

Biofilm: A New View of Plaque

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Continuing Education Units: 2 hours

This continuing education course is intended for general dentists, dental hygienists and dental assistants. The primary learning objective for this course is to increase your general knowledge of the various ways that dental professionals have viewed plaque throughout the years, highlighting the current view of plaque as a biofilm and the ramifications for periodontal therapy.

Conflict of Interest Disclosure Statement

The author has done consulting work for P&G.

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Overview

Dental researchers have attempted to understand the microbial nature of oral diseases over the past 120 years. Their view of plaque and its constituent microorganisms has shifted from a specific plaque hypothesis to a non-specific plaque hypothesis and back again to a theory of specific periodontal pathogens in plaque. Changes in the way plaque and its microorganisms are viewed affect the strategies used to prevent and control periodontal diseases. In recent years, dental researchers have begun to

view plaque as a biofilm. This shifting view of plaque has important implications for future efforts in prevention and treatment. This course addresses the various ways that dental professionals have viewed plaque throughout the years, highlighting the current view of plaque as a biofilm and the ramifications for periodontal therapy.

Learning Objectives

Upon the completion of this course, the dental professional should be able to:

- State a definition of biofilm.
- Discuss the positive and negative aspects of biofilm formation in nature. Include examples of how biofilm provides benefits and harms.
- Compare and contrast the differing views of bacterial plaque at three points in time: 1880-1930; 1930-1960; and 1960 to current times.
- Compare and contrast the behavior of bacteria as grown on culture plates with their behavior in biofilms.
- Describe strategies used currently for control of oral biofilm.
- List the strategies that are under consideration for control of oral biofilms.

Course Contents

- Introduction
- Biofilm
- Changing Views of Plaque
- Plaque as a Biofilm
- New Frontiers
- Summary
- Course Test
- References
- About the Author

Introduction

Despite the best efforts of dental health professionals, oral infections are still widespread. The average adult in the U.S. has from 10 to 17 decayed, missing or filled permanent teeth.¹ The majority of the U.S. population experiences gingivitis, with a smaller proportion experiencing moderate to severe periodontal disease (Figure 1).²

There is universal recognition these oral infections are multifactorial, with specific bacteria residing



Figure 1. Gingivitis

in intraoral plaques as a necessary, but not sufficient cause of disease. Exactly how these plaque-dwelling microorganisms (Figure 2) cause oral diseases is not completely clear. How dental plaque and its resident microorganisms are viewed is dictated by the analytical tools used to study it. Consequently, this influences the strategies used to control and prevent dental diseases.³ During the past two decades newer scientific methods have changed the view of dental plaque so dental scientists now see it as a biofilm.¹

Biofilm

A biofilm is a well organized, cooperating community of microorganisms.^{4,5} The slime layer that forms on rocks in streams is a classic example of a biofilm (Figure 3a). So is the plaque that forms in the oral cavity. Biofilms are everywhere in nature. They form under fluid conditions. It is estimated over 95 percent of bacteria existing in nature are in biofilms.⁵



Figure 2. Plaque-dwelling microorganisms
Oral microorganisms in dental plaque showing typical "corn-cob" structure of bacterium.

Sometimes biofilms are seen as positive, such as their use for detoxification of waste water and sewage. More often biofilms provide a challenge for humans.^{3,5}

The slime layer that forms in dental unit water lines is an example familiar to most dental professionals. Biofilms can also be found lining oil pipelines, fish tanks, indwelling catheters, internal implants, contact lenses, and prosthetic devices (Figure 3b). Biofilms are responsible for the majority of infections in humans.⁶ Occasionally biofilms are deadly. Legionnaires disease that killed 29 persons in Philadelphia in 1976 was ultimately traced to bacteria in the biofilm of the air conditioning system. Millions of dollars are spent each year working to control these biofilms.^{3,7}

