Dental Plaque Biofilm: An Invisible Terror in the Oral Cavity

Sangeeta Singh[1], **Parul Sharma**[2] and **A.K. Shreehari**[1]

[1] Dept. of Dental Surgery, Armed Forces Medical College, Pune-411040, India
[2] 21 Air Force Dental Centre, Air Force Station, New Delhi, Race Course, New Delhi -110010, India

Biofilms form on most surfaces exposed to the natural environment. The oral cavity being continuously exposed to saliva forms the perfect habitat for biofilm formation. Recent advancements in the field of molecular diagnosis have led us to the understanding that there are more than 500 species of microorganisms in the oral cavity. Thus the oral cavity presents a structurally and dynamically complex ecosystem favouring the formation of dental plaque, which has been redefined as a host associated biofilm. It is initially formed by the bacterial adherence to both the tooth as well as prosthesis and later physical and physiological interactions among different species in the microbial mass. Bacteria found in the biofilm may be influenced by external environmental factors that may be host mediated. The biofilm allows bacteria to co aggregate with both facultative and obligate anaerobic species facilitating their survival .eg. F.nucleatum + P.nigrescens & P.gingivalis. Recent analysis of > 13000 plaque samples of 40 SG microorganisms using a DNA hybridization methodology was used to define complexes of periodontal ligament microorganisms (Socransky, 1998). From the pioneering experimental gingivitis experiments of Loe et al (1965) it can be deduced that supragingival plaque is the main etiological factor for gingivitis. Though not all gingivitis progresses to periodontitis, the presence of gingivitis resulting from the inflammatory changes of supragingival plaque is a strong predisposing factor for disease progression. Gingivitis provides the ecological niche for changes in the ecology of plaque which may become more pathogenic, could lead to periodontal destruction (Christenson et al, 1989). Biofilm provides resistance to antimicrobial agents, allows limited diffusion of substance in the biofilm matrix. Thus plaque which is a host derived biofilm is the key to understanding the etiopathogenesis of periodontal disease which can help develop better treatment protocols aimed at preventing recurrence of periodontal disease.

Keywords: Dental plaque; Quorum sensing; Periodontal pathogens

1. Introduction

Ever since Robert Hooke and Anton Van Leeuwenhoek first discovered microorganisms, microbes have been found to be almost omnipresent. They may be living harmoniously in a symbiotic relationship in oral cavity in new born infants within few hours after birth. Over a period of time abundant, diverse and complex microbes inhabit the mouth. Oral cavity provides an ideal ecological environment for bacteria to thrive. It provides moist surface with abundance of nutrition, ideal temperature, non-shedding tooth surface as well as differential redox potential in gingival sulcus/periodontal pocket area for sustenance of both aerobic and anaerobic microbes. Even prosthesis surface of removable as well as fixed partial dentures and implant surface provide niches for microbial growth by formation of biofilms. Costerton and colleagues proposed the term “biofilms” in 1978.

Biofilm is defined as microbially derived sessile community, which is characterized by cells that are irreversibly attached to a substratum or to each other and is embedded in a matrix of extracellular polymeric substances that they have produced. Growth rate and gene transcription changes are usually evident as a result of altered phenotype [1].Bacteria in biofilm assist growth of each other and multiply and cause disease in human tissues by producing toxic products as well as altering host immune response and becoming resistant to antibiotics. Infected dental tissues show presence of biofilms and planktonic bacteria are found to be almost completely absent [2]. Dental caries and periodontitis are the most common oral infections where dental plaque biofilm has been implicated. The inherent resistance of biofilms to antibiotics explains the enigma in which a relatively accessible tissue as periodontium, is usually a challenge to treat, with various conventional techniques available.

2. Composition of biofilm

Oral cavity forms an ideal atmosphere for development of dental plaque biofilms. About 97% of biofilm matrix is water [3]. More than 700 species of microbes have been identified in oral plaque. These microbes are present on all accessible surfaces of the mouth. The resident microflora helps in maintaining the health of the host by preventing potentially pathogenic, microbes from multiplying and growing in oral cavity. This is known as colonization resistance. Normal commensal bacteria also regulate the inflammatory host response. Destruction or modification of the resident oral microflora can result in overgrowth of pathogenic microbes. The biofilm matrix also contains exopolysaccharides produced by bacteria, various polymers, nutrients and metabolites and products formed from cell destruction. The composition of cellular material is about 2–15%, while the majority component is extracellular.

