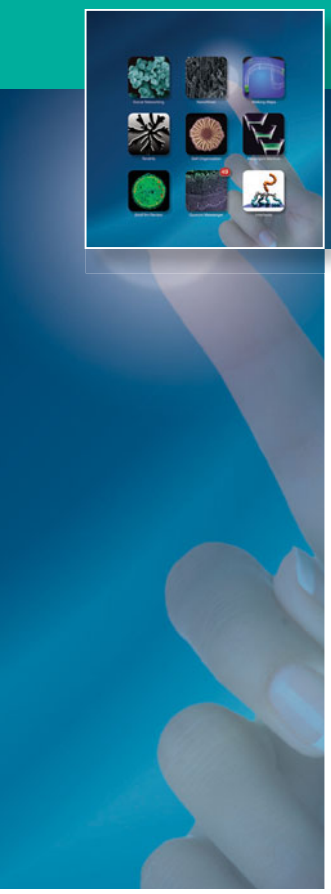

All together now: Integrating biofilm research across disciplines

Gerard C.L. Wong and George A. O'Toole, Guest Editors

Bacterial biofilms are integrated, multi-species communities of cells that adhere to almost any surface and are fundamental to the ecology and biology of bacteria. Not only do biofilms contribute to human health and disease, they also play important roles in the context of energy and the environment. The formation of biofilms requires interactions between bacteria and the surfaces they colonize, and both microbe and surface can impact the structure, function, and composition of these communities. Bacteria in biofilms exhibit surprisingly sophisticated social behavior, both cooperative and competitive, made possible by their cell biology. However, they are also hierarchically organized systems governed by complex physical and chemical interactions. Because of this, the study of bacterial biofilms has recently attracted the attention of materials scientists, physicists, chemists, and nanotechnology experts who import not only new tools, but also new concepts and perspectives. This issue reviews recent progress in multidisciplinary studies of biofilms.

Introduction

The majority of bacteria in both natural and clinical settings are organized into surface-associated, integrated communities known as biofilms. Biofilms are highly structured. Cells produce a matrix of extracellular polymeric substances (EPS), which include polysaccharides, proteins, lipids, lipopolysaccharides, and other materials that serve as a scaffold holding the biofilm together. Cells embedded in this EPS matrix communicate with one another through complex signaling networks and can cooperatively restructure the biofilm through different types of cell motility and matrix remodeling. This communal form of cellular organization, which functions via social concepts, plays a number of roles. Biofilms promote genetic diversity and maintain the high cell density needed for efficient genetic exchange. Perhaps most importantly, the community provides microbes protection from many forms of environmental insult, such as predatory stress (protozoan grazing, host immune system) and chemical stress (antibiotics, chlorine-based disinfection). In fact, it is not uncommon for biofilms to be three orders of magnitude more resistant to antibiotics compared to free-swimming, planktonic bacteria (i.e., those bacteria not attached to a substratum).

Biofilms contribute to a broad range of problems in human health and disease, such as tooth decay or cavities, biofouling of surgical implants and biomedical devices, and lethal chronic infections in cystic fibrosis–affected airways. Biofilms also impinge on a variety of industrial settings. Biofouling due to biofilms increases the hydrodynamic drag on ships, leading to increased fuel consumption. They also contribute to corrosion and scaling in reactors and increase costs in oil recovery and food processing.

Biofilms are not all bad. They can also be beneficial or even essential. Biofilms of bacteria that co-evolved and are accommodated to human niches are important for the establishment of the human microbiome, symbiotic microbial communities found at different sites of the human body, such as the gastrointestinal tract. Biofilms are used to digest organic contaminants in waste water treatment plants. Communities of "hydrocarbonoclastic" bacteria can help reduce petroleum from contaminated marine systems.

The impact of surface-associated communities of bacteria was likely first documented in the late 1920s or early 1930s based on their impact in a practical setting—their ability to increase the hydrodynamic drag on ships.[1] Subsequent studies by some of the early pioneers in biofilm research, such as Zobell and Henrici, described for the first time in the literature that bacteria could attach to and thrive on surfaces.[2–5] In the late 1970s, Geesey and colleagues developed qualitative and quantitative measures for biofilm bacteria recovery in aquatic systems.[6,7] Subsequent pioneering studies of biofilms (1970s and 1980s) were primarily the province of engineers and chemists.[8,9] After that, microbiologists revolutionized the

