THE BIOFILM: FORMATION AND REMOVAL

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REZUMAT
Cari și boala parodontală reprezintă bolile cu cea mai mare prevalență la om. Ambele sunt asociate cu bacteriile biofilmului dentar, care reprezintă o structură complexă, bine organizată. In biofilmul dentar au fost identificate peste 500 de specii bacteriene. Pentru sănătatea orala și generală a pacienților, formarea acestui biofilm trebuie impiedicată, prin îndepărtarea critică. Îndepărtarea sau reducerea biofilmului se face prin metode mecanice, asociate uneori cu metode chimice. În general, substanțele chemoterapeutice singure nu penetrează grosimea biofilmului, de unde rezultă importanța identificării și îndepărtării riguroase a biofilmului.

Cuvinte cheie: biofilm, boală periodontală

ABSTRACT
Dental caries and periodontal disease are among the most prevalent diseases known to man. Both are associated with the bacteria of the dental biofilm. The dental biofilm is complex, with a well-organized structure. Up to 500 bacterial species have been identified in the dental biofilm. Studies have shown that plaque accumulates rapidly on clinically plaque-free teeth. To maintain the oral and the systemic health, the development and maturation of dental biofilm should be impeded and the dental biofilm needs to be regularly and meticulously removed. The removal and reduction of the biofilm can be achieved by mechanical means or mechanical and chemical means. Current chemotherapeutics in general do not effectively penetrate thick biofilms, which underscores the importance of the identification and rigorous mechanical removal of the dental biofilm.

Key Words: biofilm, periodontal disease

INTRODUCTION
A biofilm is a complex community of bacteria adhering to an inert or living surface.1,2 Biofilms are the predominant mode of bacterial growth in nature. Many microbial species exist as sessile bacteria (attached bacteria in the biofilms), but release into dispersion small numbers of planktonic cells (free-floating single cell bacteria). At the start of the biofilm formation process, the bacteria “swim” to find an appropriate surface to adhere to. After they attach, the bacteria “move” on the surface to find other bacteria to form small aggregates. Next, the bacteria produce a large amount of matrix and the bacterial groups increase in size and thickness until they form a mature biofilm. After maturation, biofilms may release planktonic cells to begin another cycle of biofilm formation.

The step-by-step, gene regulated biofilm formation process results in a bacterial community with distinct mushroom-like structures, that consist of a variable distribution of cells and cell aggregates, the associated matrix, void spaces, and water channels that allow the diffusion of nutrients and waste products. Many bacteria are able to communicate with each other through a process called quorum sensing gene regulation.3 This process explains how the cells in a biofilm are able to activate certain genes.

Understanding the biofilm is important because the periodontal clinicians need to know the enemies in order to defeat them. Research in biofilms may help identify new strategies to combat bacterial infections. Since the quorum-sensing systems (QSS) play a critical role in the formation of biofilms, disrupting such systems should diminish the bacterial pathogenic ability.

Thus, a different approach is to use compounds that interfere with the cell-to-cell communication of the bacteria. Many superior organisms produce natural compounds to mount a chemical warfare against bacterial pathogens. Some of the compounds actually work by disrupting the QSS and have the potential to treat the bacterial infections.
Biofilm bacteria are several hundred to thousand times more resistant to antimicrobial agents than planktonic cells of the same species. This may help explain why some chronic bacterial infections are difficult to treat with antibiotics, even though *in vitro* antimicrobial susceptibility tests predict that the antibiotic will be effective.

While the mechanism of antimicrobial resistance is currently unknown, a more rationale and effective strategy of antimicrobial therapy will be identified and the mechanism is understood.