The dental plaque biofilm formation occurs in an orderly manner [4]. The teeth rapidly becomes coated with a variety of salivary constituents like albumin, glycoproteins and proline-rich proteins forming pellicle within few

minutes of cleaning of tooth surface either personally or professionally. This pellicle provides many different receptors which are recognized by colonizing bacteria. The primary colonizers of microorganisms attach to these receptors. They are mostly gram positive cocci , some gram-positive rods and filaments and few gram-negative cocci. The gram-positive cocci species which are amongst the first to colonize include various species of Streptococcus like S mutans, S mitis, S sanguis and S oralis, other bacteria including Rothia dentocariosa, and Staphylococcus epidermidis, Gram-positive rod and filament like Actinomyces israelis and Actinomyces viscosus. These early colonisers multiply and and make conditions suitable for colonisation by modifying the environment resulting in colonization by more fastidious bacteria, mostly obligate anaerobes. Cell surface like fimbriae and flagella present in many microbes help in adhesion amongst each other as well between microbes and host pellicle. Attached microbes secrete exopolymers such as glucans, which form the biofilm matrix that acts as a scaffold for the biofilm, and is biologically active and able to retain molecules within plaque.

Over a period of time a diverse community of interacting microorganisms develops. Socransky et al (1998) [5] examined more than 13,000 subgingival plaque samples and demonstrated presence of specific microbial groups within dental plaque. Six closely associated groups of bacterial species were recognized. The early colonizers included Actinomyces forming the blue complex, a yellow complex consisting of members of the genus Streptococcus, a green complex consisting of Capnocytophaga species, Aggregatibacter actinomycetemcomitans serotype a, Campylobacter, Eikenella corrodens and a purple complex consisting of Veillonella parvula and Actinomyces odontolyticus. As the biofilm becomes more mature, anaerobic bacteria live deeper within the thick biofilm which protects them from environment in the oral cavity which is predominantly aerobic. The microorganisms which are primarily secondary colonizers form the green, orange or red complexes. These are mostly pathogenic. The Red complex consists of Porphromonas gingivalis, Tannerella forsythia, and Treponema denticola. The red complex is of particular interest because it is often seen in patients who have bleeding on probing, an important parameter of active destructive phase in periodontal diseases (Fig1).

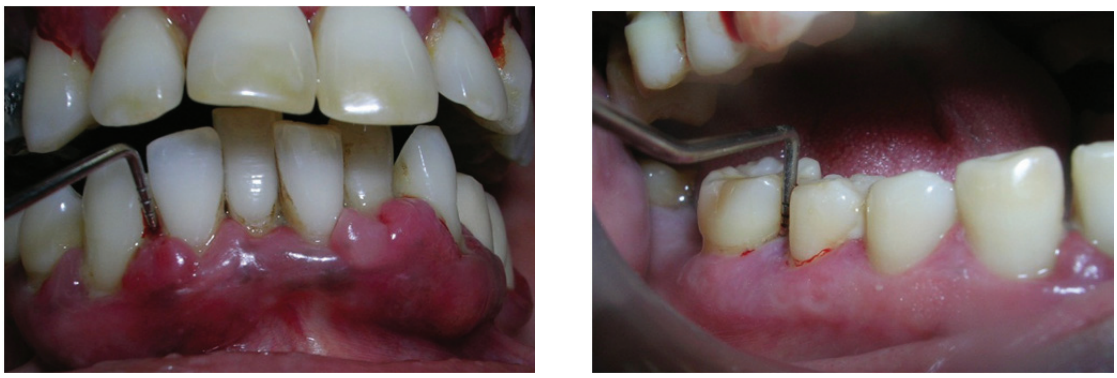

Fig. 1 The destructive phase of periodontal disease characterized by bleeding on probing and attachment loss.

