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Endodontic Bio ilm - An Enigma to the Dentist: A Review Article

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Abstract:

Microbiota are found in highly organized and complex entities, known as biofilms, the characteristics of which are fundamentally different from microbes in planktonic suspensions. Bacterial etiology has been confirmed for common oral diseases such as caries and periodontal and endodontic infections. Root canal infections are biofilm mediated. The biofilm community not only gives bacteria effective protection against the host's defense system but also makes them more resistant to a variety of disinfecting agents used as oral hygiene products or in the treatment of infections. Understanding the virulence of these endodontic microbiota within biofilm is essential for the development of novel therapeutic procedures for intracanal disinfection.

Keywords: Biofilm, Endodontic, Microorganism, Intracacanal

1 INTRODUCTION:

Biofilm can be defined as a sessile multi-cellular microbial community characterized by cells that are firmly attached to a surface and enmeshed in a self produced matrix of extracellular polymeric substances. [1, 2] These are very prevalent in the apical root canals of teeth with primary and post-treatment apical periodontitis. [3] It can be categorised as intracanal biofilms, extra radicular biofilms, periapical biofilms and biomaterial centered infections. [4] The infected root canal harbors a polymicrobial population of aerobic, anaerobic, Gram-positive and Gram-negative bacteria in a biofilm mode of growth. Bacterias involved are *E. faecalis*, Coagulase–negative *Staphylococcus, S. aureus, Streptococci, P.aeruginosa, fungi, F. nucleatum, P. gingivalis, T. forsythensis, Actinomyces species* and *P. Propionicum.* These are commonly isolated by culture, microscopy, immunological methods and molecular biology methods [3] . The microbial communities grown in biofilm are remarkably difficult to eradicate with antimicrobial agents. Therefore, different antimicrobials ranging from antimicrobial irrigants to advanced antimicrobial methods such as lasers, photoactivated disinfection, and nanoparticles are employed in the management of infected root canal systems. [4]

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2 DISCUSSION:

Bacteria which are known to be the main agents of tooth decay [5] always show the ability to aggregate in adherent microbic communities. The biofilm forms on any surface that comes in contact with natural liquids. The formation of biofilm follows a series of developmental stages. [6]

Stages in biofilm formation:

Biofilms can be formed on any surface that is bathed in a nutrient-containing fluid. The three major components involved in biofilm formation are bacterial cells, solid surface and fluid medium. [3, 4] Formation of biofilm includes four stages:

1. Deposition of conditioning film
2. Adhesion and colonization of planktonic microorganisms
3. Bacterial growth and biofilm expansion
4. Detachment of biofilm microorganisms into their surroundings.

Stage I : Deposition of conditioning film:

The earliest stage of biofilm formation involves the adsorption of inorganic and macromolecules in the planktonic phase to the surface, leading to the formation of a conditioning film. This film contains proteins and glycoproteins from saliva and gingival crevicular fluid along with some secreted microbial products. [4]

Stage II: Adhesion and colonization of planktonic microorganisms:

Attachment may be strengthened through polymer production and unfolding of cell surface structures. [6]

Stage II involves three phases:

1.Microbes are transported to substrate surface and get attached

2.Initial non specific microbial-substrate adherence phase.

3.Specific microbial–substrate adherence phase [3]

Stage III : Bacterial growth and biofilm expansion:

The third stage involves multiplication and metabolism of attached microorganisms that ultimately will result in a structurally organized mixed microbial community. At the end of this stage biofilm is seen as corncob structure. [6]

Stage IV: Detachment of biofilm microorganisms into their surroundings:

Detachment of biofilm microorganisms is of two types – seeding disposal and clumping dispersal. [7]

3 METHODS TO EXAMINE BIOFILM:

Traditionally endodontic bacteria have been studied by means of cultivation based techniques. The past decade has brought many advances in methods based on detection and analysis of microbial nucleic acids. Findings from cultivation based method with regard to the microbiota living in diverse ecosystem have been supplemented and significantly expanded with molecular biology techniques.

Various methods used to examine biofilm are:

A. Culture

B. Microscopy

C. Immunological methods

D. Molecular biology methods

A. Culture [8, 9]:

Culture is defined as the process of propagating microorganisms in the laboratory and provide them with proper environmental conditions. Ingredients necessary for microbial pathogens can be supplied by living systems or artificial means.

