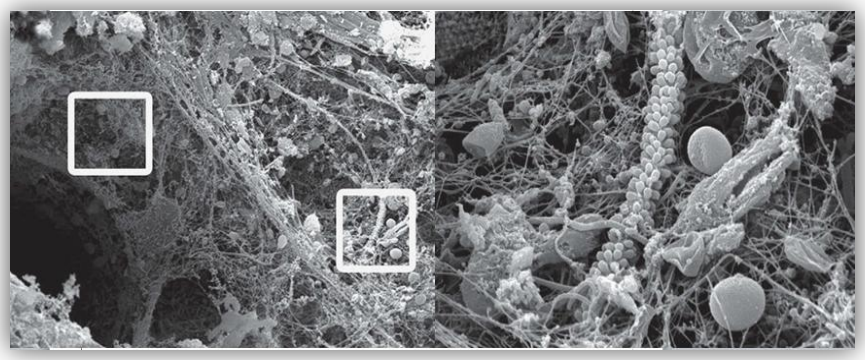

Fig. 4: Scanning electron microscopy views of biofilm at the extraradicular surface

C. Periapical microbial biofilms

Periapical microbial biofilms (Figure 5) are isolated biofilms in the periapical region of endodontically infected teeth. Microbial species in the root canal are opportunistic species that do not have the ability to survive host defense mechanism in the periapical tissues. Genus *Actionomyces* and the species *P. propionicum* may be seen in asymptomatic periapical lesions.[3] Aggregation of cells facilitates the formation of biofilm structure that differentiates, communicate, cooperate, and increase resistance against antimicrobials. Periapical region is patrolled by PMNs and macrophages.

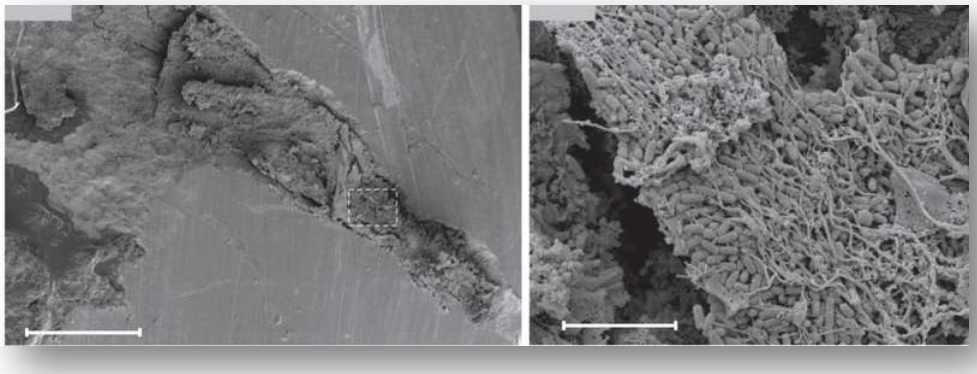

Fig. 5: Scanning electron microscopic views of the periapical microbial biofilms.

D. Biomaterial-centered infection (BCI)

Biomaterial-centered infection is caused when bacteria adhere to an artificial biomaterial surface and forms biofilm structures. The presence of biomaterials in close proximity to the host immune system can increase the susceptibility to BCI.[3] BCI constitute opportunistic invasion by nosocomial organisms such as coagulase-negative *Staphylococcus, S. aureus, enterococci, Streptococci, P. aeruginosa,* and fungi.[8] Bacterial adherence to a biomaterial surface in seen in three phases:

- Phase 1: transport of bacteria to biomaterial surface
- Phase 2: initial, non-specific adhesion phase
- Phase 3: specific adhesion phase

In root canal system, biomaterial-centered biofilms form on the root canal obturating materials. BCI can be intraradicular or extraradicular biofilm and harbors*E. faecalis, S. sanguinis, S. intermedius, S. pyogens, S. aureus, F. nucleatum, P. acues, P. gingivalis, P. intermedia.*[3]

Ecological determinants for microbial growth in root canal system

In dental plaque adherence and co-adherence are key ecological determinants for the survival and persistence of oral bacteria in the root canal environment. Low oxygen tension and the level of nutrients available from the host are important ecological factors determining the success or failure of microorganisms entering the root canal to survive and grow.[5] In a biofilm, developing an appropriate physiochemical environment for the different bacteria facilitates processing and uptake of nutrients, cross-feeding, and removal of potentially harmful metabolite products.[3] Nutrients may be derived from the oral cavity, degenerating connective tissue, dentinal tubule contents, or a serum-like fluid from periapical tissue. These factors in the root canal environment permit the growth of anaerobic bacteria dependent on fermenting amino acids and peptides, whereas bacteria that primarily obtain

energy by fermenting carbohydrates are restricted due to lack of available nutrients. As a consequence, 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. The succession of strict over facultative anaerobes with time is due to changes in available nutrition, as well as a decrease in oxygen availability. Facultative anaerobic bacteria grow well in anaerobiosis and their prime energy source is carbohydrates. A decrease in the availability of carbohydrates in the root canal occurs when there is no direct communication with the oral cavity, this restricts the growth of facultative anaerobes.[9]Ecological determinants[5] in the root canal system include:

1. Adhesion to root canal tissues and co-aggregation
2. Low oxygen concentration and redox potential
3. Nutrition
4. Microbial interactions
    a) Synergistic
    b) Antagonistic

