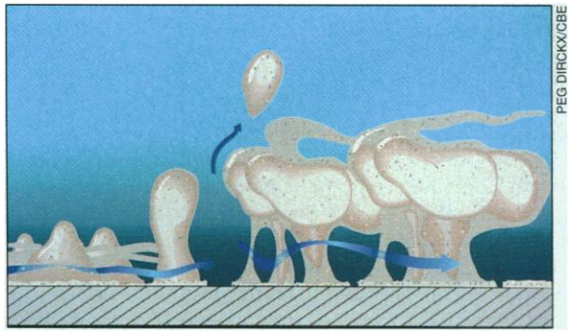
Biofilms Invade Microbiology

After focusing on free-floating bacteria for decades, microbiologists are now recognizing that many bacteria aggregate in tough biofilms that can foul pipes, infect medical implants, and even kill humans

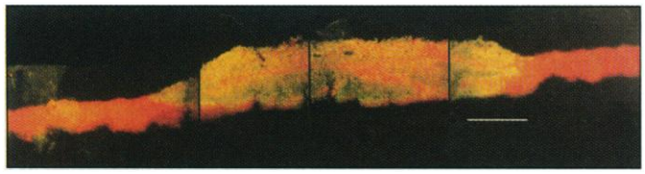
In late 1993 and early 1994, a mysterious bacterial infection struck hundreds of asthmatics throughout the United States, and 100 people died. A common denominator soon emerged: They had all been treating their asthma with the same generic albuterol inhalant, and investigators suspected that the infection could be traced to the manufacturer's albuterol processing tank. The tank had been treated with chemical disinfectants, but this standard treatment—and the antibiotics given to the patients themselves—apparently failed to subdue the virulent infection.

The deadly outbreak spawned more than 50 court claims, and a lawyer for the plaintiffs called in microbiologist William Costerton, who examined the records submitted to the Food and Drug Administration and noted the presence of a particular species of bacteria, *Pseudomonas aeruginosa*, floating freely in the tank. Not only can this species cause pneumonia, but Costerton knew that *P. aeruginosa* is notorious for forming biofilms—large clumps of bacteria surrounded in slime—that resist chemical disinfectants, antibiotics, and the immune system. If the asthmatics had inhaled pieces of this biofilm, they wouldn't have had a chance, says Costerton, director of the Center for Biofilm Engineering (CBE) at Montana State University in Bozeman, and one of the world's few experts on biofilms.



PEG DIRCKX/CBE

Biofilm blueprint. In a biofilm, water currents *(blue arrows)* flow through clumps of bacteria stuck to a surface throughout.



CHING-TSAN HUANG/CBE

Deadly mixture. Stained biofilm shows distribution of bacterial species with *P. aeruginosa* in red and *Klebsiella pneumoniae* in green.

It was not the first time that biofilms had played a role in high-profile cases—Costerton has testified in court about the presence of biofilms on intrauterine devices, for example—but the asthmatics' tragic experience highlights a gap in the way researchers generally view the microbial world. Microbiologists have traditionally focused on free-floating bacteria growing in laboratory cultures; yet they have recently come to realize that in the natural world most bacteria aggregate as biofilms, a form in which they behave very differently. And that fact has consequences for everything from medical technology to oil recovery. "Microbiologists have been barking up the wrong tree since the time of Pasteur," says Costerton.

As a result, biofilms, once considered odd curiosities, today are one of the hottest topics in microbiology. They have spawned a flurry of patents, a new online journal,* their own Medline heading, and several symposia, including the first meeting devoted entirely to biofilms, to be held from 30 September to 4 October in Snowbird, Utah. Researchers are finding creative ways to conquer biofilms, from new antibiotics to jolts of electric current; others are finding new uses for beneficial biofilms. The field is unusually interdisciplinary, with researchers exploring biofilms that decay teeth (dental plaque is one of the most common biofilms), clog water pipes, and contaminate medical devices ranging from contact lenses to artificial hearts. "The microbiology community is finally realizing that everything they know was circumstantially gained by investigating suspended cells," says chemical engineer James Bryers, co-director of the CBE. "Unfortunately, 99% of all microbial activity in an open ecosystem is [in biofilms] stuck to surfaces."

