Biofilm Formation, Identification and Removal
A Peer-Reviewed Publication
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Educational Objectives
Upon completion of this course, the clinician will be able to do the following:
1. Understand the components that make up the structure of biofilm and its biochemistry
2. Understand the process resulting in biofilm formation and maturation, including the role of adhesion
3. Understand the process of identification, whether unaided or chemically-aided, and, as a result, understand the differences in plaque-disclosing agents available for use in this step
4. Assess the appropriate type of disclosing agent and method by which to efficiently remove plaque
5. Understand how to educate patients and what information is most needed to help prevent and fight the development of biofilm in the intraoral environment

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

Introduction
Dental caries and periodontal disease are the most common intraoral diseases and among the most prevalent disorders known to man. Both are associated with bacteria contained in dental biofilms. Studies, including one that compared sucrose-restricted and sucrose-supplemented diets, have shown that plaque accumulates rapidly on clinically plaque-free teeth. In the absence of any oral hygiene measures, plaque accumulated on 52 percent to 73 percent of surfaces. When poor oral hygiene is causally related to the presence of dental biofilm and gingivitis, there is not a strong correlation between poor oral hygiene and severe periodontitis, nor does poor environment. Unfortunately, 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 found to occur.

Biofilm Structure and Biochemistry
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. One study found different patterns in the adsorption of salivary proteins onto various components of orthodontic appliances — modules, brackets, springs, and elastics — and found differing levels of bacterial accumulation. Acquired pellicle formation provides oral bacteria with binding sites, resulting in bacterial adhesion, the first step in the formation of dental biofilm. Surface modification can inhibit the development of the acquired pellicle and dental biofilm. When ceramic crown surfaces were treated in vivo with hydrophobic coatings, using disclosing agents it was found that almost no plaque formed on the hydrophobic crown surface and there was almost no detectable pellicle, even after seven days.

The Role of Adhesion
Dental biofilms accumulate on hard and soft surfaces in the oral cavity, including nonbiological surfaces such as implant surfaces, orthodontic appliances and amalgams, as well as on other bacteria. Bacterial adherence is essential for formation of dental biofilm. Following adhesion, the bacteria divide, grow, and accumulate. The bacteria contain a factor called an “adhesin” that meets its counterpart receptor 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 fimbrae that act as receptors on subgingival bacteria, or collagen and residues of sialic acid and galactosyl that serve 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. When the adhesion of streptococci is selectively reduced by preventing the buildup of sialic acid, more P. gingivalis and P. intermedia adhere in the space. It is believed that this is due to galactosyl residues acting as receptors for the Porphyromonas species.

For a subgingival biofilm to exist, the 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 periodontal disease and the subsequent development of true periodontal pockets, which in turn provide extra space for the anaerobes and an oxygen-poor environment. Unfortunately, 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 found to occur.
Biofilm Formation and Maturation

Biofilm development involves selection and adaptation by bacteria already present in the intraoral environment. Acid-producing Strep. mutans and lactobacilli are selected in a low-pH environment, leading to acid production by the bacteria in the subsequent biofilm and the initiation of the caries process. Similarly, anaerobic microorganisms are selected when there is an increase in gingival crevicular fluid, nutrients, and pH, all of which contribute to the establishment of periodontopathogens and periodontal disease. In addition to the selection of specific bacterial species in a dental biofilm, the bacteria adapt to their environment.

In vivo 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 — the host response and susceptibility determine disease. Only 20 percent of periodontal disease is attributed to bacterial variances, with host response being the key factor for most periodontal disease. Within periodontal pockets, between 30 and 100 species can be isolated from one periodontal site, and over 300 species have been identified by culturing samples from human periodontal pockets.

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

CLSM combined with fluorescence of plaque harvested from in situ enamel blocks worn 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 periodontal disease — and Treponema denticola are often found together in dental biofilms.

