Cardiovascular Device Infections and the Role of Biofilm

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Summary:

In the presence of a foreign body (e.g., prosthetic heart valve, ventricular assist device driveline, pacemaker), the microorganism inoculum necessary to initiate abscess formation is 10,000-fold less than in the absence of a foreign body, largely due to the advantages afforded by development of biofilm. A biofilm is a multicellular consortium of microbial cells that is irreversibly associated with a surface and enclosed in a self-produced extracellular matrix composed primarily of polysaccharides, and allows for survival in hostile environments. The advent of the electron microscope allowed detailed analysis of surface biofilms, first in environmental (e.g., rocks in a stream) and industrial (e.g., water pipes) arenas, and later on medical devices. Biofilms also grow on native tissue surfaces, examples being dental plaque and *P. aeruginosa* biofilms forming in the airways of patients with cystic fibrosis. The common organisms responsible for medical device infections both inside and outside the cardiovascular system are ones that are proficient in biofilm formation, including bacteria (e.g., *P. aeruginosa, S. epidermidis, S. mitis, S. aureus, E. coli*) and fungi (e.g., *Candida, Aspergillus*).

Organisms within a biofilm behave in a fundamentally different manner than their free-floating (planktonic) counterparts. When a planktonic organism attaches to a surface and begins to form a biofilm, there is a significant change in gene and protein expression that allows for increased survival of the complex biofilm community. Gene expression profiling and proteomic techniques, common research tools in the study of cancer and other human diseases, have been used to study differences between organisms in a biofilm and planktonic organisms under a variety of different environmental conditions and as a function of time over the development and growth of the biofilm. Therefore, many current definitions of biofilm include the provision that the organisms exhibit an altered phenotype with respect to growth rate, metabolism and gene transcription, adding functional characteristics to the physical ones described above.

The formation of a biofilm can be broken down into several processes. First, a conditioning film is deposited onto the material surface, providing an initial substrate for microbial adhesion. This conditioning film is formed by host molecules such as fibronectin, vitronectin, fibrinogen and other proteins, glycoproteins, proteoglycans, polysaccharides, lipids, and inorganic ions. This is essentially what the microorganisms "see" as they approach the surface, and allows for their initial attachment. Secondly, the organisms bind *reversibly* to the surface, with initial interactions being nonspecific via electrostatic, van der Waals and hydrophobic interactions. Some organisms have specific moieties that allow for binding to the human components of the conditioning film. Third,

when the appropriate environment is sensed (via quorum sensing), organisms undergo a fundamental switch in gene expression to allow for production of extracellular polymeric substances (EPS) and for irreversible attachment to the surface. Quorum sensing, mediated by small diffusible signaling molecules, allows microbes to sense population density and couple these data to gene expression and therefore behavior. The perception of a threshold concentration of the signaling molecule indicates that the population density is sufficient to make a behavioral decision, such as to form a biofilm. EPS is composed of polysaccharides, proteins and nucleic acids that help the organisms adhere to the surface, and forms the basis for some of the protective effects of biofilms. The EPS matrix plays a major role in the ability of the biofilm to evade both the host immune system and the therapeutics used to treat device-associated infections. Fourth, the biofilm grows and matures into a complex three-dimensional structure of pillars and channels to allow for enhanced mass transport from the fluid phase. Finally, some organisms within the biofilm revert to the planktonic gene expression profile and disperse into the surrounding environment, potentially to begin the cycle again in a new location.

Organisms in biofilm use several mechanisms to evade the host defense system. The EPS matrix impedes the penetration of opsonizing antibodies so they do not reach the underlying organisms, making uptake and killing by phagocytes less efficient. The EPS matrix also reduces the phagocytic ability of macrophages and polymorphonuclear leukocytes both on material surfaces and even after organisms have been released from the biofilm, promoting sepsis. There is also evidence that the EPS matrix may confer resistance to reactive oxygen species produced by the phagocytes. Biofilm also hampers the efficacy of antibiotics in the treatment of material-associated infections. Organisms in a mature biofilm divide at a slower rate, making them less susceptible to certain antibiotics. In addition, the organisms in biofilms tend to be organized within the EPS matrix rather than on its surface, making them less accessible to therapeutic antibiotics. Antibiotic concentrations between 1,000 and 15,000 times greater are needed to kill biofilm-associated organisms than their planktonic counterparts as a result of the physicochemical properties of the matrix and slower diffusion rates and delayed penetration within the EPS matrix. The difficulty in eradicating organisms from a biofilm often necessitates prolonged periods of antibiotic use, furthering the development of resistant organisms. Bacteria within a biofilm can become resistant to certain antibiotics through the accelerated acquisition of resistance plasmids (extrachromosomal DNA) through the process of conjugation, which occurs with greater ease within the physically protective environment of the biofilm. Biofilm-associated organisms also express different cell wall proteins, furthering resistance to antibiotics. Finally, the presence of persister cells may allow the biofilm to regrow, even after essentially all of the susceptible organisms have been killed.

The elucidation of the mechanisms of biofilm formation and quorum sensing, and the realization that surface-associated organisms are behaving in a fundamentally different manner than their planktonic counterparts is advancing the understanding of material-associated infections and providing insight into potential therapeutic targets. For example, one could interfere with production or sensing of the diffusible signal molecules, or

enhance the degradation of these molecules in the environment around a material. In fact, some species of bacteria are able to interfere with the quorum sensing mechanisms of other species through the production of signal molecule degrading enzymes (e.g., HSL hydrolase) to give themselves a competitive advantage, a process that is termed quorum quenching. Material-based strategies to prevent infection are also being investigated, such as materials with better tissue integrative properties to provide fewer free surfaces for organisms to attach, materials that limit the ability of organisms to adhere to their surfaces, and materials that release antibiotics in a controlled manner.

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