The Role of Dental Plaque Biofilm in Oral Health

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Overview. Microbial biofilms are complex communities of bacteria and are common in the human body and in the environment. In recent years, dental plaque has been identified as a biofilm, and the structure, microbiology, and pathophysiology of dental biofilms have been described. The nature of the biofilm enhances the component bacteria's resistance to both the host's defense system and antimicrobials. If not removed regularly, the biofilm undergoes maturation, and the resulting pathogenic bacterial complex can lead to dental caries, gingivitis, and periodontitis. In addition, dental biofilm, especially subgingival plaque in patients with periodontitis, has been associated with various systemic diseases and disorders, including cardiovascular disease, diabetes mellitus, respiratory disease, and adverse pregnancy outcomes.

Clinical Implications. An understanding of the nature and athophysiology of the dental biofilm is important to implementing proper management strategies. Although dental biofilm cannot be eliminated, it can be reduced and controlled through daily oral care. A daily regimen of thorough mechanical oral hygiene procedures, including toothbrushing and interdental cleaning, is key to controlling biofilm accumulation. Because teeth comprise only 20% of the mouth's surfaces, for optimal oral health, the use of an antimicrobial mouthrinse helps to control biofilm not reached by brushing and flossing as well as biofilm bacteria contained in oral mucosal reservoirs.

Keywords: Antimicrobial mouthrinse, biofilm, dental plaque, oral health, periodontal disease

Introduction

In contrast to an accumulation of individual bacteria, a biofilm is a complex, communal, 3-dimensional arrangement of bacteria. Bacterial biofilms are ubiquitous and are potentially found in a variety of sites within the human body. For example, they can grow on indwelling catheters, ports, and implants; external surfaces of the eye; artificial heart valves; endotracheal tubes; and contaminated prosthetic joints. Abacterial biofilm is often the cause of persistent infections and has been associated with osteomyelitis, pneumonia in patients with cystic fibrosis, and prostatitis.[1]

In areas related to oral health care, bacterial biofilms are found in dental unit water lines, on tooth surfaces and dental prosthetic appliances, and on oral mucous membranes. Biofilm in the form of supragingival and subgingival plaque is the etiologic agent in dental caries and periodontal diseases (Figure 1).[2-5] The pathogenicity of the dental plaque biofilm is

enhanced by the fact that in biofilm form, the component bacteria have increased resistance to antibiotics and other chemotherapeutic agents and are less able to be phagocytized by host inflammatory cells. Therefore, control of the dental plaque biofilm is a major objective of dental professionals and critical to the maintenance of optimal oral health. This article reviews the characteristics of dental biofilm, its role in the etiology of periodontal diseases, and strategies for controlling the biofilm to promote health.

Figure 1. Scanning electron micrograph of biofilm grown from the subgingival plaque of a healthy subject for 10 days anaerobically on saliva-coated hydroxyapatite discs. (Grown by Michael Sedlacek, PhD, and Clay Walker, PhD, at the University of Florida College of Dentistry Periodontal Disease Research Center. Image taken by the University of Florida Electron Microscopy Core Facility.)

Changing Views of Dental Plaque

Over the past 50 years, the understanding and characterization of dental plaque have undergone significant evolution. Loesche[6] proposed both a nonspecific and a specific plaque hypothesis for periodontal disease initiation and progression.

The *nonspecific plaque hypothesis* proposed that the entire microbial community of plaque that accumulated on tooth surfaces and in the gingival crevice contributed to the development of periodontal disease. Plaque bacteria produced virulence factors and noxious products that initiated inflammation, challenged the host defense system, and resulted in the destruction of periodontal tissues. Under this hypothesis, the quantity of plaque was considered to be the critical factor in the development of periodontal disease. Thus, increases in the amount of plaque (quantity), as opposed to specific pathogenic microorganisms (quality) found in the plaque, were viewed as being primarily responsible for inducing disease and disease progression.[7,8]