**THE STRUCTURE AND THE BIOCHEMISTRY OF BIOFILMS**

Prior to the development of dental biofilm, the salivary (or “acquired”) pellicle forms. This occurs through the adsorption of proteins from saliva onto the clean tooth surface. The acquired pellicle formation provides oral bacteria with binding sites, resulting in bacterial adhesion - the first step in the formation of the dental biofilm. Surface modifications can inhibit the development of the acquired pellicle and of the dental biofilm. Dental biofilms generally accumulate on hard and soft surfaces of the oral cavity, including non-biological surfaces such as implant surfaces, orthodontic appliances and fillings, as well as on other bacteria. Bacterial adherence is essential for the formation of dental biofilm. Following adhesion, the bacteria divide, grow and accumulate. The bacteria contain factors called adhesins that meet their counterparts receptors at the site of adhesion, resulting in the adhesion of the bacteria to the surface. Different receptors and adhesins are present on different bacteria and surfaces, such as fimbriae that act as receptors on subgingival bacteria, or collagen and residues of sialic acid and galactosyl, which display a similar function on tissue surfaces.

Adhesion by one species of bacteria may limit the amount of adhesion by another species in the same space. For a subgingival biofilm to develop, bacteria must be able to adhere to one or more subgingival surfaces. This is enabled by an early inflammatory process that creates a pseudopocket at the gingival margin, allowing for the development of the anaerobic species associated with the periodontal disease and the subsequent development of true periodontal pockets, which in turn provide extra space for the anaerobes and an oxygen-poor environment. The presence of subgingival calculus provides an excellent adhesion site for bacteria and for the retention of subgingival plaque.

Bacterial adhesion to soft tissue within the periodontal pocket may also play a role in the deeper invasion of periodontal tissues. Adhesion to the basement membrane and collagen has been also noted.

*In vivo*, the dental biofilm is colonized by up to 500 species. The bacteria are tightly bound to each other and to a solid substrate, interspersed with fluid-filled channels. The majority of these species are present in health, and the host response and its susceptibility determine the onset of the disease. Only 20 percent of periodontal disease is attributed to bacterial variances, with the host response being the key factor for most of the forms of the infectious periodontal disease. Within periodontal pockets, between 30 and 100 species can be isolated from one site, and over 300 species have been identified by cultures.

Young dental biofilm, assessed for 40 strains of bacteria, has been found to consist mainly of *Actinomyces* until between two and six hours after the formation begins. At this point, the numbers of *Streptococci* increase when compared to *Actinomyces*, especially *S. mitis* and *S. oralis*. Periodontal pathogens are found at extremely low levels in the up to six hours-old biofilm. Until day three, mostly Gram-positive *Streptococci* and rods are present. The next phase maturation involves the apparition of filamentous bacteria on the biofilm surface, which then invade the body of the biofilm after day seven.

CLSM (confocal laser-scanner method) combined with fluorescence of plaque harvested from *in situ* enamel blocks worn intraorally for five days has demonstrated that bacterial vitality increases with the depth of the biofilm and the depth of the bacteria within. CLSM has also demonstrated voids together with layers of live bacteria packed with nonvital materials of bacterial origin.
The composition of the biofilm depends upon its location and its degree of maturity.

Between three and 12 weeks after supragingival plaque starts to form, subgingival biofilm is well differentiated with predominantly Gram-negative anaerobic bacteria. Porphyromonas gingivalis, strongly associated with the infectious periodontal disease, and Treponema denticola are often found associated in dental biofilms.\textsuperscript{11,12}

The composition of subgingival plaque has been extensively researched. Socransky and Haffajee defined five bacterial complexes in the subgingival plaque. In addition, specific complexes were found to be associated with other specific complexes within the group, demonstrating relationships between bacterial species. While specific bacteria are known to be pathogenic, it is also now recognized that the presence of periodontopathogens does not predict the long-term progress of periodontitis.\textsuperscript{13}

Recent research has identified clonal sub-groups within species of bacteria, the relevance of which is not yet fully understood. It has been hypothesized that the virulence within a particular bacterial species may depend upon the clonal subtypes present.\textsuperscript{14}

