ENDODONTIC BIOFILM: A REVIEW

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Abstract:

An important part of endodontic microbiology is detection and identification of the micro-organisms associated with initiation and progression of this polymicrobial infection. This has helped us to modify the conventional treatment plans and effectively combat the microorganisms. Now, studies are aiming to explore the characteristics of the "most" resistant organism and the methods to eliminate them. This review describes the type of microbial flora in the untreated root canal and the root-filled canal with persistent infection

Introduction:

The bacteria of the healthy oral cavity, the bacteria of a carious cavity as well as the bacteria associated with periodontal disease and endodontic infections, are seen to reside within biofilms. Biofilms are the preferred method of growth for many and perhaps most species of bacteria.[1] Most of the pathologies of the pulp and the periapical tissues are directly or indirectly related to the microorganisms. One of the most important features of a biofilm is that embedded bacteria display a marked decrease in susceptibility to antibacterial agents and to host defense systems. There are several mechanisms of resistance of bacterial endodontic biofilms to antibiotics. This review of "Endodontic Biofilm" tries to describe microorganisms in endodontic biofilm which are of the resistant root canal microflora.[1]

Bacterial Pathways Into The Pulp:

Microbes and their products may reach the pulp through several pathways.

These include the following: 1] Dental caries 2] Pulp Exposure 3] Through dentinal tubules 4] Through periodontal ligament and gingival sulcus 5] Anachoresis 6] Through coronal leakage and faulty restoration.[2]

Endodontic Microflora:

Most of the bacteria in an endodontic infection are strict anaerobes. The flora is usually polymicrobial, dominated by obligate anaerobic bacteria. Gram-positive organisms are found in approximately 75% of the samples; the most predominant are streptococci (28%), staphylococci (15%), corynebacteria (10-25%), yeasts (12%), and others. The Gram-negative bacteria (24%) include spirochetes (9-12%), neisseria (4%), bacteroides (7%), fusobacterium (3%), pseudomonas (2%), coliform bacteria (1%), and others.[2]

Other species include dark-pigmented bacteria such as peptostreptococcus, Eubacterium, Fusobacterium and Actinomyces. It is established that Prevotella Nigrecens is the commonly identified dark pigmented bacteria from root canals and the periapical abscesses of

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endodontics origin. Gram-negative 'black pigmented bacteria' have received a lot of attention in the field of endodontics.[3]

Types of Endodontic Infections

Endodontic infections can be classified as intraradicular and extraradicular according to the anatomic location of infection in relation to the root canal.

I] Intraradicular Infection

Primary intraradicular infections:

Microorganisms that initially invade and colonize the necrotic pulp tissue cause primary intraradicular infection. Primary infections are characterized by a mixed consortium composed of 10 to 30 bacterial species and 10^3 to 10^8 bacterial cells per canal. The involved microbiota is conspicuously dominated by anaerobic bacteria, but some facultative or microaerophilic species can also be commonly found in primary intraradicular infections.[4]

Secondary Intraradicular Infections:

Microorganisms that were not present in the primary infection but that were introduced into the root canal system at some time after professional intervention cause secondary intraradicular infections. The entry can be during treatment, between appointments, or even after root canal filling. Species involved can be oral or nonoral microorganisms, depending on the cause of infection.[4]

Persistent Intraradicular Infection:

Microorganisms that can resist intracanal antimicrobial procedures and endure periods of nutrient deprivation in a prepared canal cause persistent intraradicular infections. This is also termed recurrent infection. Involved microorganisms are remnants of a primary or secondary infection. E faecalis are predominant and is a persistent organism. It is commonly found in a high percentage of root canal as a single organism or as a major component of the flora. Persistent and secondary infections are clinically indistinguishable and are responsible for persistent exudation, persistent symptoms interappointment exacerbation and failure of endodontic treatment characterized by persistent apical periodontitis.[5]

II] Extraradicular Infection:

Microbial invasion of the inflamed periradicular tissue is invariably a sequel of interradicular infection. Acute alveolar abscess is an example of extraradicular extension or a sequel to interradicular infection. Sometimes extraradicular infection can be independent of intraradicular infections. For example, apical actinomycosis caused by Actinomyces species and P. propionicum is a pathological disease which can be treated only by periapical surgery.[6]

Microbiological Diagnostic Techniques

They are based on the specificity of the antigen-antibody reaction. They detect the immunoglobulins specific to target the microorganisms. The reaction can be visualized using a variety of techniques and reactions, including direct and indirect immun-ofluorescence tests, flow cytometry, and enzyme linked immunosorbant assay (ELISA).