Studies on the microbial etiology of various forms of periodontitis support the *specific plaque hypothesis*, which proposes that only certain microorganisms within the plaque complex are pathogenic. Despite the presence of hundreds of species of microorganisms in periodontal pockets, fewer than 20 are routinely found in increased proportions at periodontally diseased sites. These specific virulent bacterial species activate the host's immune and inflammatory responses that then cause bone and soft tissue destruction.[6,8,9]

Socransky and colleagues[4,10] recognized that early plaque consists predominantly of gram-positive organisms and that if the plaque is left undisturbed it undergoes a process of maturation resulting in a more complex and predominantly gram-negative flora. These investigators assigned the organisms of the subgingival microbiota into groups, or complexes, based on their association with health and various disease severities (Figure 2).[4,10] Color designations were used to denote the association of particular bacterial complexes with periodontal infections. The *blue*, *yellow*, *green*, and *purple* complexes designate early colonizers of the subgingival flora. *Orange* and *red* complexes reflect late colonizers associated with mature subgingival plaque. Certain bacterial complexes are associated with health or disease.[10,11] For example, the bacteria in the red complex are more likely to be associated with clinical indicators of periodontal disease such as periodontal pocketing and clinical attachment loss.
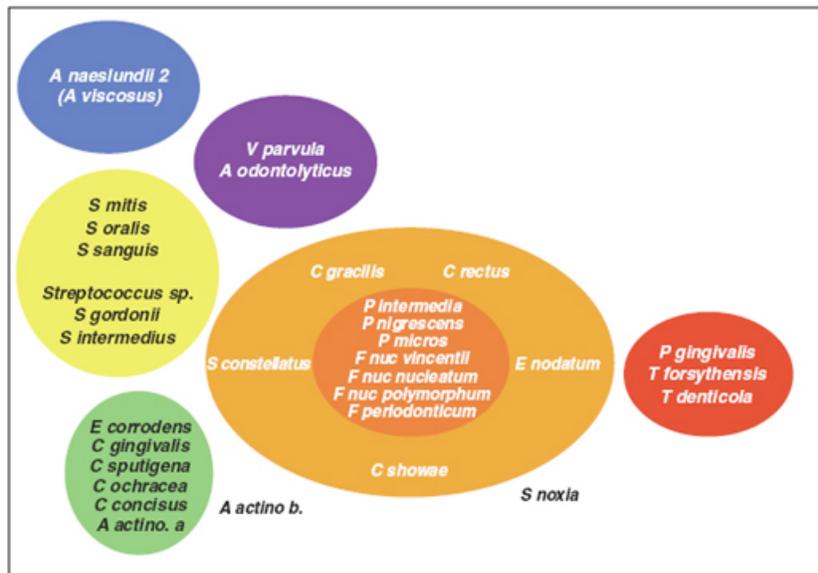

Figure 2. Microbial complexes in subgingival biofilm.[4,10] (Modified from Socransky SS, Haffajee AD, Cugini MA, et al. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134-144. Reprinted with permission from Blackwell Publishing.)

Plaque Recognized as a Biofilm

Research over the past decade has led to the recognition of dental plaque as a biofilm - a highly organized accumulation of microbial communities attached to an environmental surface. Biofilms are organized to maximize energy, spatial arrangements, communication, and continuity of the community of microorganisms.

Biofilms protect bacteria living within their structures and thereby provide an advantage over free-floating (planktonic) bacteria. The slimy extracellular matrix produced by biofilm bacteria encloses the microbial community and protects it from the surrounding environment, including attacks from chemotherapeutic agents. Chemotherapeutic agents have difficulty penetrating the polysaccharide matrix to reach and affect the microorganisms.[1,11-13] Thus, the matrix helps to protect bacteria deep within the biofilm from antibiotics and antiseptics, increasing the likelihood of the colonies' survival. Furthermore, the extracellular matrix keeps the bacteria banded together, so they are not flushed away by the action of saliva and gingival crevicular fluid. Mechanical methods, including toothbrushing, interdental cleaning, and professional scaling procedures, are required to regularly and effectively disrupt and remove the plaque biofilm. Antiseptics, such as mouthrinses, can help to control the biofilm but must be formulated so as to be able to penetrate the plaque matrix and gain access to the pathogenic bacteria.

