

Biofilm: A New View of Plaque



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Disclaimer: Participants must always be aware of the hazards of using limited knowledge in integrating new techniques or procedures into their practice. Only sound evidence-based dentistry should be used in patient therapy.

Conflict of Interest Disclosure Statement

- The author has done consulting work for P&G.

Introduction – Biofilm

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.

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Overview

Dental researchers have attempted to understand the microbial nature of oral diseases over the past 130 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 research, treatment and prevention. 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 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.

Introduction

Despite the best efforts of dental health professionals, oral infections are still widespread. Nearly 92% of U.S. adults between 20 and 64 have had dental caries, and 26% of adults in that age group have untreated dental caries.¹ A study by the Centers for Disease Control and Prevention (CDC) estimates that over 47% of American adults have mild, moderate, or severe periodontitis.²

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



Figure 1. Gingivitis.



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

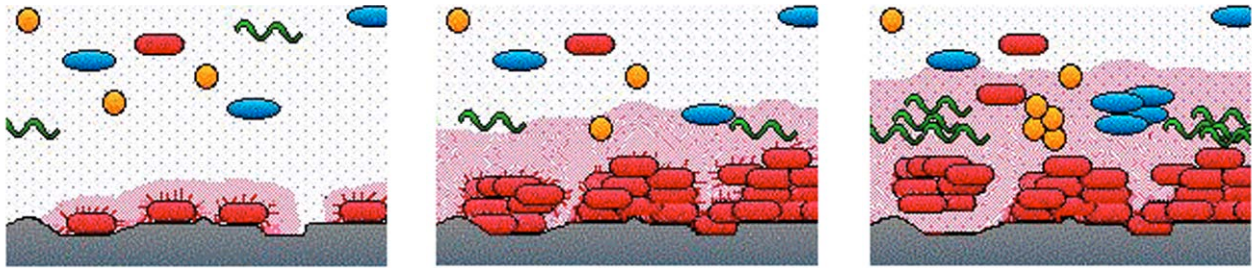


Figure 3a. Formation of biofilm on rock surface.

Consequently, this influences the strategies used to control and prevent dental diseases.³ During the past three decades newer scientific methods have changed the view of dental plaque so dental scientists now see it as a biofilm.⁴

Biofilm

A biofilm is a well organized, cooperating community of microorganisms.^{5,6} 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.⁶ Sometimes biofilms are seen as positive, such as their use for detoxification of waste water and sewage. Humans have a symbiotic relationship with their microbiome. Our resident microorganisms can provide benefits. More often biofilms provide a challenge for humans.^{3,6}

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.^{6,7} Occasionally biofilms are deadly. Legionnaire's 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.^{3,8}

Changing Views of Plaque

In 1996, the National Institute for Dental and Craniofacial Research hosted an international conference on microbial ecology. This meeting

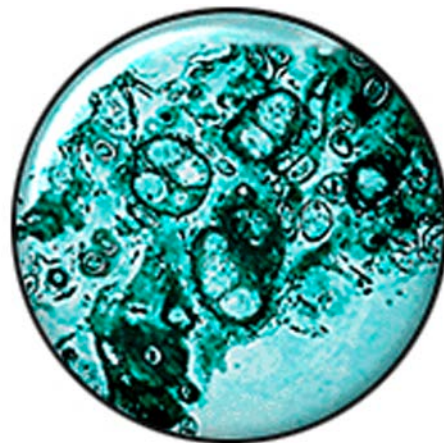


Figure 3b. Biofilm found on dental equipment.

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.⁴ This is not the first time in history dental professionals have shifted their thinking about plaque. Over the past 130 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).⁹ 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,

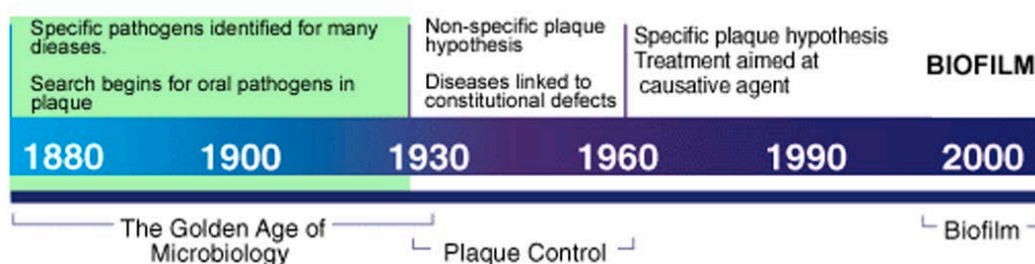


Figure 4. The changing views of plaque and periodontal diseases (1880 - 1930).

