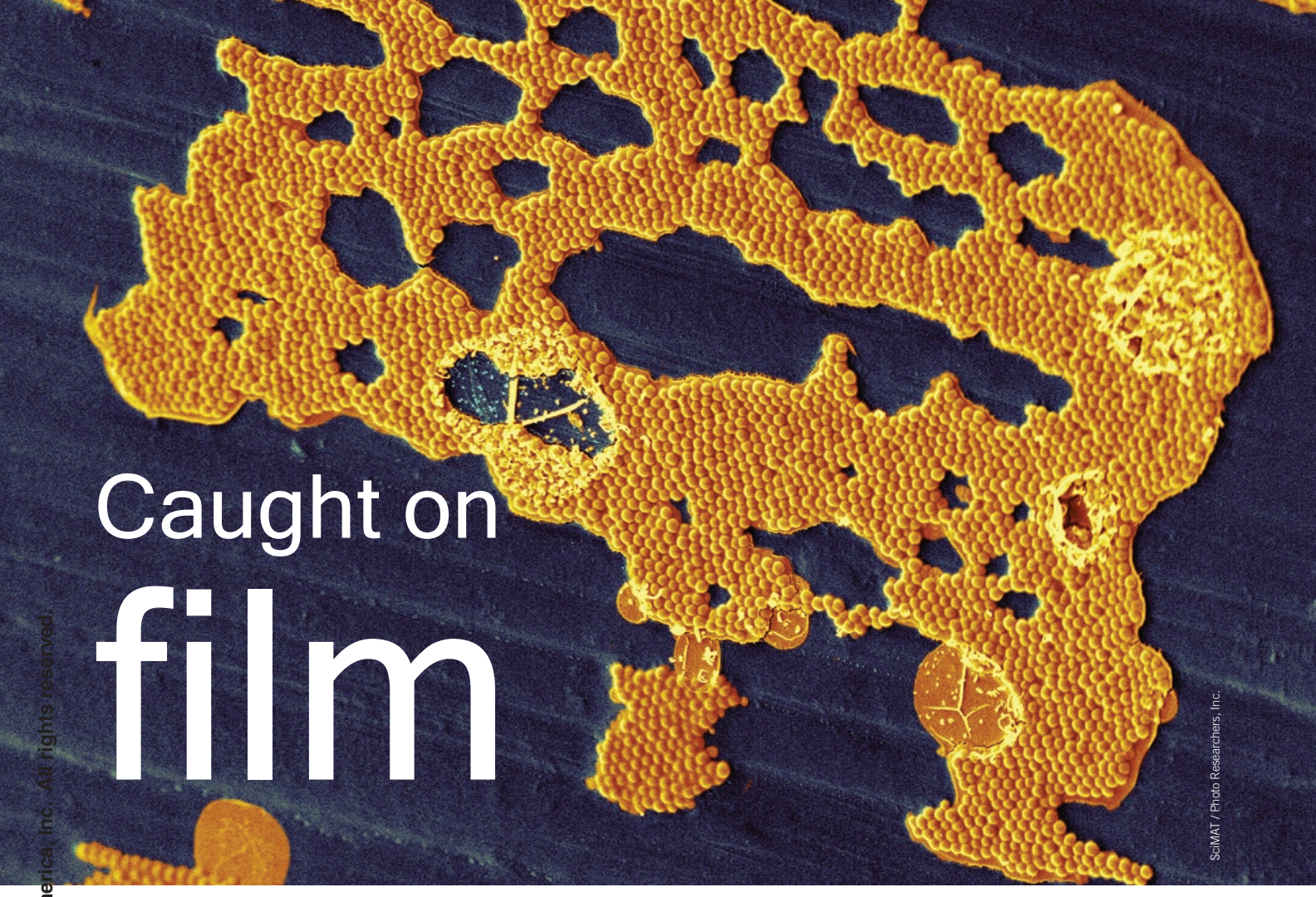


Caught on film

Scummy surface layers known as biofilms can coat everything from teeth to pacemakers. In addition to the yuck factor, these slimy fortresses offer a menacing safe haven for deadly bacteria such as *Staphylococcus aureus*. **Michelle Pflumm** reports on new vaccines that aim to prevent microbes from building biofilms in the first place.