Research using CLSM and blot analyses to assess gene expression has shown that P. gingivalis forms a synergistic biofilm with T. denticola, and has concluded that other bacteria present in dental biofilm may interact synergistically. Other bacterial species have been shown to engage in, in effect, chemical warfare by producing substances that either prevent adhesion by another species or are bactericidal for a second species. Hydrogen peroxide produced by S. sanguis inhibits A. actinomycetemcomitans; conversely, A. actinomycetemcomitans produces a bactericide for S. sanguis.

The composition of subgingival plaque has been extensively researched. Socransky and Haffajee defined five complexes as bacterial complexes in subgingival plaque. In addition, specific complexes were found to have an association with other specific complexes within the group, demonstrating the presence of relationships between bacterial species. While specific bacteria are known to be pathogens, it is also now recognized that the presence of periodontopathogens does not predict long-term progress of periodontitis. Recent research has identified clonal subgroups 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.

Subgingival plaque is not subject to the same assaults as supragingival plaque and is relatively protected from saliva and mastication forces. A further factor is the morphology of bacteria, both intraspecies and interspecies. In vitro evaluation of biofilm formation with A. actinomycetemcomitans has found that variants with a rougher morphology resulted in greater biofilm formation than in vitro smooth morphology variants. It was also concluded...
that a rough morphology in the biofilm in vivo may help protect bacteria from assault.21

The role of Candida albicans in dental biofilm is of particular interest given the increase in opportunistic C. albicans infections in immunocompromised patients. The growth and survival of C. albicans in dental biofilms has been shown to be influenced by the concentration of aerobic and anaerobic bacteria present. Research by Samaranayake et al. has shown that culturing various bacteria together with C. albicans results in varying biofilm formation and that higher concentrations of Actinomyces israelii, Prevotella nigrescens or Pseudomonas aeruginosa result in lower concentrations of C. albicans. A statistically significant negative correlation was found between concentrations of P. gingivalis or E. coli, and C. albicans. This effect was not seen with Strep. Intermedius, found between concentrations of P. gingivalis or E. coli, or Pseudomonas aeruginosa result in lower concentrations of Actinomyces israelii, Prevotella nigrescens or Pseudomonas aeruginosa result in lower concentrations of C. albicans. A statistically significant negative correlation was found between concentrations of P. gingivalis or E. coli, and C. albicans. This effect was not seen with Strep. Intermedius, Strep. Mutans, or L. acidophilus.32

Biofilm Identification

The meticulous and regular removal of dental biofilm is important for oral and systemic health, given the strong associations between the presence of periodontal disease and cardiovascular disease,23 diabetes, respiratory disease, disseminated infections and other conditions. Metabolic removal of biofilm is hindered by its relative lack of visibility, 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 present24 with poor penetration into the inner layers of the dental biofilm, which underscores the importance of frequent and adequate plaque removal.

By removing supragingival plaque in the early phase of biofilm maturation, the microbial load can be maintained at a relatively low level and colonization by anaerobic species can be contained. In the absence of adequate oral hygiene, subgingival plaque will develop, at which point elimination and effective control of the bacterial environment is considerably more difficult. One of the difficulties for both dental professionals and patients in eliminating plaque is its identification.

Unaided Detection

Plaque is not always visible even supragingivally. This is a factor in incomplete removal of supragingival plaque by patients and clinicians alike. One study compared the efficacy of removal of 48-hour-old plaque by three groups of dental hygienists with three different combinations of instruments. The first group used an ultrasonic cleaner and prophy cups, the second added dental floss to the armamentarium, and the third group used Gracey curettes and prophy cups. Regardless of the method employed, use of fluorescein and UV light afterwards revealed that more residual plaque remained interproximally than buccally or lingually and that not one person in any group was able to remove 100 percent of supra-gingival plaque in a patient in the absence of a disclosing agent.25

Chemically Aided Detection — Disclosing Agents

Chemically aided visual identification of plaque through the use of disclosing agents is a useful patient educator and motivator, and, as demonstrated by Checchi et al., a useful tool for dental hygienists. Disclosing agents have been in use since the early 20th century, starting with the introduction of iodine by Skinner in 1914.26 More recent plaque disclosing agents are based upon approved food colorants.27 Basic fuchsin and erythrosin, a coal-tar derivative,28 were used as early as the 1960s. Recent online research suggests that about half of dental hygienists use disclosants.29 The majority of disclosing agents in current use are red dye disclosants. Disclosing agent vehicles include solutions used as rinses, tablets that are chewed to mix with saliva moved around the teeth, and solutions applied using an applicator.

