Detachment and other dynamic processes in bacterial biofilms

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Objectives

Biofilms (populations of microorganisms) accumulate on virtually all wetted industrial and environmental surfaces. Biofilm microorganisms commonly produce an extracellular polymeric slime (EPS) matrix that appears to play both protective (i.e. increasing antimicrobial resistance and resisting desiccation) and mechanical (attachment of the biofilm to the surface and maintenance of mechanical stability) roles. Biofilms are a well known concern in many types of industrial systems where they are responsible for such problems as the fouling of surfaces, product contamination and corrosion. Moreover, the formation of biofilm on domestic and industrial surfaces is also a significant problem in public health. Detachment from biofilms in food production facilities and drinking water systems may result in the potential transmission of pathogens via contaminated food[1], drinking water[2] or aerosols[3]. Microbial contamination of an industrial system is putatively identified as a biofilm problem if the contamination is, 1) chronic, and 2) difficult to control through conventional heat, mechanical, or chemical treatment procedures. These same criteria are increasingly being used in the medical field to implicate biofilm formation in many types of chronic infections of indwelling prosthetic devices and on host tissue[4]. In a medical context biofilm formation itself may be considered a significant virulence factor that enables the infection to persist or spread in the host.

Although the initial events of biofilm formation, from the attachment of planktonic cells to the formation of complex biofilm structures, are reasonably well understood very little is known about the behavior of mature biofilms. Mature biofilm behavior includes biofilm detachment and the movement of biofilms over solid surfaces. Both are potentially important mechanisms in the dissemination or transfer of contamination and infection. It is the goal of this work to relate the material properties of biofilms to various dynamic biofilm behaviors, including shear-induced detachment through adhesive and cohesive failure, spontaneous detachment, and shear induced biofilm flow over solid surfaces.

Methods

Pure (*Pseudomonas aeruginosa* and *Staphylococcus aureus*), defined mixed culture (*P. aeruginosa, P. fluorescens, Klebsiella pneumoniae, and Stenatrophomonas maltophilia*) and undefined mixed culture (tap water) biofilms were grown in square glass capillary flow cells under fluid shears ranging from 0.007 N/m^2 (Re = 17) to 5.01 N/m^2 (Re = 3,600). Biofilms were grown on various minimal salts media with either glucose (40 or 400 mg/l or succinate (1000 mg/l) as the sole carbon source. The flow cells were positioned on the stage of an upright microscope for digital time-lapse microscopic (DTLM) imaging[5]. Biofilm development was assessed by surface area coverage and thickness measurements[6] over time. Biofilms were grown from periods of between 5 and 31 days. DTLM was used to reveal dynamic biofilm behaviors over a wide range of time scales. Under steady nutrient and shear conditions the spontaneous detachment of cell clusters from the biofilm was

monitored by subtracting each image from each subsequent image to reveal which clusters had left the surface in the intervening interval. Image analysis was used to quantify the size distribution of the detaching cell clusters. Transient shear experiments were conducted to investigate the short-term influence of fluid shear on biofilm deformation and detachment. Stress-strain and creep curves were used to yield both qualitative and quantitative data on the material properties of attached biofilm[7]. Shear induced detachment of single cells and cell clusters from biofilms that had accumulated under either laminar or turbulent flow was quantified using a detachment rate coefficient.

Results and Discussion

Biofilm strength and material properties

Stress-strain and creep curves demonstrated that the various pure and mixed species biofilms had a complex rheology and behaved in a similar manner to Bingham fluids (Fig. 1). Below a yield fluid shear stress the biofilms behaved like soft viscoelastic solids but when the yield point was exceeded they behaved like viscoelastic fluids and exhibited non-recoverable strain in response to elevated shear.

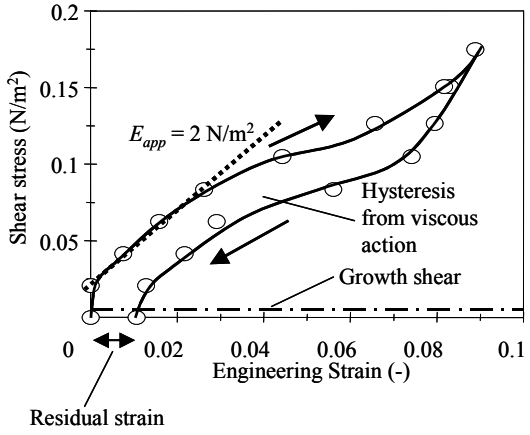

Figure 1. Characteristic stress-strain curve for attached biofilm micro-colony. Biofilm was grown from a CF isolate, *P. aeruginosa* FRD1. When strained beyond the yield point the biofilm flowed as indicated by the hysteresis and residual strain. Creep curves showed that biofilms can undergo necking and can accumulate strains of up to 20% before failure. Failure of the matrix or "cohesive failure" should be distinguished from "adhesive failure" which occurs when the whole cluster detaches from the surface. An apparent elastic (E_{app}) modulus, analogous to Youngs modulus can be determined from the linear region (dashed line).

