MICROBIOLOGY

Forging a Link Between Biofilms and Disease

The sticky conglomerations of bacteria known as biofilms are being linked to common human diseases ranging from tooth decay to prostatitis and kidney infections

"United we stand, divided we fall" was a watchword of the American Revolution—with good reason. The rebels who fought off the yoke of British colonialism recognized that, although individually they might be no match for the well-equipped British soldiers, they might prevail if they stuck together. Now, scientists are coming to recognize that, to a much greater degree than previously thought, bacteria also adopt this strategy, allowing them to counter both the might of the body's immune system and the weapons that physicians use against them.

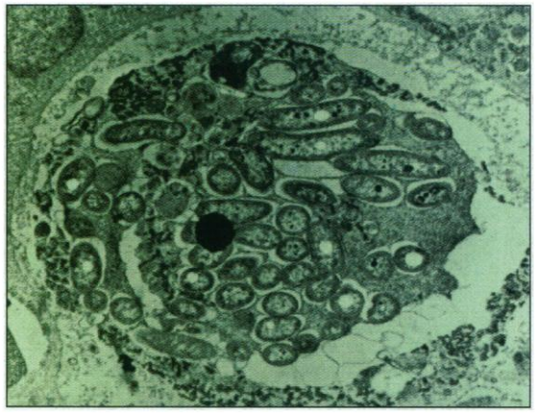
Until recently, the slimy conglomerations of bacteria known as biofilms were recognized mostly for their propensity to coat—and corrode—pipes. But within the past few years, mounting evidence has shown that they cause a host of medical problems as well. Biofilms foul tubing and implants, such as heart valves and artificial hips, and they attack body tissues, such as the teeth and gums, the lungs, the ears, and the urogenital tract. Indeed, infectious disease experts at the Centers for Disease Control and Prevention (CDC) estimate that 65% of human bacterial infections involve biofilms.

The heavy toll these cohesive bacterial troublemakers take on human health has caught the attention of the National Institutes of Health (NIH). It will soon begin awarding the first grants in a new coordinated biofilm program involving eight of the 23 institutes. "We want researchers to know that we recognize the importance of biofilms and [want to] bring people together to work on the problem," says Dennis Mangan of the National Institute of Dental and Craniofacial Research (NIDCR), who is spearheading the effort. Biofilms require a coordinated attack by researchers with expertise in everything from microbiology and immunology to materials science and mathematical modeling, Mangan explains. The goal is to understand how and why biofilms form so that researchers can identify their Achilles' heel and devise better treatments, which are badly needed.

Bacteria sequestered in biofilms are shielded from attack by the host's immune system and are often much harder to kill with antibiotics than their free-floating or "planktonic" counterparts, says William Costerton, director of the Center for Biofilm Engineering at Montana State University in Bozeman. That may be why it's so hard to rid the body permanently of some infections, such as those of the ear or urinary tract.

Biofilms' links to diseases

The first inklings that biofilms could be a health problem came in the mid-1960s when dental researchers Johannes Van Houte and Ronald Gibbons of the Forsyth Dental Center in Boston, Massachusetts, recognized that bacteria living in the mouth synthesize gummy adhesives that accumulate on the teeth, gums, and tongue. They proposed that bacteria attach themselves to solid surfaces in areas such as the mouth where they might otherwise be washed away. But although that helps the bacteria hang on, it can be bad for the body. In the mouth, for example, it results in dental plaque, tooth decay, and gum disease.



Clogging the airways. *P. aeruginosa* bacteria clump together in the lung of an animal with pneumonia similar to that in cystic fibrosis patients.

Evidence supporting the idea that bacteria attach themselves to surfaces throughout the body began appearing about a decade later when Thomas Marrie of Dalhousie University in Halifax, Nova Scotia, using the then recently developed scanning electron microscope, detected a biofilm coating a heart pacemaker removed from a patient. The following year, he also saw the films creeping up urinary catheters. Such biofilms often lead to infections of the bladder or other organs.

Since then, biofilms have been implicated in numerous infections. Perhaps the most notorious is Legionnaire's disease, named after the infection that killed 29 members of the American Legion attending a convention in Philadelphia in 1976. The culprit turned out to be chunks of biofilm containing the bacterium *Legionella pneumoniae* that had wafted out of air conditioners.