3. Cell to cell communication (quorum sensing)

The bacterial cells continue to divide until a three-dimensional biofilm forms containing different species of microbes that are arranged in specific manner and are functionally organized. The development of the biofilm is facilitated by communication between bacterial species. Pheromones released by microbes allow cell-to-cell communication which helps the biofilm-forming bacteria unify against external stress. This communication system is called Quorum Sensing (QS). Each individual bacterium produces signaling molecule which is called inducer and each bacteria has a receptor for the inducer. Binding of inducer to the receptor activates the transcription of certain genes including those required for self-activation of inducer. QS allows bacteria to relate with each other and show quick response to environmental changes like presence of nutrients, toxic products or other bacteria. Gram-negative bacteria have numerous QS systems having signal molecules as acyl homoserine lactone that activate transcriptional regulatory protein. In contrast, QS systems in Gram-positive bacteria typically use signal molecules that are secreted peptides and a regulatory system to detect the peptide and accelerate the changes in gene expression [6].

Bacteria grown in biofilms are more resistant to antibiotics than are the same cells grown in a planktonic state showing 1000 to 1500 time's greater resistance. Resistance of bacteria to antibiotics is affected by various physical characteristics like their nutritional status, growth rate, temperature and pH as well as by prior exposure to lesser concentrations of antimicrobials [7]. Slower rate of growth of bacterial species in biofilms results in higher resistance. Deeper cells in the biofilm have different hydrogen ion concentration or redox potentials, than cells at the periphery or cells growing planktonically. This results in slower growth rates of these deeper cells allowing them to survive better than faster-growing cells at the periphery in presence of antimicrobial agents. The matrix of a biofilm has certain

chemical properties that can retard diffusion. Highly charged or reactive agents do not reach the deeper zones of the biofilm because biofilm acts as an ion-exchange resin and removes such molecules from solution [8].

Detection of biofilm: Standard plaque disclosing agents contain dyes like erythrosine and fluorescein or sanguinarine salts which stain plaque biofilm in oral cavity in bright colours. These dyes are not specific and stain soft tissue also (Fig 2).

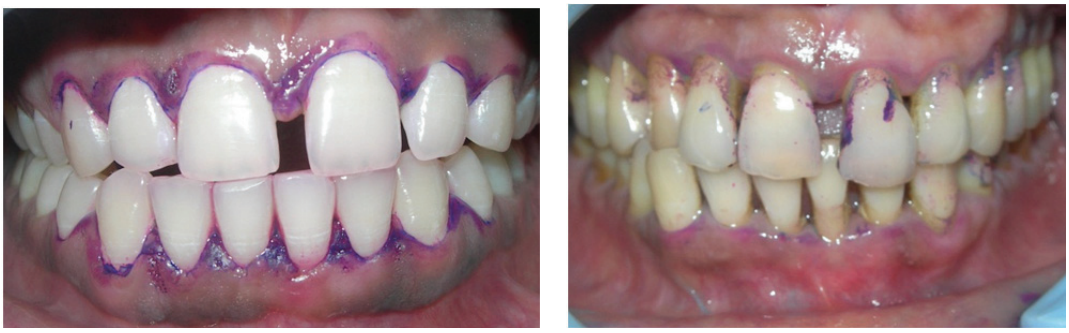

Fig. 2 Dental plaque biofilm made visible after application of disclosing agent.

Bacterial culture was considered the gold standard and is a common quantification method that allows the determination of the number of culturable cells but this technique is both labor and time-intensive (fig 3). Culture methods have another shortcoming that it is useful only for counting viable cells that are capable of growth in various nutrient media. They are not suitable for detecting non-viable cells. Moreover all viable cells may not be culturable as many require special growth conditions.