Erythrosin and basic fuchsin both stain dental plaque red. A further variant stains 24-hour plaque red and 48-hour plaque a purple/blue color. Erythrosin is a simple, inexpensive and readily available home care disclosing agent. It lacks specificity and will stain the gingivae, pellicle, calculus and oral mucosa in addition to dental plaque — thereby making it harder to identify the plaque around the gingival margin because dye penetration is similar on plaque and gingival tissue.30 This could result in particularly motivated patients brushing too hard to remove what they perceive to be dental plaque, instead creating a soft tissue abrasion at the gingival margin. The non-specificity of staining may also be esthetically displeasing to patients.

An alternative disclosing agent, fluorescein is safe and effective in disclosing plaque. It is specific and will not stain the soft tissue or pellicle. Fluorescein disclosing systems, such as Plak-Check® (Sunstar Butler), use liquid fluorescein and a blue light. After the liquid fluorescein has been applied, the fluorescein is not visible to the naked eye. Using the blue light gives the plaque a yellowish tinge after fluorescein application, making it visible to patients and clinicians. While erythrosin was found in one study to stain twice as well as fluorescein,31 fluorescein is specific for the plaque and does not stain the gingival margin or oral mucosa, reducing the risk of abrasion by overzealous cleaning by the patient. Fluorescein has been shown to be more effective in disclosing interproximal plaque than other disclosing agents.32

Plaque disclosing methods include the use of a plaque probe. Access to interdental areas can be difficult, and the use of a plaque probe with a contrasting color at the tip may aid access and enable plaque to be more easily detected.33

Plaque disclosing agents containing red dye may stain tooth-colored restorative materials. A recent in situ study found that a single use of a basic fuchsin solution noticeably changed the color of resin-modified glass ionomers in children. In contrast, there were no statistically significant changes when a fluorescent dye was used.34 While the composite resin changes were not significant with either disclosing method according to the methodology on visually acceptable
values, the study concluded that basic fuchsin should be used with caution in the presence of both resin-modified glass ionomers and composites, and that the use of fluorescent dye was preferable.

Staining of clothing has on occasion been an issue. Most disclosing agents can be removed completely from clothing, although silk shows more resistance. In this regard, the least residual stain was found with the use of fluorescein—an anticipated result since the dye is not normally visible and the clothing was not tested under ultraviolet light.

### Dental Biofilm Identification

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### Rationale for Use and Results

#### Using Disclosing Agents

Plaque disclosing agents are used in both children and adults to help patients see the areas of plaque coverage and educate them on plaque removal. Disclosing plaque during a dental hygiene appointment helps the clinician motivate and educate patients. These agents are useful visual feedback tools, showing patients the areas they have missed while brushing during home care. Some studies suggest that the improvement is due to oral hygiene motivation and not due to the use of disclosing agents; however, other studies have suggested otherwise.

One study compared the use of a toothbrush and an erythrosin plaque disclosing tablet (Signal® Integral, Unilever®), with the use of a toothbrush, and the effect of visual feedback. The use of the disclosing tablets increased brushing time by over 20 percent for supragingival plaque removal. A disadvantage of red dye disclosants is that patients may be reluctant to have them applied or to apply them themselves at home, due to cosmetic concerns. Another study concluded that using a fluorescent disclosing system “results in a healthier mouth” and reduces periodontal disease progress.

