Killer dialogue

When bacteria are told to do so they form biofilms on solid surfaces which reduce the effectiveness of antibiotics. **Richard Corfield** looks at ways of inhibiting these instructions

The idea of alien intelligences calmly watching our every move and discussing the right time to take the Earth from us is the stuff of books, movies and nightmares. But how would you feel if you knew that every time you sat in the dentist's chair your dentist was fighting a losing battle with exactly such a foe?

One of the most startling discoveries to come out of the burgeoning world of microbiology in the past two decades is the fact that bacteria can communicate. In fact, not just communicate but converse on a level where they can induce each other to switch on dormant genes that then have the capacity to do you harm. Dental plaque, it turns out, is the least of our worries. The superbug MRSA is part of the same phenomenon, called 'quorum sensing'.

Quorum sensing is the ability of bacteria to communicate and coordinate behaviour using signalling molecules called autoinducers. Autoinducers are continuously produced by bacteria but when their concentration reaches a certain threshold – that is to say, when the bacteria producing it have a quorum – they switch on transcription genes within the bacteria's DNA telling it to do two things: to produce more autoinducer and, crucially, to change behaviour.

It is the behaviour change that does the damage. Most bacteria spend their lives as free-swimming, planktonic organisms but when mandated to do so by the autoinducer will switch to a sessile lifestyle, dropping out of the swimming phase and anchoring to the nearest solid surface, be it a tooth, contact lens or the newly-minted plastic ball-joint of a hip replacement. There they form biofilms – bacterial mats that reduce the effectiveness of antibiotics and where the local concentration of autoinducer goes through the roof. Once a biofilm is established it is very difficult to get rid of – witness the ubiquity of dental plaque and the dogged resistance of MRSA to treatment.

Dietrich Mack at the University of Swansea and his group have been active in discovering just how quorum sensing works. In *Staphylococcus aureus* and *S. epidermidis* – the microbes responsible for both MRSA and implantation rejects – they have identified at least two major gene expression pathways responsible for initiating biofilm formation, one based on a polysaccharide and the other on a peptide. But as Mack acknowledges, 'Our research shows that the expression of these pathways is not straightforward. In certain cases where we inhibit a gene responsible involved in quorum sensing it can actually increase the amount of biofilm formed'.

Quorum sensing, however, does more than merely initiate biofilm formation. It is a potent weapon of war between bacteria. For example, the four most common strains of *S. aureus,* including MRSA, use four slightly different autoinducers to initiate biofilm formation, all of which also aggressively inhibit the receptor sites of the other strains. The strain that reaches its critical quorum level first not only gets to put down its biofilm inducing roots first, it also gets to silence its competitors, preventing them from building up more of their own autoinducer.

Smile and polish: biofilm forms on teeth

In brief

- **Bacteria can communicate using signalling behaviour molecules called autoinducers**
- **This phenomenom is called quorum sensing**
- **Bacteria can form biofilms responsible for MRSA and implantation rejection**
- **80% of hospital-acquired infections are associated with implants or in-dwelling devices**

Production of orthopaedic implants – from artificial hips to hip and knee joints – is an expanding industry worth $2.5bn in 2005 in Europe, according to Frost & Sullivan. Eighty per cent of hospital-acquired infections are associated with implants or other 'in-dwelling' medical devices, while 60% of hospital infections generally involve biofilms. Since MRSA and other biofilm-infections are frequently fatal, there are compelling financial and ethical reasons to find ways of preventing biofilm formation.

Just how biofilms heighten resistance to antibiotics is not straightforward. It may be simply because the ability of antibiotics to penetrate the biofilm to the bacterial cells themselves is impaired, or it may be that the lifestyle change from planktonic to sessile changes the metabolic state of the bacterial cell and therefore its resistance to antibiotics. A more extreme suggestion is that the bacteria are fundamentally altered in some way so that they behave more like a multicellular tissue than a loose agglomeration of co-operating single cells.

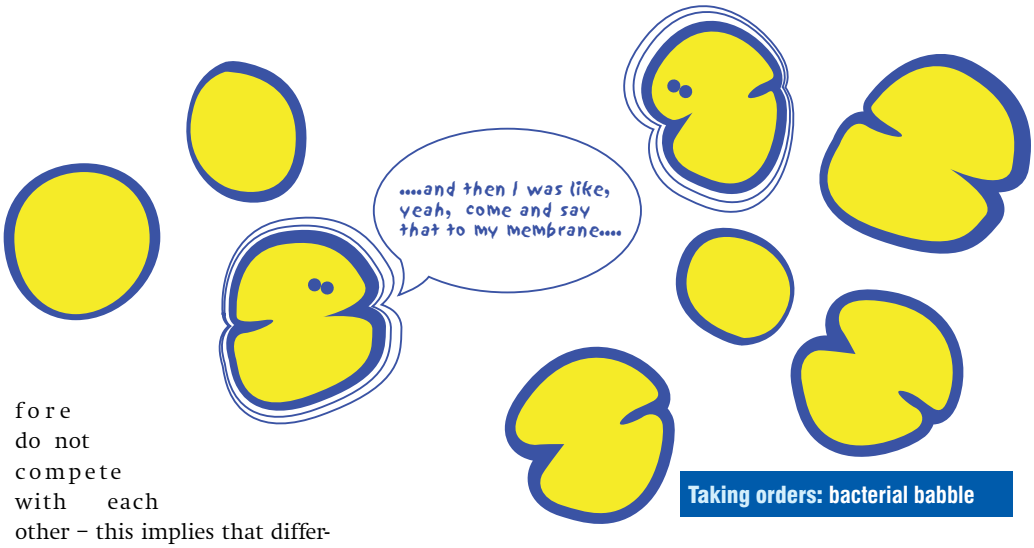
For years, the approach to tackle biofilms has been to incorporate antimicro-

