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Bacterial Biofilms No Match for Engineered Virus

Scientists have found that they can modify naturally occurring viruses to create targeted weapons against biofilms, tenacious bacterial communities that wreak havoc in both clinical and industrial settings.

No treatment has a chance of clearing an infection if it is unable to reach the bacteria. But bacteria are often protected by biofilms, complex matrices of proteins, lipids, polysaccharides, and nucleic acids - as well as bacteria themselves. Antibiotics do little to deter them, and biofilms -- which can be found in dental plaque, water pipes, industrial systems, and in medical devices, such as catheters - must usually be removed mechanically.

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New research, however, suggests a better way. Howard Hughes Medical Institute predoctoral fellow Timothy K. Lu, working in the laboratory of Boston University professor James J. Collins, has now engineered a virus that employs a two-pronged strategy to eradicate bacterial biofilms with remarkable efficiency. The two researchers reported their results a paper published in the July 3, 2007, issue of the *Proceedings of the National Academy of Sciences*, and online on June 25, 2007.

Scientists have long turned to bacteriophages - viruses that infect bacteria - to combat infections. Lu says there are even accounts of Soviet soldier being injected with bacteriophage to treat wound infections. More recently, the U.S. Food and Drug Administration approved a mix of six bacteriophages as a food additive in ready-to-eat meats and poultry.

When phage-infected bacteria die, the surrounding biofilm loses some of its structure - but bacteriophage do not normally target the biofilm matrix itself. There are enzymes that do this, however, and Lu and Collins reasoned that by adding such an enzyme to bacteriophage, they could destroy biofilms far more effectively. So Lu inserted a gene for an enzyme that degrades biofilms into a well-characterized virus known as T7, which infects *E. coli*.

After growing biofilms in on pegs, Lu introduced the bacteriophage. Within 48 hours, the engineered bacteriophage had destroyed nearly 100 percent of the biofilms. The bacteriophage without the added enzyme, on the other hand, was about one hundred times less effective.

Lu's results suggest a widespread applicability for designing enzymatic phages. "This is a simple thing to do," said Lu, who is now a Ph.D. student in medical engineering and medical physics in the Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology. "In the future, we might be able to create libraries of phage that are effective against a wide range of biofilms."

Other research has explored the use of enzymes to destroy biofilms, but enzyme delivery has been a challenge. According to the researchers, it can be difficult to achieve the high levels of the enzyme needed to disperse biofilms, especially for biofilms in sequestered locations.

By putting an enzyme-coding gene in the viral genome, high doses of the enzyme are delivered, even with low doses of bacteriophage. The enzyme is produced only when the virus replicates inside the bacteria, and the replicative power of viruses means that it is produced in large quantities. That could minimize side effects if bacteriophages are used to treat clinical infections, Lu said. And because bacteriophages infect only bacteria, human cells are not at risk for viral infection.

Bacteriophages are abundant - they can be found in soil, water, and in the human gut. Each phage infects only one species, or a few species, of bacteria. This specificity, coupled with the ability to genetically engineer phages to produce specific enzymes, makes the area a promising one for countering contamination and infections.

"Enzymatic phages are a good way to go after biofilms," Lu said. "This can be easily done with modern-day technology. If we have a library of enzymes

that we know will destroy a certain type of bacteria, and a library of phage types, you can combine them any way you wish.”