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GUEST COMMENTARY

The EPS Matrix: The "House of Biofilm Cells"[▽]

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In response to a suggestion by the *Biofilms 2007* organizing committee to hold an evening session on biofilm extracellular polymeric substances (EPS), an exceptionally inspiring event followed contributions by Ken Bayles, Alan Decho, Martina Hausner, Jan Kreft, Thomas Neu, Per Nielsen, Ute Römling, Sarah Schooling, Ulrich Szewzyk, Gideon Wolfaardt, and Liang Yang. The essence of the evening's discussion, moderated by Hans-Curt Flemming and Daniel J. Wozniak, is summarized below.

If biofilms can be metaphorically called a "city of microbes" (24), the EPS represent the "house of the biofilm cells." The EPS determine the immediate conditions of life of biofilm cells living in this microenvironment by affecting porosity, density, water content, charge, sorption properties, hydrophobicity, and mechanical stability (6).

EPS are biopolymers of microbial origin in which biofilm microorganisms are embedded. In fact, archaeal, bacterial, and eukaryotic microbes produce the biopolymers. Contrary to common belief, EPS are certainly more than only polysaccharides. They comprise, in addition, a wide variety of proteins, glycoproteins, and glycolipids, and in some cases, surprising amounts of extracellular DNA (e-DNA). In environmental biofilms, polysaccharides are frequently only a minor component (7). Unfortunately, it remains a substantial challenge to provide a complete biochemical profile of most EPS samples. It is often difficult to purify EPS matrix constituents apart from other components such as cells or other macromolecules transiently associated with the EPS (16). In addition, carbohydrate chemical analyses remain difficult, due to the diversity in sugar monomers, linkages, and unique structures present in the carbohydrate fraction of the EPS matrix material. Nonetheless, all EPS biopolymers are highly hydrated and form a matrix, which keeps the biofilm cells together and retains water. This matrix interacts with the environment, e.g., by attaching biofilms to surfaces and through its sorption properties, which allow for sequestering of dissolved and particulate substances from the environment, providing nutrients for biofilm organisms. The EPS influence predator-prey interactions, as demonstrated in a system consisting of a predatory ciliate and yeast cells, in which grazing led to an increase in biofilm mass and viability, with EPS as the preferred food source (9).

Some EPS components deserve particular attention. Alginate, a polyanion polysaccharide, is the best-investigated component of mucoid *Pseudomonas aeruginosa* biofilms. However, several recent reports have shown that other polysaccharides contribute to biofilms formed by nonmucoid *P. aeruginosa* strains, which are believed to be the first to colonize cystic fibrosis patients. A recent example is the expression of the *psl* operon, which is required in order to maintain the biofilm structure after attachment. Overproduction of the Psl polysaccharide led to enhanced cell surface and intercellular adhesion of *P. aeruginosa*, which translated into significant changes in the architecture of the biofilm (14). Nevertheless, other polysaccharides produced by *Pseudomonas* spp., such as levan, may have a role in biofilm formation (13). *P. aeruginosa* has been widely used by many biofilm researchers; it is, metaphorically speaking, the *Escherichia coli* of biofilm research. However, during this session, it was clearly pointed out that this perspective is limited and that extrapolation from *P. aeruginosa* biofilm results to biofilms in general, while tempting, is not suitable. Environmental biofilms contain surprisingly low levels of alginate. Even charged polysaccharides seem to be relatively rare in nature, as determined by uronic acid analysis. In environmental biofilms, it is extremely difficult to isolate and characterize specific polysaccharides in detail, as Alan Decho pointed out, expressing the experience of many researchers. The production of EPS in natural biofilms is dynamic and can follow cyclic patterns, as demonstrated in marine stromatolites (5). Currently the only in situ approach to EPS glycoconjugates is the use of fluorescently labeled lectins (15). An interesting role in influencing biofilm structure may be that of cellulose, formed by a variety of organisms. Cellulose is also important in infectious processes when coexpressed with curli fimbriae in *E. coli* (23). Curli as proteinaceous fibrils have attracted interest beyond infection, because curli-like fibrils have also been found to play an important role in natural biofilms produced by a variety of microorganisms. Larsen et al. (12) have found an abundance of amyloid adhesins in natural biofilms, which may contribute considerably to their mechanical properties. Strengthening of the biofilm structure is crucial for the stability of the "house" and the continuation of synergistic interactions based on the spatial proximity of various biofilm organisms. Cellulose has been found to be a component of EPS in amoebae, algae, and bacteria. In agrobacteria, cellulose is involved

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in attachment. It appears that cellulose plays an underestimated role in environmental EPS.

