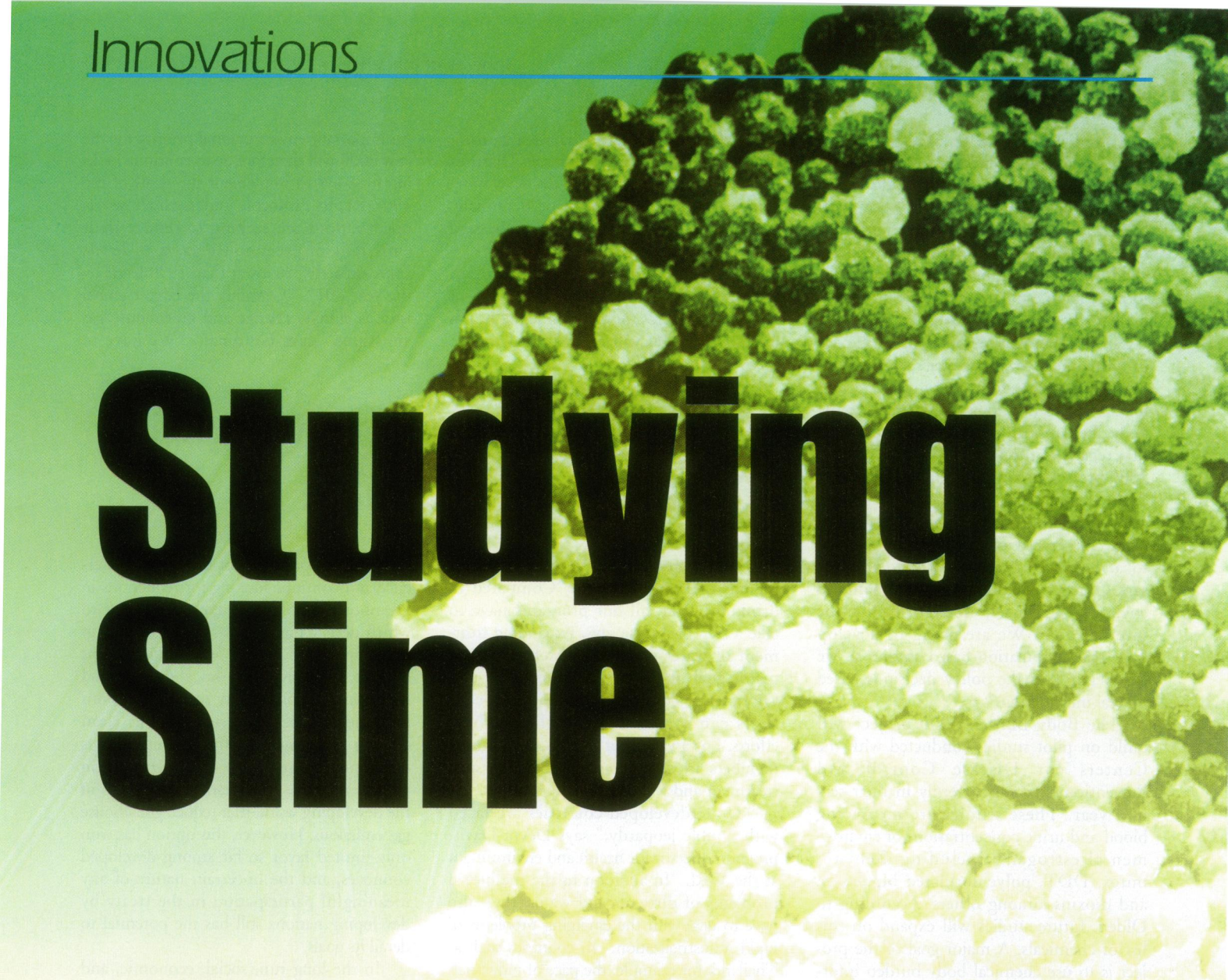


Studying Slime

Microbiologists have traditionally based their knowledge of bacteria on the behavior of colonies grown from a single species in laboratory culture plates. These so-called "planktonic" or free-floating specimens have been used to determine how well antibiotics and disinfectants kill bacteria. In nature, however, 99% of bacteria aggregate as biofilms—complex colonies composed of billions of bacteria that pool their resources to resist being killed by antimicrobial agents. "Microbiologists have been barking up the wrong tree since the time of Pasteur," says William Costerton, a microbiologist and director of the Center for Biofilm Engineering (CBE) at Montana State University in Bozeman.