A culture medium or growth medium is a substance in which cells or organisms can grow.

Advantages:

1. Broad-range nature, identification of unexpected cases.
2. Allow quantification of all major viable microorganisms in the sample.
3. Antimicrobial susceptibilities of the isolates is determined
4. Physiological and Pathological studies are possible.

Types of culture medium [?]:

1. According to the consistency:

- Liquid media.
- Semi-solid media.
- Solid media.

2. According to the constituents:

. Simple media-nutrient broth (peptone, meat extract, NaCl and H2O).

. Complex 2% agar

. Synthetic (peptone water medium)

. Special - a) Enriched media – blood agar, chocolate agar

b) Enrichment media – tetrathionate broth.

3. Aerobic media or anaerobic media:

. Aerobic media : Bower's thioglycolate broth

. Anaerobic media: fat free minced cooked meat in broth with a layer of sterile Vaseline.

Common ingredients of culture media [10]:

1. Water.
2. Agar.
3. Casein hydrolysate.
4. Peptone

Anaerobic bacterial techniques [10]:

They are important in endodontics since strict aerobic bacteria are not present in the root canals. The safest way to protect anaerobic bacteria is to avoid exposure to O_2 during the lab work.

Two methods have made this possible:

1) The prereduced anaerobically sterilized technique by Hungate. Simplified and further developed by Moore. It is based on production of low reduction oxidation potential by gassing the media with O_2 free gas and affording protection from oxygenation during sterilization and subsequent handling.

2) To use anaerobic glove box. The atmosphere in this box is usually a mixture of N_2-90%, CO_2-10% and H_2O-10%.

Limitations of culture technique:

1. Impossibility of culturing a large number of bacterial species.
2. Not all viable bacteria can be recovered.
3. Once isolated, bacteria require identification using a number of techniques.
4. Misidentification of strains with ambiguous phenotypic behavior.
5. Low sensitivity.
6. Strict dependence on the mode of sample transport.
7. Sample require immediate processing.
8. Costly, time consuming, and laborious.

Reasons for bacterial 'unculturability:'

1. Lack of essential nutrients or growth factors in the artificial culture medium.

2. Overfeeding conditions
3. Toxicity of the culture medium itself
4. Production of substance inhibitory to the target microorganisms by other species present in a mixed consortium
5. Metabolic dependence on other species for growth
6. Disruption of bacterial intercommunication systems introduced by separation of bacteria on solid culture media.
7. Bacterial dormancy or 'viable but non-cultivable state'.

B. ***Microscopy [11]:***

Direct microscopic examination represents a quick, easy and inexpensive means of screening microbial samples for major morphotypes and staining patterns. However, microscopy has limited senstivity and specificity to detect microorganisms in clinical samples.

- Scanning electron microscope
- Environment scanning electron microscope
- Transmission electron microscopy
- Confocal laser scanning microscope (CLSM)
- Epifluorescence microscopy

C. ***Immunological methods [12]:***

Immunological methods employ antibodies that recognize specific microbial antigens to directly detect target species.

Antibodies targeting host immunoglobulins specific to a target species can also be used for indirect detection assays. The reaction can be visualized using a variety of techniques and reaction including direct and indirect immunofluorescence, flow cytometry and ELISA.

Advantages:

1. Rapid –no more than a few hours to identify a microbial species.
2. Easily standardized
3. Low cost
4. Detect dead micro-organisms.

Limitations:

1. Detect only the target species
2. Low sensitivity
3. Specificity is variable and depends on the type of antibodies

D. ***Molecular biology methods:***

The basic strategy for diagnostic molecular microbiology is using microbial DNA or RNA to detect a relatively short sequence of nucleotide bases that is unique to the organism being detected or identified. This is accomplished by using a complementary sequence of nucleotide bases known as the probe or primer.

There are plethora of molecular methods for the study of microorganisms such as:

PCR (Polymerase Chain Reaction) / Genetic Xeroxing [13, 14]:

Principle: The PCR method is based on the in vitro replication of DNA through repetitive cycles of denaturation, primer annealing and extension steps carried out in automated devices called thermocyclers.

