Breaking the biofilm barrier

By Kai-Jye Lou, Staff Writer

Bacterial biofilms compromise the use of implanted medical devices such as stents, catheters and ventilators because they can shed bacteria and cause infection elsewhere in a patient. These surface-attached bacterial communities are very difficult to eradicate as they are held together by a polymeric matrix, creating barrier-like properties that protect the bacteria from antimicrobials and the host immune system.

A group of European researchers has now developed glycopeptide dendrimers that target a virulence factor of *Pseudomonas aeruginosa*, which enabled the scientists to disperse *P. aeruginosa* biofilms *in vitro*, thus rendering the bacteria vulnerable to antibacterial agents in suspension.¹

P. aeruginosa is a common cause of opportunistic nosocomial infections and can establish colonies in a broad range of environments and surfaces, including open wounds and burns, the pulmonary and urinary tracts and implanted medical devices. In addition to being naturally resistant to certain antibiotics, *P. aeruginosa* prefers to grow into biofilms.

Biofilm formation can be prevented with antimicrobial compounds and surfaces, which kill the bacterium outright or interfere with its ability to attach to a surface. Dispersal of an existing biofilm, however, is a much more difficult problem to tackle—most antimicrobials cannot penetrate the biofilm matrix and those that do are generally too toxic or too rapidly degraded to be practical for systemic use. There are no FDA-approved drugs that effectively disperse *P. aeruginosa* biofilms within the body.

“Currently available agents are effective at preventing biofilm formation,” said Richard Scott, VP of research at **PolyMedix Inc.** “The question is, can you get in and break up one that is already established?”

PolyMedix’s lead compound is PMX-30063, a synthetic defensin mimetic antibiotic in Phase I testing to treat Staphylococcal infection. PolyMedix is also developing polymer biomaterials with antimicrobial properties.

In the new paper in *Chemistry & Biology*, a research group led by Jean-Louis Reymond at the **University of Berne** showed that dendrimers targeting the fucose-binding lectin PA-IIL (lecB) may be up to the task.

Like other lectins, lecB, a known virulence factor of *P. aeruginosa*, plays a role in biofilm formation. However, because lecB is only one

of multiple lectins involved in biofilm formation, it had been unclear whether it would be a good individual target.

Reymond’s group has now shown that lecB-targeting dendrimers can prevent biofilm formation and disperse existing biofilms.

The dendrimers used for this application had highly branched peptidic polymer backbones.

In vitro, one of the highest affinity lecB-binding dendrimers, called FD2, inhibited biofilm formation by 100% at 50 μ M and by 55% at 10 μ M. The higher dose of FD2 also decreased surface coverage of *P. aeruginosa* biofilms by about 70% compared with that seen in untreated controls. A reference lecB ligand decreased surface coverage by only about 40%.

FD2 was not toxic to either the bacterium or human cell lines. “The key novelty here is that a ligand targeting only lecB can reduce and clear biofilms,” said Reymond, who is a professor of chemistry and biochemistry at the university. “The idea is that you clear the biofilm and let antibiotics and the body’s immune system do the rest.”

Debating dendrimers

Researchers contacted by SciBX had mixed opinions on whether the lack of toxicity to bacteria was a selling point for the lecB-binding dendrimers.

On one hand, dendrimers do not exert direct selective pressure against the bacteria, which should lower the odds of resistance. “The data presented show that the dendrimers do not work by killing the bacteria or affecting their growth and hence the risk of resistance is lower than for compounds like antibiotics,” Scott Rice, a senior microbiologist at **Biosignal Pty. Ltd.**, told SciBX.

Biosignal’s BOS313, an anti-biofilm furanone, is in preclinical development for *Escherichia coli* and *P. aeruginosa* infections. The company’s anti-biofilm compounds interfere with bacterial cellular signaling pathways to prevent colonization without actually killing the bacteria.

“One potential advantage is that these dendrimers seem to be specifically working without any adverse effects upon the bacteria,” said Kim Lewis, director of the Antimicrobial Discovery Center and a professor of biology at **Northeastern University**.