Biofilms, more commonly called slime, can corrupt any solid surface. The best known and most studied biofilm is dental plaque, which secretes acids that destroy teeth and gums. Biofilms also contaminate contact lenses, intrauterine devices, catheters, pacemakers, artificial hearts, joint implants, and proctoscopes. The resulting infections often defy high doses of antibiotics, making it necessary in some cases to surgically remove an artificial hip or pacemaker. In the drug industry, biofilms foul manufacturing tanks. In 1994, several people with asthma died after inhaling a biofilm that had tainted a company's therapeutic inhaler. Legionnaire's disease spreads as an airborne biofilm, and biofilms clog the lungs of many cystic fibrosis patients. New evidence suggests that biofilms may also be the culprit behind chronic ear infections.

Biofilms wreak havoc in industrial settings, corroding everything from water pipes to computer chips. Some bacteria in biofilms convert sulfur to hydrogen sulfide, which burns holes in metals and concrete. Biofilms also plug pipes, interfering with heating and cooling systems. Once an industrial water supply becomes infected with a biofilm, seemingly pure water can befoul the computer chips or artificial limbs that it was intended to cleanse. In addition to the direct problems they cause, biofilms can also damage the environment indirectly due to the toxicity of the chemicals used to clean them up and keep them from coming back.

Studying Slime

Before the 1980s, biofilms were the province of a few microbiologists studying slime in water and industrial engineers plagued by biofilms corroding and blocking water systems. William Characklis, an engineering professor at Montana State University, recognized the need to bring engineers and microbiologists together to share their specialized knowledge of biofilms. Characklis set a personal example by cross-training himself in microbiology, attending meetings of both camps, and teaching diverse graduate students. In 1990, Characklis won National Science Foundation support to establish the Center for Microbial Interfacial Process Engineering at Montana State University, which drew students and faculty from

engineering, science, mathematics, and agricultural disciplines (the center gained its current name in the mid-1990s). Understanding and taming biofilms is the chief goal of the CBE.

A new breakthrough has identified how bacteria "talk" to each other when building a biofilm. A team led by CBE microbiologist David Davies reported in the 10 April 1998 issue of *Science* that *Pseudomonas aeruginosa* bacteria communicate with each other through chemical signals called homoserine lactones (HSLs) when forming biofilms. Biofilms of *P. aeruginosa* pose particular environmental health concerns, because they are the primary cause of hospital-acquired infection and the chief bacterial species growing in the lungs of cystic fibrosis patients. Knowing that HSLs can modify biofilm development "means we have one key to turn off the development of biofilms," says Davies. Potential drugs that block HSLs have been created and shown in preliminary trials to block biofilm formation. At least 40 other types of bacteria produce HSLs, suggesting that the natural messenger is widespread among microbes.

The means of halting HSL-dependent biofilm communication are just starting to be understood. One goal is to block the key bacteria responsible for periodontal disease. An HSL analog that disrupts the first stages of biofilm formation on teeth could be added to toothpaste or mouthwash as a prophylactic ingredient, proposes Costerton. Molecular geneticist John Geiger, group leader for biotechnology at the Olin Corporation, a manufacturer of industrial biocides in Cheshire, Connecticut, says the HSL findings offer a new, environmentally benign way to disrupt biofilms. For example, paint used to prevent biofilm fouling on boats currently contains toxic heavy metals, which could be replaced by safer HSL analogs. Industrial water systems, a prime location for biofilm contamination, could be made slime-free by adding analogs that block biofilm congregation. Such analogs might be safer and healthier than the chlorine currently used to kill biofilms in water.