Method: There are several methods to check if the intended PCR product was generated. The most commonly used method for detecting PCR product is electrophoresis in an agarose gel.

Uses: PCR has unrivaled sensitivity, it can detect as few as 1 to 10 bacterial cells in a sample, making it atleast 10 to 100 times more sensitive than any other scientific method.

Advantage:

PCR can have remarkable specificity because each distinct microbial species has unique DNA signature sequence.

Derivatives of PCR:
Touchdown PCR [15]
Nested PCR [14, 16]
Multiplex PCR [17]
Reverse transcriptase PCR [18]
Real-Time PCR [19]
PCR based microbial typing [20, 21]
Broad-Range PCR [22, 23]

Denaturing Gradient Gel Electrophoresis (DGGE):

DNA fragments of the same length but with different nucleotide sequence can be separated in polyacrylamide gels containing a linearly increasing gradient of DNA denaturants. [24, 25]

Terminal Restriction Fragment Length Polymorphism (T – RFLP):

It provide insight into the structure and function of bacterial communities. T-RLP analysis measures the size polymorphism of terminal restriction fragments from a PCR amplified marker. [26, 27]

DNA – DNA Hybridization:

It is the process of annealing the complimentary bases of two single stranded DNA molecules. It employs labeled DNA molecules that can locate and bind to a target sequence forming a new duplex molecule. The labeled duplex can then be detected [28] .

Types of DNA-DNA hybridization:
Checkerboard DNA-DNA hybridization [29]
DNA microarrays [30]

FISH (Fluorescence In Situ Hybridization):

This method uses fluorescently labeled rRNA probes and fluorescence microscopy to detect intact microbial cells directly in clinical specimens. It gives information about pres-

ence, morphology, number, organization and spatial distribution of microorganisms. [31, 32]

4 *MICROBES IN ROOT CANAL INFECTION: [33]*

Almost 700 bacterial species can be found in the oral cavity, with any particular individual harboring 100–200 of these species. Infection progress to apical once the root canal is infected coronally until bacterial products or bacteria themselves got the capability to infect the periapical tissues, which leads to apical periodontitis. Endodontic infections have a polymicrobial nature, with obligate anaerobic bacteria conspicuously dominating the microbiota in primary infections. Intra-radicular and extraradicular infections are associated with many microorganisms which are involved in persistent infection

A. Intraradicular infections:

Primary intraradicular infections are associated with following organisms.

B .melaninogenicus P. intermedia, P. nigrescens, P. tannerae, P. multissacharivorax, P. baroniae, P. denticola, P. endodontalis and P. gingivalis.

Tannerella forsythia D. Pneumosintes , D. invisus , F. nucleatum, F. periodonticum, T. denticola, T. sacranskii, T. parvum, T. maltophilum, T. lecithinolyticum, P. alactolyticus, F. alocis, Actinomyces spp, P. propionicum, Olsenella spp, S. exigua, M. timidum, Eubacterium spp., P. micra (previously called Peptostreptococcus micros or Micromonas micros), Streptococcus spp. which include S. anginosus, S. mitisi, S. sanguinis, E. faecalis.

Other microorganisms in endodontic infections

- Fungi – particularly Candida spp. (e.g.) C. albicans
- Viruses –human cytomegalovirus and Epstein–Barr virus

B. Extraradicular infections:

The extraradicular infections may or may not dependent on intraradicular infection. The dominant microorganisms present are anaerobic bacteria like *Actinomyces* spp., *P. propionicum, Treponema spp., P. endodontalis, Porphyromonas gingivalis, T. forsythia Prevotella spp. and F. nucleatum.*

C. Bacteria persisting intracanal disinfection procedures and after root canal treatment:

Microorganisms which are resistant to antimicrobial treatment will survive in the root canal even after biomechanical preparation.

The most common Gram negative anaerobic rods are : *F. nucleatum, Prevotella spp. and C. rectus.*

The most common Gram positive bacteria are : *Streptococci (S. mitis, S. gordonii, S. anginosus, S. oralis), Lactobacilli (L. paracasei , L. acidophilus), Staphylococci, E. faecalis, O. uli, P micra, P. alactolyticus, Propionibacterium spp., Actinomyces spp., Bifidobacterium spp.* and *Eubacterium spp.*

Sometimes, yeasts, commonly *C. albicans*, are also found in small amounts.