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field with state-of-the-art molecular biology techniques.[10] For example, applying bacterial genetics to the study of these communities identified numerous genes involved in biofilm formation, and sequencing of microbial genomes revealed the conservation of these biofilm-related genes across many organisms. Most recently, physicists, materials scientists, and nanotechnology experts are starting to make an impact, not just with new tools, but also with new concepts and perspectives. Even with the short introduction just given, one can see that biofilms are ubiquitous, and the study of biofilms is inherently multidisciplinary rather than simply interdisciplinary; to make further progress, one needs to employ the full resources of each constituent field, rather than just accommodating oneself in the interstices between fields. So, to echo the commonplace John F. Kennedy exhortation: ask not what biofilms can do for your field, but what your field can do for biofilms! Good examples can be found in this issue. We are fortunate to have an outstanding group of investigators working with us to produce the articles appearing in this issue, representing contributions from medical schools, microbiology, environmental engineering, bioengineering, chemistry, physics, as well as materials science. This is an exciting time for synthesis, and this synthesis is in progress as we write these articles.

Overview

In the present "standard model" of biofilms, the life cycle of a biofilm is characterized by five steps: (1) attachment of planktonic bacteria reversibly to a surface or by migration or division of sessile cells to cover an empty region of the surface; (2) production of EPS to adhere cells irreversibly to the substrate; (3) formation of micro-colonies; (4) formation of a mature, spatially structured biofilm via a complex process involving additional EPS production, signaling, cellular motility, reproduction, and the expression of biofilm-specific properties (such as antibiotic resistance); and (5) release of free-swimming planktonic bacteria to repeat the process.[11]

The existence of a surface is perhaps the most important prerequisite for biofilm formation, and, indeed, the first step of biofilm formation involves the bacterial detection of a surface and its response to a surface. This broad topic, which involves physical, chemical, and structural aspects of surfaces, has a long history and is reviewed by Renner and Weibel. This is not an easy process to isolate and study. Bacterial detection of a surface is currently not well understood. In addition, bacteria can respond in complex ways to a surface, including morphological changes upon surface contact. It is known that many non-specific physical forces contribute to initial cell attachment, including electrostatic, van der Waals, and steric interactions. In fact, bacteria can attach to hydrophilic and hydrophobic surfaces via various mechanisms. Because of this, chemical modification of surfaces comprises an important class of strategies for influencing interactions between bacteria and surfaces. Examples include self-assembled monolayers that present gradients of diverse functional groups, PNIPAAm (poly(*N*-isopropylacrylamide)) surfaces that shed EPS and bacterial cells, and surfaces with antimicrobial or anti-fouling oligomers.

The work presented by Khoo and Grinstaff details the state of the art for efforts focused on new coating technologies, and, in effect, this article outlines the ultimate translational goal of all the research covered in this issue's articles. That is, medical implants represent a major advance in the treatment of disease, but how do we work to prevent such implants from serving as the source of hospital-acquired (nosocomial) infections? Khoo and Grinstaff review recent advances in anti-infective coatings for medical materials. This research area is driven both by the rise in antibiotic resistant opportunistic infections in the clinic, as well as by economic forces; such infections are expensive, and this expense is borne increasingly by the hospitals where they occur. Khoo and Grinstaff describe the use of coatings that block colonization of medical implants rather than using coatings comprised of traditional antimicrobial agents. Such antimicrobial-based coatings, especially using frontline clinical antibiotics, raise the risk of developing more resistant bacterial strains. These authors present a number of advances in non-antibiotic coatings. Complementing Khoo and Grinstaff's coverage of methods to modify existing surfaces in the context of medical devices, Renner and Weibel explore the role of various surface properties in bacterial colonization and discuss means to directly engineer surfaces that could have anti-colonizing properties. While some anti-fouling technologies currently work short term (on the order of hours or days), can we develop materials and/or coatings that protect from bacterial colonization in the long term?

Bacteria in biofilms can coordinate their activities via signaling. "Quorum sensing" (QS), or the regulation of bacterial gene expression in response to the local concentration of a detected signal, is a good example of cell-cell signaling in the context of biofilm communities, including during early events in biofilm formation. A minimal critical cell density is required for QS-controlled genes. Shrout et al. review QS and motility in *Pseudomonas aeruginosa*, the "fruitfly" of biofilm forming bacteria, which is also an important opportunistic human pathogen in immunocompromised individuals, such as patients with cystic fibrosis and burn victims. It is estimated that QS controls ~5% of the genes in *P. aeruginosa*, including many virulence factors. For example, QS is linked to rhamnolipid production, which is important for bacterial migration to form mature biofilms. Controlling biofilm development artificially via QS manipulation has not proven to be successful to date, perhaps because, as shown by Shrout et al., environmental conditions can drastically impact the influence of QS. These observations and many others have led to widespread, current interest in QS and related concepts in sociomicrobiology.

