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Focus on the physics of biofilms

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Abstract

Bacteria are the smallest and most abundant form of life. They have traditionally been considered as primarily planktonic organisms, swimming or floating in a liquid medium, and this view has shaped many of the approaches to microbial processes, including for example the design of most antibiotics. However, over the last few decades it has become clear that many bacteria often adopt a sessile, surface-associated lifestyle, forming complex multicellular communities called biofilms. Bacterial biofilms are found in a vast range of environments and have major consequences on human health and industrial processes, from biofouling of surfaces to the spread of diseases. Although the study of biofilms has been biologists' territory for a long time, a multitude of phenomena in the formation and development of biofilms hinges on physical processes. We are pleased to present a collection of research papers that discuss some of the latest developments in many of the areas to which physicists can contribute a deeper understanding of biofilms, both experimentally and theoretically. The topics covered range from the influence of physical environmental parameters on cell attachment and subsequent biofilm growth, to the use of local probes and imaging techniques to investigate biofilm structure, to the development of biofilms in complex environments and the modeling of colony morphogenesis. The results presented contribute to addressing some of the major challenges in microbiology today, including the prevention of surface contamination, the optimization of biofilm disruption methods and the effectiveness of antibiotic treatments.

Introduction

Biofilms [10, 11] are complex systems in which cells organize both structurally and functionally (by differentiation), as a result of biophysical processes that remain largely unknown. An important step forward in this respect is the ability to accurately control the physical and chemical environment in which biofilms are studied, using microfluidic technology coming from physics and engineering [12]. This is exemplified in the article by Lambert *et al* [1], who studied biofilm formation in 'small habitat patches'. These are microfluidic chambers where bacteria are physically confined but have access to nutrients. Bacteria in the chambers formed non-adhering biofilms called flocs, and the microfluidic setup allowed the authors to investigate the effect of cell–cell communication, cell density and nutrient concentration on biofilm initiation. Bacteria are known to form biofilms under 'stress' conditions, for instance in the absence of essential nutrients. The results of Lambert and coworkers suggest that increased cell density, rather than nutrient depletion, triggers biofilm formation in *Escherichia coli*. The authors also succeeded in studying the competition between two fluorescently-labeled strains forming a biofilm in the same microenvironment—a case relevant to most biofilms outside the lab, which are multi-species consortia—and in imaging the *in situ* organization of biofilms by scanning confocal microscopy. This work illustrates how microfabrication techniques can be a powerful tool to investigate multiple aspects of biofilm formation and the value of coupling this microenvironmental control with imaging techniques such as confocal microscopy.

The spatial organization of biofilms continues to be a major, fascinating question for physicists, and represents a critical phenotype of biofilms determining access to nutrients, resistance to insults, and the ability to spread. Deng *et al* [2] studied biofilms of *Pseudomonas aeruginosa* grown on agar plates at the solid–air interface.

They confirmed that biofilms in this environment grow as complex fractal structures and showed that the observed swarming patterns can be described by a coarse-grained lattice model, involving short-distance expansion of colonies due to cell division and long-distance repulsion between colonies due to nutrient depletion, without explicit involvement of any biophysical parameters such as bacterial motility or surfactant concentration. This approach, directly borrowed from population ecology, reveals an intriguing analogy between bacterial colonies and groups of cells in other contexts, such as cells forming tissues or organs.

Understanding how cell adhesion depends on the physical properties of the substrate or the environment is essential to comprehending the mechanisms by which bacteria colonize surfaces. Physicists and chemists have long addressed this question, in particular by functionalizing the substrate to modify its chemical surface properties [13]. More recently, patterning techniques have allowed the fabrication of spatially heterogeneous surfaces, with properties that are controlled down to the micrometer scale. Möller *et al* [3] studied biofilm growth on chemically heterogeneous surfaces (adhesive spots embedded in a non-sticky background surface), highlighting the importance of bacterial filamentation for surface colonization. When subjected to a treatment promoting filamentation, *E. coli* cells created a confluent layer faster than their non-filamentous counterparts, and could bind patches up to 80 μm apart in less than half the time needed for the latter. A mathematical model suggested that the impact of filamentation was even more pronounced at high flow rates. Interestingly, some antibiotics promote filamentation and could thus counterproductively facilitate surface colonization. An alternative to chemical patterning is topographic patterning. Epstein *et al* [4] report a novel approach for reducing biofilm attachment based on physical rather than chemical factors: the synergy between mechanical strain and micro-wrinkled surface topography. These authors assessed the impact of physical parameters (the amplitude and timescale of the cyclic mechanical strain; the wrinkle length scale) on biofilm formation onto dynamic substrates, and identified conditions that reduced *P. aeruginosa* attachment by up to 80% after 24 h of growth. Although the efficacy was strongly dependent on the bacterial species, these results suggest new means of selective biofilm inhibition without reliance on toxic or frequently ephemeral surface chemical treatments.

