Volume : 2 | Issue : 12 | December 2013 • ISSN No 2277 - 8179

Research Paper

Medical Science

KEYWORDS :

Endodontic Biofilm - A Review

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ABSTRACT

Most of the resident oral microbial flora is consistent with dental health. The predominant microbial diseases of the oral cavity, caries and periodontal disease, develop at sites where a microbial biofilm, plaque, is already established and disease occurs with a change in the environmental conditions, the type and mix of microbial flora. Thus, changes at the tooth surface with a buildup of acidogenic or acid uric bacteria result in demineralization at the tooth surface, leading to caries. An endodontic pathogen is therefore defined as a microorganism capable of inducing the tissue destruction of apical periodontitis. This raises the question as to whether all microorganisms that inhabit the root canal space cause apical periodontitis, or whether specific organisms of the microbial flora are considered to cause the disease. Biofilms have great importance for public health because of their role in certain infectious diseases and importance in a variety of device-related infections. A greater understanding of biofilm processes should lead to novel, effective control strategies for biofilm control and a resulting improvement in patient management.

Introduction

Microorganisms have primarily been characterized as planktonic, freely suspended cells and described on the basis of their growth characteristics in nutritionally rich culture media. Rediscovery of a microbiologic phenomenon, first described by van Leeuwenhoek, that microorganisms attach to and grow universally on exposed surfaces led to studies that revealed surface-associated microorganisms (biofilms) exhibited a distinct phenotype with respect to gene transcription and growth rate.

These biofilm microorganisms have been shown to elicit specific mechanisms for initial attachment to a surface, development of a community structure and ecosystem, and detachment. In 1894, WD Miller published his findings on the bacteriological investigation of pulps. He observed many different microorganisms in the infected pulp space and realized that some were uncultivable when compared with the full range observed by microscopy, and that the flora was different in the coronal, middle and apical parts of the canal system. Due to limitations of his sampling and cultivation technique, Miller was unable to verify this observation and it was not until 1982 that this could be shown by culturing.

Kakehashi et al. (1965) exposed the dental pulps of conventional and germ-free rats to the oral cavity and reported that pulp necrosis and periradicular lesions developed only in conventional rats with an oral micro biota. Bacteria located in areas such as isthmuses, ramifications, deltas, irregularities and dentinal tubules may sometimes be unaffected by endodontic disinfection procedures.

A biofilm is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix. Van Leeuwenhoek, using his simple microscopes, first observed microorganisms on tooth surfaces and can be credited with the discovery of microbial biofilms. A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material. Noncellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix.

Biofilm Structure

Extracellular Polymeric Substances

Biofilms are composed primarily of microbial cells and EPS. EPS may account for 50% to 90% of the total organic carbon of biofilms and can be considered the primary matrix material of the biofilm. EPS may vary in chemical and physical properties, but it is primarily composed of polysaccharides. Some of these polysaccharides are neutral or polyanionic, as is the case for the EPS of gram-negative bacteria. The presence of uronic acids (such as D-glucuronic, D-galacturonic, and mannuronic acids) or ketal-linked pryruvates confers the anionic property.

This property is important because it allows association of divalent cations such as calcium and magnesium, which have been shown to cross-link with the polymer strands and provide greater binding force in a developed biofilm. In the case of some gram-positive bacteria, such as the staphylococci, the chemical composition of EPS may be quite different and may be primarily cationic. Hussain et al. found that the slime of coagulase-negative bacteria consists of a teichoic acid mixed with small quantities of proteins.

Gene transfer

Biofilms also provide an ideal niche for the exchange of extra chromosomal DNA (plasmids). Conjugation (the mechanism of plasmid transfer) occurs at a greater rate between cells in biofilms than between planktonic cells.

Quorum Sensing

Cell-to-cell signaling has recently been demonstrated to play a role in cell attachment and detachment from biofilms. The signaling is mediated by diffusible molecules which, when present in sufficient concentrations, serve to modify gene expression in neighboring microorganisms.

Biofilm formation

Thus, biofilm formation will occur at surfaces in any system that comes in contact with natural liquid. Although the structural organization of biofilms, the composition and activities of the colonizing microorganisms in various environments may be different, the establishment of a micro-community on a surface seems to follow essentially the same series of developmental stages, including deposition of a conditioning film, adhesion and colonization of planktonic microorganisms in a polymeric matrix, co-adhesion of other organisms, and detachment of biofilm microorganisms into the surroundings.

Enterococcus faecalis

Enterococcus faecalis is a microorganism commonly detected in asymptomatic, persistent endodontic infections. Its prevalence in such infections ranges from 24% to 77%. Factors that may contribute to a persistent periradicular infection after root canal treatment include intraradicular infection, extraradicular infection, foreign body reaction, and cysts containing cholesterol crystals. It is generally believed that the major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth. Enterococci are gram positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. Enterococci survive very harsh environments including extreme alkaline pH and salt concentrations. They resist bile

salts, detergents, heavy metals, ethanol, azide, and desiccation. They can grow in the range of 10 to 45°C and survive a temperature of 60°C for 30 min. E. faecalis is able to suppress the action of lymphocytes, potentially contributing to endodontic failure.

E. faecalis in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide for over 10 days. E. faecalis is able to form a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than nonbiofilm producing organisms. 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.

The following reasons have been proposed to explain why E. faecalis is able to survive intracanal treatment with calcium hydroxide:

(a) E. faecalis passively maintains pH homeostasis. This occurs as a result of ions penetrating the cell membrane as well as the cytoplasm's buffering capacity.
(b) E. faecalis has a proton pump that provides an additional means of maintaining pH homeostasis. This is accomplished by "pumping" protons into the cell to lower the internal pH.
(c) At a pH of 11.5 or greater, E. faecalis is unable to survive.

Conclusion

Our challenge as endodontic specialists is to implement methods to effectively eliminate this microorganism during and after root canal treatment. Currently, use of good aseptic technique, increased apical preparation sizes, and inclusion of full strength sodium hypochlorite and 2% chlorhexidine irrigants are the most effective methods to eliminate E. faecalis. Recent studies have helped us better understand E. faecalis and the mechanisms that enable it to cause persistent endodontic infections.

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