Mark Shirtliff never intended to be a vaccinologist. What he wanted to do when he went to graduate school at the University of Texas in Galveston was to help people suffering from bone infections, a condition known as osteomyelitis that can arise after procedures to fix a break or repair a joint. This diseased bone tissue must be surgically removed, so as part of his doctoral work, he developed an experimental bone-stabilizing cement to fill in the gaps of leg bones of rabbits that had undergone such a procedure. But the cement ended up doing more harm than good. The animals started to limp and lose weight just days after surgery.

Eventually, he discovered that the surface of the cement was the ideal meeting ground for *Staphylococcus aureus*, the microbe infamous for causing fatal hospital-acquired infections. These clever bacteria adhered onto the gluey acrylic substances and formed gunky layers of protein and sugar called biofilms to dodge the concurrently administered antibiotics. Try as he might, Shirtliff couldn't break the cycle of recurrent bone infections.

"It annoyed the hell out of me that you couldn't cure a biofilm infection," he recalls.

Shirtliff, who took up a professorship at the University of Maryland Dental School in Baltimore in 2003, thinks the best bet is to create a vaccine to stop the formation of biofilms before they get out of hand. And for the last decade, he has narrowed in on which biofilm proteins to immunize against and developed an experimental vaccine that has shown some early signs of success in animal trials. The idea is to ultimately have a vaccine to train people's immune systems to specifically hunt out *S. aureus* while it's in the process of forming deadly films on the surface of tissues and medical devices. But skeptics say that it's still impossible to know which biofilm proteins to target and that the approach may not pan out.

Nothing to smile about

One smile in the mirror is all it takes to come face to face with biofilms. The stubborn plaque that can form on teeth at the gumline is a quintessential biofilm, housing many kinds of microbes including the tooth decay-causing *Streptococcus mutans*. But these biofilms can lead to a lot more than an uncomfortable visit to the dentist's office.

Over half a million people die each year due to biofilm infections. The lungs of people with cystic fibrosis can clog up with biofilms, resulting in respiratory failure. Superbugs that hide in these slimy fortresses can also reemerge from diseased tissues or devices and flood the bloodstream, resulting in toxic shock.

Additionally, people with devices such as pacemakers can get sick from bacterial biofilms that form on the medical devices soon after they are implanted. Last year, more than one million people came down with device-associated biofilm infections, according to the US Centers for Disease Control and Prevention. And antibiotics are often unable to penetrate the slime and mop up the bacteria that compromise the slimy

surface coatings, thereby failing to clear up these infections.

"The likelihood of curing a prosthetic device infection without removing the device itself is relatively low, maybe 10–20%," says infectious disease specialist Frank Lowy of New York's Columbia University College of Physicians and Surgeons.

Antibiotic-based therapies, which preferentially kill free-flowing microbes in the body, often fail to penetrate the sugary matrix surrounding biofilms. With few good strategies to treat biofilm infections, drug manufacturers in the late 1990s turned toward the development of *S. aureus* vaccines to protect people from getting these stubborn bacterial diseases.

"The trick of any vaccination approach," says Steve Projan, senior vice president of research and development at MedImmune, a vaccine manufacturer based in Gaithersburg, Maryland, "is to prevent a biofilm from forming in the first place."

To train the immune system to hunt for biofilm-forming pathogens, a few years ago New Jersey's Merck developed a vaccine directed against a key *S. aureus* cellular surface protein. The vaccine, known asV710, showed promise in rodent experiments, in which it protected against a catheter-associated biofilm infection (*Hum. Vaccin.* doi:10.4161/hv.7.6.15407, 2011). But this past April, Merck halted the late-stage testing of its V710 vaccine in 7,700 people undergoing heart surgery pending the review of the intervention's benefits by an independent monitoring committee. Maryland-based Nabi Pharmaceuticals and Pennsylvania's Wyeth (now part of Pfizer) have upped the ante by introducing multicomponent vaccines. Their vaccines—dubbed PentaStaph and SA3Ag, respectively—both simultaneously target the microbe's outer surface and the toxins it produces (*Vaccine* **73**, 3276–3280, 2009).