The use of micropatterning techniques is all the more relevant because biofilms often grow in environments with complex geometries [14]. Porous media have for instance been the focus of much attention, in particular due to their relevance in industrial and environmental processes. For biofilms developing in a flowing environment the geometry of the flow plays an important role and can lead to the formation of filamentous biofilms called 'streamers' [15]. Using microfluidic experiments, Kim *et al* [5] discovered that *Staphylococcus aureus* rapidly forms streamers in curvy channels of different sizes and in branched network channels. When surfaces were coated with human blood plasma, streamers appeared within minutes and grew to clog the channels more rapidly than if the channels were uncoated. Using mathematical models, these authors showed how flexibility and tethering conditions of the streamers affect their orientation in curved flow fields.

The growth of a biofilm depends on multiple physical processes, including shear forces from ambient flow, nutrient transport to the colony, oxygen diffusion within the colony, and the dispersion of molecules involved in chemical communication. Researchers have developed theoretical models taking into account these different processes, which has not proven to be an easy task. The problem is all the more complicated because these chemical or physical processes can induce bacterial responses, for example affecting matrix production and thus biofilm growth and structure. Studying *Bacillus subtilis* colonies growing on agar plates, and monitoring simultaneously the biofilm size and the production of an extracellular matrix, Zhang *et al* [6] found that colonies reach a critical thickness above which matrix production is up-regulated due to nutrient deprivation. Using results from experiments on planktonic cells in liquid cultures, for which a similar response to starvation was found, and applying the conditions in the biofilm, these authors showed that the thickness of the colony at the point of starvation can be predicted by a balance between nutrient diffusion and consumption. Thus, the potential benefit that matrix production confers to the biofilm is to overcome mass transport limitations by creating an osmotic pressure that expands the colony and thus provides fresh nutrients.

Microbial communities can sometimes be used as chemical catalysts, for instance in wastewater treatment or industrial processes [16]. In another study of biofilms in a complex environment, Zhang and Klapper [7] considered biofilm-induced mineralization in a porous medium under flow, and how mineralization can trigger clogging. Considering a 'tri-phasic' system (biomaterial, solvent and calcite) and using an advection-diffusion-reaction model, these authors suggest the existence of a critical pressure drop below which clogging happens rapidly, and above which it is strongly delayed.

Finally, efficient biofilm disruption and removal requires better knowledge of the structural and rheological properties of the biofilm matrix and the transport mechanisms by which antibiotics can penetrate the colony. Particle tracking techniques are a valuable tool to gain new insights into the structure of biofilms and their local mechanical response, as illustrated in two contributions to this 'focus on' series. Combining single particle tracking, statistical analyses of bead trajectories, surface functionalization and confocal microscopy, Birjiniuk *et al* [8] found that *E. coli* forms biofilms with physical density and charge density that vary both spatially and temporally. This work also revealed inter-connecting channels that run throughout the biofilm and allow for the

passage of small molecules and micron-scale objects while limiting passage of larger objects. Zrelli *et al* [9] address the question of the impact of an antibiotic treatment on the mechanical properties of biofilms. Biofilms have shown significant multifactorial resistance to antibiotics but the role of physical factors is still poorly understood. These authors took advantage of a recently developed technique enabling the *in situ* characterization of the mechanical properties of *E. coli* biofilms by tracking the response of magnetic microparticles seeded into the biofilm. They found that several hours of antibiotic treatment at high concentration, while killing the majority of the cells, did not alter the biofilm's mechanical properties. At a time when antibiotic resistance is being recognized as one of the major societal challenges of the 21st century, this latter aspect is of particular importance, and highlights the need for a better understanding of biofilm physics at multiple levels. Physicists trained in material science, complex fluids, soft matter physics, polymer physics or statistical mechanics have begun to provide much needed experimental and theoretical insights into the different stages of biofilm formation, a welcome development that will contribute to a better understanding of these complex biological systems.

We would like to thank all the contributing authors for their submissions, and we are grateful to all the referees for their time and helpful comments during the review process. We hope that, by providing a glimpse into the richness of the physics involved in biofilm formation and the importance of understanding physical processes in biofilms, this 'focus on' series will serve to attract an even broader range of researchers from a wide range of areas in physics, to augment today's interdisciplinary efforts towards understanding and controlling the formation of biofilms.

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