How do the mechanics and three-dimensional structures of bacterial biofilms impinge on their biology?

Biofilms, which are communities of interacting microbes embedded in a polymeric matrix, take a large and increasing toll on health, civic infrastructure, and industry. Biofilms resist many of the treatments that are successful against free-swimming, "planktonic" bacteria, and this resistance arises largely from the multicellular structure of biofilms. Here we characterize the mechanics and the three-dimensional structure of biofilms grown from *Pseudomonas aeruginosa*, an opportunistic human pathogen. *P. aeruginosa* produces chronic, and ultimately fatal, biofilm infections in the lungs of patients with cystic fibrosis. Here, we show that evolutionary changes in the polymer production by different infecting sub-populations can result in softening and weakening of the biofilm if alginate production increases, but if production of a second polymer, "Psl," also increases, the biofilm's stiffness and strength is maintained. This suggests a mechanical fitness benefit to increasing Psl production. Psl promotes clumping of bacteria into multicellular aggregates, which are widely found in natural settings. However, the impact of pre-existing multicellular aggregates on biofilm growth and development is not known. Here, we use a combination of experiment and simulation to show that the three-dimensional structure of an aggregate can give a growth advantage to the aggregate if the level of competition for growth resources is high. This suggests that the progeny of pre-existing aggregates could dominate biofilms and provides one selection mechanism by which this form of multicellularity might be evolutionarily maintained.