The subgingival plaque is not subject to the same hygienic/therapeutic assaults as the supragingival plaque and is relatively protected from saliva and from the masticatory impacts. A further factor of resistance is the morphology of bacteria, both intraspecies and interspecies. In vitro evaluation of the biofilm formation with A. actinomycetemcomitans has found that variants with a rougher morphology resulted in more biofilm formation than in vitro, smooth variants. It was also concluded that a rough morphology in the biofilm in vivo may help protect bacteria from exterior assaults.\textsuperscript{15}

**THE REMOVAL OF THE BIOFILM**

The meticulous and regular removal of dental biofilm is important for the oral and systemic health, giving the strong associations between the presence of periodontal disease and cardiovascular disease, diabetes, respiratory diseases, disseminated infections and other conditions.\textsuperscript{16} The meticulous removal of biofilm is hindered by its relative lack of visibility, by mechanical or physical difficulties, and by its chemical resistance. The use of current antimicrobial agents is known to be effective in the outer surface where a thick dental biofilm is present with poor penetration into the inner layers of the dental biofilm, which underscores the importance of frequent and adequate plaque removal.\textsuperscript{12}

By removing the supragingival plaque in the early phase of biofilm maturation, the microbial load can be maintained at a relatively low level and the colonization by anaerobic species can be contained. In the absence of adequate oral hygiene, the subgingival plaque will develop, at which point elimination and the effective control of the bacterial environment is considerably more difficult. Daily removal of dental plaque is essential in the prevention of oral disease as well as in the continued rehabilitation and maintenance of patients with preexisting periodontal disease. Reducing the amount of biofilm and/or or selectively reducing the number and proportion of pathogenic species will help prevent caries and periodontal disease.\textsuperscript{17}

**Mechanical Removal**

In developed countries, patients brush for less than one minute on average. The education and motivation of patients to mechanically remove as much plaque as possible is a priority.\textsuperscript{18} The use of soft-bristled brushes, interdental brushes, floss, toothpicks, and rubber tips have all been recommended for the removal of plaque from the various surfaces of the teeth during home care.

Toothbrushes and tooth-brushing techniques have evolved over time. Using of a soft-bristled brush will achieve the best results for plaque removal and will help prevent abrasion associated with the use of hard-bristled brushes or overzealous brushing. While a straight horizontal brushing motion may remove the plaque, the Bass technique is well accepted as optimal. By brushing at a 45 degree angle, the brush is more able to reach under the gingival margin into the gingival crevice to remove plaque in this area. Without this, the inflammation will appear with the formation of the pseudopocket, making it a more attractive environment for the anaerobic colonization and for the further formation of a true periodontal pocket. Toothbrushes are available with ergonomically-designed handles. Some have handle designs with thumb grips that are
positioned to tilt the bristles at a 45 degree angle to the sulcus.

Electric toothbrushes are a further option. Clinical research into the effectiveness of these toothbrushes has produced widely varying and often contradictory results. The selection is based upon the preference, the ability and the willingness of patients to brush manually. Where a dexterity problem exists, the use of an electric toothbrush may be easier or more effective.

Interdental brushes, floss, and toothpicks are all available as cleaning aids. Both flossing and interdental brushing are effective with an appropriate technique. The choice will depend upon the clinician’s and patient’s preference, the compliance of the patient with the method chosen, the space available, and the patient’s dexterity.

Studies have pointed out that subgingival plaque in deeper pockets is not responsive to home-care oral hygiene measures alone. Professional cleaning (e.g., root planing and scaling, professional prophylaxis) and home care (tooth brushing and either flossing or an interdental brush, at a minimum) combined are required to remove subgingival plaque. In the absence of subgingival plaque control and therapy, supragingival plaque control does not prevent the progression of the periodontal disease.19

**Scaling & root planing**

The overall goals of periodontal treatment are to stop the progression of the disease and to obtain clinical attachment gains. Supra- and subgingival scaling are the standard non-surgical treatment for periodontal disease, and may be supplemented with systemic or local antimicrobial therapy or other adjunctive therapy.20,21 The objectives of scaling are to disrupt the dental biofilm and to remove the maximum possible amount of dental biofilm, dental calculus, periodontal bacteria, and debris from the root surfaces and soft tissue.22 A further objective is that the root surfaces be biocompatible and smooth upon completion of scaling, thereby reducing the risk of recolonization and subgingival biofilm adhesion and retention on biocompatible surfaces. The dental calculus provides a distinct prominent or rough site for the adhesion of bacteria and for biofilm retention, and also contains endotoxins.