5 *IMPLICATIONS OF BIOFILM IN ENDODONTICS : [3, 4, 6]*

Primary source of biofilm formation within the root canal are the microorganisms present in the oral cavity and the anatomical complexities in the root canal system provide shelter to these microorganisms.

As bacteria in biofilm survive the unfavourable environmental and nutritional conditions, bacterial biofilm can be easily seen beyond the apex of the root. Infectious processes in root canal gain sufficient power to cause subsequent destruction of the pulpal tissues only after biofilm formation. Pulp chamber is invaded by planktonic oral organisms after some tissue breakdown, then only biofilm formation is intiated. At this point, the inflammatory lesion frontage that moves successively towards the apex will provide the fluid vehicle

for the invading planktonic organisms so that these can multiply and continue attaching to the root canal walls. The necrotic pulp tissue becomes a favorable environment for microbial proliferation due to the presence of organic residue or nutrients, which act as substrate or culture medium. Gram-negative bacteria are more frequently associated with biofilm formation than Gram- positive bacteria. Facultative or strict anaerobic microorganisms are more frequent than aerobic microorganisms, and the presence of bacilli and filaments is equivalent to that of cocci.

5.1 *Endodontic bacterial biofilms are categorized as: [34]*

- Intracanal biofilms
- Extra radicular biofilms
- Periapical biofilms
- Biomaterial centered infection

5.2 *Ultrastructure of biofilm*

A fully developed biofilm is described as a heterogeneous arrangement of microbial cells on a solid surface. [3]

5.3 *Characteristics of biofilm*

Bacteria in a biofilm can survive tough growth and environmental conditions due to presence of following unique features:

1. Biofilm structure protects the residing bacteria from environmental threats such as UV radiation, osmotic shock, metals and toxins.

2. Structure of biofilm permits trapping of nutrients and metabolic cooperativity between resident cells of same species and/or different species.

3. Biofilm structure 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.(*quorum sensing). [3]*

5.4 *Interactions in biofilm [3]*

Interaction in biofilm is of two types:

- Interaction between microorganisms and the host

Interaction between microorganisms in biofilm

Methods to eradicate biofilm:

1. Sodium hypochlorite (NaOCl)

NaOCl is the most widely used irrigating solution. It is a potent antimicrobial agent, and effectively dissolves pulpal remnants and organic components of smear layer. NaOCl ionizes in water to produce Na^+ and the hypochlorite ion, OCl^- , which establishes an equilibrium with hypochlorous acid, HOCl. Hypochlorous acid is responsible for bacterial inactivation by chlorine release. It disrupts oxidative phosphorylation and inhibits DNA synthesis. [35]

2. Chlorhexidine (CHX)

CHX gluconate has been in use for a long time in dentistry because of its antimicrobial properties its substantivity, and its relatively low toxicity. Presence of organic matter reduces capacity of CHXas its activity is ph dependent. [36]

3. QMix

2in1 is a single solution that provides one-step smear layer removal and disinfection. This saves time as compared to using 17% EDTA and 2% Chlorhexidine sequentially. antibiotic-free formula shows that upto 99.99% of *E. faecalis* can be killed, which provides the best practice irrigation protocol for proven and effective irrigation in 60 to 90 seconds. [37]

4. *Irrigation with iodine compounds like* iodine potassium iodide (IPI). [37]

5. EDTA

EDTA (17%, disodium salt, pH 7) has little if any antibacterial activity. Bacterial death occur as EDTA extracts bacterial surface proteins by combining with metal ions from the cell envelope. [38]

6. Citric acid : Citric acid is used for irrigation of the root canal. To remove the smear layer. concentrations ranging from 1% to 50% have been used. [39]

7. MTAD: Bio Pure MTAD (Dentsply, Tulsa, OK) is a mixture of a tetracycline isomer, an acetic acid and Tween 80 detergent (MTAD)—was designed to be used as a final root canal rinse before obturation. [40]