In a further study in 10-year-old children, knowledge of oral hygiene and use of disclosing tablets resulted in a 20 percent reduction in plaque over a four-month period compared to the control group.40

#### Removal of Biofilm

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 biofilm mass and/or selectively reducing the numbers and proportion of pathogenic species will help prevent caries and periodontal disease.

#### Mechanical Removal

In industrialized countries, patients brush for less than one minute on average.41 The education and motivation of patients to mechanically remove as much plaque as possible is a priority. 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 toothbrushing techniques have evolved over time. Use of a soft-bristled brush will achieve the best results for plaque removal and will help prevent abrasion associated with use of hard-bristled brushes or overzealous brushing. While a straight horizontal brushing motion may remove plaque, the Bass technique is well accepted as optimal. By brushing at a 45 degree angle, the brush is better able to reach under the gingival margin into the gingival crevice to remove plaque in this area. Without this care, inflammation will occur in the gingival crevice with the formation of the pseudopocket, making it a more attractive environment for the development of anaerobic colonization and the formation of a true periodontal pocket. Toothbrushes are available with ergonomically-designed handles to help patients brush (Cross-Action®, Oral-B®; Colgate Wave, Colgate-Palmolive), and some have handle designs with thumb grips that are positioned to tilt the bristles at a 45 degree angle to the sulcus (GUM® Technique Toothbrush, Sunstar Butler).

Electric toothbrushes are a further option. Clinical research into the effectiveness of these toothbrushes has produced widely varying and often contradictory results. Selection is based upon preference and the ability and 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. Floss requires less interdental space; however, its use requires more control/dexterity than interdental brushes to clean on either side of the interdental papillae, and tight contact points may make flossing difficult. One-handed flossing devices (Eez-Thru Flossers, Sunstar Butler; Glide Flosser, Crest) may help solve the dexterity problems for some patients. Flossing is often inadequately and infrequently performed. A survey of dental hygienists found that none reported all of their patients flossing daily, and 54 percent reported that only 20 percent to 30 percent of their patients flossed daily.43 Many patients return for maintenance appointments—such as for implants—without ever
flossing. When used properly, interdental brushes placed below the contact point are effective interdentally and even subgingivally, provided sufficient space is available. Smaller-head interdental brushes are easier to use than large ones and facilitate access. Both flossing and interdental brushing are effective with an appropriate technique. The choice will depend upon clinician and patient preference, the likelihood of the patient complying with the method chosen, the space available, and the patient’s dexterity.

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

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.

Essential oils, fluoride, chlorhexidine, zinc citrate, and triclosan have all been used as antimicrobial rinses and dentifrices. At low concentrations, fluoride starts to affect acid production, and at high concentrations has been shown to be bactericidal. Triclosan copolymer dentifrice (Colgate® Total®) has been shown to significantly reduce plaque and gingivitis and inhibit the progress of periodontal disease.

The effectiveness of chlorhexidine used as a chemotherapeutic is well documented. Twice-daily 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. Sekino et al. found that twice-daily 60-second rinsing with 0.2 percent CHX as well as gargling and using 1 percent CHX gel on the tongue resulted in significant reduction in 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 less following CHX use. Once rinsing ceases, the microbial flora composition in individuals can revert to the pre-CHX flora within four days. The use of a single CHX one-minute rinse was recently studied to assess antimicrobial activity in situ. Compared to the control group, a single rinse of CHX was found to be significant at six hours. However, in 48-hour-old plaque, a single rinse only had a significant effect in the outer layer. Studies have been conducted on both 0.2% and 0.12% CHX rinses. When the rinsing time and dosage are taken into consideration, these two formulations are equivalent. In the U.S., CHX rinse is available as 0.12 percent (Periogard®, Colgate Oral Pharmaceuticals; Periogard®, Colgate Oral Pharmaceuticals; and recently an alcohol-free CHX was introduced (GUM®, Sunstar Butler).

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 formation of the biofilm. Other areas of research include the use of bacterial macrophages, bacterial inhibitors, vaccines, and antimicrobial peptides.

