The Evolution of the Biofilm Concept: A Long and Winding Road

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"There is a tide in the affairs of men which, taken at the flood, leads on the fortune. Omitted, all the voyage of their lives is bound in shallows and in miseries."

William Shakespeare

The Origins

In a recent newspaper article in Boston, Roberto Kolter recounted to a breathless reporter how he had discovered biofilms by watching a cloudy film develop on the front glass plate of his tropical aquarium. But several decades before Roberto had gotten depressed, and found much too much time on his hands, dentists had surveyed plaque in people's mouths and sanitary engineers had carefully followed the accretion of slimy films on surfaces exposed to wastewater. These intrepid pioneers had taken the plaque or the slime, placed it under simple microscopes, and found that they were completely composed of bacterial cells, separated by very large amounts of amorphous matrix material that dampened Brownian movement. If we extend the use of our senses from the visual to the tactile, we can feel slippery slime on rocks in streams, and the mobilization of our olfactory senses allows us to detect anaerobic bacteria in the dark brown rings that develop at the air-water interface in neglected toilets. Biofilms are all around us, and the first descriptions of the bacterial communities that form on surfaces exposed to sea water date back to 1933 (Henrici) and 1935 (Zobell and Allen). Sea water provides endless fascination to the microscopist, because floating or swimming

(planktonic) cells settle on surfaces to form multi-species of considerable complexity, and Zobell described a “bottle effect” that removes 99 % of planktonic cells from suspension if the sample is simply held in a vessel for one hour.

The Medical Apostasy

While Henrici and Zobell were pondering the ways of sea-going bacteria, their much more numerous medical colleagues were stamping out the last of the great epidemic bacterial diseases, using vaccines developed against pathogens grown in culture. During the Great War the armamentarium of Medical Microbiology was expanded by the addition of antibiotics, also developed against bacteria grown in culture, and Koch’s Postulates deservedly reigned supreme (Grimes, 2006) because they had provided the intellectual rationale for the conquest of acute bacterial disease. After the confetti from the victory parade was swept up, a number of niggling questions emerged. Why did children with cystic fibrosis die of pulmonary infections, when the infecting organism (*Pseudomonas aeruginosa*) was not really a recognized pathogen, and showed exquisite sensitivity to antibiotics when cultured? Why did bacterial infections associated with orthopaedic prostheses fail to respond to conventional antibiotic therapy, when the bacteria that caused them showed sensitivity when tested in culture? Why did the physical removal of the prosthetic hardware allow resolution of the infection? After victory over bacterial diseases was announced, nagging problems remained unexplained, and it became obvious that a piece was missing from the puzzle.

The Reductionist Apostasy

Single species cultures of planktonic bacteria growing in defined media provided Microbiologists with the prokaryotic equivalent of the ubiquitous fruit fly. If your interest lay in the Byzantine complexities of the nucleic acids that comprise the ribosome, or in

the 14th elongation factor that accomplishes protein synthesis, you needed a consistent source of fast-growing bacterial cells and the liquid culture was your “oyster”. So reductionist Microbiologists could join their “molecular” counterparts in other fields of Biology in discussing, with due credit to the theologians of the University of Paris, how many proteins could dance on the head of a strand of 16 S rRNA. But doubts began to emerge, even in these arcane circles, when it was noted that the lab-evolved K 12 strains of *E. coli* had, in the course of hundreds of transfers, lost 32.5 % of the genome that had allowed them to function as wild strains in the real world (Fux et al., 2005). Reductionist science has dominated the second half of the 21st century, and filled our universities with biologists who have never even seen their chosen subjects in the ecosystems in which they function. The operative assumption has been that their laboratory models mimic natural systems, and the revelation that cells in the biofilms that predominate in nature express phenotypes that differ from those of their planktonic counterparts by as much as 70 % (Sauer et al., 2002) shakes this assumption to its foundations.