Biofilms have a definite architectural structure. The bacteria are not uniformly distributed throughout the biofilm; rather, there are aggregates of microcolonies that vary in shape and size. Channels between the colonies allow for circulation of nutrients and by-products and provide a system to eliminate wastes.[14,15] Microorganisms on the outer surface of biofilms are not as strongly attached within the matrix and tend to grow faster than those bacteria deeper within the biofilm. Surface microorganisms are more susceptible to detachment, a characteristic that facilitates travel to form new biofilm colonies on nearby oral structures and tissues.

Bacteria in biofilm communicate with each other by a process called *quorum sensing*. This dynamic, sophisticated communication system enables bacteria to monitor each other's presence and to modulate their gene expression in response to the number of bacteria in a given area of the biofilm.8 In addition, as a result of quorum sensing, portions of the biofilm can become detached in order to maintain a cell density compatible with continued survival.

Stages of Biofilm Formation

The growth and development of biofilm are characterized by 4 stages: initial adherence, lag phase, rapid growth, and steady state. Biofilm formation begins with the adherence of bacteria to a tooth surface, followed by a lag phase in which changes in genetic expression (phenotypic shifts)occur. A period of rapid growth then occurs, and an exopolysaccharide matrix is produced. During the steady state, the biofilm reaches growth equilibrium. Surface detachment and sloughing occur, and new bacteria are acquired.

Initial Adherence and Lag Phase

The first phase of supragingival biofilm formation is the deposition of salivary components, known as *acquired pellicle*, on tooth surfaces. This pellicle makes the surface receptive to colonization by specific bacteria. Salivary glands produce a variety of proteins and peptides that further contribute to biofilm formation. For example, salivary mucins, such as MUC5B and MUC7, contribute to the formation of acquired pellicle,[16,17] and statherin, a salivary acidic phosphoprotein, and proline-rich proteins promote bacterial adhesion to tooth surfaces.[18] Acquired pellicle formation begins within minutes of a professional prophylaxis; within 1 hour, microorganisms attach to the pellicle. Usually, gram-positive cocci are the first microorganisms to colonize the teeth. As bacteria shift from planktonic to sessile life, a phenotypic change in the bacteria occurs requiring significant genetic up-regulation (gene signaling that promotes this shift). As genetic expression shifts, there is a lag in bacterial growth.

Rapid Growth

During the rapid growth stage, adherent bacteria secrete large amounts of water-insoluble extracellular polysaccharides to form the biofilm matrix. The growth of microcolonies within the matrix occurs. With time, additional varieties of bacteria adhere to the early colonizers - a process known as *coaggregation* - and the bacterial complexity of the biofilm increases. These processes involve unique, selective molecular interactions leading to structural stratification within the biofilm. Coaggregation and subsequent cell division also increase the thickness of biofilm.[19-21]

Steady State/Detachment

During the steady state phase, bacteria in the interior of biofilms slow their growth or become static. Bacteria deep within the biofilm show signs of death with disrupted bacterial cells and other cells devoid of cytoplasm; bacteria near the surface remain intact. During this phase, crystals can be observed in the interbacterial matrix that may represent initial calculus mineralization.[22] As noted above, during the steady state stage, surface detachment and sloughing also occur, with some bacteria traveling to form new biofilm colonies.

