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EDITORIAL

Understanding biofilms – are we there yet?

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Modern biofilm research was initiated by two independent observations of aggregating bacteria, one consisting of bacterial aggregates on rocks from an alpine stream (Geesey *et al.*, 1977) and the other in sputum from patients with cystic fibrosis (CF) suffering from chronic lung infection (Høiby, 1977). Other well-known scientists had already described bacterial aggregates (van Leeuwenhoek, 1684; Pasteur, 1864; Henrici, 1933), but Høiby and Costerton were the first to realize the significance of this bacterial behavior in infections. Over the years, the biofilm growth phenotype has been well accepted, and many investigators have developed tools to study biofilms under controlled conditions in the laboratory. Tools such as the flow cells and high throughput screens such as the 96-microtiter plate crystal violet assay have been developed for noninvasive, continuous observation and for measuring bacterial aggregation, respectively. *Pseudomonas aeruginosa* biofilms are the most frequently studied, and most of our current knowledge on biofilm biology is based on the behavior of this bacterium. Other bacterial species have also been studied, but none close to the extent of *P. aeruginosa.* Many experimental animal models have also been developed, both for implant-related biofilm infections, CF, chronic wounds, and for many other chronic infections. *P. aeruginosa* is indeed involved in many of these chronic infections, thus justifying its use as a biofilm model organism. More recently, many studies have used state-of-the-art molecular techniques to identify the microbiota in different chronic infections. While *P. aeruginosa* is still thought as being a relevant system, some of the newer studies claim that numerous different bacteria do play a role in chronic infections.

Today, we know from the thousands *in vitro* and *in vivo* biofilm-related observations that aggregates of bacteria are at the source of most persistent infections. We know that the bacteria in these aggregates are physically joined together and that they have an extracellular matrix consisting of many different extracellular produced substances such as proteins, DNA and polysaccharides. We also know that these aggregates withstand very high doses of antibiotics that will normally kill planktonic cells and that their tolerance toward host immune defenses is dramatically increased. Numerous studies of *in vitro P. aeruginosa* biofilms have also revealed a characteristic stepwise series of events including cellular attachment to any available surface, growth and proliferation of bacterial aggregates (microcolonies), and embedding in a self-made, protective matrix of exopolymers. The *in vitro*, surface-based, biofilm developmental process can be divided into the different stages of (1) attachment, (2) maturation and (3) dispersion. Bacteria attach to the surface and form microcolonies of the biofilm upon clonal growth. The surface becomes covered with motile bacteria, by means of twitching motility. Spectacular three-dimensional structures resembling mushrooms are formed by these motile bacteria, which eventually climb up the stalks using type IV pili, over time forming the caps of the mushrooms. The development of mushroom structures has been identified as wild-type behavior of *P. aeruginosa* growing in biofilms. However, these structures depend on nutritional and environmental conditions.

The hallmark of chronic, biofilm-based infections is an extreme tolerance to the action of antibiotics as well as a number of conventional antimicrobial agents and an almost infinite capacity to evade host defenses. These mechanisms have also been intensively studied in the laboratory, mostly using *P. aeruginosa*, and we know that quorum sensing (QS), slow growth, limited oxygen, matrix protection, etc., all contribute to the tolerance.

Do we now understand bacterial biofilms? Yes, we probably understand much of the developmental processes and matrix production of surface *in vitro*-generated *P. aeruginosa* mono-species biofilms and to a lesser extent of *Staphyloccocus aureus*, but little else. Our knowledge today can be termed 'Biofilm version 1.0' and is probably sufficient for academic purposes. 'Biofilm version 2.0 and higher' should be developed over the next decades and is direly needed within the medical field of biofilm research and in the clinic. But first, we need to remove the 'bugs' from version 1.0. These include for instance the notions that QS and solid surfaces are necessary for biofilm formation and that mushroom structures are formed *in vivo*. QS is a bacterial cell density–dependent regulatory mechanism, and as in biofilms the density of bacteria is high, QS is likely to occur. QS is also known to regulate the antibiotic tolerance and the production of virulence factors. However, biofilms can form without QS both *in vitro* and *in vivo* and will even persist without QS in

infections such as CF lung infections. Additionally, QS regulation can be initiated in planktonic cultures in the absence of biofilm formation. Regarding the need for a surface, biofilms can easily form without either QS or a surface both *in vitro* and *in vivo*, while many chronic infections occur without a surface. As for the mushroom structures, these are strictly a wild-type behavior of *P. aeruginosa* biofilms grown in *in vitro* flow cells with glucose as the sole carbon source. Mushroom structures have never been observed in experimental animals or in specimens from chronically infected patients.

What will 'Biofilm version 2.0' contain? We can of course only speculate about this. An obvious place to start is to investigate whether the *in vitro*-derived knowledge applies to experimental animal models and in chronic infections. With improved molecular tools such as *in vivo* and *in situ* transcriptomics and proteomics, we are now able to obtain data from less and less material and at a much higher resolution than before. This will help to elucidate the true role of QS and cyclic di-GMP *in vivo*. Do the identified developmental processes and matrix production of biofilms apply to animals and humans? How do chronic infections even initiate and what differences in developmental processes are there between acute and chronic infections, if any? The improved molecular tools will also enable us to understand whether only a few or all bacteria present in the biofilm microbiota play a role in the pathogenesis of chronic infections. Investigations into the tolerance mechanism and matrix production *in vivo* in the presence of an active immune response are also needed. Do the bacteria only encapsulate themselves in a 'self-produced' matrix or do they also utilize DNA from dead leukocytes? Is *in vivo* tolerance because of slow growth, or to matrix protection, or to other unknown factors?

Most importantly, for the benefit of biofilm-relevant medicine, we need to develop improved, rapid diagnostic methods for biofilm infections as well as improved prevention and treatment strategies. Here, the *in vitro* models can again be a suitable starting point, but basic researchers and medical practitioners need to closely collaborate to ensure that the right questions are asked and to draw the correct conclusions. *In vitro*-derived results should constantly be related back to clinical findings, as well as tested and evaluated in experimental animals.

In conclusion, we do not know how biofilms initiate and know little of what they do *in vivo*, except that their persistence provokes a continuous inflammatory response. Although we are not able to diagnose chronic biofilm infections easily, we can to some extent prevent some chronic infections, but we cannot treat biofilm infections efficiently. So are we there yet? No, but with new tools already available, and those yet to come, it is predictable that the biofilm field is primed for major advances in the next decade.

This thematic issue covers the trends in biofilm research as presented in Copenhagen at EuroBiofilms 2011. We hope to see you all at the third EuroBiofilms meeting to be held in Gent, Belgium, in 2013.

The Thematic Issue is dedicated to J. W. Costerton who died May 2012.

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