Traditional efforts to kill bacteria have spawned problems such as antibiotic-resistant strains. "Now we can't even kill them," says Costerton. "But perhaps we can coexist with bacteria and manipulate their bothersome ways."

Medical Advances

Until recently, microbiologists and industrial engineers assumed that biofilm bacteria behaved like their planktonic counterparts. Early microscopic studies of biofilms used dead, stained cells in which the biofilm structure had been destroyed. In the early 1990s, the technique of confocal scanning laser microscopy, which magnifies live cells in real time without destroying them, revolutionized ideas about biofilms.

The rule of thumb is that 1,500 times more of an antimicrobial agent is needed to kill a biofilm than a planktonic bacteria.

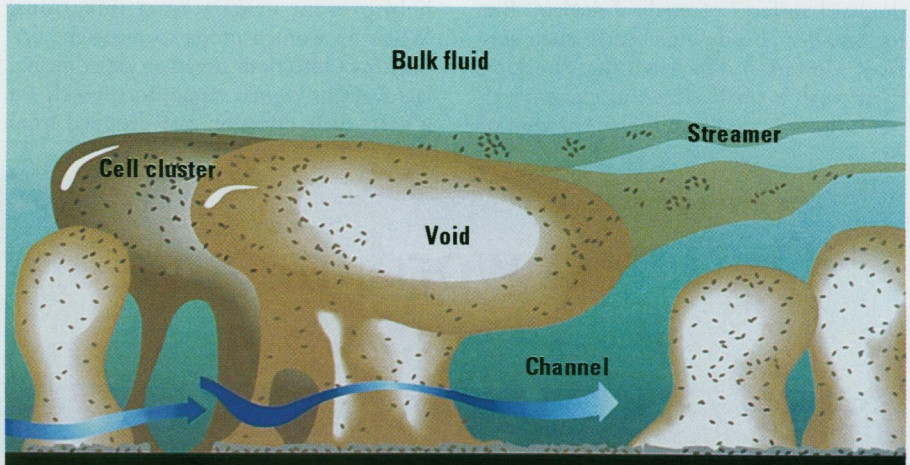
–William Costerton

Using this technique, researchers found that biofilms are highly organized structures that resemble mushrooms, surrounded by circulating water channels that supply nutrients and remove wastes. A biofilm can contain a single species of bacteria or several species living synergistically. Any bacteria can transform itself into a biofilm once it attaches to a hard surface in a moist environment. Genetic studies confirm that bacteria switch on different genes, depending on whether they're living as free-floating microbes or clustering as biofilms.

Biofilms secrete a sticky carbohydrate coating to protect themselves from antibiotics and disinfectants. Moreover, up to 40% of the proteins in cell walls differ between planktonic and biofilm forms of bacteria as a result of different gene expression. So cell targets for traditional antibiotics may not be present in biofilm bacteria, making biofilms notoriously difficult to kill. "The rule of thumb is that 1,500 times more of an antimicrobial agent is needed to kill a biofilm than a planktonic bacteria," says Costerton.

Microbiologist Garth Ehrlich, director of the Center for Genomic Sciences at the Allegheny University of the Health Sciences in Pittsburgh, Pennsylvania, conducted a study on ear fluid from children with a condition called otitis media with effusion (OME), or the persistence of fluid in the middle ear. OME leads to hearing loss and developmental problems in speech and socialization. Erlich discovered that the fluid contained genetic material from bacteria weeks after antibiotic treatment. When he analyzed ear fluid from 82 pediatric patients with chronic OME, Ehrlich found that 35% were positive for *Haemophilus influenzae*, the pathogen most commonly cultured from effusions. Ehrlich theorized in the 28 January 1998 issue of the *Journal of the American Medical Association* that *H. influenzae* persists in the ears of OME patients despite antibiotic treatment because it's a biofilm.