Biofilm and Oral Disease

Biofilms can cover surfaces throughout the oral cavity. Microcolonies exist on oral mucosa, the tongue, biomaterials used for restorations and dental appliances, and tooth surfaces above and below the gingival margin (Figure 3). It is important for oral health professionals to communicate to their patients that both dental caries and periodontal disease are infectious diseases resulting from dental plaque biofilm accumulation. Each of these diseases requires specific strategies for prevention and treatment.
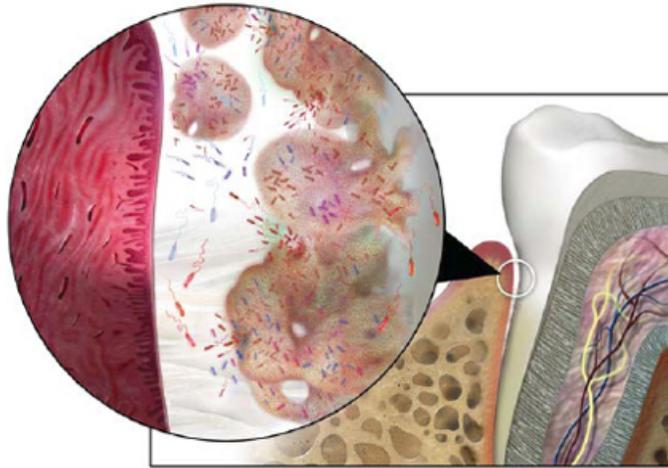

Figure 3. Biofilm lodges in the crevices around the teeth both above and below the gingival margin. Accumulation of dental plaque biofilm can result in dental caries and periodontal disease. (Figure copyright 2006 Keith Kasnot, MA, CMI, FAMI.)

With respect to periodontal disease, dental plaque biofilm demonstrates a succession of microbial colonization with changes in bacterial flora observed from health to disease. Researchers studied over 13,000 plaque samples from 185 patients with conditions ranging from oral health to periodontal disease.[4,23] As noted above, based on their findings, a number of microbial complexes were identified that were associated with various stages of disease initiation and progression. Bacterial species contained in the yellow, green, and purple complexes appear to colonize the subgingival sulcus first and predominate in gingival health. In contrast, orange complex bacteria are associated with gingivitis and gingival bleeding. Interestingly, bacteria of the orange complex may also be associated with red complex microorganisms including *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Treponema denticola*, organisms found in greater numbers in diseased sites and in more advanced periodontal disease.[10,24]

Bacterial communities living in a biofilm possess resourceful survival strategies, including a broader habitat for growth, nutrition, waste elimination, and new colonization; environmental niches for safety; barriers to thwart antimicrobial drug therapy; protection from the host's defense system including phagocytosis; and enhanced pathogenicity.[1,8] These strategies account for the ongoing challenge of successfully controlling periodontal infection and disease progression.[25]

As the biofilm matures and proliferates, soluble compounds produced by pathogenic bacteria penetrate the sulcular epithelium. These compounds stimulate host cells to produce chemical mediators associated with the inflammatory process[26] (see Figure 4).
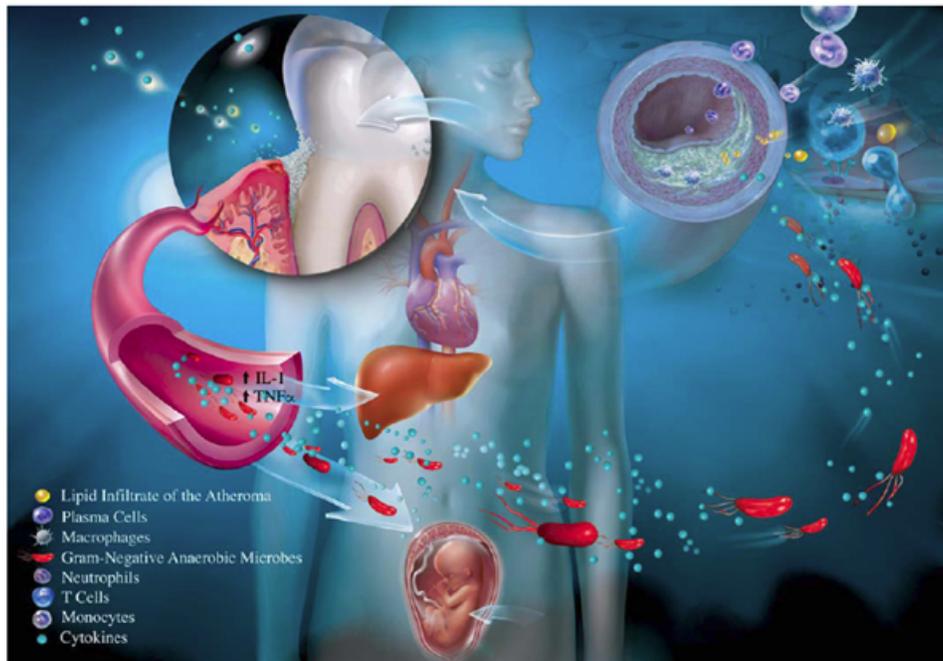
Figure 4. Subgingival plaque bacteria and/or their products may gain access to distant sites in the body through the circulatory system and may potentially contribute to systemic inflammation; in this way, a dental biofilm infection may potentially contribute to various systemic diseases and conditions. (Illustration owned by McNEIL-PPC, Inc. and provided for educational purposes only. May not be reproduced without the prior written permission of McNEIL-PPC, Inc.)