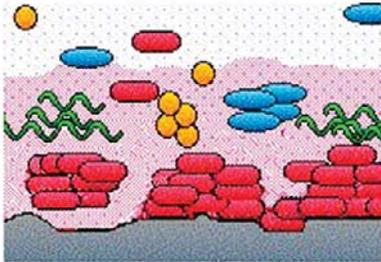


Figure 3a. Formation of biofilm on rock surface

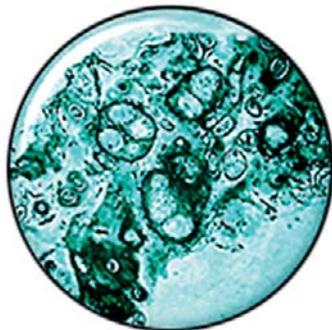


Figure 3b. Biofilm found on dental equipment

Changing Views of Plaque

In 1996, the National Institute for Dental and Craniofacial Research hosted an international conference on microbial ecology. This meeting focused on a new view of plaque as a biofilm. The conference highlighted the importance of this shift in thinking about dental plaque and its role in oral diseases.¹ This is not the first time in history dental professionals have shifted their thinking about plaque. Over the past 120 years the view of dental plaque has gone through several changes.

The period from 1880 to 1930 was called the golden age of microbiology (Figure 4).⁸ During this period, the pathogens that caused many systemic infections of medical importance were identified. Researchers also looked for a single, specific cause of oral diseases. Assuming plaque contained the microorganism that caused periodontal disease, dental scientists studied plaque in search of the causative agent. Using the techniques available at that time (wet mounts or stained smear microscopy), scientists identified four different groups of potential etiologic agents for periodontal diseases. *Amoebae*, *spirochetes*, *fusiforms* and *streptococci* were isolated from patients with periodontal diseases and, therefore, suggested as possible etiologies. Periodontal treatments of those times varied according to the suspected causative agents and included dyes, systemic administration of arsenic-containing antimicrobial preparations, intramuscular injection of mercury as well as vaccines.⁹

The 1930s ushered in a different view of the role of plaque and its microorganisms in the etiology of periodontal disease (Figure 5). Dental scientists believed that periodontal disease was linked with some constitutional defect in the individual.⁹ Mechanical irritants such as calculus and overhanging restorations were also thought to play a major role in the pathogenesis of periodontal disease.¹⁰

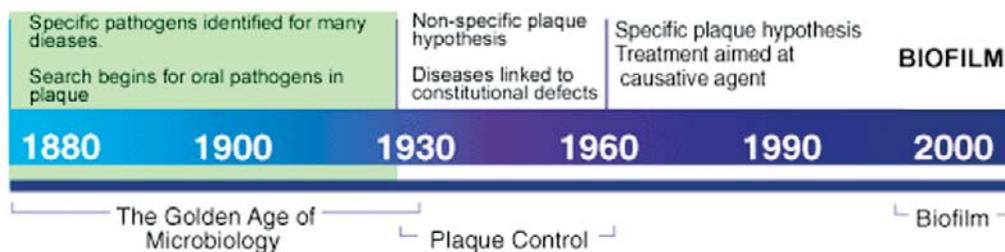


Figure 4. The changing views of plaque and periodontal diseases (1880 - 1930).

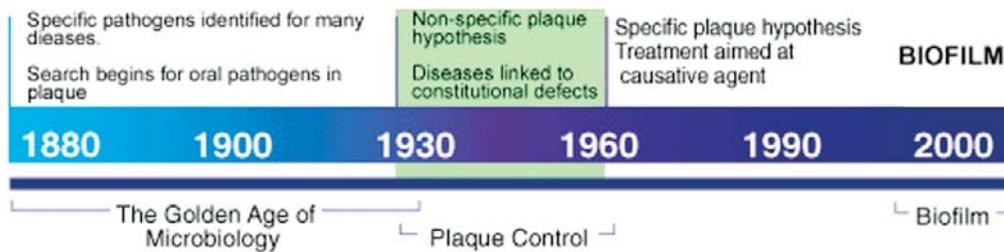


Figure 5. The changing views of plaque and periodontal diseases (1930 - 1960).