The Stage is Set

The stark simplicity of the approach that developed in Microbial Ecology, in the 1960s and 1970s, belied its significance. Robust young men like Gill Geesey and Gordon McFeters would shoulder their packs and trot in to mountain lakes, Staffan Kjelleberg and Kevin Marshall would clamber over the seashore, and we would simply determine the number and location of the bacteria in aquatic ecosystems. How many cells, and where were they located? Our first conclusion was that cultures were useless, because less than 1% (Colwell and Huq, 2001) of the species present in these ecosystems would grow on the media we had, so we unlimbered our microscopes. The conventional light microscope was limited by the fact that we have to study optically tractable

surfaces, but we could recover bacteria from the bulk fluid (filtration) and from surfaces (scraping), and stain them with acridine orange. We could confirm the presence of very large numbers of bacteria on surfaces, by scanning electron microscopy (SEM), and examine their adhesive mechanisms (Costerton et al., 1978) by transmission electron microscopy (TEM) of ruthenium red-stained preparations (Figure 1).

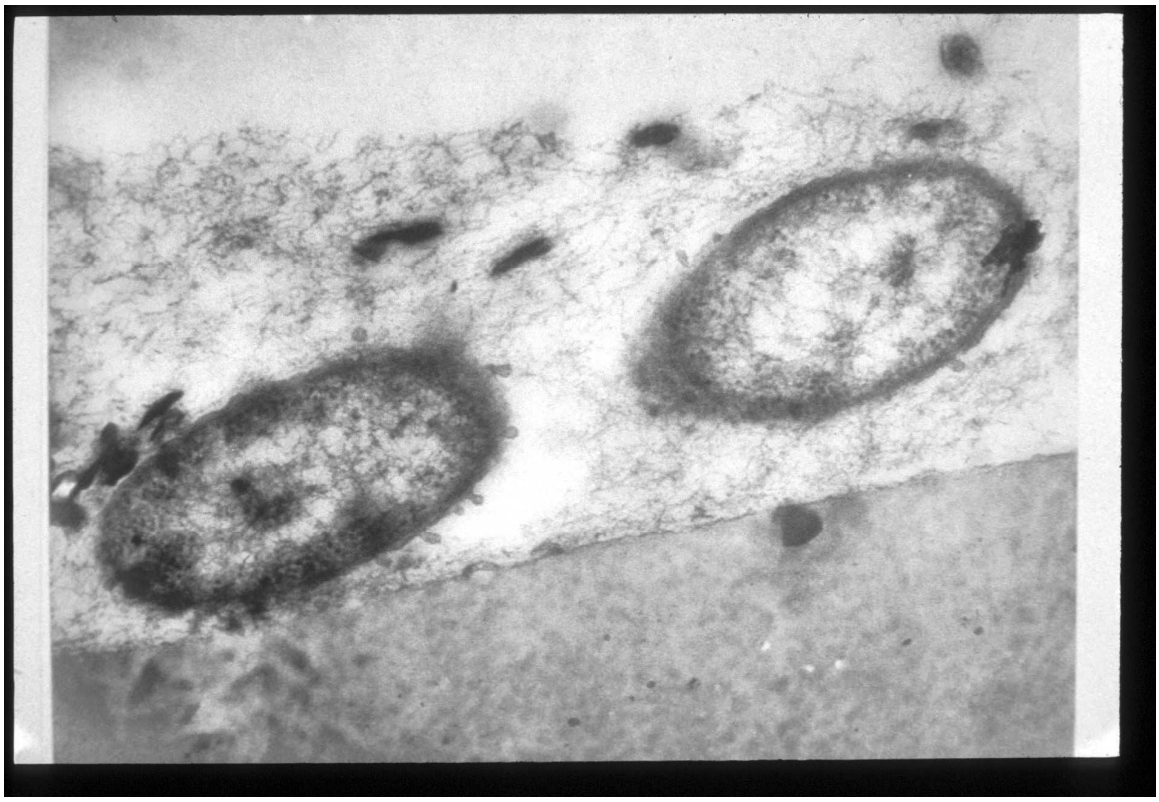

Figure 1: Transmission Electron Micrograph (TEM) of a ruthenium red stained section of a biofilm formed on the surface of a methacrylate disc placed in a mountain stream for 20 minutes. Note the cross sections of two gram-negative bacterial cells, the fibrous matrix material that binds these cells to each other and to the surface, and the electron dense clay platelets trapped in the matrix material.

