Imaging of Biofilms: Spatial distribution

An alternative view of the 3D heterogeneous mosaic structure of mono and polymicrobial species biofilms

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Scanning electron microscopy created a dogma lasting 25 years that biofilms were flat, confluent films. Subsequent developments in scanning confocal laser microscopy (SCLM) and use of fluorescent stains revealed that biofilms have a complex 3D architecture. The majority of early work studied the "biofilm type organism", *Pseudomonas aeruginosa*, which ironically also helped create a dogma that all biofilms have a mushroom structure. Parallel to use of SCLM has been the invention and use of novel light microscopy techniques such as episcopic differential interference contrast (EDIC) microscopy which does not require use of cover slips nor oil immersion or water lenses for high magnification imaging; consequently it can visualise highly complex, convoluted surfaces, biotic or abiotic, in real time without the use of fluorescent stains. The technique can also be coupled with epifluorescence (EF) microscopy for combined imaging to facilitate identification of individual species using antibody or rRNA fluorescence *in situ* hybridisation (FISH), or vital staining for membrane integrity (live/dead), protonmotive force generation and other ion gradient studies. EDIC microscopy has shown that the majority of environmental and clinical biofilms studied are more complex than the simple mushroom model where some assume that the mushroom is a microcolony. Instead, monospecies or polymicrobial biofilms consist of a thin basal layer of cells on the substratum decorated with stacks or columns containing many microcolonies aggregating together and separated by water channels. Video imaging shows that these are predated by eukaryotic grazers in polymicrobial communities where amoebae graze the basal layers and rotifers and higher species graze the upper stacks of microcolonies. Exopolysaccharides and eDNA which help consolidate fusion of the microcolonies are also clearly seen and provide elasticity in shear flow regimes. Anaerobic reporter constructs of *Escherichia coli* show that heterotrophic metabolism of the stacks of microcolonies in drinking water systems creates anaerobic micro-zones which facilitate the colonisation of facultative and anaerobic species, including *Legionella pneumophila*, *Helicobacter pylori* and *Campylobacter jejuni*. Moreover, the biofilm represents a complex physico-chemical mosaic whose footprint creates electrochemical gradients which drive pitting corrosion processes on metal surfaces. Current studies using the direct viable count (cell elongation) technique are describing the presence of viable but nonculturable (VBNC) species within the biofilm which is beginning to shed new light on biofilm physiology and community interactions.

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