Bacterial Biofilms: a review of current research

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Résumé • Summary

Les biofilms protègent les cellules bactériennes contre les antibiotiques et les mécanismes de défense du receveur. Afin de comprendre comment contrôler les biofilms, il est important de connaître la complexité de son fonctionnement. Ceci est une vue d'ensemble de quatre domaines de la recherche actuelle sur le biofilm : la résistance du biofilm aux antimicrobiens et aux mécanismes de défense, la complexité de la structure du biofilm, l'existence possible d'un phénotype du biofilm et les ramifications de communication cellule – cellule à l'intérieur du biofilm.

Mots clés : Biofilm – Résistance – Mécanismes de défense.

Biofilms provide bacterial cells with a protective environment that allows for survival from antibiotics and host defense mechanisms. In order to understand how to control biofilms, it is important to understand the complexity of the biofilm system. This is in overview of four areas of current biofilm research: biofilm resistance to antimicrobials and host defense mechanisms, the complexity of biofilm structure, the possible existence of a biofilm phenotype, and the ramifications of cell cell communication within the biofilm.

Key words: Biofilm – Resistance – Host defense mechanisms.

Introduction

Biofilms are the preferred mode of growth for most bacteria. Existence within a biofilm provides bacteria with a protective environment that effectively prevents attack by antimicrobials, biocides, and host defense mechanisms. It is just these biofilm survival strategies that make treatment difficult and erradication almost impossible. Biofilms play a role in the infection and failure of medical implants[1] and in diseases such as dental caries, otitis media, osteomyelitis, endocarditis and cystic fibrosis.[2]

Research into biofilm formation began in the early 1970's based on the assumptions that biofilms were relatively simple systems of homogeneous slime composed of bacteria physiologically indistinct from their planktonic or free-floating counterparts. Improvements in technology have allowed biofilm scientists to determine the complex nature of the biofilm system.[2,3] This presentation is an overview of current biofilm research emphazing four topic areas: biofilm resistance to antimicrobials and host defense mechanisms, the complexity of biofilm structure, the possible existence of a biofilm phenotype, and the ramifications of cell cell communication within the biofilm.

Recalcitrance of biofilm bacteria

The development of a biofilm is a very effective survival strategy for bacteria. In fact, the potential problem of device-related infections such as pacemakers, hemodialysis catheters and heart valves points out just how efficiently bacteria can survive within the biofilm matrix.[2] Electron micrographs of the surfaces of medical devices often show thick layers of bacterial cells embedded in a polysaccharide matrix.[4] Studies have shown biofilm cells can exhibit an increased resistance to biocides, antimicrobials, and host defense mechanisms in comparison to planktonic cells.[2,5] A number of hypotheses have been put forth to attempt to explain this phenomenon. In some cases, there can be a limitation to the penetration of the antimicrobial into the biofilm due to the physical barrier of the exopolysaccharide matrix.[6] In addition, the presence of microenvironments within the biofilm causes the bacterial cells to exist at different physiologic states[3] thereby affecting the rate of uptake of the antimicrobial into the cell.[7] These differences in bacterial cell physiology within the biofilm will reduce the susceptibility of cells to some antimicrobials such as growth-dependent antibiotics.[8] However, diffusion and physiologic differences alone may not account for the entire decrease in susceptibility to antimicrobials seen in biofilm cells. This suggests that additional changes in the planktonic bacterial cell during the process of becoming a biofilm cell may somehow also affect its susceptibility to various antimicrobials.