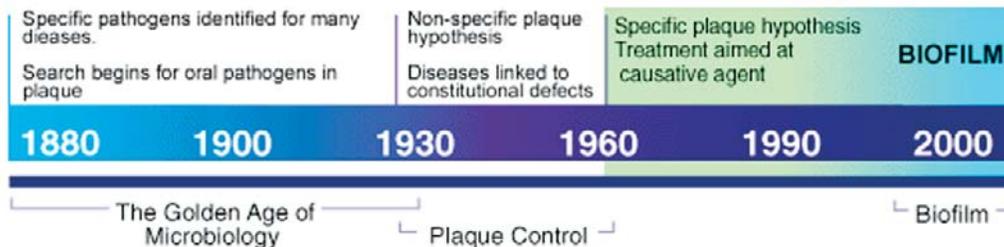


Figure 6. The changing views of plaque and periodontal diseases (1960 - 2000).

The belief there was a single microbial agent that caused periodontal disease was replaced by non-specific plaque theories.⁹ Non-specific plaque hypothesis held that the entire bacterial flora in plaque played a role in periodontal destruction rather than specific bacteria. All plaque was viewed as bad plaque. Furthermore, more plaque meant more disease. Plaque control was viewed as essential to limit the production of gingival irritants that lead to inflammation and periodontal destruction.¹¹ Identification of specific microorganisms was not important. Stringent plaque control was important, and it became the focus of periodontal therapy.

The 1960s marked a return to specific plaque hypotheses (Figure 6). Researchers were successful in showing that periodontal disease could be transmitted between hamsters.¹² The electron microscope confirmed spirochetes were in the connective and epithelial tissues of patients with acute necrotizing ulcerative gingivitis in contrast to healthy controls.¹³ Believing there were differences in plaque brought about by different species, scientists again returned to the search for a specific microbial periodontal pathogen and treatment aimed at the causative agent.⁹

Newer methods of microbial analysis such as darkfield microscopy, transmission electron microscopy, scanning electron microscopy, DNA

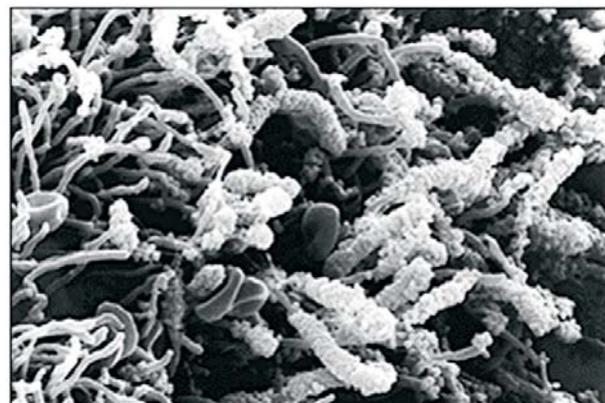


Figure 7. SEM of mature human dental plaque demonstrating corn cob formation. Bar = 10 microns at an original magnification of 2,020. Image courtesy of Dr. Charles Cobb. University of Missouri-Kansas City

probes, BANA hydrolysis and immunoassay aided the search.¹⁴

Since that time, scientists have continued to search for the specific etiologic agent with mixed success. Haffajee and Socransky¹⁵ have detailed the reasons for the difficulties in pinpointing specific pathologic agents. Some of these difficulties are related to microbial sampling and culturing and include: obtaining a sample from a periodontal pocket, the difficulty cultivating some organisms, and the large number of possible periodontal pathogens that may be found and cultivated from a periodontal pocket.



Sampling is further complicated by the fact that periodontal pockets contain not only pathogens, but also opportunistic species. Other difficulties in pinpointing periodontal pathogens are related to the nature of periodontal diseases themselves. First, periodontal disease is not a single disease, but a collection of different diseases. Secondly, these diseases produce periods of disease activity and inactivity and variations in disease activity in different sites within an individual. A final difficulty in identifying specific periodontal pathogens is the variation in individual host response.¹⁶