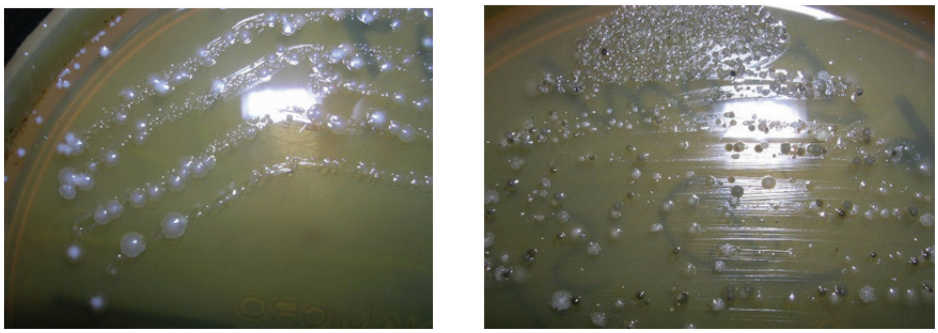

Fig. 3 Periodontal pathogens cultured from dental plaque biofilm on Dentaid media using anaerobic culturing.

Both viable and nonviable cells can be detected using technique like the polymerase chain reaction (PCR) in a shorter period of time. PCR is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence hence making detection easier (Fig 4). Real-Time PCR uses various fluorescent dyes which react with the amplified product and can be measured by an instrument. This also facilitates the quantification of the DNA. Direct light and electron microscopic observation clearly shows that biofilm bacteria are enveloped in large amounts of exopolysaccharide matrix which is fibrous and has high water content and whose chemical composition was specific for that particular species [9]. The disadvantage of electron microscopy is that sample preparation results in dehydration resulting in collapse of biofilm. As a result a true three dimensional picture is not evident. Confocal Scanning Laser Microscope (CSLM) allows the visualization of fully hydrated samples and has therefore revealed the elaborate three-dimensional structure of biofilms. CSLM has been used very effectively to monitor biofilm development in flow cells. Flow cells are small continuous-flow systems with a viewing port that allows direct observation of the biofilm without disrupting the community [10]. Fluorescently labeled chemical probes like lectins are used as non-invasive probes to localize carbohydrate-containing polymers within biofilms. Identification of specific cell types within the matrix using green fluorescent protein has been found to be highly useful technique for detection of microbes in biofilm [11]. Various microsensor probes can be used to determine dissolved oxygen and pH.

4. Dental plaque biofilm: Role in etiopathogenesis of periodontal disease

It is well accepted that dental plaque biofilm plays a major role in the initiation and progression of periodontal disease. However, extent of breakdown of the periodontal tissues depends upon the composition of the plaque. The factor that

contributes to the breakdown of periodontal tissues is the periodontopathic bacterial invasion of periodontal tissues. Periodontopathogens residing in the dental plaque biofilm express different proteases which are important in the initiation and progression of periodontal tissue destruction [12]. Under normal conditions, the synthesis and degradation of connective tissue is well balanced. Once the balance between the matrix metalloproteinases and their inhibitors; i.e. tissue inhibitors of MMPs (TIMPs) is disturbed, it results in connective tissue matrix breakdown leading to periodontal destruction, signalling progress of disease[13].Increased matrix metalloprotease levels and decreased levels of tissue inhibitors of matrix metalloprotease is a hallmark of severe periodontitis [14].

Dental plaque biofilm has a polymicrobial composition that remains relatively stable in health. The species from diseased sites are different from those found in healthy sites, although the putative pathogens can often be detected in low numbers at normal sites. In dental caries, there is a shift toward dominance by acidogenic species such as streptococcus mutans and lactobacilli. The present day concept of microarrays by which the presence of all of the species of microorganisms in plaque can be determined may help in identification of microbial profiles (molecular "signatures") that correlate with caries or periodontal disease [15].

5. Current hypotheses to explain the role of plaque bacteria in the etiology of diseases

The role of plaque bacteria in the etiology of caries and periodontal diseases has been evaluated using two main hypotheses. The "Specific Plaque Hypothesis" suggests that only a few species are actively involved in disease [16]. The "Non-Specific Plaque Hypothesis" suggested that disease is the outcome of the activity of the total plaque microflora [17]. An alternative hypothesis has also been proposed i.e. the "Ecological Plaque Hypothesis" that explores the key elements of the earlier two hypotheses [18]. The latest concept of plaque-mediated diseases is attributed to imbalances in the resident microflora resulting from enrichment within the microbial community of these "oral pathogens."