The article by Chai et al. details some of the recent efforts in understanding bacterial biofilms by one of the pioneers in the use of genetic studies to dissect these communities. Working with long-time collaborator Richard Losick, these investigators have exploited one of the best-studied and most experimentally tractable bacterial developmental systems—*Bacillus*

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subtilus—to study bacterial biofilms. A few key themes arise from this work. First, these investigators highlight clear evidence of sub-population differentiation of cells within the biofilm at the molecular level. The important implication of such a finding is that there may be no single "magic bullet" to effectively eliminate all bacteria in a biofilm. These various subpopulations clearly have different properties in regard to their gene expression patterns, physiology, and functional properties and, as such, may require a diverse set of strategies to effectively control the properties. Furthermore, as also highlighted in the article by Nealson and Finkel, as well as Shrout and colleagues, there is a clear role for cell-to-cell signaling in the context of these communities, and such "chatter" is required to drive critical properties of these communities. What has not been considered extensively, and is highlighted in the work of Wilking and colleagues, in particular, is that there are several fundamental properties of biofilms that might have profound impacts on the functioning of diffusible bacterial signals. We will touch on this point again later.

Mature biofilms are complex entities par excellence. Fortunately, there is a branch of physics that deals with just that. Wilking et al. offer an insightful perspective from soft condensed matter physics, which over the last few decades has developed conceptual tools to deal with heterogeneous complex fluids—arrangements of matter that combine solid-like and liquid-like characteristics. Biofilms are essentially anisotropic colloids embedded in a cross-linked polymer gel. The polymer strands in the cross-linked gel prefer to maximize their entropy, and therefore resist mechanical deformations that constrain their motions and thereby reduce their entropy. What are the physical implications of this besides structural integrity? From the physics of cross-linked polymer gels, we know that there is an equilibrium water content for a given polymer concentration and cross-link density in the EPS matrix. Via this analogy, we can therefore see that bacteria can adjust the water content of the biofilm by remodeling the EPS matrix. The implications are far reaching. Biofilms grown in direct contact with a reservoir of water (such as those in catheters) can imbibe water freely, so high water content biofilms are formed. In contrast, biofilms in the airways of cystic fibrosis patients can imbibe water only by doing work to dehydrate the surrounding material, so water content is set by external osmotic pressure, leading to lower water content biofilms. In the case of cystic fibrosis, it is interesting to think about how a defect in CFTR (cystic fibrosis transmembrane conductance regulator), the Cl ion transporter that is the molecular cause of the disease, can guide bacteria to develop into a phenotypically distinct type of biofilm specific to this environmental context, and how this relates to various proposed therapies such as inhalation of hypertonic saline.

Biofilms are characterized by heterogeneity. Here also, soft matter physics offers insights. Spatial heterogeneity in secreted surfactants can lead to gradients in surface tension, leading to spreading forces in biofilm colonies. These effects, referred to as "Marangoni flows," can be seen in the behavior of high alcohol content liquor: If you roll Scotch whisky around in a clean glass, you will observe that the Scotch will stick to the walls and form finger-like patterns, known to connoisseurs as the "legs" of the whisky. (The longer the legs, the stiffer the drink.) This is caused by the alcohol concentration gradients that develop from evaporation and is analogous to effects from surfactant gradients recently observed in the spreading of *B. subtilis*, a system discussed in more detail by Chai et al. In fact, when viewed in this way, the strange patterns observed at the edges of spreading colonies look a bit more familiar.