Biofilms are also an ideal place for exchanging genetic material and maintaining a large and accessible gene pool. Horizontal gene transfer is facilitated, since the cells are maintained in close proximity to each other, are not fully immobilized, and can exchange genetic information. In 1999, Hausner and Wuertz reported significantly higher rates of conjugation in bacterial biofilms than in planktonic populations (8). Recently, however, nucleic acids have attracted more attention. A focus of particular interest during the evening session was the observation of e-DNA within biofilms. Although e-DNA has been reported as a component of biofilms for quite some time, it is commonly considered a remnant of lysed cells. However, e-DNA occurs in sufficiently high quantities to raise some doubt. In fact, Palmgren and Nielsen reported in 1996 the accumulation of DNA in the EPS matrix of activated sludge and pure cultures of *Pseudomonas putida* (17). Allesen-Holm et al. (1) found that in *P. aeruginosa* biofilms, the e-DNA is likely derived from whole genomic DNA. Surprisingly, e-DNA was organized in distinct patterns in the biofilms of this organism, forming grid-like structures, suggesting a structural role for e-DNA. The observations of Böckelmann et al. (3), who report the formation of e-DNA as a spatial structure forming a filamentous network in biofilms of an aquatic bacterium, strongly support and differentiate such considerations. The e-DNA had similarities to genomic DNA but also distinct differences. It seemed as if the cells could move along these filaments, using them as nanowires. In the discussion session, speculations were welcome; one was that the cells might use the filaments for electron transfer and even for communication. Yang et al. (25) also found that e-DNA was one of the major matrix components in *P. aeruginosa* biofilms, functioning as an intercellular connector; they supported the concept of the stabilizing role of e-DNA for the biofilm matrix. In *P. aeruginosa*, the release of e-DNA is under the control of quorum-sensing systems as well as iron regulation (1, 25). In *Staphylococcus aureus* biofilms, *cidA*-controlled cell lysis plays a significant role during biofilm development and releases genomic DNA, which serves as an important structural component of *S. aureus* biofilms (18). A very interesting question is the mobility of DNA in the matrix. After all, from an energetic point of view, DNA is an expensive molecule, and Matt Parsek put the very justified question of what advantage makes such an effort affordable for the cells.

Not only is the EPS matrix composed of a variety of components, but these components are able to interact. One example is the retention of extracellular proteins such as lipase by alginate (6). Such mechanisms are crucial for preventing the washout of enzymes, keeping them close to the cells that produced them and allowing for effective degradation of polymeric and particulate material. This leads to the concept of an "activated matrix." Activation is made even more dynamic and versatile by the release of membrane vesicles (MV). These highly ordered nanostructures act as "parcels" containing enzymes and nucleic acids, sent into the depth of the EPS matrix (20). Such MV, along with phages and viruses, which are similar in size, can serve as carriers for genetic material, thereby enhancing gene exchange. Through their chemistry, the MV may bind extraneous components; their enzymes may help degrade polymers, provide nutrients, and permit enhanced resistance to certain inimical agents by inactivating them. Furthermore, MV seem to be part of "biological warfare" within biofilms, occurring as predatory vesicles containing lytic enzymes. This biological warfare is also long-range; in common with other matrix material, MV are shed from the biofilm and thus may deliver, among other things, virulence factors and cell-to-cell signals.

TABLE 1. EPS functionality[a]

Effect of EPS component	Nature of EPS component	Role in biofilm
Constructive	Neutral polysaccharides	Structural component
	Amyloids	Structural component
Sorptive	Charged or hydrophobic polysaccharides	Ion exchange, sorption
Active	Extracellular enzymes	Polymer degradation
Surface-active	Amphiphilic	Interface interactions
	Membrane vesicles	Export from cell, sorption
Informative	Lectins	Specificity, recognition
	Nucleic acids	Genetic information, structure
Redox active	Bacterial refractory polymers	Electron donor or acceptor?
Nutritive	Various polymers	Source of C, N, P

[a] Discussion contribution of T. Neu, based on a prior version published by Allison et al. (2) and completed by the results of the discussion.

The composition, architecture, and function of the EPS matrix, as discussed in this evening session, revealed a very complex, dynamic, and biologically exciting view. The matrix is a network providing sufficient mechanical stability to maintain a spatial arrangement for microconsortia over a prolonged period. This stability is provided by hydrophobic interactions, cross-linking by multivalent cations, and entanglements of the biopolymers (6, 10), with e-DNA as a newly appreciated structural component. Thomas Neu and John Lawrence tried to systematically arrange the information on EPS components and functions (Table 1).

While Table 1 is not complete, it reflects the variety of functions of EPS in the matrix. The discussion section revealed that many aspects of EPS remain to be addressed. An example is their function in biocide resistance (21). Also, the manifold ways in which the biofilm cells can modify their matrix, including production of EPS by various organisms, extracellular enzymatic EPS turnover and modification, and the resulting spatial and temporal heterogeneity, have not been addressed. In this context, species dynamics is crucial, since different species produce different EPS.

In the context of this complexity, modeling of EPS seems to be an almost impossible task, although it would be extremely helpful in predicting and controlling biofilm processes. Consequently, Jan Kreft asked for EPS research to provide more data, which would help to establish and optimize the existing models (11). Determining the rate of EPS production would be a useful task providing such data and giving information about

parameters such as the creation of more biovolume. Some of the methods developed by EPS research, such as the use of fluorescent biomarkers, may contribute to the data demanded by modelers.

In conclusion, it appears that "slime" has been very much underestimated. It may turn out that the EPS matrix is considerably more than simply the glue for biofilms. Rather, it appears to be a highly sophisticated system, which endows the biofilm mode of life with particular, successful features. More information about the specific components of the biofilm EPS, as well as their localization and stability, will likely support Costerton and Irvin's visionary concept of "biofilm as a tissue" in 1981, when EPS still was called the "glycocalyx" (4). In this regard, there exciting evidence is emerging that in several microbial communities, EPS often has a defined macromolecular "honeycomb" structure (19, 22).

We are only beginning to understand the construction principles, functions, and dynamics of the "house of the cells." Further understanding requires more-sensitive and less-destructive new methods, which will allow for the investigation of biofilm processes in situ over time. Then we can better understand what the "house of biofilm cells" is built of and what its functions are.

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