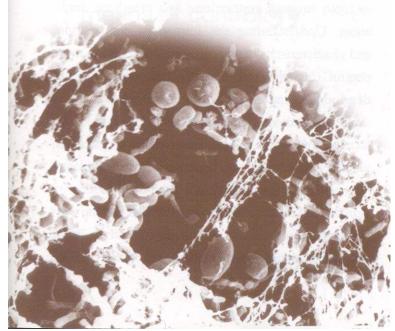
By Jill S. Nield-Gehrig, RDH, MA*

Bacteria are the primary etiologic agents in periodontal disease. More than 500 bacterial strains may be found in dental plaque.¹ These bacteria have evolved to survive in the environment of the tooth surface, gingival epithelium, and oral cavity. Recent technical advances have led to the recognition that dental plaque is a biofilm. Changes in thinking about the structure of dental plaque have improved our understanding of why periodontitis is so difficult to treat and will affect the strategies used to prevent and control periodontitis in the future.

Bacterial Lifestyles

Bacteria may be free-floating or attached to a surface. Recent advances in research technology have allowed researchers to study bacteria in their natural environment. These studies have revealed that most bacteria live in complex communities called biofilms. A biofilm is a well-organized community of bacteria that adheres to surfaces and is embedded in an extracellular slime layer. Once a bacterium attaches to a surface, it activates a whole different set of genes that gives the bacterium different characteristics from those that it had as a free-floating organism. It has been estimated that more than 99% of all bacteria on the earth live as attached bacteria.² Biofilms can

Figure 1. Contact Lens Case Biofilm. This scanning electron micrograph shows the biofilm formation on the inside of a contact lens case. (Micrograph courtesy of Drs. Lousie McLaughlin-Borlace and John Dart of the Institute of Ophthalmology, University College, London).



be found on medical and dental implants living in intravenous and urinary catheters, contact lenses, and prosthetic devices, such as heart valves, biliary stents, pacemakers, and artificial joints (Figure 1). Bacteria can also be lifethreatening; Legionnaire's disease, which killed 29 people in 1976, was the result of a bacterial biofilm in the hotel's air conditioning system.

The Structure of Biofilm Communities A biofilm community comprises bacterial microcolonies, an extracellular slime layer, fluid

channels, and a primitive communication system. The basic properties of the biofilm

structure are summarized in the Table. As the bacteria attach to a surface and to each

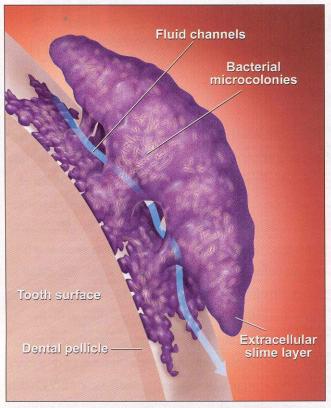


Figure 2. Dental Plaque Biofilm. Conceptual illustration of the biofilm structure.

е

ntrations) within which bacteria with different physiological needs can survive.

The extracellular slime layer is a protective barrier that surrounds the mushroomshaped bacterial microcolonies. The slime layer protects the bacterial microcolonies from antibiotics, antimicrobials, and host defense mechanisms. A series of fluid channels penetrates the extracellular slime layer (Figure 4). These fluid channels provide nutrients and oxygen for the bacterial micro colonies and facilitate movement of bacterial metabolites, waste products, and enzymes within the biofilm structure. Each bacterial microcolony uses chemical signals to create a primitive communication system used to communicate with other bacterial microcolonies (Figure 5).

other, they cluster together to form sessile, mushroom-shaped microcolonies that are attached to the surface at a narrow base (Figure 2). Each microcolony is a tiny, independent community containing thousands of compatible bacteria. Different microcolonies may contain different combinations of bacterial species. Bacteria in the center of a microcolony may live in a strict anaerobic environment, while other bacteria

at the edges of the fluid channels may live in an aerobic environment (Figure 3). Thus, the biofilm structure provides a range of customized living environments (with differing pHs, nutrient availability, and oxygen

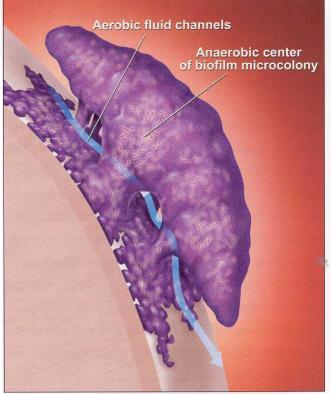


Figure 3. Biofilm Environments. Conceptual illustration of the biofilm indicating the aerobic environment at the edges of the fluid channels and the anaerobic environment at the center of a microcolony.