- Interleukin-1 beta (IL-1), prostaglandins, tumor necrosis factor alpha (TNF-α), and matrix metalloproteinases are mediators that recruit neutrophils to the area via chemotaxis and cause increased permeability of gingival blood vessels, permitting plasma proteins to migrate from within the blood vessels into the tissue.

- As the gingival inflammatory process continues, additional mediators are produced, and more inflammatory cell types such as neutrophils, T cells, and monocytes are recruited to the area.

- Proinflammatory cytokines are produced in the tissues as a response to the chronic inflammatory process, and these proteins may further escalate the local inflammatory response and affect the initiation and progression of systemic inflammation and disease.

The result of this chronic inflammation is a breakdown of gingival collagen and accumulation of an inflammatory infiltrate, leading to the clinical signs of gingivitis. In some individuals, the inflammatory process will also lead to the breakdown of collagen in the periodontal ligament and resorption of the supporting alveolar bone. It is at this point that the lesion progresses from gingivitis to periodontitis, continuing the same challenge from proinflammatory mediators as with chronic gingivitis. Thus, controlling dental plaque biofilm is essential to preventing and reversing gingivitis as well as preventing and managing periodontitis.

Periodontal Biofilm Infection and Systemic Health

In recent years, studies have demonstrated an association between periodontitis and various systemic diseases and conditions, including cardiovascular disease, diabetes mellitus, respiratory disease, adverse pregnancy outcomes, obesity, pancreatic cancer, and Alzheimer's disease.[27-57] While several of these associations have not been definitively established, biological mechanisms explaining some of the more extensively studied relationships are emerging.

The association between periodontal disease and some systemic diseases may relate to the ability of subgingival plaque bacteria and/or their products to gain access to the systemic circulation through the ulcerated epithelium of the periodontal

pocket. For example, environmental niches like a subgingival pocket that contains anaerobic gram-negative microorganisms can potentially seed orange and red complex bacteria and/or their products to distant sites through the circulatory system. In this way, a dental biofilm infection can potentially contribute to both oral and systemic inflammation.[25]

Research on Periodontal Microorganisms

Atheromas. Direct evidence for the role of dental biofilm infection in systemic inflammation comes from findings of periodontal microorganisms in human carotid atheromas. Studies of atheromatous lesions in carotid arteries revealed that over 40% of atheromas contain antigens from periodontal pathogens including P gingivalis, T forsythensis, and Prevotella intermedia. [28,58] In addition, P gingivalis is known to induce platelet aggregation, a component of atheroma and thrombus formation,[29] and invade endothelial cells in cell cultures.[59] While such findings suggest a possible invasion of atheromas by oral pathogens as well as possible contribution to their development, it is important to note that causality has yet to be established. Preterm Birth. Research suggests that periodontal pathogens may travel via the bloodstream from the oral cavity to the placenta initiating preterm birth. In an animal model, Han and coworkers60 found that periodontal bacteria, including *Fusobacterium nucleatum*, entered the bloodstream from ulcerated gingival sulci or periodontal pockets and negatively influenced the normal birth process.