Adam Zewe, courtesy of University of Maryland Dental School

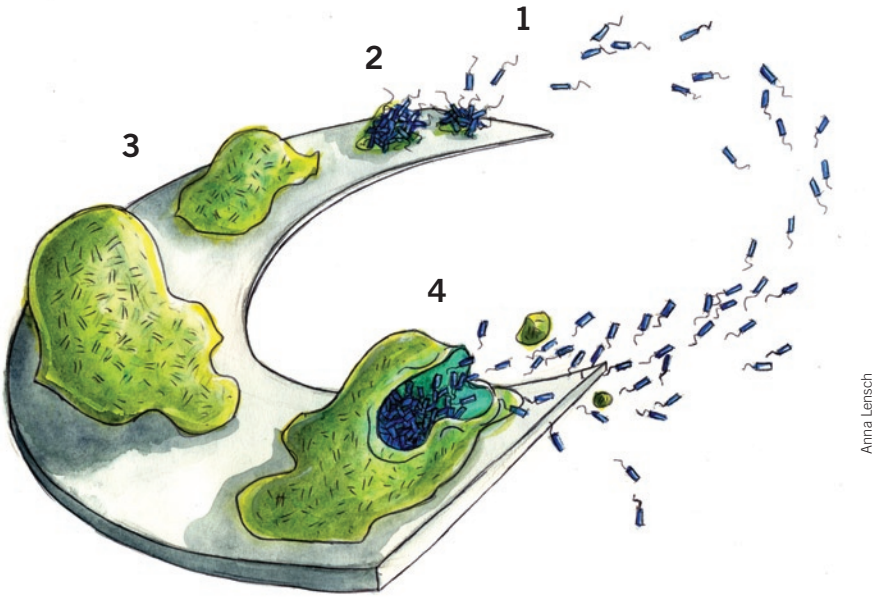
Seeing it through: Mark Shirtliff observes bacteria being flushed through to create biofilms on tubing.

"We are making sure that bacteria can't speed into organs or onto prostheses and really prevent disease," says Annaliesa Anderson, senior director of microbial vaccine research at Pfizer, headquartered in New York.

But infectious disease specialist Stan Deresinski of California's Stanford University School of Medicine cautions that such efforts are tricky because these interventions are designed to prime production of antibodies found to be raised by people who survived *S. aureus* infections. "The fact of the matter is there is no good evidence that any of these antibodies are protective," says Deresinski.

Plus, says University of Chicago microbiologist Olaf Schneewind, *S. aureus* has the potential to evade even bolstered immune systems. These wily microbes produce proteins that cut off production of antibodies by obliterating the immune system's B cells. They also produce toxins that kill neutrophils, key immune cells that target these microbes for destruction.

Schneewind is nevertheless determined to beat *S. aureus* at its own game. He is developing vaccines that counter such microbial evasion measures. Using so-called reverse vaccinology, Schneewind's team is ferreting out the microbe's major immune countermeasures by injecting virulence proteins into mice and looking for protection against *S. aureus* infection. Last August, he found that mice vaccinated against protein A—a component of the bacterium known to disrupt the host's immune response—did better when exposed to virulent strains of *S. aureus*. The vaccinated



Anna Lensch

The vicious cycle of biofilms: When bacteria cling to surfaces in the body, it happens in a multi-stage process. First the bacteria land on the site and become initially attached (1). Next the microbes on the surface begin releasing extracellular polymeric substances, which act as a sort of chemical glue to keep them in place (2). Then the bacteria grow into a three-dimensional structure, covering even more surface (3). Last, after maturation, the biofilm begins releasing individual microbes (4), which start the cycle all over again.

group developed only one abscess, on average, compared with the controls, which developed an average of four (*J. Exp Med.* **207**, 1863–1870, 2010). In the future, he hopes to incorporate these proteins into conventional vaccines to more effectively protect people from acquiring *S. aureus* infections.