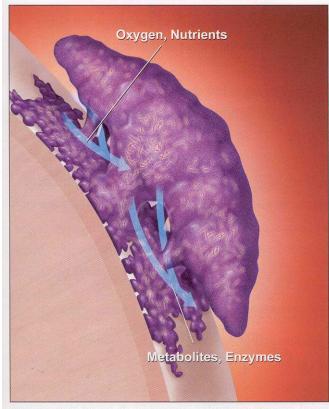


Figure 4. Fluid Channels. Fluid channels that facilitate the movement of nutrients, oxygen, bacterial metabolites, and enzymes within the biofilm structure penetrate the extracellular slime layer.

The initial attachment of bacteria begins with pellicle formation. The pellicle is a thin coating of salivary proteins that attach to the tooth surface within minutes after a professional cleaning. The pellicle acts like double-sided adhesive tape. adhering to the tooth surface on one side and on the other side, providing a sticky surface that facilitates bacterial attachment to the tooth surface. Following pellicle formation, bacteria begin to attach to the outer surface of the pellicle. Bacteria connect to the pellicle and each other with hundreds of hairlike structures called fimbriae. Once they stick, the bacteria begin producing substances that stimulate other freefloating bacteria to join the community. Within the first 2 days in which no further cleaning is undertaken, the tooth's surface is

Formation of Dental Plaque Biofilms The moment a baby passes through the birth canal and takes its first breath, microbes begin to reside in its mouth. Later, as the teeth erupt, additional bacteria establish colonies on the tooth surfaces. Dental bacterial plaque is a biofilm that adheres tenaciously to tooth surfaces, restorations, and prosthetic appliances. Understanding the formation, composition, and characteristics of the plaque biofilm assists in its control (Figure 6). The pattern of plaque biofilm development can be divided into three phases:

1. Attachment of bacteria to a solid surface;

2. Formation of microcolonies on the surface; and

3. Formation of the mature, subgingival plaque biofilms (Figure 7).

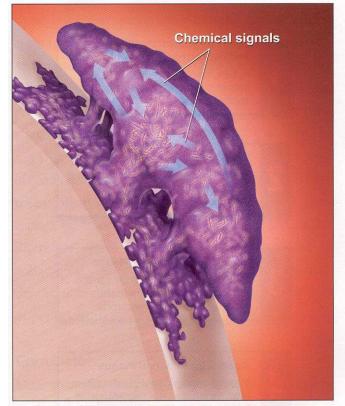


Figure 5. Primitive Communication System. The bacteria in a biofilm use chemical signals to communicate with each other.

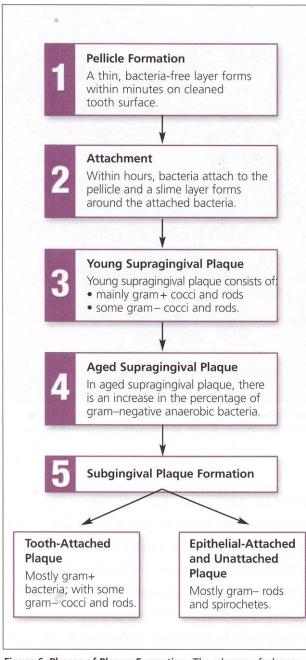


Figure 6. Phases of Plaque Formation. The phases of plaque formation are: pellicle formation, attachment, young supragingival plaque, aged supragingival plaque, and subgingival plaque formation.

colonized predominantly by grampositive facultative cocci, which are primarily streptococci species. It appears that the act of attaching to a solid surface stimulates the bacteria to excrete an extracellular slime layer that helps to anchor them to the surface and provides protection for the attached bacteria.

Microcolony formation begins once the surface of the tooth has been covered with attached bacteria. The biofilm grows primarily through cell division of the adherent bacteria, rather than through the attachment of new bacteria. Next, the proliferating bacteria begin to grow away from the tooth. Plague doubling times are rapid in early development and slower in more mature biofilms. Bacterial blooms are periods when specific species or groups of species grow at rapidly accelerated rates. A second wave of bacterial colonizers adheres to bacteria that are already attached to the pellicle. Coaggregation is the ability of new bacterial colonizers to adhere to the previously attached cells. The bacteria cluster together to form sessile, mushroom-shaped micro colonies that are attached to the tooth surface at a narrow base. The result of coaggregation is the formation of a complex array of different bacteria linked to one another.