Supra- and subgingival scaling can be performed with hand instruments or with power scalers. An alternative is a procedure combining the use of both hand instruments and power scalers. Considerations on the choice of method include efficacy, efficiency, safety, patient comfort, and ergonomics. The use of hand scalers requires great care to achieve a satisfactory result, and takes a considerable amount of time. It is now generally held that hand scalers and ultrasonic scalers are similar in their effectiveness in removing subgingival biofilm.23

Hand scalers have been found to be ineffective in removing calculus deposits in furcation areas whether an open- or closed flap technique is used.24,25 Ultrasonic scalers are considered superior to hand instruments for the treatment of moderate and advanced furcations. The precision thin tips of ultrasonic scalers are significantly thinner than the working end of the curettes, enabling them to enter narrow furcation areas.26 Traditionally, scaling and root planing used a selection of manual instruments such as curettes, chisels and hoes. Increasingly, ultrasonic scaling becomes a preferred choice for the initial periodontal treatment and for the periodontal maintenance protocols as the instruments, the comfort of the patient and of the operator improved. Clinicians can select hand scaling, ultrasonic scaling or a two-step protocol, whereby ultrasonic scaling removes deposits and hand scaling is then used for fine calculus or biofilm removal. Ultrasonic scalers have been found to be effective in removing also subgingival biofilm and calculus.27

The aggressive instrumentation of the root surface was believed to be necessary to remove the embedded endotoxins, but it is now known that endotoxins are only loosely adsorbed on the root surface and their removal requires only the light use of ultrasonic inserts. Based on these considerations, a two-step protocol involving ultrasonic scaling followed by extensive hand scaling (rather than spot touch-ups in specific areas) may offer little benefit.28,29

**Ultrasonic Scalers**

Ultrasonic scaling offers several advantages over hand scaling. It is less fatiguing and less time consuming, requires less force, results in less tooth substance loss, is more effective in areas with narrow difficult root morphology, and provides irrigation of the pocket during instrumentation. Regarding the damage of the root surface, Ritz et al. have found that hand scaling with a curette resulted in a loss of 109 microns of cementum, when compared to 12 microns of loss with an ultrasonic scaler, after 12 working strokes.30 The whole root surface must be instrumented with gentle and precise overlapping strokes of the instrument.

Piezoelectric and magnetostrictive ultrasonic scalers differ. The movement of a piezoelectric tip results from the action of the alternative electricity on ceramic discs, while the magnetostrictive ultrasonic
insert movement is obtained by metal stacks of ferromagnetic material that create a magnetic field.\(^{31}\)

**Procedure and technique of the ultrasonic removal of the dental biofilm**

Piezoelectric tips move linearly, mimicking the motion of hand scaling. Piezoelectric inserts include beveled, bladed, slim and probe-like designs, depending on each particular ultrasonic unit. For most units, several inserts are used during the procedure. The scaler inserts are mostly active on the two lateral surfaces (for Piezon® Master - EMS; Mini Piezon® - EMS; Symmetry IQ™ - Hu-Friedy; Varios 350 - Brasseler; Suprasson P-Max - Satelec/Acteon). Only these active lateral surfaces should be used against the root surface. Positioning the inserts to the tooth surface at a 90-degree angle would result in root surface damages. Narrow, slim and probe-like tips offer better adaptation to areas such as severe root curvatures and molar furcations, and improve penetration to the base of pockets.\(^{32}\)