1. Adhesion to root canal tissues and Co-aggregation

After invading into the root canal space, microorganisms must first colonize through a series of interactive elements. To begin with colonization, bacteria must adhere to the root tissue surface. Adhesion to the dentin requires the cell to attach to the proteinaceous portion of the dentin matrix such as deposited salivary or tissue proteins or glycoproteins.[5] Oral streptococci bind to collagen type I when adsorbed onto hydroxyapatite surfaces to unmineralized collagen and to root dentin.[1] Binding to collagen is mediated by expression of oral streptococci antigen I/II polypeptide adhesins on the surface of the bacteria in serine protease and the collagen-binding protein (Ace) contributes to cell adhesion to radicular dentin.[1]Streptococcal protein adhesins that can interact with salivary molecules include the antigen I/II family polypeptides, amylase-binding proteins, surface lectins, fimbrial adhesins, EP-GP binding protein, and glucan- binding proteins GBP74 and GBP59.[1,10] Strains of *P. gingivalis*bind to collagen-coated hydroxyapatite with the adhesion fimbriae that bind strongly to collagen.[1]

2. Low oxygen tension and Redox potential

Generally, the oxygen tension and redox potential in root canals are low, favouring growth and survival of obligate anaerobic microorganisms. Any oxygen entering the root canal, e.g. with saliva, will be consumed by the facultative anaerobes, which tolerate oxygen due to their enzymes that catalyzethe removal of toxic oxygen product.[5] The extracellular matrix of biofilm allows the diffusion of oxygen. The oxygen tension in the inner parts is low because it is actively consumed by facultative anaerobes in the biofilm. Thus, in dense microbial biofilms, low oxygen levels and a low redox potential is suitable for obligate anaerobes to prevail and survive.[5]

3. Nutrition

The necrotic tissue, tissue fluid and inflammatory exudates from the apical tissue provide the basic requirements for carbon, nitrogen, salts and energy as well as the special requirements for amino acids, nucleotides, vitamins and hemin.[5] Nutrients can be derived directly from these sources or from the degradation of macromolecules such as proteins and glycoproteins. The carbohydrates, small peptides and amino acids then serve as nutrients and an energy source for many inhabitants in the root canal environment. The organisms growing in a complex microbial interaction of biofilm may also benefit from inter-microbial food chains where metabolic end-products (e.g. ammonia, carbon dioxide and organic acids) of one species serve as nutrients for others as illustrated in Figure 6.[7] Small amount of carbohydrate available directly or liberated from degradation of glycoproteins in the root canal favours the growth of proteolytic and amino-acid degrading microorganisms. As a result, high proportion of proteolytic bacteria may be present in the endodontic microbiota. In clinical situations where the necrotic tissues in root canals have been removed and the apical inflammatory process controlled, nutrients will be scarce, so the microorganisms are likely to regulate their metabolic balance away from multiplication, towards the acquisition of energy for survival.[5]

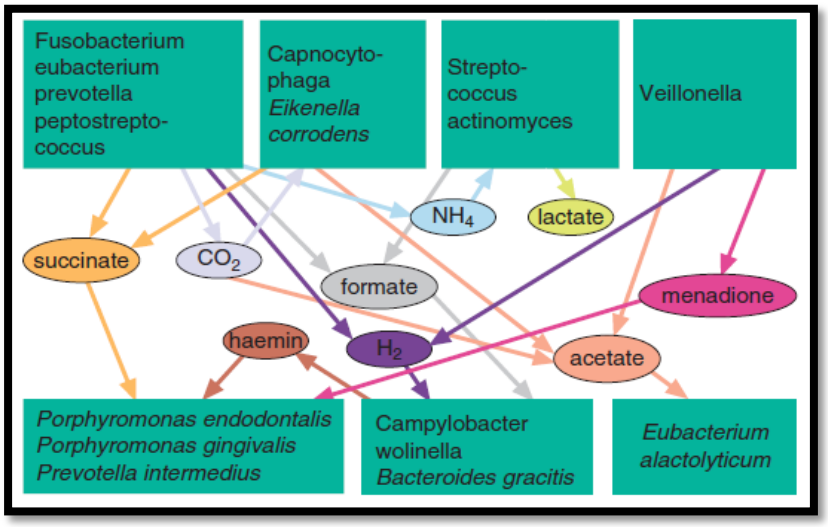

Fig. 6: Nutritional interactions between microorganisms

4.Microbial interaction

It is most commonly seen in root canal biofilms involving multiple oral species. Members of the microbial community in biofilms are actively involved in a wide range of metabolic, molecular and physical interactions essential for the attachment, growth and survival of species at a site, enabling microorganisms to persist in harsh environments. Microbial interactions in a poly-microbial community can be synergistic and antagonistic.

a) Synergism

Some microorganisms utilize oxygen combined with the accumulation of reduced metabolic end-products creating an environment suitable for obligate anaerobes. Synergistically interacting microorganisms metabolize complex host glycoproteins and proteins which are normally resistant to catabolism by individual microorganisms but can easily be broken down by the concerted action of the microbes in microbial communities.[3] Bacterial population in biofilm offer protection to other microorganisms from competition, host defense mechanisms and toxic substances such as antiseptics and antibiotics. Colonizing species in polymicrobial community undergoes genetic exchange and become resistant to antimicrobial agents.[3]

b) Antagonism

Antagonism is seen in polymicrobial community as metabolic end-products (e.g. Hydrogen Peroxide, fatty acids and sulfur compounds) may accumulate in concentrations that are inhibitory or toxic for other species.[11] Bacteriocins and antimicrobial peptides generated by microbial speciestarget others that are sensitive, so that their growth is suppressed.[12]

Conclusion:

Infection of the root canal is not a random event. Microbial flora in the root canal system develops in response to the surrounding environment. Microorganisms that establish in the untreated root canal experience an environment of nutritional diversity. In contrast, well-filled root canal offers the microbial flora a small, dry, nutritionally limited space. Therefore, root canal system acts as a 'privileged sanctuary' for the bacterial growth and survival of microorganisms. Root canal system acts as a perfect host for different microbes to cause endodontic infections.

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