In spite of these challenges, current researchers continue to agree that periodontal diseases are infections caused by specific pathogens. Recently, attention has turned to *Tannerella forsythensis* (formerly known as *Bacteroides forsythus*), as well as, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* as primary pathogens for most periodontal infections with moderate evidence linking another subset of microorganisms (*C. rectus*, *E. nodatum*, *F. nucleatum*, *P. intermedia*/

nigrescens, *P. micros*, *S. intermedium*, and *T. denticola*) as possible pathogens.^{14,17}

Researchers are working to develop diagnostic tests for detection and treatments designed to target periodontic pathogens. Systemic antibiotics such as amoxicillin, metronidazole, tetracycline, doxycycline, and Augmentin have been proposed.¹⁵ Local delivery of antimicrobials (tetracycline fibers, metronidazole and minocycline gels, chlorhexidine chips, and doxycycline polymer) have also been introduced.¹⁸ While these approaches have enhanced our ability to manage periodontal diseases, they have still failed to provide uniform success. Viewing plaque as a biofilm promises to aid in the effort to effectively manage periodontal disease.

Plaque as a Biofilm

Previously, bacteria have been studied as they grew in colonies on culture plates in the laboratory. Newer and more sophisticated microscopy, such as confocal scanning laser and two-photon excitation microscopy, has permitted examination of the biofilms in their natural states.^{5,19}

Microorganisms in biofilm behave differently than bacteria in a culture medium (see Table 1).

Seen through a microscope, bacteria in a biofilm are not distributed evenly. They are grouped in microcolonies surrounded by an enveloping intermicrobial matrix (Figure 8).

The biofilm matrix is penetrated by fluid channels that conduct the flow of nutrients, waste products, enzymes, metabolites, and oxygen. The microcolonies within the biofilm have micro environments with differing pHs, nutrient availability, and oxygen concentrations (Figure 9).

Table 1. Basic Biofilm Properties^{20,21}

- Cooperating community of various types of microorganisms
- Microorganisms are arranged in microcolonies with channels between the microcolonies
- Microcolonies are surrounded by protective matrix
- Differing environments within the microcolonies in the biofilm
- Microbial gene expression differs when microorganisms are in a biofilm
- Microorganisms have primitive communication system
- Microorganisms in biofilm are resistant to antibiotics, antimicrobials, and host response

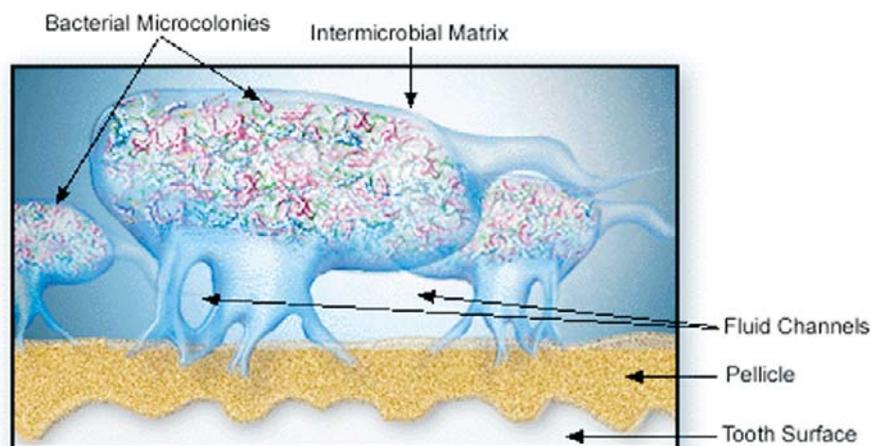


Figure 8. Artistic Depiction of Plaque Biofilm

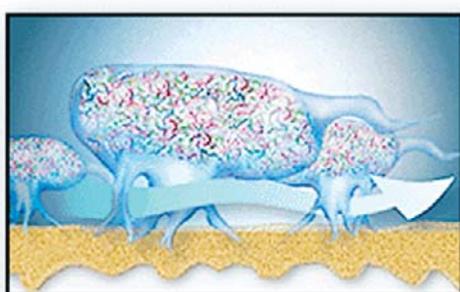


Figure 9. Fluid Channels

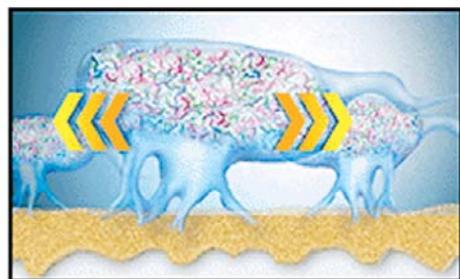


Figure 10. Biofilm bacteria communicate by sending out chemical signals.

