

# Biofilms: sensing and signaling

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**abstract** Biofilms are a community of surface-attached microorganisms that can have far-reaching effects. Biofilms are costly to industry and affect human health in a variety of ways. Research is only now beginning to discern the complexities of biofilm formation.

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**t**he problem of bacterial contamination of dental waterlines is an excellent illustration of a basic precept in biofilm science: Biofilms are the preferred mode of growth for most bacteria. Existence as a biofilm provides bacteria with a protective environment that effectively prevents attack by antimicrobials, biocides, and even immunologic factors. Biofilms are costly for industry due to their biofouling potential, which can cause a pressure drop or product degradation.<sup>1</sup> The detachment of biofilms has been implicated in the contamination of food and household products during manufacturing and processing. Biofilms are also associated with public health

issues beyond the problem of dental waterline contamination. For example, biofilms in drinking water systems may act as a reservoir for potential pathogens.<sup>2</sup> In the human body, there is a direct relationship between the presence and severity of dental plaque biofilm and an increase in the potential of suffering a heart attack.<sup>3</sup> Despite the growing body of research into biofilm formation, relatively little is known about the metabolism and physiology of biofilm bacteria.<sup>4</sup>

Antony van Leeuwenhoek could be considered one of the first biofilm researchers when, in the late 1600s, he scraped dental plaque from his mouth and looked at it with his microscope. In 1943, ZoBell published a study of the affinity of marine bacteria for attaching to surfaces.

However, it was not until the 1970s that research in the formation of biofilms really started. Two assumptions were pervasive in early biofilm research: that biofilm bacteria and planktonic or free-floating bacteria are the same and that biofilms were relatively simple systems of homogeneous slime. The more-traditional microbiological methodologies of plating and broth culturing of bacteria have, until recently, warped the view of how bacteria really live and survive in the environment. Improvements in technology have allowed biofilm scientists to prove otherwise.<sup>5</sup>

#### current research

When a bacterial cell comes in contact with a surface, it may or may not stick immediately. The confocal scanning laser microscope allows for the visual examination of biofilms in real time with minimal preparation. Using the confocal scanning laser microscope, individual cells of *Pseudomonas aeruginosa* containing a genetic insert called green fluorescent protein were followed as they attached to a surface. The green fluorescent protein genes, which come from jellyfish, cause the cells to fluoresce, allowing for the visualization of bacteria without the use of fixatives or stains that kill the cells. Results indicated that some bacteria will permanently attach to the surface while others will attach briefly and then move on to another position.<sup>6</sup> During this time of initial adhesion, there are a number of changes taking place within the bacterial cell. Bacteria that are dividing at the rate of minutes in culture will stop dividing for hours when first attached to a surface.<sup>6</sup> During this time, there are numerous changes occurring as that bacterial cell makes the transition from a planktonic to a biofilm cell. Eventually, the biofilm bacterial cell will be metabolically and physiologically very different from its planktonic counterpart to the point that there may even exist what is now termed the biofilm phenotype.<sup>7</sup>

Attached bacteria produce an exopolysaccharide matrix that can act

as a protective polymer for the cells embedded within. As the biofilm grows and thickens, it begins to develop into a heterogeneous matrix interspersed with channels that allow nutrients and oxygen to penetrate into the depths of even the thickest biofilms. Researchers have shown that the cells within the biofilm matrix exhibit differences in physiology depending on their location. This concept of spatial heterogeneity within a biofilm has been applied to oxygen limitations (from aerobic to anaerobic), pH, nutrients, and rates of growth.<sup>8-10</sup> Within a thick biofilm, there are various microniches that allow for numerous types of metabolic processes to take place. Dental plaque is an excellent example of the complexity of microorganisms that can exist within a biofilm with a range of metabolic capabilities.<sup>10</sup>