Magnetostrictive ultrasonic inserts move elliptically. Magnetostrictive scalers (Cavitron® - Dentsply; Dual Select™ - Dentsply) use a variety of inserts shapes during scaling and root planing. Straight inserts are used for gross calculus removal, and other designs such as beavertail, chisel, probe-like, slim, furcation and curved tips are used for specific areas. As with piezoelectric inserts, the points of the inserts must not be directed towards the tooth, to prevent root damage. The force required with magnetostrictive inserts is greater than with piezoelectric inserts. Curved universal inserts are counter indicated in pockets of 4 mm or more, to prevent the gouging of the root surface. Unlike most piezoelectric units, the inserts can be used on the back, face and lateral surfaces, enabling the clinician to select the active surface depending on their angulations and the area being treated. This makes the procedure less technique-sensitive.

Currently, only the magnetostrictive scaler XO Odontogain® (XO Care, Denmark) has the instrument tips equally active on all sides, rotating elliptically in a three dimensional movement. The instrument tip oscillates at a very high frequency (42 kHz) and it moves with an amplitude of only 10-20 microns. Thin-profile inserts can be used at a higher power setting, thus a reduced risk of burnishing subgingival calculus, when compared to the use of low power, and without risking the insert breakage. Tips made of titanium also reduce this risk. When used with the appropriate technique and with appropriate inserts, the ultrasonic scaling and root planing is highly effective.\(^{33}\) One of the real advantages of ultrasonic scaling is that, unlike Gracey curettes, slim and probe-like ultrasonic insert tips can reach the vault of the furcations and other narrow morphological details for thorough instrumentation. Inserts with straight surfaces offer poorer adaptation than rounded surfaces to a concave or convex root surface. Lack of adaptation can be a reason for hand scaling following ultrasonic scaling.

**Figure 4.** The XO Odontogain (XoCare, Denmark) ultrasonic unit.

**Chemotherapeutic Reduction and Removal of Biofilm**

Chemotherapeutics can be used to reduce the number of bacteria in the dental biofilm, to reduce the numbers of specific pathogenic species, and to inhibit adhesion of microbes to the tooth surface.\(^{34}\) Essential oils, fluoride, chlorhexidine, povidone-iodine, zinc citrate, and triclosan have all been used as antimicrobial
rinses and dentifrices.

The Triclosan (the copolymer included in the Colgate Total® toothpaste) has been shown to significantly reduce the plaque and gingivitis and to inhibit the progress of the periodontal disease.²³

The effectiveness of chlorhexidine (CHX) is well documented. The twice-a-day use of CHX for 21 days in the absence of any other oral hygiene measures has been shown to completely prevent plaque and gingivitis from developing.³⁶ Sekino et al. found that twice-daily 60-second rinsing with 0.2 percent CHX as well as gargling plus using 1 % CHX gel for tongue brushing resulted in significant reduction of the microbial load over a four-day period in the absence of any mechanical oral hygiene measures. Some microorganisms are influenced more than others, and the presence of Actinomyces is substantially decreased following the use of CHX.³⁷

Promising advances are being made in the development of antimicrobial agents, including research into agents that alter the surface of the tooth or the acquired pellicle, to prevent the adhesion of bacteria and alter the formation of the biofilm. Other areas of research include the use of bacterial macrophages, bacterial inhibitors, vaccines, and antimicrobial peptides.³⁸

CONCLUSIONS

1. Periodontal disease relies upon the presence of a mature biofilm rich in periodonto-pathogens.
2. The progression of periodontal disease is highly variable and dependent largely upon the host response, with bacterial variances between individuals accounting for only 20% of cases.
3. Nonetheless, the removal of bacteria and their byproducts is essential to prevent and stop the periodontal disease.
4. Home care measures can be effective in removing the supragingival biofilm when properly performed.
5. However, once a mature subgingival biofilm has developed, or dental calculus is present, home care is ineffective and clinical care is required.
6. In the absence of a clinical intervention, the periodontal disease progression in individual patients leads to attachment and bone loss.

REFERENCES