8. Tetraclean

Tetraclean (Ogna Laboratori Farmaceutici, Muggiò (Mi), Italy), like MTAD, is a mixture of an antibiotic, an acid, and a detergent. However, the concentration of the antibiotic, doxycycline (50 mg/mL), and the type of detergent (cetrimide and polypropylene glycol) differ from those of MTAD. [41]

9. Calcium hydroxide:

Calcium hydroxide, a commonly used intracanal medicament, has been shown to be ineffective at killing E. faecalis on its own, especially when a high pH is not maintained. [42]

10. Ozone

Ozone is a very powerful bactericide that can kill microorganisms effectively. A very low concentration of 0.1 ppm, is sufficient to inactivate bacterial cells including their spores. [43]

11. Electrochemically activated water

Anolyte and Catholyte solutions are 2 types of ECAs produced. [44]

12. Lasers

Infrared lasers such as CO2, Nd:YAG, diode and Erbium lasers have been used for endodontic disinfection. The laser-induced thermal effect produces an alteration in the bacterial cell wall leading to changes in the osmotic gradient and cell death. [44]

13. Photon-activated disinfection

Oscar Raab firstly reported the lethal effect of acridine hydrochloride on Paramecia caudatum. [44]

14. Antibacterial nanoparticles

Antibacterial nanoparticles have been found to have a broad spectrum of antimicrobial activity and a far lower propensity to induce microbial resistance than antibiotics. Nanoparticles synthesized from powders of silver (Ag), copper oxide (CuO), and ZnO are currently used for their antimicrobial activity. [45]

15. Herbal alternatives

Morinda citrifolia (MCJ) is an herb that has a broad range of anti bacterial, antiviral, antifungal, analgesic, anti-inflammatory, and immune-enhancing effects. [46]

16. Endovac system

It is a novel new irrigation system in which a delivery/evacuation tip is attached to a syringe of irrigant and the high speed suction of the dental chair. A small tube attaches either a macro- or microcannula to the suction. The delivery/evacuation tip places irrigant in the chamber and siphons off the excess to prevent overflow. The macrocannula is plastic with an open end that measures size 55 with a .02 taper. Stainless steel being the microcanula has 12 small, laterally positioned, offset holes in 4 rows of 3, with a closed end. As these cannulas are placed in the canal, negative pressure pulls irrigant from a fresh supply in the chamber, down the canal to the tip of the cannula, into the cannula, and out through the suction hose. [47]

17. Ultrasonic irrigation

Two types of ultrasonic irrigation have been described: one where irrigation is combined with simultaneous ultrasonic instrumentation (UI) and another so called passive ultrasonic irrigation (without simultaneous instrumentation). [47]

18. Endoactivator

The EndoActivator System is comprised of a handpiece and variously sized polymer tips. This system is sonically-driven to safely activate various intracanal reagents and vigorously produce the hydrodynamic phenomenon. [47]

19. Rotary Endodontics

This is used for cleaning and shaping of the root canal which helps in elimination of intra radicualr biofilm also. [48]

6 SUMMARY:

The infected root canal harbors a polymicrobial population of aerobic, anaerobic, Gram-positive, and Gram-negative bacteria in a biofilm mode of growth. These are commonly isolated by culture, microscopy, immunological methods and molecular biology methods. Pulp chamber is invaded by planktonic oral organisms after some tissue breakdown which initiates biofilm formation.

Biofilm bacteria might not express the drug target as they have been found to be more resistant to amoxicillin, doxycycline, and metronidazole. NaOCl, chlorhexidine, Qmix, IPI, EDTA, citric acid, MTAD, Tetraclean, ozone, Electrochemically activated water, lasers, Photon activated disinfection, antibacterial nanoparticles, herbal alternatives (turmeric, triphala), endovac system, ultrasonic and endoactivator are used for biofilm removal with varying degree of efficacy

7 CONCLUSION:

Root canal environment is a challenging locate for eliminating surface-adherent biofilm bacteria. Different antimicrobials are being employed in the management of infected root canal systems with varying success. However, sodium hypochlorite has been found to be the most effective irrigant against biofilm. However, further research on endodontic biofilms is required so as to provide a better understanding of its physiology, ecology, pathogenicity, and its response to treatment.

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