* Biofilms Online, at: http://www.erc.montana.edu

A world of their own

The renegade bacteria in biofilms bind together in a sticky web of tangled polysaccharide fibers, which connect cells like strands of spaghetti and anchor them to a surface and to each other. Within this microcosm, anaerobic and aerobic bacteria can thrive alongside each other, sharing water passageways and a complex structure. Water flows in convective patterns through the channels, which deliver nutrients and remove wastes like a circulatory system. Some microbes release hydrogen, while others ingest it in order to reduce carbon dioxide to methane; still other bacteria dine on dead cells. "The polysaccharide coating is like a coat of armor," and the different types of bacteria "collaborate to make themselves much more powerful," explains biofilm pioneer David C. White, executive director of the Center for Environmental Biotechnology at the University of Tennessee, Knoxville.

By 1990, researchers confirmed that biofilm bacteria are morphologically and metabolically distinct from free-floating ones, and that any bacterium can form a biofilm, once it finds a place to stick. Slamming up against a hard surface sets off a genetic cascade that turns on specific genes to make polysaccharides and other substances needed to establish the biofilm colony. Even bacteria that have long floated in test tubes will stick if given the chance. "They don't forget for many generations," says Bryers.

Biofilms' sticky habit creates problems for industry in everything from corroding water pipes to computer-chip malfunctions. For example, anaerobic bacteria in biofilms reduce sulfur to hydrogen sulfide, a corrosive agent that burns holes in pipes, and aerobic bacteria corrode metal by oxidation. On computer chips, biofilms serve as conductors and so interfere with electronic signals. "I have called biofilm-induced corrosion the venereal disease of industry," says White. "It's expensive, it's painful, everybody has it, and nobody admits it." Understanding and taming biofilms on industrial surfaces is a chief goal of the CBE (see box), a hub for biofilm research.

In addition to biofilms' role as fouling agents, researchers are also increasingly aware of their devastating effects on people. "Much of the biofilms in medicine are a disaster," says White. Not only do biofilms resist antibiotics, they are also big enough

Linking Microbiology and Engineering

Biofilms were once considered chiefly a practical problem, the province of industrial engineers concerned about corroded pipes and fouled equipment. So in the 1980s, when William Characklis spoke about biofilms at microbiology conferences, the biologists he addressed were not a receptive audience: He was considered a "microbiologist who had fallen amongst engineers," recalls biofilm expert William Costerton.

PHOTOS BY CBE

Building bridges. Engineer Characklis *(right)* began the biofilm center, and microbiologist Costerton *(left)* now leads it.

In reality, Characklis, who died an untimely death in 1992 at age 50, was a chemical engineering professor at Montana State University in Bozeman. But he had become convinced that a more biological perspective was needed to understand the puzzling nature of biofilms. Costerton and other biofilm leaders credit Characklis for almost single-handedly bridging biology and engineering, tirelessly crisscrossing the boundary between the two camps by training himself in microbiology, attending meetings, and teaching diverse graduate students. In 1990 Characklis won National Science Foundation (NSF) support to start an Engineering Research Center on biofilms at Montana State. Under his leadership, the center, then called the Center for Microbial Interfacial Process Engineering, drew students and faculty from engineering, science, mathematics, and agricultural departments. After Characklis's death, the center's name changed to the Center for Biofilm Engineering (CBE), but his interdisciplinary vision is thriving under the leadership of microbiologist Costerton. The center's original 5-year NSF grant was renewed in June, for $7.6 million for the next 5 years.

One of the center's crucial jobs is to develop tools to study biofilms. For example, early researchers thought that the bacteria grew uniformly in a polysaccharide envelope, a view that stemmed from drying or treating biofilms before studying them. Then in 1991, researchers at the CBE and the University of Saskatchewan in Canada used confocal scanning laser microscopy (CSLM) to examine successive planes in living biofilms in real time. CSLM clearly showed that biofilm bacteria grow as remarkably complex microcolonies, crisscrossed by water channels that comprise up to 60% of the biofilm's volume. CBE scientists, led by Zbigniew Lewandowski, are now further refining this method with new probes and software for mapping interactions among bacteria.

Today the CBE is a haven of interdisciplinary work, with graduate students from 10 departments working in interdisciplinary teams. Engineering students clone genes, microbiologists construct mathematical models, and mathematicians learn biochemistry, all to solve real-world problems. "The center has evolved a system that works," says Costerton, who thinks the key to success is the interdisciplinary research teams. A microbiologist may help an engineer do taxonomic mapping of a mixed-species biofilm, then the engineer might help the microbiologist scan the surface of a medical device with a scanning vibrating electrode to measure the extent of biofilm-induced corrosion.

