Letters

# Multicellular resistance: biofilms

In 1987, Bill Costerton and colleagues published a landmark review article that was the first to articulate the general phenomenon of biofilm resistance to antimicrobial agents[1]. Fourteen years later, the question of how bacteria in biofilms manage to evade killing by antiseptics, antibiotics and antimicrobial components of the host defenses remains unanswered. Recently, Mah and O'Toole provided an excellent overview of the current hypotheses and recent data on mechanisms of biofilm resistance[2]. Recognition of the significance of this problem is bound to grow as the role of biofilms in chronic infections becomes increasingly clear[3,4].

Why has the underlying basis of reduced susceptibility of bacteria in biofilms proven to be such a difficult nut to crack? Mah and O'Toole register the possibility that multiple resistance mechanisms operate in concert within a single biofilm community. I would like to offer two further perceptions, both admittedly speculative, about the general nature of the resistance of bacterial biofilms to antimicrobial agents. Although these ideas are probably only partially correct, perhaps they can guide some fresh approaches to solving the problem of biofilm recalcitrance.

The first perception is that the mechanisms of resistance at work in a biofilm are different from those that confer resistance in free-floating bacteria. It is very tempting to look to known mechanisms of bacterial resistance in search of an explanation for biofilm resistance. As Mah and O'Toole discuss, preliminary experimental forays in these directions (e.g. multidrug-efflux pumps) have not been particularly fruitful. My guess is that other well-established antibiotic resistance mechanisms, such as target mutations and modifying enzymes, are likewise not at the root of biofilm resistance. The most compelling observation in support of this view is that even susceptible strains of bacteria, which lack a known genetic basis for antimicrobial resistance, mount protection in the biofilm mode of growth[5,6]. To solve the problem of biofilm resistance, we must identify entirely new genes and explore novel protective strategies.

The second perception is that the mechanisms of biofilm resistance to antimicrobial agents are inherently multicellular. We are accustomed to thinking about bacteria as single-celled entities, yet they persist in biofilms by communal strategies that depend on multicellular structures and on cooperation between differentiated cells within a population. For example, hydrogen peroxide fails to penetrate fully into biofilms because of the action of catalases expressed by the bacteria[7]. It is only by the concerted enzymatic action of aggregated bacteria that this phenomenon is manifested. Individual bacterial cells are overwhelmed by the same concentration of oxidant, even though they express similar levels of catalase. It is interesting to note that catalase activity is independent of viability. It therefore seems plausible that bacteria in the surface layers of the biofilm could continue to degrade the antimicrobial agent and shield their more deeply embedded neighbors even after they have expired.

Consider another example of the multicellular nature of the biofilm defense. Some antibiotics require aerobic conditions for effective killing. If bacteria in the outer layer of a biofilm consume the available oxygen, those bacteria in the depths of the biofilm will be protected. This defense cannot be implemented by planktonic bacteria because a lone cell simply does not exert sufficient respiratory activity to deplete oxygen from its immediate environment. In the aerobic zone of the biofilm, cells will suffer damage from the antibiotic, but these same cells might continue to consume oxygen, and hence protect their neighbors, long after they have lost the ability to reproduce.

Might bacteria in a biofilm differentiate, with some cells entering a static, spore-like state in which they are highly protected from many physical and chemical challenges? Other cells of the same species, perhaps even in the same cell cluster within the biofilm, could grow and reproduce. This division of labor would be a very effective way of surmounting occasional catastrophic events. The majority of cells, which are in a growing state, would be vulnerable to an antimicrobial challenge. Their differentiated siblings, the numerical minority, would survive the challenge and reseed the biofilm. Like the examples above, such a strategy is inherently multicellular. No single cell can occupy both states (growing and spore-like) at the same time.

Biofilm formation is just beginning to be recognized as a multicellular developmental process[8]. New strategies for dealing with the stubborn persistence of bacteria in the biofilm state will emerge when the multicellular nature of the biofilm defense is better understood.

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