Soon the verdict was in: the vast majority of bacteria (> 99.9%) in aquatic ecosystems grow in matrix-enclosed biofilms on all available surfaces. The realization that sessile communities predominate in microbial ecosystems, and that the morphological complexity of these communities rivals that of eukaryotic tissues, prompted us to use

the well established technique of confocal microscopy (Lawrence et al., 1991). This microscope uses a laser beam that scans surfaces, without respect to their opacity, and requires neither fixation nor dehydration, and the rest is (as they say) history (Figure 2).

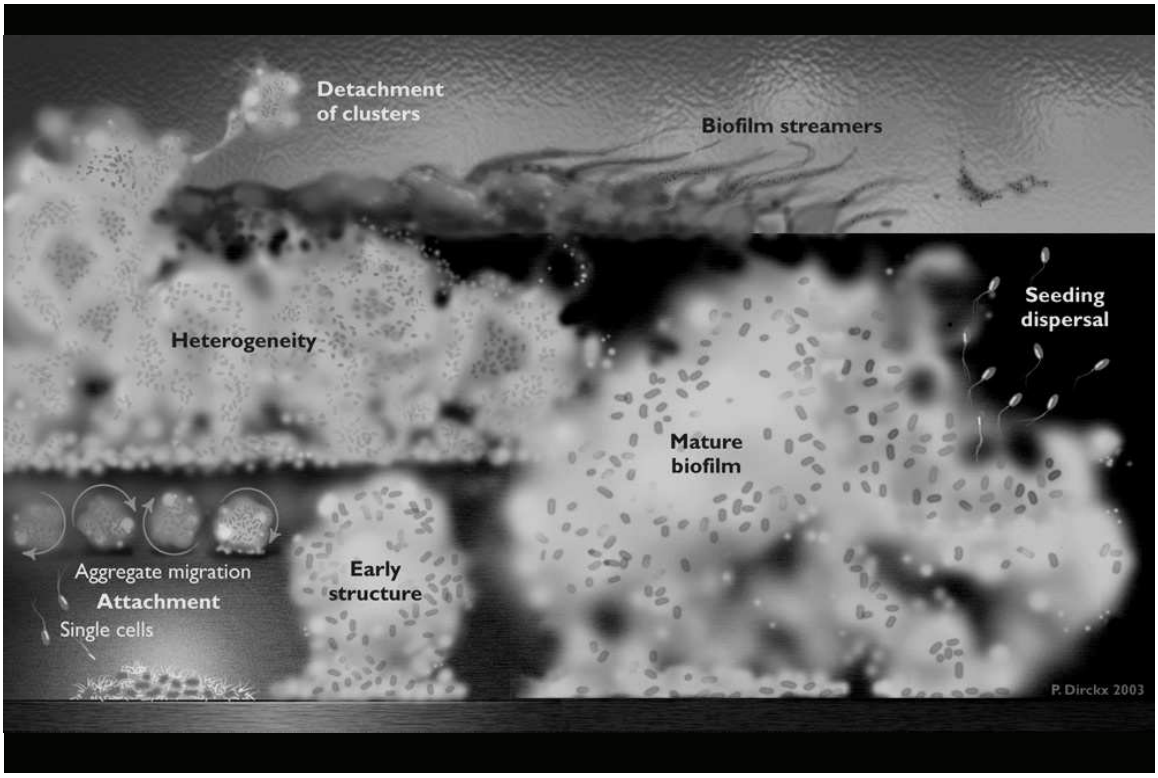

Figure 2: Diagrammatic representation of the structure of a biofilm, based on images obtained by confocal scanning laser microscopy (CSLM). Note the formation of a complex biofilm with water channels and detachment zones, in which bacteria comprise +/- 15 % of the mass of the community, and in which cells are distributed in a highly-ordered species-specific pattern. Multi-species communities show metabolically cooperative zones, and the biofilms are dynamic in terms of movement and detachment.

