PARTICLE TRACKING FOR UNDERSTANDING THE PROPERTIES AND DYNAMICS OF BACTERIAL BIOFILMS

by

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ABSTRACT

Bacterial biofilms consist of surface adherent bacteria that surround themselves with a polymer matrix which provides environmental protection and antibiotic resistance. Biofilms can grow on most implanted medical devices, on heart valves, and in the lungs of patients with cystic fibrosis, resulting in difficult to treat infections that can become blood-borne and spread throughout the body. They also pose problems in industry by growing in pipes, on membrane reactors and on ship hulls. Understanding the physical properties and dynamics of biofilms is therefore of interest as such insight may lead to methods for their disruption and removal.

Biofilms have been characterized biochemically, as the general composition of the matrix is known, as are the specific polysaccharides forming the bulk of the matrix for some species. Insight into physical properties of biofilms, such as elasticity and deformability, has been limited to macroscale techniques that assess averaged values. These techniques do not provide details on the spatial gradients of physical properties within a biofilm nor do they allow for evaluation of properties over time. In addition, while some methods have been used to understand the adhesive forces of bacteria leading to biofilm formation, little effort has been put forth to understand how bacteria that are natively non-motile can reach a surface to which they adhere. Particle tracking is a technique in which probe particles are placed in a material and observed using microscopy. The observed trajectories can be analyzed in various ways, for example to determine physical properties and structure of the material they are embedded in. Trajectories can also be analyzed to better understand motion patterns such as those of motile probes or to assess for diffusive behavior. In this work, particle tracking was used in different contexts to assess various biofilm systems. The overall goal was to gain an understanding of the structure, physical properties, and dynamics of biofilms.

We first developed a method by which we performed single particle tracking in biofilms with beads of varying surface functionalization. With a combination of single particle tracking and microrheological concepts, it was found that Escherichia coli biofilms display height dependent charge density that evolves over time. Statistical analyses of bead trajectories and confocal microscopy showed inter-connecting micron scale channels that penetrate throughout the biofilm, which may be important for nutrient transfer through the system. This methodology provides significant insight into a particular biofilm system and can be applied to many others to provide comparisons of biofilm structure. The elucidation of structure provides evidence for the
permeability of biofilms to microscale objects, and the ability of a biofilm to mature and change properties over time.

Second, we applied particle tracking to elucidate the motions of non-motile bacteria in the presence of a motile species. In static co-culture, *Pseudomonas aeruginosa* and *Staphylococcus aureus* formed multispecies biofilms at an air-liquid interface, while monocultures of *S. aureus* were not capable of forming a biofilm at the interfacial region. Based on these observations, we tested if *P. aeruginosa* could facilitate the transport of *S. aureus* to the air-liquid interface by a motility-based mechanism. Using a cell tracking method, we compared the motion behavior of *S. aureus* in the presence or absence of *P. aeruginosa*. Our data revealed a shift in *S. aureus* motility, which changed from random motion in monoculture to directed horizontal and vertical migration when cultured with *P. aeruginosa*. Additionally, we observed a similar behavior between *P. aeruginosa/S. epidermidis* and *E. coli/S. aureus* co-cultures. Our results suggest that non-motile bacteria perhaps leverage motility from other species to promote exploration of new ecological niches. We envision that this observed behavior perhaps has significant implications during the establishment and dissemination of polymicrobial infections in the host organism.

By using multiple techniques to assess trajectories of either bead or bacteria probes, we were able to improve understanding of biofilm dynamics. The first technique can be applied to other biofilm systems, such as those formed by genetically modified bacteria, to promote a comparison of biofilm structure and properties. The second can allow for further assessment of interspecies interactions, perhaps to probe the specific mechanisms by which bacteria can attach to one another to improve motility.

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