The development of a biofilm appears to be a very effective survival strategy for bacteria. The cells within the biofilm exhibit an increased resistance to biocides and antimicrobials in comparison to planktonic cells. A number of hypotheses have been put forth to attempt to explain this phenomenon. In some cases, there is a limitation to the penetration of the antimicrobials into the biofilm matrix. Since cells within the matrix are living at different physiologic states, the rate of uptake into the cell of the antimicrobial can be affected. The exopolysaccharide of the biofilm matrix may provide a physical barrier to the penetration of antimicrobials.<sup>11</sup> The differences in bacterial cell physiology within the biofilm will reduce the susceptibility of cells to some antimicrobials such as growth-dependent antibiotics.<sup>12</sup> However, diffusion and growth limitations alone may not account for the entire decrease in susceptibility to antimicrobials seen in biofilm cells. A study of the effects of antibiotics on *Klebsiella pneumoniae* biofilms grown on microporous polycarbonate membranes showed that ampicillin, unable to penetrate the biofilm matrix, cannot kill *K. pneumoniae* biofilm

cells. In contrast, ciprofloxacin was shown to be able to diffuse through the *K. pneumoniae* biofilm in as little as 20 minutes. However, *K. pneumoniae* cells were resistant to ciprofloxacin at even 10 times its established minimal inhibitory concentration.<sup>13</sup> This suggests that the genetic changes the planktonic bacterium undergoes as it becomes a biofilm cell may somehow also affect its susceptibility to various antimicrobials.

Ongoing research at the Center for Biofilm Engineering at Montana State University in Bozeman has been working to delineate the changes that occur in *P. aeruginosa* during initial attachment. Proteomics involves the analysis of differentially expressed (induced or repressed) proteins and allows researchers to analyze the protein expression of an organism at a particular point in time or under a particular condition. Results indicate that cells of *P. aeruginosa* attaching to a surface begin to express changes in their protein profiles (when compared to planktonic cells) in as little as 10 minutes after inoculation. These changes in protein expression continue during the time of the experiments (three hours).<sup>14</sup> These differences in protein expression during initial adhesion indicate physiologic changes are taking place within cells as they attach to a surface.

As the biofilm develops, bacterial cells within the matrix will release chemical signals. These signal molecules may enable the bacterial colonies to develop the characteristics of a more mature biofilm. A number of bacterial species, both gram-positive and gram-negative, use these chemical signal molecules to coordinate activity.<sup>15</sup> The action of these signal molecules relies on a process called quorum sensing. In quorum sensing, the ability of the molecule to cause an action is dependent on its concentration within the environment. That concentration can increase only when there is a sufficient number of bacterial cells producing that particular signal. Probably some of the best-known quorum sensing systems are

found in marine bacteria of the genus *Vibrio*. Species of this bacterial genus symbiotically colonize the light organs of certain fish or squid and will emit luminosity only when the population density has reached sufficient quorum density numbers.<sup>16</sup>

The cell-to-cell signaling systems of *P. aeruginosa* have been extensively studied as a model for quorum sensing during biofilm development by gram-negative bacteria. Mutant strains of *P. aeruginosa* deficient in one of the quorum sensing systems (*lasR*) have been shown to produce biofilms that lack the towers and channels often seen in *P. aeruginosa* biofilms. In addition, these mutant biofilms lack the resistance to treatment by sodium dodecyl sulfate seen in wild-type biofilms.<sup>17</sup> Recently, researchers have isolated quorum-sensing molecules produced by *P. aeruginosa* from the sputum of cystic fibrosis patients, suggesting that this is a biofilm disease of the lungs.<sup>18</sup>

Research into the cell-to-cell signaling capabilities of gram-positive biofilm-forming bacteria has also been ongoing. Mutants of *Streptococcus gordonii*, a gram-positive oral bacterium that initiates the formation of dental plaque, were assayed for defective biofilm formation. In this particular study, nine mutants shown to have defects in genes of known function could not form biofilms. One of the genes identified, *ComD*, is a known component of the cell-to-cell signaling system in gram-positive bacteria.<sup>19</sup>

## conclusion

The majority of bacteria in the environment are found attached to surfaces rather than as unicellular, freely suspended planktonic cells. Biofilms are found in almost every environmental system studied and in nearly every industrial and medical setting where microbial contamination is a problem. Dental water lines can provide just the environment conducive to biofilm growth. The quality of dental water

is obviously critical. To successfully minimize contamination, it is important to understand the physiology and metabolism of biofilm bacteria. That bacteria do not usually live in the environment in suspensions of single cells has significant ramifications both for the relevance of how most bacterial species are studied and for the treatment options utilized for biofilm control.

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