Layers of complexity

To banish biofilms, some scientists say you have to develop a truly biofilm-specific strategy rather than just go after *S. aureus* in any form. This philosophy gained ground in the late 1990s, when Gerald Pier, a microbiologist at Brigham and Women's Hospital in Boston, suggested an *S. aureus* vaccine directed against the sugar poly-*N*-acetyl glucosamine (PNAG), which coats the surface of bacterial biofilms. This sticky substance is an especially good choice because it is integral to the pathogen's immune evasion strategies and therefore can potentially be used to protect against all *S. aureus* infections.

> "It annoyed the hell out of me that you couldn't cure a biofilm infection."

In 1999, the Brigham and Women's team showed that the vaccine protected mice from a systemic *S. aureus* infection. And preinjection of PNAG-specific antibodies completely protected mice from a lethal bacterial challenge (*Science* **284**, 1523–1527, 1999). But even though Pier took biofilms into consideration when developing the vaccine, it has yet to be shown to clear biofilm infections. The problem, according to some scientists, is that PNAG is not produced by every strain of *S. aureus*, and therefore a vaccine against it might not necessarily work against all types of these stubborn infections.

Now, Shirtliff hopes to one-up Pier's PNAG-targeting vaccine by introducing a new approach directed toward *S. aureus* residing in the slime. His team has been hunting for key biofilm antigens, growing biofilms on medical tubing in the lab and then doing microarray analysis to tease apart which proteins are most active in the formation process on the basis of genetic signatures. Ultimately, they identified 22 cell surface proteins produced by biofilm-building microbes (*Infect. Immun.* **74**, 3415–3426, 2006). The researchers zeroed in on four proteins that were highly upregulated throughout the infection cycle and that together covered the entire biofilm. One of these, called glucosaminidase, is speculated to help break down cells surrounding the biofilm matrix to provide the bacteria there with chemical building blocks for growth.

This past April, his team reported that 87% of antibiotic-treated rabbits that previously

Persistence may pay off for antibiotics innovators

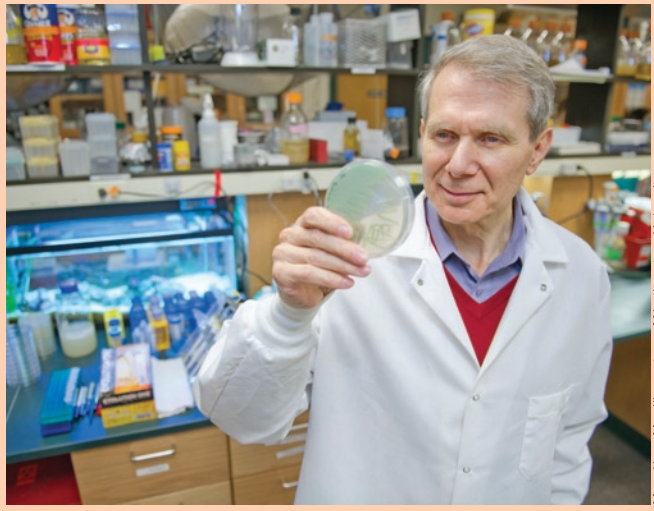
Among people with cystic fibrosis, nearly 90% experience growth of biofilms that build up in their airways and clog their lungs. Existing therapies can help affected individuals breathe easier but stop short of clearing the infections. Now, scientists think they understand why: the microbes behind these infections develop a characteristic known as 'persistence'. Unlike 'resistant' microbes that can grow in the presence of antibiotics, so-called 'persistent' microbes can only tolerate these antibacterial medicines when they are dormant, which often occurs when they are in biofilms. Some experts speculate that persistent bacteria are able to escape antibiotics by turning down their internal metabolic activity.