In many ways, the article by Nealson and Finkel encompasses the direction in which biofilm researchers must move going forward. These authors describe the intimate interaction between bacterial cells and the surface to which they attach. In this case, the surface has two roles—as the substratum on which the biofilm forms and the material that they "breathe," that is, the ultimate electron acceptor used by these microbes to generate energy. In effect, the microbe described in this article, *Shewanella*, uses a solid metal substratum as its terminal electron acceptor in a respiratory pathway, analogous to the way humans use oxygen. Nealson's groundbreaking work exploring the physiology of microbes that grow on solid metals has opened a new world in regard to the way we think about biofilms. Nealson and Finkel discuss the important implications of electron flow both in the growth of the microbe and in harnessing this fundamental aspect of microbial physiology to generate energy in microbial fuel cells. These authors hammer home the point that studying such systems (as they do themselves in the context of a large research team) requires expertise ranging from microbial physiology and metabolism to evolutionary biology and from chemistry to fundamental engineering principles. To understand and optimize these microbial fuel cells, investigators must take into account heterogeneity in the communities (as highlighted in the articles by Chai et al. and Shrout et al.), complexities of the surface (see the article by Renner and Weibel), and considerations of biofilm properties as viewed by a physicist (as outlined in the article by Wilking et al.). Perhaps by exploiting the surface coating technologies outlined by Khoo and Grinstaff, there may be the opportunity to engineer specific community compositions and structures.

Conclusions

The six review articles in this issue suggest themes that provide the opportunity to transform the study of biofilms to a more integrated field. This list is by no means exhaustive but highlights some areas of scientific common ground.

One central theme illustrated by several articles is the concept of cell-to-cell signaling in the context of bacterial biofilms. This communication is both "autocrine," that is, talking to oneself, and "paracrine," or more simply stated, communicating directionally. In either case, such communication, as currently understood, relies on diffusible small molecules that may be both generated and sensed in the context of spatial and temporal gradients. A number of factors could impact such communication, including but not limited to, cell density, community structure, chemical properties of the biofilm matrix, and chemical

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composition and potency of the signals. All of these factors might be aspects of the endogenous features of a biofilm, but as we increase our understanding of how these communities work, will we have the ability to engineer such properties? As suggested by Wilking et al., it may be possible to manipulate core properties of biofilms, and thus modulate the diffusive properties of bacterial signals, which could potentially alter communication in these communities.

In a parallel line of inquiry, could we use our increased knowledge of surface engineering to manipulate signaling to test our models of how the underlying biological systems work in the context of densely packed biofilm communities, rather than planktonic systems that have traditionally been the workhorse of microbiologists? As an example of such interdisciplinary studies, a recent report teamed a surface chemist with a microbiologist to construct picoliter scale microcavities, so-called "lobster traps," to grow communities of predetermined populations to test theories regarding QS signaling.[12]

The article by Shrout and colleagues on QS and surface motility suggests another theme. How does motility impact the structure and function of biofilms? A subset of microbes has the ability to both attach to a substratum and move across the same substratum, invoking the need for the microbe to regulate these two behaviors. How do bacteria modify their motility mechanisms and motility decisions as the surface itself evolves, as bacteria progressively deposit various types of polysaccharides? Work along these lines has begun.[13]

Biologists have often looked to the physical sciences and engineering for new tools. Examples are legion and include techniques such as protein crystallography and single molecule manipulation. Indeed, methodological advances and cross-fertilization have great potential in the study of biofilms and have already been described in several of the articles in this issue. However, it should also be clear in this issue that tools can take the form of concepts imported from another field, and not just instrumentation.

Where do we go from here? When we say a "field of knowledge," we are using a revealing metaphor that goes at least as far back as Cicero.[14] Fields are cultivated, of course, but they are also defended and battled over. In fact, there is a long sociological history of scholars defending their fields of study against encroachments of multidisciplinary neighbors. How do we incentivize interactions across lines of disciplines in the present climate, and how do we make these interactions beneficial to the study of biofilms?

A great way to start would be to get investigators of different disciplines in the same conferences. Indeed, the editors of this special issue, a physicist and a microbiologist, first connected at the recent 2009 ASM Biofilms conference. We challenge conference organizers to look beyond the usual cast of characters invited to speak about biofilms and serve on biofilm panels—both at specialized biofilms conferences and at biofilms sessions of larger meetings. In our experience, the best collaborations are formed by people with contrasting skill sets but common interests. Cultivation of such relationships requires time and effort, but also opportunity.

Directed funding opportunities for multidisciplinary work in biofilms can have a major impact. The NSF and some smaller funding initiatives through the U.S. Air Force and Navy have been the first to do this. We hope other funding agencies will also experiment along similar lines. Having new NIH study sections composed of microbiologists as well as physical scientists will go a long way toward promoting multidisciplinary approaches in the bacterial biofilm field. In the past, biofilm research has been criticized for its tendency to investigate broad phenomenology at the expense of understanding detailed underlying mechanisms. We feel that cross-disciplinary approaches, driven by the goal to answer scientific questions large enough to justify the effort, are the key to future progress.

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