Biofilms are Phenotypically Distinct, Communities

The first road block in the acceptance of the predominance of biofilms in aquatic system came in the form of a suggestion that these sessile populations were simply an accumulation of dead cells, and that the planktonic cells still represented the vital population. This was disposed of quickly when we showed that virtually all sessile cells

in biofilms are alive and functional, and when we showed that the sediments and other surfaces in rivers carry out > 99% of organic transformations in natural ecosystems (Wyndham and Costerton, 1981). Our tactful suggestions that planktonic bacterial cells are a minor population in nature, and a *de facto* laboratory artefact that has monopolized the attention of Microbiologists for far too long, was then countered by the assertion that biofilm cells are identical with planktonic cells except that they are piled up on surfaces. Inspired by the well-established observation that cells in biofilms are resistant to antibiotics at levels hundreds of times higher than those that kill their planktonic counterparts, we then began to examine protein production/gene expression in biofilms versus planktonic cultures. Both 2 D gel studies of protein production, and array-based studies of gene expression, have shown very large differences in the biofilm and planktonic phenotypes of all species examined to date, and Karin Sauer et al. have recently shown that the biofilm phenotype varies with the age of the community (Southey-Pillig et al., 2005). If the biofilm phenotype differs from the planktonic phenotype, in terms of more genes than are necessary for spore formation, we must conclude that sessile cells differ from their planktonic counterparts in many respects more profound than simple resistance to antibiotics.

Biofilms are Complex Integrated Communities

When Paul Stoodley and Dirk deBeer spent long hours in the lab with single species biofilms growing on surfaces in flow cells, revelations popped out at an amazing rate. Biofilms were not amorphous accretions of cells embedded in a slimy matrix, but they were composed of an architecturally distinct array of “towers” and “mushrooms” interspersed with open water channels. Water from the bulk fluid was entrained into the network of water channels, to set up a convective flow pattern (Stoodley et al., 1994), and this flow carried nutrients (including oxygen) to the tower-like microcolonies, in

which cells were distributed in a species specific pattern (Figure 2). We detected oxygen limitations in the centres of microcolonies, using microelectrodes, and we noted that these anaerobic centres of the towers and mushrooms often hollowed out when the microcolony reached a certain critical size. All of our thought processes slowly distilled into questions: how do water channels remain open, when random growth by adjacent microcolonies would close them? How do the bacterial cells that find themselves in the anaerobic centres of microcolonies revert from the biofilm to the planktonic phenotype and swim away? Even more tentatively, we began to consider the heretical notion that bacteria in biofilms have developed the ability to communicate by means of cell-cell signals, and we enlisted the help of Peter Greenberg of quorum sensing fame. Our subsequent discovery (Davies et al., 1998) that biofilm architecture, and even the process of biofilm formation itself, are controlled by several systems of chemical signals established the fact that biofilms are integrated communities within which individual bacterial cells can communicate with each other. Others have discovered that cells within biofilms can also transmit electrical signals, via nanowires (Gorby et al., 2006), that certainly constitute a method of power sharing and possibly represent yet another means of communication. These totally unexpected and highly sophisticated communications between sessile cells, perhaps ironically, may present the most practical amongst us with opportunities to control biofilm processes, including chronic biofilm diseases, by interfering with these communications. Plans are afoot to block chemical signalling by specific inhibitors, and electrical signalling by voltage clamps, and the sophistication of their communications may prove to be their Achilles' heel.

The Arrested Development of Microbiology

The logical development of Microbiology, as a modern science, is recovering from the Medical Apostasy and from the Reductionist apostasy, and many of us now study

bacteria *in situ* within the communities of which they are integral members. We now realize that mutations that affect the performance of a species as a member of an integrated community may be just as important as mutations that affect the survival of planktonic cells of the same species. We realize that communications within a biofilm community may allow it to respond to a stress (e.g. beta lactam antibiotic) applied to one location in the community, by initiating changes (e.g. beta lactamase production) throughout the community. Biofilms can now be represented as multi-cellular communities that have a primitive circulatory system, a degree of cell specialization, and an unexpectedly sophisticated communication system. Four decades have brought many changes in the way Microbiologists conceive of the organisms that we study: These are not your Grandfather's bacteria!

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