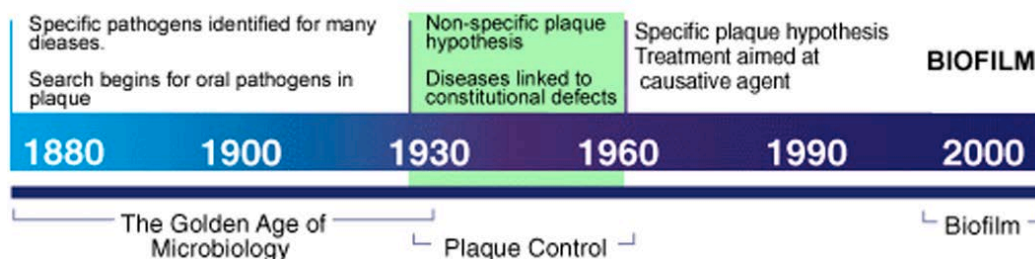


Figure 5. The changing views of plaque and periodontal diseases (1930 - 1960).

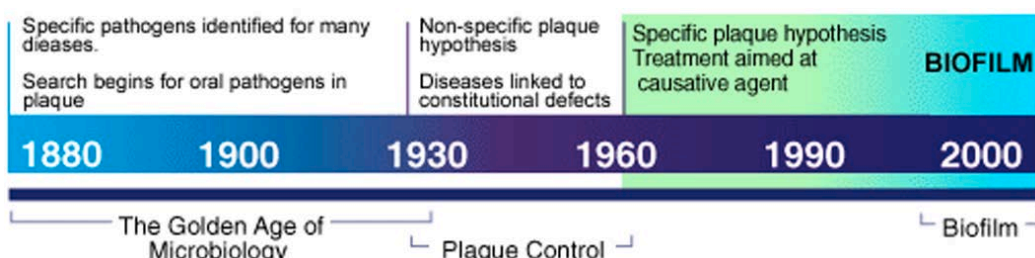


Figure 6. The changing views of plaque and periodontal diseases (1960 - 2000).

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.¹⁰

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.¹⁰ Mechanical irritants such as calculus and overhanging restorations were also thought to play a major role in the pathogenesis of periodontal disease.¹¹

The belief that there was a single microbial agent that caused periodontal disease was replaced by non-specific plaque theories.¹⁰ 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.¹² 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.¹³ The electron microscope confirmed spirochetes were in the connective and epithelial tissues of patients with acute necrotizing ulcerative gingivitis in contrast to healthy controls.¹⁴ 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.¹⁰

Newer methods of microbial analysis such as darkfield microscopy, transmission electron microscopy, scanning electron microscopy, DNA probes, BANA hydrolysis and immunoassay aided the search.¹⁵

Since that time, scientists have continued to search for the specific etiologic agents with mixed success. Haffajee and Socransky¹⁶ have detailed the reasons for the difficulties in pinpointing specific periodontal pathogens. 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.¹⁷

In spite of these challenges, researchers continued to agree that periodontal diseases were infections caused, in part, by specific pathogens. Attention has been given 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. intermedius*, and *T. denticola*) as possible pathogens.^{15,18} Researchers have sought to develop diagnostic tests for detection and treatments designed to target periodontal

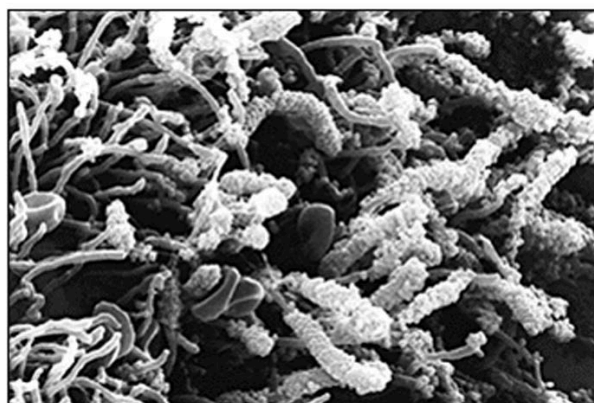


Figure 7. SEM of mature human dental plaque demonstrating corn cob formation. Bar = 10 microns at an original magnification of 2,020.

Image courtesy of Dr. Charles Cobb, University of Missouri-Kansas City

pathogens. Systemic antibiotics such as metronidazole, clindamycin, doxycycline, ciprofloxacin, azithromycin alone or in combination have been proposed.¹⁸ Local delivery of antimicrobials (tetracycline fibers, metronidazole and minocycline gels, chlorhexidine chips, and doxycycline polymer) have also been introduced.¹⁹ 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 technology, such as confocal scanning laser and two-photon excitation technology, as well as molecular analysis methods, such as DNA-DNA hybridization and gene expressions and metabolomics, has permitted examination and understanding of oral biofilms in their natural states.^{6,20,21}

Microorganisms in biofilm behave differently than planktonic (free-floating) bacteria or those in a culture medium (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).