Another part of the CBE's mission is to advance industrial competitiveness, by transferring technology to industrial associates such as oil companies or municipal water districts. Microbiologist Rod Donlan of Calgon Corp. in Pittsburgh, for example, has used the center's expertise to find chemical disinfectants that can kill biofilms in industrial water systems. He adopted a CBE-developed staining technique to identify the kinds of bacteria and their metabolic activity inside a biofilm, and this helps him determine which bacteria are harmed by disinfectants. "That's why we're part of the center—because they do basic research that helps us develop new applications," he says.

And it's clear that Characklis's cross-disciplinary spirit lives on. During a recent NSF site review, an eminent microbiologist was convinced that two students presenting a project about biofilms degrading trichloroethylene were microbiologists. "They were, in fact, both engineers," says Costerton. –C.P.

to defeat the immune system. That's why the asthmatics faced slim odds, explains Costerton. White blood cells, typically about 15 micrometers in diameter, can track down and engulf free-floating bacteria of a micrometer or so, but they choke on biofilms, which may reach 50 to 100 micrometers in diameter. "If you're unfortunate enough to aspirate a biofilm, the bacteria have a 100% chance of surviving in your lungs," says Costerton.

And because they cling to surfaces, biofilms contaminate just about any device inserted into the body. Conventional drugs, even at thousands of times the normal dose, often can't kill these tenacious implant-associated infections, which may progress to systemic, life-threatening conditions. "The resistance of these biofilms to antibiotics is phenomenal," says Costerton, although researchers are still figuring out exactly why. Bryers and his colleague, chemical engineer Steven Peretti at North Carolina State University in Raleigh, have preliminary evidence suggesting that different bacteria within a biofilm can trade genes—perhaps including genes for antibiotic resistance.

Shock therapy

Meanwhile, Costerton is convinced that antibiotics must be developed that specifically target biofilms, and he has worked with Hoffmann-La Roche Inc. to do just that. Two years ago, targeted screening uncovered a new fluoroquinolone antibiotic, called fleroxacin, that in a rabbit model can kill a common biofilm on urinary catheters, although no one knows why it works, says Costerton. Clinical tests are now under way. But there is a long way to go before such antibiotics are widely applicable. And because biofilm infections are so tenacious, other researchers are trying to stop infections before they start, especially in medical implants. Once a biofilm is established, "it is extremely hard to cure, and you have to remove the device," says Rabih Darouiche, an infectious-disease specialist at the Veterans Administration Medical Center in Houston. "Prevention is the best way to go."

Darouiche is using conventional antibiotics to do the job, and last year he headed a multicenter study comparing vascular catheters pretreated with the antibiotics rifampin and minocycline, both of which have been available for decades, with untreated ones. Although Darouiche doesn't know why these well-known antibiotics work, they significantly reduced the rate of bacterial colonization of the devices from 30% to 9% and cut the rate of infection in patients from 4% to 0. The Food and Drug Administration approved the antibiotic-coated catheters in February.

Yet another approach, developed by Costerton, zaps existing biofilms with low-dose electric current. Patented in 1994, the method disrupts the electrical charge of the polysaccharide coating, rendering the underlying bacteria more susceptible to antibiotics. Costerton and his colleagues have treated lab-grown biofilms with electric charges and antibiotics simultaneously. When they zapped the biofilms first, they could kill them with just 0.1% of the antibiotic required to wipe out an untreated biofilm. Costerton plans to use the technology, which is now licensed to a Montana firm, to sterilize repeat-use sigmoidoscopes (instruments used to explore the colon for tumors), to prevent spreading of biofilms. In the long term, infected implants may someday be treated in vivo with electric currents.

While the new work in biofilms should lead to better ways to control them, the same research is also helping find ways to use beneficial biofilms. "Biofilms have a good and bad side," says Bryers. For instance, biofilms have been used for 50 years to degrade common contaminants in waste water. In the last few years, they have also become a hot item for in situ bioremediation of toxic contaminants like jet fuel and carbon tetrachloride. Researchers simply gather bacteria found at toxic sites, then select for species with an appetite for the targeted contaminant and cultivate them to thrive as biofilms, explains Bryers.

Beneficial biofilms also serve as biobarriers in the oil industry. To pump oil out, water is pumped in. Once the oil is removed, water tends to flow into the empty space, which reduces the water pressure available for pumping more oil. Oil-industry engineers once used hazardous chemicals to fill the holes and redirect the water. Now they pump in biofilms in a starving state, and the bacteria grow rapidly on local nutrients. They "explode like popcorn and fill the holes and make the area impenetrable," says Bryers. Such beneficial applications are continually being improved, as researchers mix biofilms of various species to maximize various bacterial activities, whether it be filling underground holes or degrading specific contaminants.

