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A CLOSER **Look**

***Dimensions of Dental Hygiene* speaks with J.W. Costerton, PhD, MS, and Paul Stoodley, PhD, of the Center for Biofilm Engineering at Montana State University, Bozeman, about the complexities of dental biofilm.**

J.W. Costerton, PhD, MS, is the center director and professor of Microbiology, and Paul Stoodley, PhD, is assistant research professor, both for the Center for Biofilm Engineering at Montana State University, Bozeman.

Q Define biofilm and discuss the differences between biofilm, plaque, loosely adherent plaque, and the plaque mass.

A Biofilms are the collections of bacteria and other microorganisms that assemble on surfaces. They are widespread in nature and can colonize natural, nonliving hard surfaces such as river rocks, man-made surfaces like the concrete found in industrial pipelines, and even plant and animal surfaces and, of course, the teeth and gums.

Within this broad definition, dental plaque falls into one of many types of different biofilms. Loosely adherent plaque and the denser, more firmly attached plaque mass are also considered biofilms. However, they are different in the types of organisms that inhabit them, their strength, and the likelihood that they might detach either spontaneously, through application of normal oral forces, or through oral cleaning.

Q How does this differ from what we learned in school and how does this change what we do in practice?

A When we start to see dental plaque as biofilm, our perceptions begin to change considerably. To many people, the term “plaque” has connotations of a static coat of dirt, eg, nonliving, simple, and something that is easily washed away. By switching to the term “biofilm,” we think of plaque in a whole new way—as living, changing, dynamic communities of microorganisms, which can flourish in sometimes hostile environments. Education plays a major role in how effectively patients maintain personal dental hygiene practices. Removing biofilm plaque everyday is like frequently mowing your yard. It not only looks better, but continual maintenance prevents the ecological succession from lawn, to weeds, to thicket. As the biofilm grows, it provides a protective environment for the harmful bacteria within. By keeping the plaque biofilm “mowed” back, we prevent other nastier and more pathogenic organisms from becoming established.

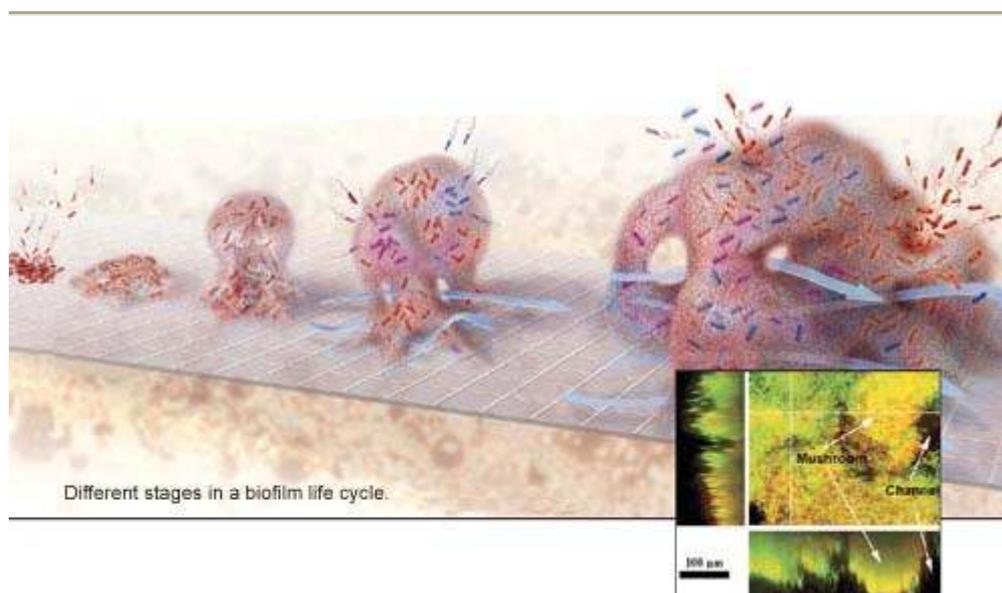
Q How complex are biofilms? How do they compare to other pathogenic biofilms? Can you have a dental biofilm that does not contribute to disease?