And in the mid-1980s, Joseph Lam of the University of Calgary in Alberta, using the transmission electron microscope, confirmed that biofilms are present in the lungs of cystic fibrosis patients. More recent studies by Nels Hoiby of the University of Copenhagen in Denmark show that biofilms containing the bacterium *Pseudomonas aeruginosa* clog the lungs of 80% to 90% of these patients. This eventually leads to death by respiratory failure. "Antibiotic [therapy] kills some cells, but biofilms hunkered down survive the onslaught," says Peter Greenberg of the Cystic Fibrosis Research Center at the University of Iowa, Iowa City.

Microbiologist Fred Quinn, chief of the Pathogenesis Laboratory at the CDC, believes that something similar may occur in tuberculosis. Sputum from patients in the late, infectious stage of the disease consists of viscous clumps of the causative bacterium *Mycobacterium tuberculosis*, resembling the *P. aeruginosa* clumps seen in cystic fibrosis patients. "Perhaps this late stage of tuberculosis is a biofilm," surmises Quinn. Chest x-rays of tuberculosis patients also show dense areas that suggest biofilm-filled cavities in the lungs, he notes.

Researchers now suspect that biofilms are also behind a number of additional medical conditions. For example, microscopic exams have shown that biofilms form the glue that binds struvite kidney stones. Accounting for a quarter of all kidney stones, struvite stones damage the kidney more than other types. They also tend to return after surgical removal. The biofilm connection has led to better surgical techniques, however. Urologists know they "must get every little bit [of the stones] or the biofilm recurs," says urology specialist Curtis Nickel of Queens University in Kingston, Ontario.

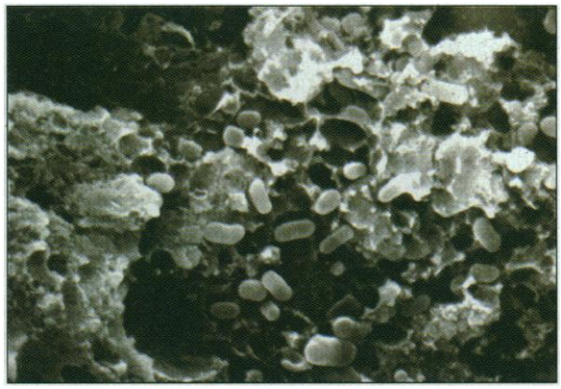
Long-lasting biofilms, undetectable by traditional culture techniques, may also cause some common recurring infections. Using the sensitive DNA detection technique known as the polymerase chain reaction, microbiologist Garth Ehrlich of Allegheny University of the Health Sciences in Pittsburgh, Pennsylvania, found evidence for gene expression by the bacterium *Haemophilus influenzae*, a common cause of ear infections, in ear fluid from children

CREDIT: CENTER FOR BIOFILM ENGINEERING

weeks after they had antibiotic treatment. At the time, planktonic cultures of the fluids were negative. Ehrlich theorizes that the ear pathogen persists in biofilms.

Further evidence for that possibility comes from Ehrlich's Allegheny colleague, Xue Wang, who detected bacterial protein synthesis in culture-negative ear fluids. "This strengthens our idea that bacteria are metabolically active in a biofilm, yet can't be cultured under planktonic conditions," says Ehrlich. If so, he says, physicians should consider using tubes made of materials that resist bacterial growth to drain the fluids from chronically infected ears.

Urologists have long battled urinary tract infections caused by biofilms creeping up catheters. Now they have evidence linking biofilms to other disorders of the urogenital tract. Most researchers had assumed that prostatitis, a common inflammation of the prostate gland that produces chronic pain and sexual dysfunction, isn't a bacterial disease because bacteria cannot be cultured from prostate fluid. But Nickel saw small amounts of biofilms in electron micrographs of prostate tissue removed from many prostatitis patients. The inflammation is always centered on the biofilms, he says, and contamination of just 1% of the prostate is sufficient to cause a serious problem. Nickel plans further studies to determine just how many cases of prostatitis stem from biofilms.



Inciting the stone. Rod-shaped bacteria fill the cavities of this struvite kidney stone. Their secretion of a biofilm may have helped form the stone.

CREDIT: J. W. COSTERTON

What makes biofilms so tough

As researchers have gathered evidence that biofilms are a common cause of infections, they have gained new respect for their powers of cohesion. In the early 1990s, Costerton and his Montana State colleague Zbigniew Lewandowski trained a confocal scanning laser microscope, which magnifies living cells in real time, on biofilms and saw that they are highly organized structures consisting of mushroom-shaped clumps of bacteria bound together by a carbohydrate matrix and surrounded by water channels that deliver nutrients and remove wastes.