Despite all the recent activity in biofilm research, plenty of unanswered questions remain, such as why some bacteria are better than others at forming biofilms, and exactly how biofilms manage to resist antibiotics. "Biofilms cause enormous problems and still aren't recognized by many who are affected by them," says White. "I don't think it's possible to go too fast in this area."

–Carol Potera

Carol Potera is a science writer in Great Falls, Montana.

AIDS RESEARCH

Receptor Mutations Help Slow Disease Progression

In a mere 9 months, a group of natural chemicals called chemokines has rocketed from obscurity to celebrity in the AIDS research community. First, researchers found that a trio of chemokines appears to potently suppress HIV's ability to infect cells and, more recently, that the cellular receptors through which these chemicals exert their effects are critical "coreceptors" to which HIV must bind in order to enter cells (*Science*, 21 June, p. 1740).

BILL BRANSON/NCI

Powerful study. Stephen O'Brien led largest study yet of CCR5 mutations.

The latest chapter in this fast-moving saga appears on page 1856. Population geneticist Stephen O'Brien of the National Cancer Institute, his NCI colleagues Michael Dean and Mary Carrington, and their collaborators provide strong confirmatory evidence that people who have two mutant copies of the gene for CCR5 (also known as CKR5), the chemokine receptor that HIV uses when it initially infects white cells, are highly resistant to HIV infection. Another, entirely new, finding is that people who do get infected with HIV, but have one mutant copy of the CCR5 gene, progress to AIDS more slowly than do people without the mutation.

"It's the most impressive data I've seen so far in this area," says Robert Gallo, head of the University of Maryland's Institute of Human Virology. Oncologist James Hoxie of the University of Pennsylvania, who has long studied how HIV enters cells, has high praise, too. "The implications are really remarkable about what it might be telling us about the risk of infection and how the natural history of disease might be affected," says Hoxie.

In addition to possibly clarifying questions of HIV pathogenesis, these discoveries have raised high hopes of developing new AIDS therapies or vaccines. It might be possible, for example, to prevent HIV from infecting cells by mimicking the way chemokines suppress HIV infectivity: blocking the receptor that they and HIV share. As molecular biologist Marc Parmentier of the Université Libre de Bruxelles in Belgium, whose lab first cloned CCR5, explains, this new work "demonstrates quite clearly that CCR5 is the most important coreceptor for strains of HIV [that establish the initial infection]." (After the initial infection, HIV mutates and different strains take over—and use different chemokine receptors.)

The first evidence that mutations in the CCR5 gene might protect people from HIV came from a team led by Ned Landau and Richard Koup at the Aaron Diamond AIDS Research Center in New York City (*Science*, 19 July, p. 302). Landau, Koup, and co-workers found two individuals who had been repeatedly exposed to HIV without becoming infected, in whom both copies of the gene were mutated. The finding suggested that these "homozygotes," who were unable to produce the receptor, were resistant to HIV because the virus could no longer enter their cells. (The results were published in the 9 August issue of *Cell*.)

Further evidence for that came from Parmentier's group, which had independently identified the same CCR5 mutation. As these researchers described in the 22 August issue of *Nature*, they found that none of 723 HIV-infected Caucasians they studied were homozygous for the mutation. The Parmentier group further reasoned that because this infected population had fewer people with one mutant gene copy (so-called heterozygotes) than did the uninfected group (10.8% versus 16.2%), even heterozygosity may offer some degree of protection from infection.

O'Brien's team at NCI has now looked for CCR5 mutations in some 1955 people, making it the largest study of its kind to date. In keeping with the Parmentier study, the O'Brien team found no homozygous, infected people. And O'Brien also saw the mutant allele much more frequently in Caucasians than in people of African descent, although the difference was less dramatic. Parmentier found the mutation in 17% of 704 Caucasians, compared to none in 124 central and western Africans, while O'Brien found the allele in 11% of Caucasians and 1.7% of African Americans analyzed.

But there were also some notable differences between the two studies. O'Brien found no indications that heterozygotes were protected against HIV infection: There were roughly the same number of heterozygotes in both infected (15%) and uninfected (14%) populations. O'Brien attributes this to the fact that Parmentier derived the bulk of his