Following a few days of undisturbed

plaque formation, the gingival margin becomes inflamed and swollen. These inflammatory changes result in the creation of a deepened gingival sulcus. The biofilm extends into this subgingival region and flourishes in this protected environment, resulting in the formation of a mature subgingival plaque biofilm. Gingival inflammation does not appear until the biofilm changes from one composed largely of gram-positive bacteria to one containing gram-negative anaerobes. A subgingival bacterial

microcolony, predominantly composed of gram-negative anaerobic bacteria, becomes established in the gingival sulcus between 3 and 12 weeks after the beginning of supragingival plaque formation. Most bacterial species currently suspected of being periodontal pathogens are anaerobic, gram-negative bacteria.

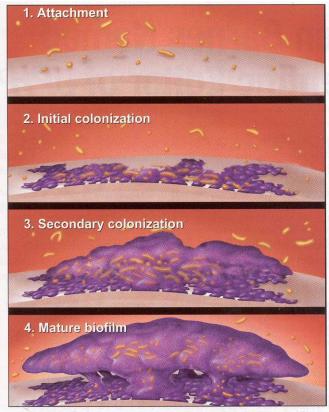


Figure 7. The Pattern of Biofilm Development. The stages of biofilm maturation are: attachment, initial colonization, second-ary colonization, and mature biofilm.

Control and Removal of Dental Plaque Biofilms The recent recognition that subgingival plaque is a biofilm helps considerably in understanding its persistence and resistance to the host's defense system. The formation of biofilms by subgingival bacteria provides the bacteria with an advantage that permits long-term survival within the sulcus or pocket environment.

Bacterial microcolonies are protected by one another and by the extracellular slime layer and are unusually resistant to antibiotics (administered systemically), antimicrobials (administered locally), and the body's defense system. Antibiotic doses that kill free-floating bacteria, for example, need to be increased as much as 1,500 times to kill biofilm bacteria (and at these high

doses, the antibiotic would kill the patient before the biofilm bacteria).^{2,3} It is likely that several mechanisms are responsible for biofilm resistance to antibiotics and antimicrobial agents. The slime layer may prevent the drugs from penetrating fully into the depth of the biofilm. Bacteria can develop resistance to antimicrobial drugs by producing a thicker protective slime layer. The slime layer may protect the bacteria against leukocytes (defensive cells of the body's immune system). Antibiotic or antimicrobial therapy usually will not kill the biofilm; the biofilm can be destroyed, however, by simply wiping them off (disrupting their attachment to a surface).

Due to the structure of biofilms, physical removal of bacterial plaque biofilms is the most effective means of control. Subgingival plaque within pockets cannot be reached by brushes, floss, or oral rinses. Therefore, frequent periodontal debridement of subgingival root surfaces by a dental hygienist or dentist is an essential component in the treatment of periodontitis.

TABLE. Biofilm Structure⁴

- The bacteria in biofilms cluster together to form sessile, mushroom-shaped microcolonies.
- Each microcolony is an independent community with its own customized living environment.
- A protective, extracellular slime layer surrounds the microcolonies.
- A series of fluid channels penetrate the slime layer and facilitate movement of nutrients and bacterial products throughout the biofilm.
- A primitive communication system of chemical signals allows communication between the bacterial microcolonies.

Conclusion

More than 500 bacterial strains have been identified in dental biofilm. Experts agree that most forms of periodontal disease are caused by specific pathogens, particularly gram-negative bacteria. The recognition that dental plaque is a biofilm helps to explain why periodontal diseases have been so difficult to prevent and to treat. Periodontal pathogens within a biofilm environment behave very differently from free-floating bacteria. The

protective extracellular slime matrix makes bacteria extremely resistant to antibiotics, antimicrobial agents, and host defense mechanisms. Mechanical removal is the most effective treatment currently available for the control of dental plaque biofilms.

References

1. Kroes I, Lepp PW, Reiman DA Bacterial diversity within the human subgingival crevice. *Proc Natl Acad Sci USA* 1999;96(25):14547-14552.

2. Coghlan A Slime City. New Scientist 1996;2045:32-36.

3. Elder MJ, Stapelton F, Evans E, DartJK. Biofilm-related infections in ophthalmology *Eye* 1995;9(Pt. 1):102-109.

4. Nield-Gehrig JS and Willmann DE. *Foundations of Periodontics for the Dental Hygienist*. Philadelphia: Lippincott Williams & Wilkins 2003:67-73.

* Textbook author, international speaker, and dental hygiene consultant, Asheville, North Carolina. She can be reached at jng_consulting@bellsouth.net.

Portions of this presentation have been adapted with permission from Nield-Gehrig JS and Willmann DE. Foundations of Periodontics for the Dental Hygienist; @ 2003 Lippincott Williams & Wilkins (<u>http://www.lww.com/</u>).