Host defense mechanisms can rarely resolve a biofilm-related infection.[2] During the formation of a biofilm, attached bacterial cells release antigens thereby stimulating the production of antibodies. Phagocytes, attracted to the biofilm will release phagocytic enzymes in response. Unfortunately, these enzymes will not completely destroy the biofilm, but can instead damage surrounding healthy host tissue.[9] In addition, some planktonic bacteria may be released from the biofilm matrix, resulting in the dissemination of bacteria.[10]

Complexity of the matrix

Attached bacteria produce an exopolysaccharide matrix that can act as a protective polymer for the cells embedded within. As the biofilm thickens, it begins to develop into a dynamic,

hetergeneous matrix interspersed with channels that allow nutrients and oxygen to penetrate into the depths of even the thickest biofilms. Microscopic images of biofilms using the Confocal Scanning Laser Microscope (CSLM) reveal a structural complexity characterized by towers and mushroom-like shapes interspersed with open channels.[11] Variations of biofilm thickness and flow rates create microenvironments within the biofilm. The use of microelectrode technology has enabled researchers to map out such variations by measuring characteristics as pH, dissolved oxygen and nitrate concentrations within a biofilm. For example, within a *Pseudomonas aeruginosa* biofilm there exists a gradient of oxygen concentrations such that some bacterial cells will be growing aerobically while others, deeper within the matrix, will be living in an anaerobic environment.[12] This variation in metabolic states has profound implications for the ability of the organisms to survive killing by certain antimicrobial agents.[13] This concept of spatial heterogeneity within a biofilm has been applied to oxygen limitations (from aerobic to anaerobic), pH, nutrients, and rates of growth.[3]

The biofilm phenotype

When a bacterial cell attaches to a surface, it undergoes a number of physiologic and metabolic changes. Bacteria that are dividing at the rate of minutes in culture, will stop dividing for hours when first attaching to a surface.[14] During this time, there are numerous changes occurring as that bacterial cell makes the transition from a planktonic to a biofilm cell. Reporter gene technology has shown that the activity of an alginate promoter (*algC*), involved in matrix production in *P. aeruginosa,* is up-regulated when the bacterial cells attach to a surface.[15]

The analysis of differentially expressed (induced or repressed) proteins or proteomics allows researchers to analyze the protein expression of an organism at a particular point in time or under a particular condition. Cells of *P. aeruginosa* attaching to a surface have been shown to express changes in their protein profiles (when compared to planktonic cells) in as little as 10 minutes after inoculation (Pulcini, work in progress). These differences in protein expression during initial adhesion indicate physiologic changes are taking place within cells as they attach to a surface. These changes are significant and have lead researchers to postulate the existence of a distinct biofilm phenotype.[10]

Cell-cell communication

It has been shown that both Gram-positive and Gram-negative bacteria use chemical signal molecules to coordinate activity among bacterial cells.[16] The action of these signal molecules relies on a process called quorum sensing defined as the ability of a molecule to cause an action dependent on its concentration within the environment. The concentration of the signal molecule can increase only when there is a sufficient number of bacterial cells producing that particular signal. As the biofilm develops, bacterial cells within the matrix have been shown to release these chemical signals. Specifically, Gram-negative bacteria have been shown to produce the quorum-density signal molecules called acyl-homoserine lactones (HSLs). Mutant strains of *P. aeruginosa* deficient in the LasR-LasI HSL quorum sensing system have been shown to produce biofilms that lack the towers and channels often seen in normal *P. aeruginosa* biofilms.[17]

Halogenated furanones, isolated from the marine red algae *Delisea pulchra,* have been shown to act as blockers on the receptors for acyl-homoserine lactones. Polymers embedded with these halogenated furanones and placed in the ocean have been shown to resist colonization by marine microorganisms for as long as five months.[18] It is entirely possible that the development of implant materials embedded with similar homoserine lactone blocking compounds may allow for a better level of biofilm control in the medical setting.[1]

Conclusion

Biofilms are found in almost every environmental system studied and in nearly every industrial and medical setting where microbial contamination is a problem. The fact that bacteria do not always live in suspensions of single cells, but instead live in complex biofilm habitats, has significant ramifications for the manner in which we study most bacterial species. In order to prevent and/or control the formation of biofilms, it is imperative that methodologies be developed that target such aspects of the biofilm system as cell signaling or phenotypic changes in the bacterial cell.

For further information on current research and trends in biofilm science, please visit the Center for Biofilm Engineering Website (www.erc.montana.edu).

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