Summary
Dental biofilm is complex, with a well-organized structure. Up to 500 bacterial species have been identified in dental biofilm, including cariogenic as well as periodontopathic bacteria. Young dental plaque consists mainly of gram-positive bacteria, and as the plaque matures and a subgingival plaque develops, an increasing number of gram-negative microorganisms are seen. For oral and systemic health, the development and maturation of dental biofilm should be impeded by regular and meticulous removal. This can be accomplished mechanically, chemically, or via a combination of the two. Disclosing agents provide clinicians and patients alike with the ability to visually identify plaque, and a variety of toothbrushes and interdental aids are available to assist with its removal. Adjunctive chemotherapeutics such as chlorhexidine and triclosan can be used to reduce and inhibit the plaque burden. Recent promising advances in the development of antibacterial therapy include altering the surface of the tooth or acquired pellicle to prevent bacterial adhesion. Generally, current chemotherapeutics do not effectively penetrate thick biofilm, underscoring the importance of identifying and rigorously removing the dental biofilm.

References


28. Ibid.


46. Van Leuvenen. J. Dent. Research 1990 (Spec Issue); 69.


Author Profile

Dr. Fiona M. Collins has over 20 years of clinical, marketing, education and training, and professional relations experience. She has practiced as a general dentist for 13 years, written and given CE courses to dental professionals and students, and conducted market research projects. Dr. Collins is a past member of the Academy of General Dentistry Health Foundation Strategy Board and has been a member of the British Dental Association, the Dutch Dental Association, and the American Dental Association. Dr. Collins earned her dental degree from Glasgow University and holds an MBA and MA from Boston University.

Acknowledgement

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Anaerobic microorganisms are influential in periodontal disease. Bacterial adhesion to soft tissue within the periodontal pocket may also play a role in the development of bacterial biofilm. Prior to the development of dental biofilm, the salivary or acquired pellicle forms. A subgingival pellicle forms followed by bacteria bind to the tooth, resulting in the formation of dental biofilm. The salivary or acquired pellicle formation is essential for the development of dental biofilm and serves as a binding site for oral bacteria. Among the factors influencing dental biofilm development, nutrients are the most crucial, followed by pH and other bacteria. The composition of the biofilm depends upon its location and its degree of maturity. Subgingival plaque is subject to the same assault as supragingival and is relatively protected from saliva and mastication forces. In the absence of adequate oral hygiene, subgingival plaque will develop in the outer surface where a thick dental biofilm is present. In the inner surface where a thick dental biofilm is present, effective control of the bacterial environment is more difficult. In the presence of subgingival plaque, enhancing the growth of biofilm mass is responsive only to home-care oral hygiene measures. Plaque is always visible even supragingivally, and disclosing agents are simple, inexpensive, and readily available tools for oral hygiene. However, disclosing agents, such as Fluorescein and Erythrosin, require careful use since they are responsive to the bacterial environment.
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**Educational Objectives**

1. Understand the components that make up the structure of biofilm and its biochemistry
2. Understand the process resulting in biofilm formation and maturation, including the role of adhesion
3. Understand the process of identifying, whether unaided or chemically-aided, and, as a result, understand the differences in plaque-disclosing agents available for use in this step
4. Assess the appropriate type of disclosing agent and method by which to efficiently remove plaque
5. Understand how to educate patients and what information is most needed to help prevent and fight the development of biofilm in the intraoral environment

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1. Were the individual course objectives met?
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   - Objective #2: Yes No
   - Objective #3: Yes No

2. To what extent were the course objectives accomplished overall?
   - 5 4 3 2 1 0

3. Please rate your personal mastery of the course objectives.
   - 5 4 3 2 1 0

4. How would you rate the objectives and educational methods?
   - 5 4 3 2 1 0

5. How do you rate the instructor’s effectiveness?
   - 5 4 3 2 1 0

6. Please rate the author’s grasp of the topic?
   - 5 4 3 2 1 0

7. Was the overall administration of the course effective?  5 4 3 2 1 0

8. Do you feel that the references were adequate?    Yes  No

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