The bacteria in a biofilm communicate with each other by sending out chemical signals (Figure 10). These chemical signals trigger the bacteria to produce potentially harmful proteins and enzymes, virulence factors that help the intraoral biofilm bypass host defense systems.^{5,6}

Our previous attempts to predict and control periodontal diseases have been based on the performance of bacteria cultured under laboratory conditions.^{1,5} Increased understanding of biofilms have demonstrated great differences between

bacterial behavior in laboratory culture and in their natural ecosystems. For example, bacteria in biofilm produce compounds in biofilm that they do not produce when in culture. Also, the biofilm matrix surrounding the microcolonies serves as a protective barrier. This helps explain why systemic and locally delivered antimicrobials have not always proven successful, even when they were targeted at specific microorganisms. One researcher has estimated that it can take 1,000 times the drug to kill a microorganism in a biofilm as it does to kill the same organism in a free floating or planktonic environment.²⁰ The protective matrix of biofilm also helps explain why mechanical plaque control and personal oral hygiene have continued to be an integral part of periodontal therapy.^{17,21} Biofilms can be removed by mechanical means. However, they immediately begin to reform, so the search continues for ways to combat biofilms.

New Frontiers

Industrial researchers are pursuing new technology to combat biofilm. One approach is to interfere with the signaling between bacteria in biofilm so they can't communicate with each other. Another tactic is to mimic the natural defenses developed by ocean creatures like whales and dolphins that don't accumulate bacterial biofilms.⁵ Dental researchers are also pursuing new strategies to control oral biofilms^{1,22} (see Table 2).

Varying the oxygen concentration, pH, and nutrient availability in plaque have been shown to modulate biofilm microflora and may prove useful. For example, periodontal pathogens require a low

redox potential for growth. Addition of a redox agent, such as methylene blue, to periodontal pockets has been shown to inhibit the growth of *P. Gingivalis*.²⁴ Since increased gingival crevicular flow (GCF) increases the nutrient supply for subgingival biofilm, control of GCF

may be used in the future to control subgingival biofilm. Use of anti-inflammatory agents may not only help inhibit destructive host pathways, anti-inflammatory agents may also reduce the nutrient supply of GCF for the biofilm community. NIDCR is currently supporting research in this area with the goal of new therapies for the future.¹



Summary

Dental researchers have attempted to understand the microbial nature of oral diseases over that past 120 years. The view of plaque and its constituent microorganisms have shifted from specific plaque hypothesis to a non-specific plaque hypothesis and back again to a theory of specific periodontal pathogens in plaque. Recently dental researchers have begun to view plaque as a biofilm. The nature of a biofilm helps explain why periodontal diseases

Table 2. Possible Strategies to Control Oral Biofilms ²³
<p>Control of nutrients</p> <ul style="list-style-type: none"> • addition of base-generating nutrients (arginine) • reduction of GCF flow through anti-inflammatory agents • inhibition of key microbial enzymes
<p>Control of biofilm pH</p> <ul style="list-style-type: none"> • sugar substitutes • antimicrobial agents • fluoride • stimulate base production
<p>Control of redox potential</p> <ul style="list-style-type: none"> • redox agents • oxygenating agents
<p>Other strategies</p> <ul style="list-style-type: none"> • interfere with communication networks • prevent colonization of selected organisms • enzymes to dissolve matrix of biofilm • replace pathogens with a less virulent strain • photoactivation of microorganisms

have been so difficult to prevent and treat. An improved understanding of biofilm will lead to new strategies for management of these widespread diseases.

To receive Continuing Education credit for this course, you must complete the online test. Please go to www.dentalcare.com and find this course in the Continuing Education section.