Table 1. Basic Biofilm Properties.^{22,23}

- 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

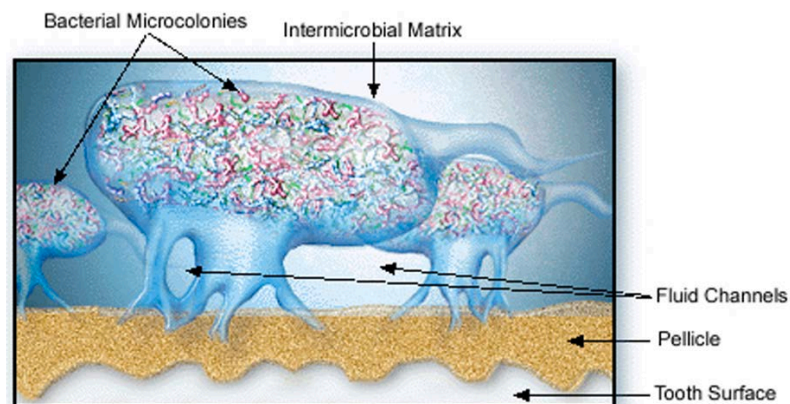


Figure 8a. Artistic Depiction of Plaque Biofilm.

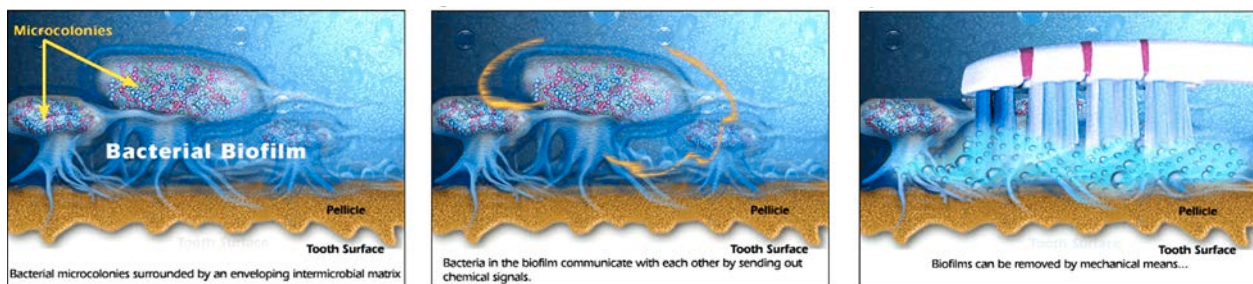


Figure 8b. Animation of Biofilm.

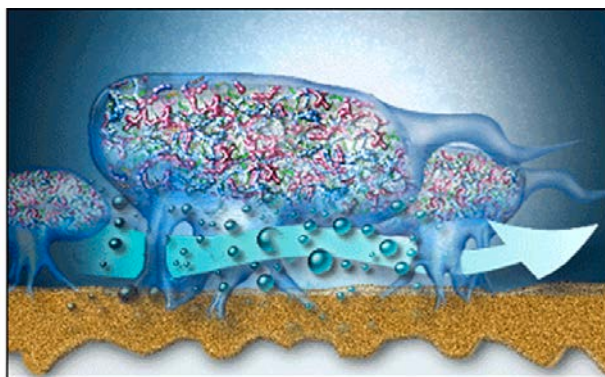


Figure 9. Biofilm Fluid Channels.

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 pH's, nutrient availability, and oxygen concentrations (Figure 9). The bacteria in a biofilm use a communication system termed quorum sensing that involves 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.

Our previous attempts to predict and control periodontal diseases have been based on the performance of bacteria cultured under laboratory conditions.^{1,6} 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. Researchers have 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.²² 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. Biofilms can be



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

removed by mechanical means. However, they immediately begin to reform, so the search continues for ways to combat pathogenic biofilms.

New Frontiers

Researchers are pursuing new technology to manage all types of biofilms, not just those in the oral cavity. One approach is to interfere with the signaling between bacteria in biofilm so they can't communicate with each other. Another tact 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 (Table 2).^{4,24,26}

Varying the oxygen concentration, pH, and nutrient availability in plaque have been show 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*.²⁵ 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.⁴

Table 2. Possible Strategies to Control Oral Biofilms.²⁴

Control of nutrients

- addition of base-generating nutrients (arginine)
- reduction of GCF flow through anti-inflammatory agents
- inhibition of key microbial enzymes

Control of biofilm pH

- sugar substitutes
- antimicrobial agents
- fluoride
- stimulate base production

Control of redox potential

- redox agents
- oxygenating agents

Other strategies

- 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

Conclusion

Dental researchers have attempted to understand the microbial nature of oral diseases over that past 130 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 have been so difficult to prevent and treat. An improved understanding of biofilm will lead to new strategies for management of these widespread diseases.

Course Test Preview

To receive Continuing Education credit for this course, you must complete the online test. Please go to: www.dentalcare.com/en-us/professional-education/ce-courses/ce42/test

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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Additional Resources

- No Additional Resources Available.

About the Author

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Dr. Pamela Overman is professor emerita of dentistry at the University of Missouri-Kansas City School of Dentistry. Throughout her academic career, Dr. Overman taught dental, dental hygiene and graduate students in oral health education and health promotion, educational methods for health professional faculty, and evidence based decision making. Her professional service included numerous positions of national leadership including chair of the ADEA's National Dental Hygiene Directors, chair of ADEA's Section on Academic Affairs, and as and the American Dental Hygienist's Association Commissioner on

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