Liquid crystals, phages and biofilms: the complex world of antibiotic resistance

Tanmay Bharat at the University of Oxford

Tanmay Bharat is the 2021 Eppendorf Young European Investigator. He works in the Sir William Dunn School of Pathology at the University of Oxford, studying the structures of prokaryotic cell surface proteins, including their role in infection, to uncover novel modes of antibiotic resistance. Here, Tanmay speaks to science journalist Geoff Marsh about his award-winning work on *Pseudomonas aeruginosa* and its peculiar extracellular matrix in biofilms.

Interview also available as a podcast at: go.nature.com/eppendorf2021

Explain the importance of the current challenges our society faces with growing antibiotic resistance.

In COVID-19 vernacular, antibiotic resistance is a slow moving pandemic. If it's not dealt with now it will lead to the return of a dark age where antibiotics don't work and we have no treatments against bacterial infections. The only option would be to physically remove infected tissues through amputation, or to admit that patients will die. In order to avoid such a future, it's extremely urgent that we invest in fundamental and translational research now.

How do biofilms fit into the fight against antibiotic resistance?

Researchers estimate that most human bacterial infections proceed with biofilm formation. These biofilms are able to take hold in different body tissues and, because they are resistant to high doses of antibiotics, they are extremely difficult to treat. This makes biofilms important in the fight against antibiotic resistance as they are the framework in which the bacteria need to be treated within a medical setting.

What exactly is a biofilm?

Biofilm formation is a process by which individual bacteria grow into a multicellular community with the secretion of an extracellular matrix. This is accompanied by those bacteria becoming tolerant to a wide variety of environmental stresses, including antibiotic treatment.

Many labs have done experiments that have shown that phages, viruses that usually infect bacteria, are rich in the biofilm extracellular matrix, so there is a new realization in the field that phages can have a symbiotic role with bacteria. These phages have been shown to form aggregates of liquid crystals in biofilms, but we wanted to understand this process, following on from work of several of our colleagues around the world.

Describe your research into these phage molecules.

We knew that they were upregulated in biofilms, but we wanted to show what they were actually doing by *in vitro* experiments. So we grew biofilms, and from those we purified the phage molecules. Then we added physiologically relevant biopolymers that would mimic the biofilm environment and we saw the phages forming liquid crystals. Then we asked if these liquid crystals actually have an effect on the cells. We grew the bacterial cells under different conditions, and only in the condition when the liquid crystals were present were the cells protected against the antibiotic. The next step was to see what was happening in single cells under the microscope.

How did you visualize individual *Pseudomonas aeruginosa* cells and what were the phages doing?

We used electron cryomicroscopy, a technique in which an electron microscope operates at liquid nitrogen temperatures, allowing us to image these bacteria and molecules at extremely high resolution. Combining these microscopy techniques with high-end image processing methods allows us to get high-resolution structures. Here, we can observe single molecules and understand the molecular mechanisms at play. What we saw was that the phages encapsulated the bacteria and formed nano compartments, a sort of shield made of liquid crystals, which protected the bacteria from antibiotics. We don't know what these liquid crystals look

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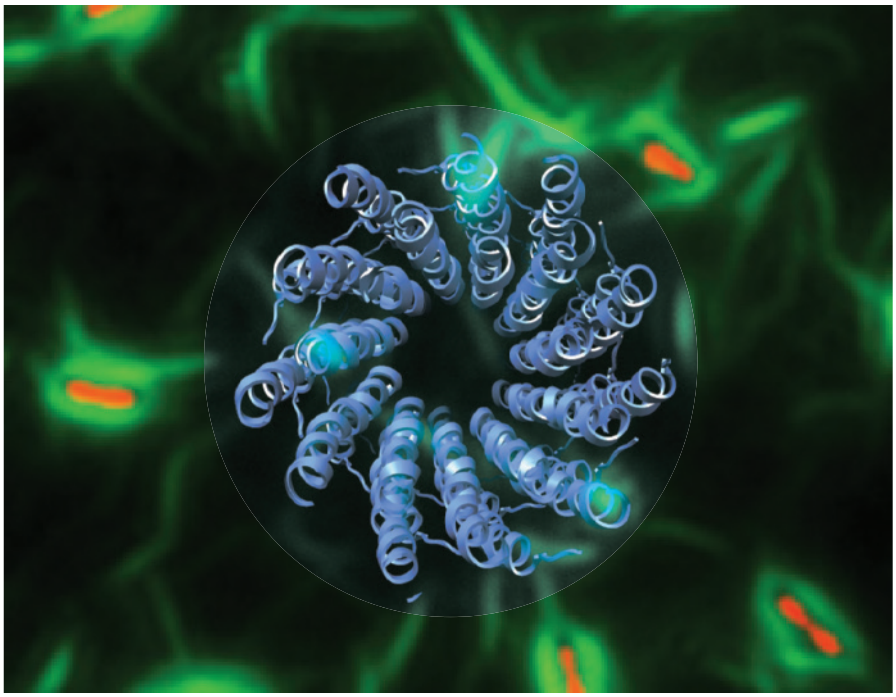

The atomic structure of a symbiotic bacteriophage (blue ribbon). In a biofilm, phage liquid crystalline droplets (green) form protective sheaths around bacterial cells (red), shielding them from antibiotic treatment.

like in an infection setting right now, but we are developing models to image that.

Do the liquid crystals physically protect the bacteria from antibiotics?

We don't know the exact mechanism behind this and whether it's purely a biophysical phenomenon or if there is a large chemical component. We have to do more research to figure out exactly what the mechanism is. But the presence of the liquid crystals absolutely protects the bacteria from antibiotic attack.

What is orchestrating this process? Do the bacteria direct the bacteriophages, or do the bacteriophages form this sheath around anything the right shape?

I think the bacterium is pulling the strings because the bacteriophage's genetic material is encoded in the bacterial genome, and prophage genes are overexpressed during biofilm formation. The phages wouldn't be there if the bacterium didn't harbour them within its genome, or produce and secrete them into the extracellular matrix

Could these bacteriophages become a therapeutic target?

Yes. If we target bacteriophages, then the protective effect they give will be removed. If that treatment is provided alongside an antibiotic, it could be very powerful in treating the bacterial biofilm disease.

There are many ways bacteria can avoid antibiotics and it depends on the type of antibiotic. Antibiotic resistance is a multifactorial, complex issue that needs a community effort with people working on it from different perspectives. So even if the bacteria are able to evade one particular antibiotic in one setting, you could use a cocktail of antibiotics to overload the system and treat the infection.

We have to keep looking and keep trying to outpace the bacteria. Bacteria are actively evolving and using new methods, which they can pick up from other bacteria or from other organisms. We have to be one step ahead.

What will this award mean for you and your group?

This award gives us an opportunity to highlight our work and validates the importance of the research we are involved in. I would like to acknowledge the encouragement we received in entering the field from Patrick Secor, Paul Bollyky and colleagues, our research builds on other stellar work. Although we don't do the work for any kind of accolade, when one does come along it is extremely nice. It suggests our original goal was a worthy one and a noble one, and something that people find as important as we do.