Microbiologists are now documenting the changes that allow bacteria to form biofilms—information that may help in designing therapies to combat the infections biofilms cause. Some of the findings, for example, point to why bacteria in biofilms are so much more resistant to antibiotics than their planktonic counterparts.

Many common antibiotics, such as penicillins, prevent planktonic bacteria from synthesizing certain of the building blocks of their cell walls. But studies of gene expression patterns by Hongwei Yu of the University of Calgary have shown that up to 40% of the cell wall proteins of bacteria in biofilms may be different from those of their planktonic brethren. So antibiotics' targets may disappear when the organisms form biofilms. And even if they are present, antibiotics may not be able to get at them: Work by Costerton and by Nels Hoiby and his Copenhagen colleague Ami Kharazmi has shown that bacteria in biofilms secrete a sticky carbohydrate armor that can't be penetrated by antibodies or many antibiotics. And biofilm bacteria can survive without dividing, making them resistant to antibiotics that attack only dividing cells.

One of the principal thrusts of the current work is to find ways to overcome these defenses. As NIDCR's Mangan points out, "we still don't know how to treat or prevent biofilms. That's a big reason for more research." Some researchers are trying to identify the biochemical signals needed for biofilm formation. Last year, for example, researchers in Greenberg's lab at Iowa and David Davies at the Center for Biofilm Engineering discovered molecules, called acylhomoserine lactones, that instruct planktonic *P. aeruginosa* bacteria to join forces and build biofilms (*Science*, 10 April 1998, p. 295). These lactones turn on a series of 40 genes that tell bacterial cells to make a slime coating and remodel their outer walls. It might be possible to find drugs that interfere with that signaling.

In a similar vein, microbiologist Richard Lamont of the University of Washington School of Dentistry in Seattle is searching for the signals involved in the formation of dental plaque. His team found that although the bacterium *Porphyromonas gingivalis* dominates in causing periodontal infections, another organism, *Fusobacterium nucleatum*, embedded in plaque, provides the anaerobic conditions needed for *P. gingivalis* to wreak its destruction. Lamont is now looking for the signals that enable *F. nucleatum* to set up housekeeping in plaque, in hopes of finding compounds that block them and make plaque inhospitable to *P. gingivalis*. "We don't know if acylhomoserine lactones are involved, but some signal definitely is," Lamont says.

Researchers are exploring a different strategy to combat biofilm infections already under way. Most likely, a signal "tells [biofilm bacteria] it's time to leave the nest," Greenberg suggests. Once identified, these dispersal signals could be used to disrupt biofilms, rendering them more susceptible to killing by antibiotics or the immune system.

Other researchers are probing biofilm microenvironments in hopes of coming up with more effective antibiotic regimens. Laboratory-grown *Pseudomonas* biofilms display marked differences in pH, chloride concentration, permeability, and oxygen supply at various locations in the biofilms. "No single antibiotic can work in all these microenvironments," says Costerton.

With the CDC's Quinn, Costerton plans on using microelectrodes to probe the lung lesions of rabbits with an experimental form of tuberculosis and then use that information to fine-tune the antibiotic cocktails used to treat the disease. For instance, if an antibiotic wipes out all bacteria except those thriving in pockets with a pH below 4.0, it could be combined with another that selectively kills acid-loving bacteria.

It may even be possible to put bacteria themselves to work in combating noxious biofilms. As it turns out, not all biofilms are bad. In looking for ways to prevent urinary tract infections, microbiologist Gregor Reid of the University of Western Ontario in London stumbled on "good" biofilms containing some 50 species of bacteria in the urogenital tracts of healthy women. Urinary tract infections disrupt this healthy biofilm, which can be restored by adding specific strains of *Lactobacillus* bacteria.

In a pilot study of 55 women who had had multiple urinary tract infections, Reid found that a vaginal *Lactobacillus* suppository, applied weekly for a year, reduced recurrences from an average of six per year to 1.6. But no drug company wants to make the suppositories. "The biopharmaceutical industry isn't ready for biological preventive therapy," Reid says.

But perhaps the NIH initiative will help catch the industry's attention by providing a better understanding of how to separate the bacterial militiamen from their comrades. Rather than waging an all-out war on biofilms with old weapons like antibiotics, Costerton says, "we have to learn how to manipulate their bothersome ways."

–CAROL POTERA

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