6. Targeting Biofim: Key to prevention of Periodontal disease

From the pioneering experimental Gingivitis experiments of Loe et al [19] it can be deduced that supra gingival plaque biofilm is the main etiological factor for gingivitis. The presence of gingivitis resulting from the inflammatory changes of supra gingival plaque is a strong predisposing factor for disease progression. Gingiva provides the ecological niche for changes in the ecology of plaque which may become more pathogenic, and could lead to periodontal destruction. Longitudinal studies have shown that oral prophylaxis, oral hygiene instruction and motivation leading to excellent individual plaque control can result in cessation of progression of PDL disease in the populations studied [20]. Proper supragingival plaque control can prevent breakdown of healthy sites and even arrest lesions. Dental plaque biofilm control is one of the key elements of practice of dentistry. Good plaque control facilitates the return to health for patients with gingival and periodontal disease, prevents tooth decay and preserves oral health for a lifetime. Every patient in every dental practice should be in a dental plaque biofilm control program - a healthy patient for preservation of oral health, in periodontal disease for optimum healing following treatment and post treatment, to prevent recurrence.

7. Methods of dental plaque biofilm control

“Forty years of experimental research, clinical trials and demonstration projects in different geographical and social settings have confirmed that effective removal of dental plaque biofilm is essential to periodontal health through life.”

7.1 Policy statement adopted in the 1998 European workshop on mechanical plaque control

Mechanical plaque control continues to be the mainstay of controlling gingivitis and therefore the occurrence/re-occurrence of periodontitis. Ineffectiveness of mechanical cleaning to specific sites using a toothbrush and limited / lack of use of interdental cleaning by many individuals hampers the long term success and benefits from oral hygiene measures. Therefore chemical dental plaque biofilm control has an important role as an adjunct to mechanotherapy in the management of gingivitis and periodontitis. Concept of chemical plaque control may be justified as a means of overcoming inadequacies of mechanical cleaning.

8. Chemical plaque control

The well-ordered and dynamic process of dental plaque biofilm formation can be interrupted, interfered with reversed or modified at several points, before the dental plaque biofilm mass complexity reaches a level where gingival health deteriorates. Chemical agents could influence plaque quantitatively and qualitatively via different processes: antiadhesive, antimicrobial, plaque removal.

Anti-adhesive: Act on pellicle to prevent initial attachment of dental plaque. e.g Delmopinol (0.1% to 0.2%). Side effects include tooth discoloration, transient numbness of mucosa (especially tongue) and burning sensation.

Anti-microbial: Inhibit bacterial proliferation, either before plaque tooth attachment or before division and bactericidal by destroying all organisms attaching/attached. E.g Chlorhexidene represents the nearest that research has come to identifying a chemical agent that works alone rather than as an adjunct to mechanical therapy in eliminating dental plaque biofilm [21].

Plaque removal (Chemical tooth brush): Hypochlorities, Enzymes

9. Systemic antimicrobial therapy

An increased interest in antibiotic therapy as an adjunct to standard periodontal treatment regimens began in the late 1970s with the realization that certain bacteria were frequently associated with the disease process. Periodontitis is quite different from most bacterial infections in that the bacterial flora present is always heterogeneous, relatively complex and varies significantly from one patient to another.

Antimicrobial therapy defined by (AAP, 1986) as the use of specific agents for the control / destruction of microorganisms either systemically / specific sites. Generally, inclusion of these agents is designed to enhance the effects of debridement and is never a substitute for professional and personal mechanical therapy. To evaluate an antimicrobial agent and its delivery system, AAP (1991) suggested 3 criteria: - Must be effective in lab against the target pathogen**,** reach the site of infection in effective concentration. Remain long enough to be effective. During the past two decades, periodontist and microbiologists have embraced periodontal antibiotic therapy as evidence for bacterial specificity in periodontitis has accumulated and strengthened. It is based on the premise that specific microorganisms cause destructive periodontal disease & the antimicrobial agent in periodontal pocket can exceed concentration necessary to kill the pathogens. The Antibiotics enter the periodontal tissue and periodontal pocket is a serum and can affect organisms outside the reach of cleaning instruments or topical anti-infective agents. They can also potentially suppress periodontal pathogens residing on tongue / other oral surfaces thereby delaying subgingival recolonization of pathogens.

