

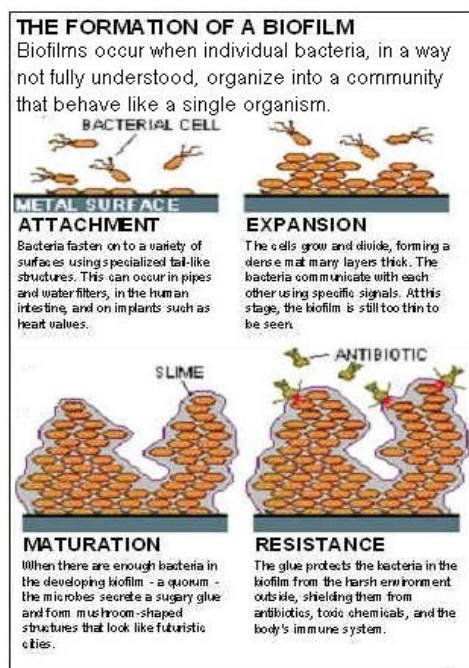
GETTING A GRIP ON BACTERIAL SLIME

Business Week: September 13, 1999
Department: Science & Technology: Biotech
Headline: Getting a Grip on Bacterial Slime
Deck: Science has declared war on biofilms, which play a part in 65% of all infections--and resist antibiotics
Byline: By Ellen Licking in New York

Science has declared war on biofilms, which play a part in 65% of all infections and resist antibiotics

In 1684, a dry-goods merchant named Anton van Leeuwenhoek discovered what he called "animalcules" in the tartar on his teeth. A thorough cleaning with vinegar killed only those "on the outside of the scurf", or dental plaque. Intrigued, Leeuwenhoek, the inventor of the microscope, took a closer look. In the "scurf," he found a sticky layer of bacteria that resisted the vinegar. It was one of the first glimpses of what are now called biofilms--bacterial "communities" that can appear almost anywhere, fouling machinery, clogging pipes, and contributing to many forms of human disease.

Researchers have found that biofilms play an important role in many medical conditions, such as kidney stones, chronic ear and urinary-tract infections, and gum disease. Experts at the Centers for Disease Control & Prevention in Atlanta now believe biofilms are involved in 65% of all human bacterial infections. And roughly 5% percent of the patients who annually receive catheters and stents develop serious infections from biofilms growing on the devices.



Biofilms also can be found on the hulls of boats, in water filters, and in drinking-water pipes, where they can

do great harm. Worldwide, industries spend \$7 billion annually on toxic chemicals that are only partly successful in blocking this bacterial scum.

CONSPIRACY. The sheer havoc wreaked by these adhesive troublemakers is spurring scientists to look for ways to fight or prevent biofilm formation. Industry is using new, long-acting enzymes to dissolve the sticky glue that anchors a biofilm to a surface. Researchers are also avidly searching for blockers to inhibit biofilm formation. One novel class of compounds, called furanones, could be on the market within the year. Scientists are even beginning to unravel the genetic basis of biofilms. "The ultimate goal is to identify a biofilm's pressure points," says E. Peter Greenberg, a microbiologist at the University of Iowa College of Medicine.

At its earliest stages, this strange thing called a biofilm is little more than a layer of cells attached to a surface. But as the bacteria grow and divide, something wondrously conspiratorial happens. When enough of them--a quorum--have gathered, they send signals around, telling each other to reorganize. They begin to arrange themselves into an array of pillars and mushroom-shaped structures, all connected by convoluted channels that deliver food and remove waste. They become, in other words, not a simple collection of bacteria, but a spooky kind of communal organism, with its own defense capabilities and communication systems. "We used to think bacteria were pretty primitive," J. William Costerton, director of the Center for Biofilm Engineering at Montana State University, says wryly.

As the biofilm matures, the bacteria become as much as 1,000 times more resistant to antibiotics and microbicides than they were when separate. So once a biofilm takes hold, getting rid of it is tough, if not impossible. Researchers believe the bacteria in the biofilm evade antibiotics by changing up to 40% of the proteins that make up their cell walls. Proteins that once served as the target of antibiotics may disappear, making the antibiotics ineffective. Even if the targets are still present, antibiotics and chemicals probably can't get through the biofilm slime to reach the microbes anyway. Costerton's group has shown that the gel-like substance secreted by bacteria in a biofilm acts like a coat of armor

that is impervious to noxious chemicals.