Reporting last December, scientists at Northeastern University in Boston discovered that airway infections in people with cystic fibrosis probably recur because the microbes in the lungs have mutations that allow them to evolve persistence, which sharply decreases the effectiveness of typically prescribed antibiotics such as ofloxacin, carbenicillin and tobramycin (*J. Bacteriol.* **192**, 6191–6199, 2010). Part of the difficulty, according to lead author Kim Lewis of Northeastern University, is that these pathogens use many mechanisms to develop persistence, making it that much harder to come up with new drugs to eradicate them.

"This Hydra has many heads," says Lewis. "Traditional, straightforward target discovery is not going to work."

The Northeastern team is now looking toward alternative strategies to develop biofilm-busting medicines. The researchers hope to develop so-called 'prodrug' antibiotics that kill pathogens only upon internalization. This is an especially good choice for persistent pathogens because the drug needs only to permeate into these microbes—the medicines eliminate these pathogens by membrane or genome disruption. Such strategies already show great promise to treat persistent tuberculosis infections.

Meanwhile, a group of researchers at Boston University School of Medicine believe that adding sugar to antibiotics might nudge



Mary Knox Merrill, courtesy of Northeastern University

Not giving up: Kim Lewis wants to thwart persister cells.

persistent bacteria into a metabolically more active state and make them more susceptible to defeat by antibiotics. Reporting last month, James Collins and his team found that sugars made lab-grown *Escherichia coli* and *Staphylococcus aureus* biofilms more vulnerable to a certain class of antibiotics called aminoglycosides. And sugar stimulated over a 90% drop of bacterial load in antibiotic-treated mice suffering from catheter-associated infections (*Nature* **473**, 216–220, 2011). In the future, Collins hopes to optimize his persister-busting regimen to clear these infections.

"We would like to expand the microbial arsenal," says Collins, "and make the tools we already have more effective."

—MP

Adam Zewe, courtesy of University of Maryland Dental School

Giving vaccines a shot: Mark Shirtliff.

received their four-component vaccine beat *S. aureus* biofilm infections as a compared to 55% of rabbits treated with antibiotics alone (*Infect. Immun.* **79**, 1797–1803, 2011). And 50% of vaccinated mice kicked these infections without any antibiotic treatment compared with none of their unvaccinated counterparts.

Dose of reality

Vaccination approaches against *S. aureus*, however, may not benefit the people that need them the most, according to Frank DeLeo, an immunologist at the US National Institute of Allergy and Infectious Diseases (NIAID) in Hamilton, Montana. The people at highest risk for biofilm infections are critically ill and often on immunosuppressive medications and thereby are unable to be protected by vaccination.

To circumvent this issue, Pier's team is developing a PNAG-specific antibody to protect patients from hospital-acquired infections upon hospital admission or before device implantation. Licensed in 2010 by Sanofi, the vaccine is soon to be tested in a phase 2 clinical trial. "We think our vaccine will work well by killing bacteria released from the biofilm to prevent a bloodstream or tissue infection," he says. Scientists at MedImmune are also ditching the vaccine approach altogether and developing protective monoclonal antibodies to *S. aureus* that can be administered before patients are wheeled into the operating room to prevent the onset of infections.

Even if vaccines are given to people while they are still healthy, some scientists remain skeptical that they can block biofilm formation. That's because biofilms are complicated structures, and infectious agents such as *S. aureus* use many repeated backup mechanisms to adhere to host tissues and construct their slimy microbial abodes. It remains unclear whether Shirtliff has identified the crucial targets that will send these fortresses tumbling down, says microbiologist Knut Ohlsen of Würzburg University in central Germany.

"At the moment, it is hard to believe it is possible to develop a vaccine against biofilm formation," Ohlsen says. "We don't know which proteins are essential for this process."

And even if the proteins identified by Shirtliff's team turn out to be key biofilm building blocks, the infectious microbes producing them are buried in these sugary structures and therefore may be shielded from antibody-based attack, according to Michael Otto, NIAID's chief of pathogen molecular genetics.

