

ABSTRACT

Zhang, Wen. Ph.D., Purdue University, August 2011. Characterization of Passive and Active Biofilm Detachment. Major Professor: M. Katherine Banks.

Biofilm is a ubiquitous aggregate of microorganisms attached to a surface which exists in nature and specialized industrial processes. When biofilm detaches from a surface, the effect is often detrimental and can result in pipe obstruction (industrial applications) or infection (biomedical implants). Our understanding of biofilm detachment is not as complete as biofilm development due to difficulties in detachment characterization and oversimplification of the biofilm removal process. In this study, a novel detachment theory is proposed, namely, the differentiation of passive and active biofilm detachment. Passive detachment describes biofilm removal resulting from external forces such as fluid shear stress and abrasion. Active detachment refers to removal due to internal changes caused by stressors such as oxygen depletion, nutrient starvation, and chemical toxicity. Three removal mechanisms were identified for *Pseudomonas aeruginosa* biofilms: fluid shear stress resulting in passive detachment; active detachment due to glutathione-gated potassium efflux (GGKE); and active detachment caused by cell death.

Confocal laser scanning microscopy was used to assess biofilm viability and in-depth composition with membrane-integrity stains. A Multisizer Coulter Counter allowed for quantification of detached particles and differentiation of cell fragments and polymers, single cells, and aggregates. Results showed that increasing fluid shear stress enhanced passive biofilm detachment without a change in cell viability. GGKE induced by N-ethylmaleimide (NEM) caused active detachment without significant viability change due to EPS structural destruction (potassium release). Cell death induced by tobramycin resulted in active biofilm detachment, with cell fragments and polymer detachment present as a result of cell lysis. Active biofilm detachment also occurred when GGKE and cell death were simultaneously induced by cadmium, although detachment did not increase cumulatively. Induction of GGKE impacted the size of the detached particles more than cell death, resulting in enhanced single cell detachment. Fluid shear produced more cumulative biofilm detachment after exposure to toxins than without exposure, indicating that the structural integrity of the biofilm was compromised. Detachment mechanisms also were observed with immature biofilms. High levels of cell fragments and polymer detachment in immature biofilms indicates an enhanced production of EPS during early stages of biofilm development.