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The polymicrobial nature of biofilm infection

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Abstract

The model of biofilm infection was first proposed over a decade ago. Recent scientific advances have added much to our understanding of biofilms, usually polymicrobial communities, which are commonly associated with chronic infection. Metagenomics has demonstrated that bacteria pursuing a biofilm strategy possess many mechanisms for encouraging diversity. By including multiple bacterial and/or fungal species in a single community, biofilms obtain numerous advantages, such as passive resistance, metabolic cooperation, byproduct influence, quorum sensing systems, an enlarged gene pool with more efficient DNA sharing, and many other synergies, which give them a competitive advantage. Routine clinical cultures are ill-suited for evaluating polymicrobial infections. DNA methods utilizing PCR methods, PCR/mass spectroscopy and sequencing have demonstrated their ability to identify microorganisms and quantitate their contribution to biofilms in clinical infections. A more robust model of biofilm infection along with more accurate diagnosis is rapidly translating into improved clinical outcomes.

Keywords: Biofilm, co-aggregation, horizontal gene transfer, metabolic cooperation, passive resistance, PCR, polymicrobial, sequencing, synergies

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Introduction

It has been over a decade since Costerton and Stewart [1] proposed a simple model of biofilm infection.The evolving biofilm infection paradigm was a significant departure from the then widely held view of infection, which envisioned single-species bacteria in a planktonic mode of growth utilizing virulence factors to cause infection [2,3]. This planktonic view of infection could explain most acute infections, but was wholly inadequate for understanding chronic infections. However, Costerton and Stewart's early innovative biofilm model of infection demonstrated, at the biochemical and cellular levels, a new bacterial strategy by which communities of bacteria produce infection.

Their biofilm model of infection explained the ineffectiveness of antibodies [4] and white blood cells [5] in combating biofilms. Other work showed the ineffectiveness of antibiotics for clearing a biofilm infection [6]. The final thread was the biofilm's ability to induce host hyperinflammation, as shown by elevated levels of proinflammatory cytokines [7] and matrix metalloproteases [8], and excessive numbers of neutrophils [9].

Over the last decade, many new studies utilizing an emerging and sophisticated science have generated a wealth of fresh insights into the nature of biofilm infection. It is hoped that, through weaving of these different threads of new information into the original biofilm model of infection, a robust tapestry will emerge that will allow for more focused and productive research going forward.

Metagenome

Kim [10], in a recent review of the molecular pathways that bacteria utilize for producing host infection, found that these molecular pathways could be grouped into two different types. One group of mechanisms was clearly for breaching

the host tissue, producing cell death and necrosis for bacterial nutrition. The other group comprised molecular mechanisms by which bacteria could attach to host cells, and inject small effector proteins that commandeered host cellular pathways to reorganize the cellular cytoskeleton [11,12], prevent migration [13], prevent mitosis [14] and, most importantly, inhibit apoptosis [15–18]. This 'new' model of infection encompasses the molecular strategies employed by biofilm phenotype bacteria.

Bacteria pursuing a biofilm strategy for infection have molecular mechanisms for recruiting other bacteria. It seems that biofilms actively attempt to become polymicrobial, apparently to improve their survivability. There has been a shift in microbiology into thinking of biofilms as systems with global regulation of the expanded gene pool provided by species diversity [19]. This new understanding suggests that a biofilm is a single entity that exerts central control over the individual members to yield the activities necessary for the colony's survival. Biofilms require gene expression that allows for attachment to the host, produces host cellular senescence to prevent shedding, and causes local inflammation that creates plasma exudate for sustained colony nutrition [20]. Microorganisms may combine their genetic resources to fulfil these requirements, so that each individual member of a biofilm need not possess all of the genes necessary to carry out each function. This has led to the proposal of functional equivalent pathogroups, which are frequently identified recurring groups of microorganisms in biofilm infections [21].

It has been demonstrated that, in *Streptococcus pneumoniae*, individual members of the community possess only a proportion of all the genes present within the culture, and this has led to the distributive genome hypothesis [3,22]. Sharing the total genes of the species (supragenome) allows each member to expend less energy in maintaining its proportion of the total gene pool, while allowing the entire community to have all of the genes present [23]. Application of this principle to polymicrobial biofilms has led to the suggestion that the genomic plurality leads to the continuous production of novel strains, fostering a persistent infection [24]. The main molecular method by which genomic plurality is accomplished within the biofilm is upregulated and highly efficient horizontal gene transfer [25,26].

In clearly defined spatial locations within the biofilm, horizontal gene transfer is optimized by quorum sensing systems, and is usually much more efficient than the planktonic phenotype. Horizontal gene transfer, much more than vertical gene transfer from the parent cell to the offspring, has been reported to be the main mechanism for distributing genes within prokaryotic bacteria [27,28]. The close spatial arrangement of the community, along with quorum sensing, allows for more efficient DNA transfer among the members, mainly through conjugation, occasionally through transformation, and rarely through bacteriophage-mediated transduction [28]. Horizontal gene transfer is more efficient in permissive regions of the biofilm and severely limited in regions of low/no growth, owing to the accumulation of metabolic byproducts [29].

It has long been known that species diversity in ecological systems provides greater ability to withstand various stresses; this is known as the 'insurance hypothesis' [30]. Boles's group [31] took this one step further, showing that *Pseudomonas aeruginosa*, through a *rec*A-dependent mechanism, self-diversifies its gene pool to become more recalcitrant in an infection. So, whether by functional equivalence, a distributive genome, self-diversification, or other methods, biofilms seek to expand their genetic diversity in order to 'insure' survival.

Synergies

Biofilm communities in most environments, including human disease, tend to be polymicrobial [32]. By including multiple bacterial and/or fungal species in a single community, biofilms obtain numerous advantages, such as passive resistance [33], metabolic cooperation [34,35], byproduct influence [36], quorum sensing systems [34], an enlarged gene pool with more efficient DNA sharing [37], and many other synergies, which give them a competitive advantage. It is best to view a biofilm as a single entity possessing multiple genetic resources that allow it to adapt and thrive regardless of the stresses that it encounters. In general, the greater the diversity, i.e. the larger the gene pool, the more robust the biofilm is in terms of its survivability [38].

Individual bacteria possess multiple molecular mechanisms to actively co-aggregate with other beneficial species. Co-aggregation mechanisms are usually reversible molecular bonds that allow the genetically distinct bacteria to select for beneficial partners within the biofilm [39]. Co-localization is a similar concept, but carries the connotation of being a more passive process. In co-localization, a species of bacteria will encourage the local growth of a beneficial partner by providing benefits for its growth rather than by utilizing physical bonds.

Unique species of microorganisms that have the ability to form biofilms usually possess species-specific quorum sensing molecules to direct the organization of the monoclonal biofilm. For polymicrobial biofilms, there are some quorum sensing molecules that can upregulate pathways in multiple