One strategy for penetrating that armor was devised by the University of Iowa's Greenberg and collaborators at the Center for Biofilm Engineering. The team identified a substance that triggers biofilm formation in *Pseudomonas aeruginosa*, the bacterium that coats the lungs of more than 80% of all cystic fibrosis patients. This substance, belonging to a class called lactones, sparks a series of genetic changes--more than 40 in all--that instruct individual, free-living microbes to remodel their cell walls and begin spewing out slime.

DISRUPTION. Greenberg believes he can block biofilm formation simply by designing compounds that interfere with the signaling. He likens his approach to an army's attempts to make its military strike more effective by disrupting an opponent's communications array. "Here we are, taking out the bacteria's communication lines--making them more sensitive to the host immune system and traditional antibiotics," he says. Six months ago, Greenberg founded Quorum Sciences Inc. to market these compounds to makers of medical devices.

According to Costerton, it is likely that each bacterial species has its own version of the *Pseudomonas* lactone. He predicts it will one day be possible to develop inhibitors that target troublesome bacteria specifically--say, compound X to prevent *Staphylococcus* biofilms or compound Z for *E. coli* colonizations. There may even be a signal blocker that is general enough to ward off all types of bacteria.

New research suggests this may be true. Staffan Kjelleberg and Peter D. Steinberg, two researchers at Australia's University of New South Wales in Sydney, have long been searching for ways to protect boat hulls and fishing nets from corrosive biofilms. They recently discovered a red-colored, salt-water plant called *Delisea pulchra* that is naturally resistant to biofilm formation. In just a few months they were able to isolate the plant's protective ingredient, called a furanone, from ground-up plant tissue. Now, Kjelleberg and Steinberg have chemically synthesized more than 60 furanone derivatives, all of which prevent the development of biofilms. They are forming a company, Biosignals Inc., to develop them

commercially. Kjelleberg believes these compounds could be used in a variety of settings--both industrial and medical. For instance, he envisions adding one or more to mouthwash to prevent biofilms from forming on teeth and gums.

Roberto Kolter, a microbiologist at Harvard University, is using a different approach to identify new biofilm blockers. He's searching for the bacterial genes that control biofilm formation in order to make a detailed road map of the process. This knowledge, says Kolter, will be used to design new compounds that specifically interfere with their development. So far, his lab has identified 50 genes in *Pseudomonas* that could serve as targets for drugs.

JUMP OFF. Other approaches include the use of weak electric currents, which somehow pass through the slime and kill the bacteria by punching microscopic holes in them. But Greenberg and Costerton believe the answer to biofilm busting lies within the biofilm itself. If there's a signal triggering bacteria to get on the biofilm bandwagon, why not a signal telling them to jump off?

Efforts are also under way to develop materials that biofilms can't stick to--

Teflon equivalent for biofilms. Roger Bayston, a researcher at Britain's University of Nottingham, has created a polymer that contains small amounts of rifampicin and clindamycin, two powerful, long-lasting antibiotics. In laboratory tests, catheters made of the polymer were able to repel large doses of bacteria--100,000 times the amount that would sicken a patient--for months at a time. To date, neurosurgical shunts made from Bayston's biomaterial have been implanted in more than 200 people in Europe. Britain's Ortho McNeil Pharmaceutical, which is the diagnostics division of Johnson & Johnson, and Cook Inc., a manufacturer of medical products in Bloomington, Ind., are developing their own versions of biofilm-resistant plastics.

More than 300 years after Leeuwenhoek's discovery of animalcules, biofilms are getting the attention they deserve. This past year, the National Institutes of Health formed a coordinated program to bring scientists from diverse disciplines together to work on the problem. Thanks to a coordinated attack by engineers and biologists, new inhibitors are on the horizon. And there's even talk of vaccines to prevent chronic infections and dental disease. "We

need to learn how to manipulate the critters' bothersome ways," declares Costerton. The war on biofilms has just begun.

HARMFUL AND DANGEROUS

Biofilms can appear almost anywhere, and they usually have undesirable consequences:

LOCATION	IMPACT
• Drinking water Pipes	• Corrosion, disease
• Household Drains	• Plugged-up sinks
• Dental plaque	• Gum disease, Cavities
• Contact lenses	• Eye Infections
• Catheters and Stents	• Disease
• Ears	• Chronic Infection
• Lungs	• Pneumonia

Business Week: September 13, 1999
 Department: Science & Technology: Biotech
 Headline: GRAPHIC: The Formation of a Biofilm
 Copyright 1999 The McGraw-Hill Companies, Inc.
 All rights reserved. By Ellen Licking in New York,
 Getting a Grip on Bacterial Slime., 09-13-1999.