> "At the moment, it is hard to believe it is possible to develop a vaccine against biofilm formation."

Shirtliff disagrees. He says the proteins he is targeting are produced by these microbes as soon as they touch down on the host tissues and start to build biofilms and therefore are still vulnerable to attack. "What we want to do in our vaccination approach is make sure the biofilm doesn't form," explains Shirtliff.

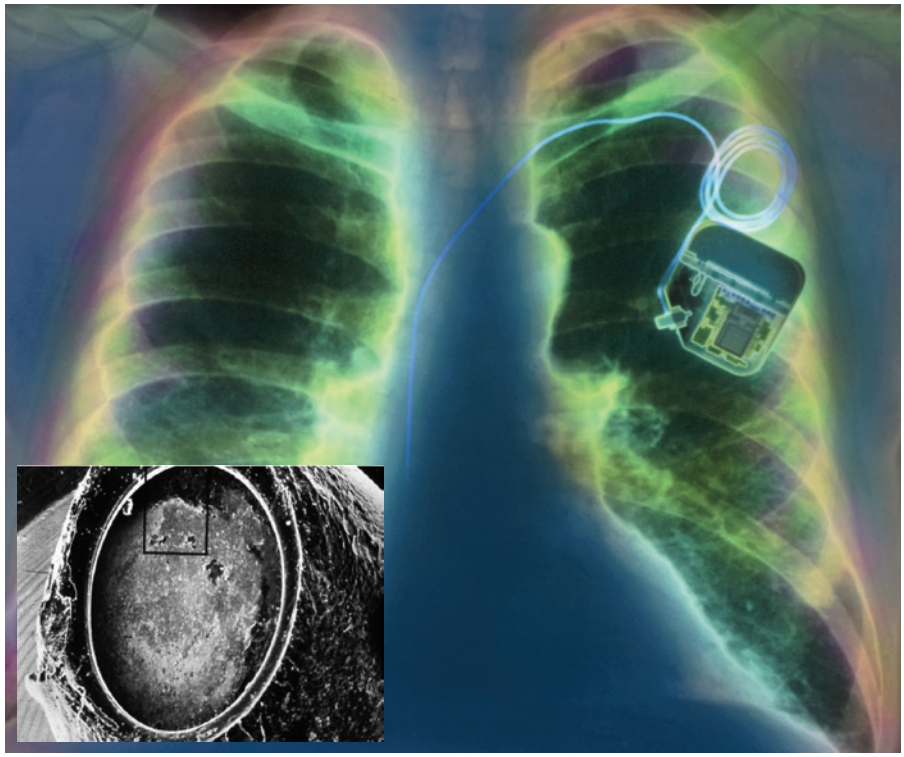
And he says antibodies raised against these proteins can penetrate even established biofilms, making them especially ideal to diagnose these stubborn infections. In 2007, his team found that fluorescently labeled antibodies were able to wiggle into lab-grown late-stage biofilms and attach to the target proteins.

Shirtliff has even added a fifth component (against cell surface proteins) to his vaccine to protect against all *S. aureus* infections. He hopes to launch phase 1 trials by the end of next year.

Meanwhile, larger pharmaceutical firms are pushing ahead with their more traditional *S. aureus* vaccines. Pfizer scientists presented phase 1 clinical trial results for their SA3Ag vaccine last month at the European Congress of Clinical Microbiology and Infectious Diseases in Milan. The race for the *S. aureus* vaccine is still at early mileposts. But scientists nevertheless remain confident that they will reach the finish line and topple the bacterium.

"It's smart, but it's only 2.8 or 2.9 megabases," says Projan, referring to *S. aureus* and its genome. "I like to think I am smarter than the damn bacteria."

Michelle Pflumm, a former intern at* Nature Medicine*, is a freelance writer based in Boston.



SPL / Photo Researchers, Inc.; *Circulation*, **66**, 1339–1341, 1982/Wolters Kluwer

Heartbreaking biofilms: Bacteria can gunk up the tips of pacemakers (inset).