Respiratory Disease. Likewise, biofilm in the oral cavity may serve as a reservoir of infection leading to respiratory disease. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and enteric bacteria have been shown to colonize the teeth of patients admitted to hospitals and long-term care facilities. These bacteria may be released into saliva and aspirated into the lower airway causing respiratory infection.[46-49,61] Intubation is another vehicle by which bacteria from the oral biofilm can be directly introduced into the respiratory system. Intubation tubes support biofilm growth contributing to nosocomial infection such as pneumonia. This is one reason why oral intubation raises the risk of nosocomial infection in intensive and critical care hospital populations.

Association With Chronic Diseases and Conditions

Research has also suggested that the association between oral inflammation and systemic inflammation may be key to understanding and managing the significant, deleterious effects on the multiple organ systems involved in some chronic diseases and conditions (Figure 4).[26]

Cardiovascular Disease. Cardiovascular disease is characterized by inflammatory plaque accumulation in blood vessels that can cause thromboses and lead to myocardial infarction. Atherosclerosis represents a chronic inflammatory process that causes endothelial dysfunction and injury to the elastic and muscular arterial tissue. Early atherosclerotic lesions contain neutrophils, monocytes, and lymphocytes. These leukocytes can affect the vascular endothelial lining and cause oxidation of low-density lipoproteins. As a result, monocytes, induced to become macrophages, take up these oxidized lipoproteins and become lipid-laden foam cells. As the lesion progresses, the extracellular matrix of the vessel wall is degraded by proteolytic enzymes and becomes susceptible to rupture. Thromboses can occlude blood flow to the heart and brain and eventually lead to infarction, heart attack, or stroke.[26]

Since atherosclerosis is inflammatory by nature, identifying inflammatory markers that correlate with disease state is important. One recognized and consistent marker of systemic inflammation and poor cardiovascular prognosis is the acute-phase protein C-reactive protein (CRP), the level of which rises with systemic inflammation.[62], Animal model studies of the relationship between cardiovascular disease and periodontal disease demonstrate that clinically induced oral infection with *P gingivalis* will increase atheroma size and elevate CRP levels in the blood.[30] Conversely, some studies have shown that treatment of periodontitis decreases CRP blood levels,[64] though this has not been a consistent finding.

Diabetes Mellitus. Diabetes mellitus is another chronic systemic disease associated with periodontitis. In fact, periodontitis has been identified as one of the major complications of diabetes.[65] Although diabetes increases the susceptibility to periodontal disease,[38,39,65] periodontitis may also increase the difficulty of maintaining satisfactory glycemic control in people with diabetes as compared with those with diabetes without periodontitis.[40] One biological mechanism proposed

to explain the increased incidence and severity of periodontal disease in individuals with diabetes is the finding of elevated levels of inflammatory mediators in the gingival crevicular fluid from periodontal pockets of patients with diabetes with poor glycemic control as compared with those with diabetes who are well controlled or those without diabetes. Those with poor glycemic control had considerable periodontal destruction with an equivalent bacterial challenge.[39,66] Of note, the proinflammatory cytokine TNF-α plays a significant role in this process. TNF-α has a major role in insulin resistance, the primary cause of type 2 diabetes, and is produced in large quantities by fat cells. Periodontitis also has been associated with increased levels of TNF-α. Elevated levels of TNF-α may lead to greater bone loss by killing cells that repair damaged connective tissue or bone. Elevated TNF-α levels also may exacerbate insulin resistance and worsen glycemic control.[44,66,67]

Adverse Pregnancy Outcomes. Studies also demonstrate that periodontal diseases are associated with the risk of adverse pregnancy outcomes, especially preterm low-birthweight infants.[50-52] Chronic infection, such as that found with chronic periodontitis, can stimulate the inflammatory process throughout the body. In the placenta, this may lead to elevated amniotic levels of prostaglandins, TNF-α, and IL-1 and IL-6, stimulating premature rupture of membranes, preterm labor, and the birth of lowbirth- weight infants. Intervention studies are currently under way to investigate a cause and effect relationship between advanced periodontitis and adverse pregnancy outcomes.