bial agents in biomaterials that are to be used within the body, but the problem is the incredible ability of bacteria to develop antibiotic resistance. Because of their short generation time and their uncomplicated genomes, the fact is that bacteria can mutate and develop resistance faster than we can develop drugs to combat them. Simply killing bacteria *in situ* can lead to dead microbial cells and associated detritus fouling the surfaces of crucial implants. Defeating biomaterial biofilm formation requires a different approach, one that does not result in the death of the bacterium but rather in the neutralisation of its malevolence.

There are several possibilities: coating the biomaterial with substances that prevent bioadhesion, developing responsive surfaces that react to bacterial invasion, controlling the orientation of surface-tethered adhesion molecules, or interfering with receptor-ligand specific adhesion. But as Llinos Harris and Geoff Richards of the AO Foundation in Davos, Switzerland, point out: 'no surface modification or coating fully prevents bacterial adhesion'. This leaves perhaps the most exciting possibility of all: disabling their quorum sensing mechanisms so that the bacteria cannot form biofilms in the first place.

Several signal molecule families involved in quorum sensing have been identified in Gram-negative bacteria – those with two sets of cell membranes – like *Pseudomonas aeruginosa,* but the most intensively studied is the N-acylhomoserine lactone (AHL) family. AHLs contain a homoserine lactone ring attached via an amide bond to an acyl side chain containing anything from four to 14 carbon atoms. Once the AHL reaches a critical threshold, concentration members of the LuxR and LuxN family of transcriptional activator genes are switched on, forming proteins that start binding the bacteria to the substrate, thereby beginning biofilm formation. Variations in the chain length and oxidation at the 3-position provide different Gram-negative bacteria with species-specific languages with which they can communicate with their own kind.

Yet, since 75 Gram-negative bacterial species are known to use AHL, and only 25 AHL varieties have been found, it must also be the case that some of these species share a common tongue and can therefore talk across species boundaries. Since biofilms usually consist of a multitude of different bacterial species – which have different niches and therefore do not compete with each other – this implies that different bacteria co-operate in biofilm formation. It is a frightening thought.



Taking orders: bacterial babble

There is some good news, however. Some natural molecules have been found to interfere with AHL-mediated quorum sensing and the most important of these are halogenated furanones produced by the large marine alga *Delisea pulchra*. Halogenated furanones are structurally similar to AHLs and interfere with the ability of AHLs to bond to biomaterial surfaces. An analogy would be the way that carbon monoxide interferes with oxygen's ability to bond with haemoglobin by occupying the haemoglobin's receptor sites first.

'There are compelling financial and ethical reasons to find ways of preventing biofilm formation'

The Australian firm *Biosignal* is leading the way in the application of anti-biofilm agents to contact lenses. Biosignal's compounds are based on the naturally occurring furanones from *Delisea pulchra*. As the trend toward long-wearing disposable contact lenses gathers momentum it is increasingly important to make sure that the lenses do not grow a biofilm and cause eye infection. An initial human safety trial of their furanone-based coating was completed last year and the results look positive. 'The potential market is enormous', says Michael Oredsson, Biosignal's ceo, 'somewhere between $5bn and $6bn per year. We plan to levy a small but meaningful royalty on the use of Biosignal's proprietary technology – we aim for around 5%.'

Gram-positive bacteria like *S. aureus* and *Staphylococcus epidermidis* – the major cause of implant biofilm infections – use peptides rather than AHLs as signal molecules. In *S. epidermidis* a single peptide, once it has reached its critical level, activates an accessory gene regulator (agr) operon that results in the synthesis of Polysaccharide Intercellular Adhesin (PIA), a molecular glue that starts the process of biofilm formation.

An inhibiting peptide, appropriately known as RIP, can inhibit biofilm formation in both *S. epidermidis* and *S. aureus* and is under investigation as a potential treatment for *Staphylococcus*-induced infections.

As yet, however, there is no magic bullet for preventing Gram-positive, quorum sensing-induced, biofilm formation, and scaling up these techniques to clinical level offers substantial technical challenges. When asked exactly how inhibition of quorum sensing can be used to stop MRSA biofilm formation, Dietrich Mack responded: 'That is a very good question. I would like to know the answer to that too.'

But there is everything left to play for. Oredsson acknowledges that contact lenses are just the tip of the iceberg, and that the impetus behind quorum sensing remains a cure for serious bacterial infections, including those caused by MRSA.

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