These material properties may explain the apparent flowing of biofilms over solid surfaces that has been revealed by DTLM[8]. We have also found that aerobic and anaerobic biofilms that were developed under high shear have a higher E_{app} and a higher yield point (i.e. are stronger) than those that were grown under lower shear[9]. Although it appears that the material properties of the biofilm are largely determined by the chemistry and physical arrangement of the polymers, it is unclear if the strength of the biofilm is genetically regulated, determined through strain selection, or is controlled by the physical environment.

Spontaneous detachment of cell clusters from biofilms

DTLM has also revealed that biofilms can continually shed particulates. We have found that such particulates cover a wide size range from single cells to an aggregate with a diameter of approximately 500 μm. We estimated that such an aggregate could contain up to $1x10^5$ CFU. There was an inverse relationship between the frequency of detachment and the size of detaching particulates so that although detached particulates containing over 1000 cells made up less than 2% of total number of detached particulates they contained approximately 20% of the total number of detached cells. The size distribution of detaching particulates has not previously been quantified and the effluent is usually homogenized prior to enumeration. Although this is useful for mass balance analysis any information on the size distribution of detached particulates is lost. If, as we hypothesize, detached aggregates retain the antimicrobial resistance commonly attributed to attached

biofilms the spontaneous detachment of aggregates from biofilms may represent a significant dissemination mechanism both in industrial contamination and infection.

Shear induced detachment – cohesive and adhesive failure

In addition to the spontaneous detachment that occurs under steady nutrient and flow conditions biofilm cell clusters and attached cells can be caused to detach by increasing the fluid shear. The rate at which the clusters or cells detach is a function of the adhesive strength between the biofilm and the surface to which it is attached. By monitoring the shear induced detachment of single cells or cell clusters from the surface with time a detachment rate coefficient can be related to fluid shear stress (Fig. 2).

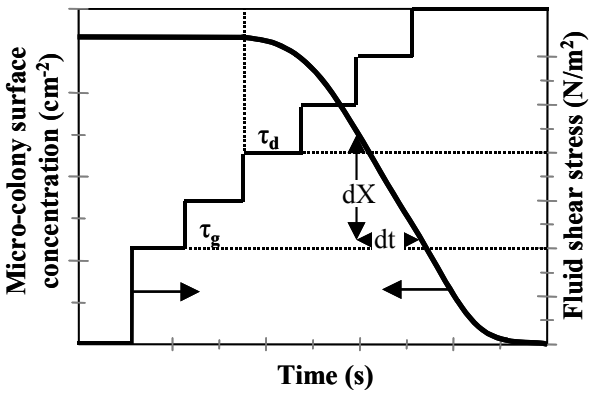

Fig. 2. Typical curve showing the shear induced detachment of cell clusters or single cells from a surface over time. τ_g is the shear at which the biofilm was initially grown. Flow was then turned off and the shear increased in a stepwise function over time. DTLM was used to determine the concentration of cell clusters on the surface of the flow cell. No detachment was observed until the shear reached a critical detachment shear τ_d.

We have found that τ_d = approximately $2\tau_g$.

A detachment rate coefficient (k_d) can be calculated for different shear stresses by determining the detachment rate (-dX/dt) at each shear increment. As expected k_d increases as a function of transient increase in shear and as a function of the shear at which the biofilm was grown. Taken together with the stress-strain data it appears that the shear at which biofilms are grown not only influences the cohesive strength but also the adhesive strength of the biofilm.

Conclusions

Biofilms are physically dynamic over a wide range of time scales from the rapid oscillation (>10 Hz) of filamentous streamers to the slow migration of biofilm structures over surfaces (μm/h). The material properties of attached biofilms remain largely uncharacterized and yet will be required for us to fully understand various biofilm behaviors as they are revealed through techniques such as DTLM. Detachment of biofilm aggregates and the motion of biofilms across surfaces both represent dissemination mechanisms of which very little is known. In part this is due to our view from the laboratory in which biofilms are usually grown from the seeding of clean sterile surfaces with single planktonically derived cells. Understanding the material properties of biofilms will also give us insight into the form and function of biofilms and help us understand how biofilms may adapt to survive in different physical environments.

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