Now Ehrlich is analyzing gene expression in samples of ear fluid from OME patients and comparing it with genes expressed in free-floating *H. influenzae*. Preliminary data reveal marked differences in gene expression. "In the natural ear environment, biofilms appear to be the predominant mode of growth," says Ehrlich. Genes unique to the biofilm state will be identified as possible targets for better



Peg Dirckx, Center for Biofilm Engineering

The structure of slime. At the microscopic level, biofilms are heterogeneous in structure and include characteristics such as cell clusters, void spaces, water channels, and slime streamers that are affected by flow of the fluid surrounding the biofilm.

antibiotics, or genes expressed only in the biofilm could form the basis of a new vaccine to eliminate the occurrence of ear infections and the resulting need for antibiotics. Ehrlich also suspects that other long-term infections that are not killed by antibiotics, such as chronic sinusitis, arise from biofilms. "OME will serve as a model for investigating the development of antibiotic resistance in chronic infections and the demonstration of biofilm communities *in vivo*," he says.

Preemptive Strikes

Because biofilm infections are tenacious, other research is aimed at preventing biofilms from taking hold. Rabih Darouiche, an infectious disease specialist at the Baylor College of Medicine and the Veterans Affairs Medical Center, both in Houston, Texas, helped to develop special catheters coated with the antibiotics rifampin and minocycline that were placed in patients for a variety of medical therapies. "The antibiotic coating cuts down on clinical infections," says Darouiche, whose research in this area was published in the 15 August 1997 issue of the *Annals of Internal Medicine*. Moreover, in a multicenter trial, a scanning electron microscope was used to examine biofilm growth on catheters inserted for 2 weeks. Mild to heavy biofilm colonies formed on 35% of the coated catheters, compared to 80% of the untreated catheters. The results, published in the February 1998 issue of *Critical Care Medicine*, support clinical observations of fewer bloodstream infections in patients treated with coated catheters. The antibiotic-coated catheters received FDA approval in 1996 and are gradually catching on, says Darouiche.

Neither an electrical current nor antibiotics alone will kill biofilms entrenched on the hard surfaces of medical devices. But for biofilms already attached to such surfaces, Costerton discovered that shocking them with a small electrical current (15 milliamperes) speeds up the penetration of the antibiotics that actually kill them. Costerton has received a patent on the electrically based method; prototypes of various sterilizers have been built for cleaning medical devices ranging from contact lenses to proctoscopes, but none are advanced enough for marketing.

In looking for ways to prevent biofilm infections in the urogenital tract, microbiologist Gregor Reid of the University of Western Ontario in London discovered a new twist—"good" biofilms. Fifty to 100 species of bacteria populate the urogenital tracts of healthy women, mostly as biofilms. Urinary tract infections disrupt the healthy biofilm population. Reid found that certain strains of lactobacillus maintain and restore the healthy biofilms, and help prevent urinary tract infections. When 55 women prone to recurrent urinary tract infections tested an experimental lactobacillus vaginal suppository weekly for a year, their infection rate dropped from 6.0 to 1.6 recurrences per patient per year (*International Dairy Journal*, in press). Women want to buy the suppositories, but "we're having problems finding a corporate partner," says Reid. "The biopharmaceutical industry isn't ready for biological preventive therapy."

Biofilms have risen from scientific obscurity to become a topic of grant requests. At the National Institutes of Health, 8 of 23 institutes are sponsoring programs and seeking proposals for biofilm research in areas ranging from the role of biofilms in ear, urinary, and other infections to making better biomaterials that will resist the growth of biofilms. To Costerton, this means that health officials are conceding that "a great many medical problems are caused by biofilms." With luck, the new research will lead to better and safer ways to control biofilms.

Carol Potera

Suggested Reading

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