Molecular Biology Methods

The limitations of culture method have led to the introduction of molecular method for identifying uncultivable species. This method relies on the fact that every living being, including microorganisms have signature sequences in their genes that can be used as targets for precise identification and phylogenetic classification. A number of bacteria, such as *Bacteroids and Treponemas* could be identified from root canals following molecular methods.[7]

Microbial community structures can be analyzed via fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RELP). Fluorescence in situ hybridization (FISH) can measure abundance of target species and provide information on their spatial distribution in tissues. Among other applications, DNA-DNA hybridization arrays, species-specific PCR, nested PCR, multiplex PCR, and quantitative real-time PCR can be used to survey large numbers of clinical samples for the presence of target species.[8.9]

Endodontic Biofilms

Biofilm is defined as a community of micro colonies of microorganisms in an aqueous solution that is surrounded by a matrix made of glycocalyx, which also attaches the bacterial cells to a solid substratum. Biofilms is one of the basic survival methods employed by bacteria in times of starvation.

Endodontic biofilms mode of bacterial growth offers other advantages such as (1) Resistance to antimicrobial agents (2) Increase in the local concentration of nutrients (3) Opportunity for genetic material exchange (4) Ability to communicate between bacterial populations of same and/or different species, and (5) Produce growth factors across species boundaries.

Endodontic biofilms could be classified as follows:

1] Intracanal biofilms that are generally present on the root canal dentine of an endodontically infected tooth.

2] Extraradicular microbial biofilms that are formed on the root (cementum) surface adjacent to the apex of endodontically infected teeth.

3] Periapical microbial biofilms that are isolated biofilms found in the periapical region of an endodontically treated tooth.

4] Biomaterial centered infection that is formed when bacteria adheres to an artificial biomaterial surface such as root canal obturating materials, thereby forming a biofilm. These biofilms could be intraradicular or extraradicular, depending on the apical extent of the obturation.[3]

Bacteria in a biofilm state show distinct capacity to survive tough growth and environmental conditions. This unique capacity of bacteria in a biofilm state is due to the following features: (1) Biofilm structure protects the residing bacteria from environmental threats. (2) Structure of biofilm permits trapping of nutrients and metabolic cooperatively between resident cells of same species and/or different species. (3) Biofilm structures display organized internal compartmentalization, which allows bacterial species with different growth requirements to survive in each compartment. (4) Bacterial cells in a biofilm community may communicate and exchange genetic materials to acquire new traits.[3]

Some microbial virulence factors implicated in apical periodontitis[10]

Colonization of root canal

- Surface components for adhesion and co aggregation
- Enzymes to get nutrients
- Microbial food chains

Evasion of host defenses

- Location
- Immunoglobulin-degrading proteases
- Complement-degrading proteases
- Capsules
- Inhibition of phagocytes

Direct tissue damage

- Proteases and other enzymes
- Cytotoxic metabolic products
- Lipopolysaccharide endotoxins

Indirect tissue damage due to inflammatory response to the microorganisms

- Cytokines
- Proteases and other enzymes from host cells.

The necessary components in the elimination of endodontic infection are:

(i) Host defense system,

(ii) In some cases, systemic antibiotic therapy,

(iii) Chemo mechanical preparation and irrigation,

(iv) Local root canal disinfecting medicaments,

(v) Permanent root filling and

(vi) Permanent coronal restoration

Summary and conclusion:

Most of our current knowledge about the microbial behavior of root canal bacteria originates from research using pure cultures, grown in nutrient-rich media under optimal conditions, extrapolation of results from such conventional studies to the real-life situation can be highly misleading. Thus, one future challenge for research in endodontology is to assess virulence expression in vivo and in situ models with microenvironments resembling the real-life condition in the root canal.

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