10. Local drug delivery

Systemic antimicrobial therapy, although effective, involves a relatively high dose with repeated intakes over a prolonged period of time to achieve the required inhibitory concentrations in the sulcular fluid. This increases the chances of development of resistance, of alterations of the commensal flora, and of increased potential for adverse effects. Advances in the technology of drug delivery systems have resulted in a number of site-specific, controlled release methods. Local delivery systems offer the advantages of high concentrations at the target site with reduced dosage, fewer applications, and high patient acceptability [22]. Thus, adjunctive use of local drug delivery may provide a beneficial response, especially in specific areas where conventional forms of therapy might fail thus targeting the dental plaque biofilm flora. The local drug delivery systems are especially indicated for patients in maintenance phase, medically compromised patients who cannot undergo surgical therapy, institutionalized patients, localized refractory sites, and failing implants to reduce the bacterial load in the dental plaque biofilm [23]. A variety of LDD devices are available commercially and are used routinely as adjuncts to mechanical therapy for dental plaque biofilm control (Fig 5,6)

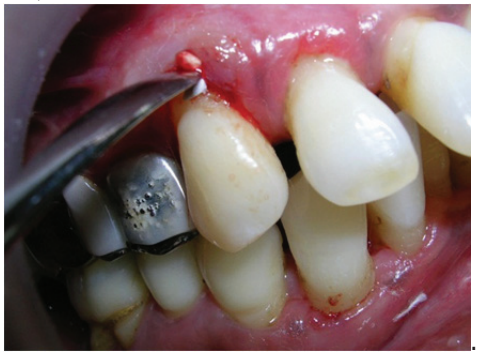

Fig. 5 Metronidazole sponge as a local drug delivery device.

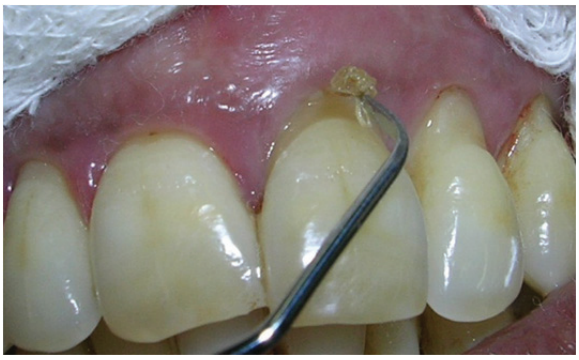

Fig. 6 Tetracycline fibers as a local drug delivery device.

11. Photodynamic therapy

Photodynamic therapy (PDT) was introduced in the 1960s and has been developing rapidly in various fields of medicine since then. PDT has been defined as "the light induced inactivation of cells, micro- organisms, or molecules [24]. As discussed earlier, periodontal bacteria in dental plaque biofilms are protected within the plaque matrix and are therefore less susceptible to systemic antibiotics. Based on the concept of using antimicrobial photodynamic therapy for the treatment of localized microbial infections, this modality of antimicrobial therapy has been tried in periodontal pocket disinfection with the aim of targeting the pathogens residing in the dental plaque biofilm. Antimicrobial photodynamic therapy acts by free radical formation and causing selective damage to the targeted organisms. Literature cites PDT as an effective means of bacterial eradication in cases of Aggressive periodontitis, Chronic periodontitits, Periimplantitis as well as residual pockets. The elimination of bacteria residing in the dental plaque biofilm using PDT in combination with conventional scaling and root planning leads to reduction in the pocket depths and gain in clinical attachment [25].

12. Conclusion

Dental plaque biofilm is invisible and appears to be innocuous till it leads to dental caries or inflammation of the periodontium which varies from mild gingivitis to a more severe and destructive form of periodontal disease. If left untreated, periodontal disease progresses into severe attachment loss and alveolar bone destruction eventually leading to loss of teeth. Untreated dental caries can lead to alveolar abscess which can be very debilitating. Thus dental plaque essentially is an invisible terror in the oral cavity and it is important to take appropriate measures for prevention of these diseases by controlling/limiting the formation of dental plaque biofilm.

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