Course Test Preview

1. **A biofilm is _____.**
 - a. a loose collection of free-floating bacteria
 - b. a calcified collection of bacteria that cannot be easily removed
 - c. an acellular translucent, homogeneous film covering moist surfaces
 - d. a well-organized, cooperating community of microorganisms

2. **A positive use of biofilm is _____.**
 - a. detoxification of human waste products
 - b. lining on indwelling catheters
 - c. coating in fish tanks
 - d. layer in dental unit water lines

3. **The specific plaque hypothesis would support the following belief:**
 - a. "Where there is more plaque, there is more disease."
 - b. "All bacteria in plaque contribute to gingivitis and periodontitis."
 - c. "Calculus plays a major role in causing periodontitis."
 - d. "The presence of bacterial plaque is necessary to develop periodontal disease, but not sufficient to guarantee disease."

4. **Researchers currently believe that all of the following bacteria play a role as periodontal pathogens EXCEPT:**
 - a. *T. pallidum*
 - b. *P. gingivalis*
 - c. *A. actinomycetemcomitans*
 - d. *B. forsythus*

5. **Scientists have had difficulty in identifying specific periodontal pathogens because _____.**
 - a. periodontal pockets contain both pathogens and non-pathogens
 - b. the different bacteria in periodontal pockets require different culture media
 - c. periodontal disease goes through active and quiescent periods
 - d. All of the above.

6. **To study biofilms, scientists have used newer microscopy techniques such as _____.**
 - a. wet mount microscopy
 - b. scanning electron microscopy
 - c. confocal scanning laser microscopy
 - d. smear microscopy

7. **Which of the following characteristics is typical of a bacteria in a biofilm?**
 - a. Bacteria communicate with each other by sending out chemical signals.
 - b. Bacteria are dispersed more or less evenly through the plaque.
 - c. The environment surrounding bacteria consists of the same or similar pHs.
 - d. Bacteria exist in isolation from each other.

- 8. Given the nature of bacteria in biofilm, which techniques may be helpful in controlling oral biofilms?**
- a. Keep bacteria from communicating with each other.
 - b. Prevent fluid flow between microcolonies of bacteria in a biofilm.
 - c. Change the oxygen concentration with the biofilm microenvironments.
 - d. All of the above.
- 9. How can a subgingival biofilm formation protect periodontal pathogens from locally delivered antimicrobial agents?**
- a. The biofilm prevents the antimicrobial agent from entering the periodontal pocket.
 - b. The biofilm matrix serves as a protective barrier.
 - c. The biofilm fluid channels direct the antimicrobial agent out of the pocket.
 - d. The biofilm changes the pH of the antimicrobial agent and inactivates the agent.
- 10. Possible new strategies to control oral biofilms include all of the following EXCEPT:**
- a. Control of biofilm nutrient sources.
 - b. Alteration of pH within biofilm microcolonies.
 - c. Varying the oxygen concentration within biofilm.
 - d. Addition of systemic antibiotics.

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About the Author

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Dr. Overman is Associate Dean for Academic Affairs at the University of Missouri-Kansas City (UMKC) School of Dentistry. Her teaching background includes oral health education and health promotion, educational methods for health professional faculty, and evidence based decision making. She currently teaches evidence based decision making methods to third year dental students and cross cultural communication to first year dental students. Her administrative responsibilities include faculty development. She brings to the program a wealth of experience in helping practitioners make the transition to their roles as new faculty. Dr. Overman received her B.S. in dental hygiene from UMKC School of Dentistry, her M.S. degree from UMKC's School of Graduate Studies, and in 2001, a doctoral degree in education policy and leadership at the University of Kansas. Her professional service has included numerous positions of national leadership including chair of the ADEA's National Dental Hygiene Directors, chair of ADEA's Council of Allied Program Directors, and American Dental Hygienist's Association Commissioner on Dental Accreditation. She is currently chair-elect of ADEA's Section on Academic Affairs as well as a peer reviewer for the *Journal of Contemporary Dental Practice*, the *Journal of Dental Education* and the *Journal of Dental Hygiene*.

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