He noted that other agents used to disperse biofilms are more detergent like, with a nonspecific mode of action that can result in undesirable effects against off-target cells.

On the other hand, Dmitri DeBabov, head of microbiology at **NovaBay Pharmaceuticals Inc.**, thinks “the development of nonlethal antibacterials, targeted exclusively against virulence factors or biofilms, is a controversial approach. Though this idea has been around for awhile, no drugs of this class have been approved. FDA likes to see compounds that kill bacteria.”

NovaBay is developing topical Aganocides, N-chlorinated broad-spectrum antimicrobial molecules that can penetrate biofilms and kill the bacteria residing within.

The company’s lead Aganocide, AgaNase NVC-422, is an antimicrobial derivative of chlorotaurine, which is an oxidizing agent pro-

duced by innate immune cells. The compound is partnered with **Alcon Inc.** and is in Phase II testing for nasal *Staphylococcus* infection and catheter-associated urinary tract infections.

Researchers did agree that benefits of the dendrimers published in *Chemistry & Biology* include specificity and the potential for combination therapy with conventional antibiotics.

John Magnani, CSO of **GlycoMimetics Inc.**, noted that the approach specifically targets a known virulence factor of *P. aeruginosa*. "This strategy targets virulence factors, which is a novel mechanism and is not microbicidal. Thus, it has the potential to show enhanced effects when used in combination with traditional standard-of-care therapies that are microbicidal," he said.

GlycoMimetics' GMI-1051, a small molecule glycomimetic antagonist of lectin PA-IL (lecA) and lecB, is in preclinical testing to treat and prevent *P. aeruginosa* infection.

The specificity of Reymond's dendrimers also presents another potential advantage. Rice noted that if the compounds don't show cross-reactivity with other cell types, "then such a treatment has significant advantages over indiscriminate treatments, such as antibiotics, which can leave the patient susceptible to secondary infections."

As antibiotics also kill beneficial bacteria, resistant pathogens like *P. aeruginosa* gain a selective advantage and proliferate.

Branching out

Both Scott and Magnani said the large size of the lecB-targeting dendrimers could decrease bioavailability and complicate synthesis during the scale-up for commercial manufacturing, thereby potentially lowering their potential.

In addition, Lewis and Magnani said the dendrimers use a peptide backbone, which can potentially decrease stability and increase immunogenicity *in vivo*. Lewis suggested a polymeric carrier like

polyethylene glycol (PEG) should be used to prevent rapid proteolytic degradation of the dendrimer in the host.

Reymond said his group has now found that the dendrimers did not show signs of immunogenicity in mice. He also said the dendrimers did not show significant proteolytic degradation in serum.

Meanwhile, according to Rice, "it would be interesting to see if the dendrimers also disperse *P. aeruginosa* in the presence of other bacteria," because *P. aeruginosa* can form biofilms with other species of bacteria.

Going forward, Reymond said, "it is well known that several lectins play a role in biofilm formation, and a dendrimer would be optimal to hit several of these simultaneously since we can display multiple copies of various epitopes on their surface."

He added that the clinical development of a single ligand that hits multiple targets "would be easier than that of a cocktail of different ligands for various targets."

Reymond said the work covering the use of lecB-targeting dendrimers to disperse biofilms is not patented.

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Contact: Jean-Louis Reymond, University of Berne, Berne, Switzerland
 e-mail: jean-louis.reymond@ioc.unibe.ch

COMPANIES AND INSTITUTIONS MENTIONED

Alcon Inc. (NYSE:ACL), Fort Worth, Texas
Biosignal Pty. Ltd. (ASX:BOS), Eveleigh, Australia
GlycoMimetics Inc., Gaithersburg, Md.
Northeastern University, Boston, Mass.
NovaBay Pharmaceuticals Inc. (AMEX:NB; TSX:NB), Emeryville, Calif.
PolyMedix Inc. (OTCBB:PVMX), Radnor, Pa.
University of Berne, Berne, Switzerland