Strategies for Managing Dental Biofilm to Promote Health

Although dental biofilm cannot be completely eliminated, its pathogenicity can be lessened through effective oral hygiene measures. Daily toothbrushing, interdental cleaning, and the use of topical antimicrobial chemotherapeutics are patient-based strategies to reduce the bacterial biofilm and to help prevent periodontal diseases. American Dental Association (ADA)-Accepted antimicrobial mouthrinses have been shown to help prevent and reduce plaque and gingivitis when added to a daily oral hygiene regimen of mechanical plaque removal. Further, bacteria from the biofilm on mucosal and tooth surfaces are shed constantly into saliva and transferred to other areas of the mouth. Since oral mucosa, which represents about 80% of the oral cavity surface,[68] can serve as a reservoir for pathogenic bacteria that can be transferred to the tooth surface and sulcus, supplementing mechanical plaque control methods with topical antimicrobials may also play an important role in reducing reservoirs of pathogens that are unaffected by brushing and flossing directed at the tooth surface.

Using Evidence in Practice

Products recommended to patients should be those that have documented efficacy and safety. Only 2 nationally branded antiseptic mouthrinses and their generic equivalents have received the ADA Council on Scientific Affairs Seal of Acceptance for control of supragingival plaque and gingivitis: Listerine® (fixed combination of essential oils) and Peridex® (0.12% chlorhexidine gluconate). However, due to recent changes in the ADA Seal Program, Peridex® and its generic equivalents no longer carry the ADASeal because chlorhexidine gluconate is a prescription product. The fixed combination of essential oils and cetylpyridinium chloride have also been reviewed by a Food and Drug Administration (FDA) advisory committee and have received a Category I recommendation, meaning they have been found to be safe and effective for the control of supragingival plaque and gingivitis. Peridex® and its generic equivalents, which are prescription products, have been approved for marketing by the FDA via the New Drug Application route (or for generics, the Abbreviated New Drug Application process). Examples of effective antimicrobial mouthrinses currently on the market appear in Table I.

Table I. Examples of Antiseptic Mouthrinses*

Active Ingredients	Brands	Indications	Contraindications
0.12% Chlorhexidine gluconate (available by prescription)	Peridex®† (3M ESPE, St Paul, MN) PerioGard®† (Colgate Oral Pharmaceuticals, Inc., Canton, MA) PerioRx®† (Discus Dental, Culver City, CA) Canton, MA) Various generics†	Gingivitis, supragingival plaque	Those hypersensitive to chlorhexidinegluconate or other formula ingredients. Long-term use: can cause moderate staining, increased calculus formation, and possible alteration of taste perception
Four essential oils: eucalyptol, menthol, methyl salicylate, thymol	Listerine® Antiseptic† (Johnson & Johnson Healthcare Products Division of McNEIL-PPC, Inc., Skillman, NJ) Various generics†	Supragingival plaque, gingivitis, oral malodor	Children under 12 years
Cetylpyridinium chloride	Breath Rx® (Discus Dental, Culver City, CA) Colgate Viadent® (Colgate-Palmolive, New York, NY) Crest® Pro-Health™ Rinse (Procter & Gamble, Cincinnati, OH)	Supragingival plaque, gingivitis, oral malodor	Children under 6 years

* For the mechanisms of actions of antiseptic mouthrinses, see pages 19 and 20.
† Has received the ADA Seal of Acceptance; note that as the ADA Seal program has recently phased out prescription products, chlorhexidine gluconate products no longer carry the ADA Seal.

Conclusion

Dental biofilm is a complex, organized microbial community that is the primary etiologic factor for the most frequently occurring oral diseases, dental caries and periodontal diseases. Although the dental biofilm cannot be eliminated, it can be controlled with comprehensive mechanical and chemotherapeutic oral hygiene practices. Teaching patients to use daily brushing, interdental cleaning, and antimicrobial mouthrinses that carry the ADA Seal of Acceptance increases the likelihood of periodontal disease prevention and reduction. Although additional research is needed, there is the possibility that these cost-effective, preventive strategies may minimize the effect of periodontal diseases on specific systemic conditions.

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