A The more we study biofilms, the more complexity we find. Even in the most simple systems of pure culture biofilms (biofilms grown from one type of bacteria), intricate patterns of attachment and the subsequent growth into elaborate structures, which optimize the flow of nutrients and enhance the survival of the microorganisms on the surface, are found. Some biofilms, such as the common human pathogen *Staphylococcus aureus* can continually shed clumps of bacteria in the protected biofilm state while the seeding dispersal strategy of motile species such as *Pseudomonas aeruginosa* is primarily through swimming motility of single cells.

Biofilms growing in flowing water can also disperse by flowing or rolling over surfaces. Their viscoelastic properties allow clumps to attach at the “leeward” side and detach at the “windward” side. When you add mixed cultures and the complexities of the host tissue and immune response, the complexity increases as bacterial cells interact with each other while also trying to avoid the immune system.

In terms of diversity, dental plaque biofilms tend to be one of the more complex associated with pathogenic biofilms. Although multispecies infections have been reported for invasive infections, these are relatively rare in comparison to the monospecies infections by organisms like *S. aureus*. Multispecies pathogenic biofilms, not surprisingly, tend to occur on the outside of the epithelia.

There is a concept where “beneficial” microorganisms can inhabit a biofilm and actually protect us from disease. These organisms are termed “commensals.” It is possible that one of the commensal biofilm’s mechanisms to keep pathogens out is to occupy a space that might otherwise be occupied by a pathogen. *In vitro*¹ and clinical studies² suggest that *Lactobacillus rhamnosus* GG (LGG) can inhibit the colonization of streptococci caries pathogens, thus reducing the incidence of caries in children.



Confocal microscopy shows the complex structure of a *Streptococcus mutans* biofilm. The square image is a plain view and the vertical and horizontal sidebars are cross sections taken through the biofilm.

Images courtesy of Joanna Heersink and Paul Stoodley, PhD, funded by Philips Oral Healthcare. Illustration by Keith Kasnot, Scientific American 2001. Courtesy of Philips Oral Healthcare.

Q What is confocal scanning laser microscopy (CSLM) and why is it important?

A CSLM allows biofilms and other thin tissues to be microscopically sectioned in the fully hydrated, live state. By recombining images taken at different depths in the biofilm, a 3D picture of biofilm structure is constructed. The use of fluorescent protein gene reporters allow us to look at the distribution of gene activity and different bacterial species within the biofilm.

Using CSLM in biofilm research provided a breakthrough in bringing the true complexity of bacterial biofilms to our attention. Prior to CSLM, our view of biofilm structure was determined largely by images from scanning electron microscopy (SEM). However, since SEM is a high vacuum technique, the biofilms had to be dehydrated, which caused them to collapse and lose much of their intricate structures. However, SEM still provides much higher resolution images than CSLM and both techniques now complement each other.

In addition to observing structure and bacterial distribution in the biofilm, CSLM also allows the real time tracking of fluid flow and the supply of nutrients in the biofilm. By specifically locating the position of microelectrodes in the biofilm, we are able to demonstrate that the channel structures running throughout the biofilm can enhance the supply of nutrients, such as oxygen, to the biofilm cells. For the first time, there is a link between the complex structure and function in biofilms. This sets the stage for our current thinking that the structural complexity is not necessarily an incidental result of the growth environment but may actually be optimized for the survival of micro-organisms in biofilms.

The development of a confocal endoscope at

Montana State University promises to expand CSLM technology out of the laboratory and into the dental office, where the effects of a dental hygiene procedure on plaque biofilm removal can be assessed by looking at the 3D structure of the biofilm on the microscopic scale.



Side view of an *S. mutans* biofilm grown on a glass surface in a drip flow reactor. *S. mutans* is an early colonizer of the tooth surfaces and is responsible for caries.

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