CSIRO PUBLISHING
Healthcare Infection, 2013, **18**, 61–66
http://dx.doi.org/10.1071/HI12059

Review

A review of bacterial biofilms and their role in device-associated infection

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Abstract. ***Background*:** Most of the world's bacteria live in biofilms, three-dimensional clusters attached to surfaces. Many hospital-acquired infections are associated with biofilm infections of implantable medical devices such as orthopaedic prostheses and intravascular catheters. Within biofilms, bacteria are significantly less susceptible to antibiotics and host defences, making biofilm infections difficult to diagnose and treat, and often necessitating removal of the infected implant.

***Method*:** In this review article we describe the process of biofilm formation, quorum sensing, and biofilm infection of the healthcare environment, surgical instruments and implantable medical devices.

***Conclusion*:** The inability to treat biofilm-infected devices means that therapies targeting biofilm-specific processes and targeting prevention of biofilm formation are required.

Additional keywords: biofilms, biomaterial-related infections, environmental contamination, implant-related infections, infection control, staphylococci, surgical infection.

Received 28 November 2012, accepted 30 January 2013, published online 23 April 2013

Introduction

Bacterial biofilms have a major impact on society, not only contributing to hospital-acquired infections (HAIs), but colonising our environment causing among other things, corrosion, fouling of water pipes, and food and pharmaceutical spoilage.[1] Bacteria can attach to and infect all medical devices and up to 60% of HAIs are associated with biofilm infections of implantable medical devices such as orthopaedic prostheses and intravascular catheters.[2]

Over 99% of the world's bacteria are thought to live in biofilms[3] so it must be assumed that they gain an advantage living in this state. Biofilms consist of three-dimensional aggregations of sessile microorganisms surrounded by hydrated, extracellular polymeric substances (EPS). The EPS is secreted by the microorganisms following surface attachment. It consists principally of polysaccharides, nucleic acids, proteins and lipids and comprises between 50 and 90% of the mass of the biofilm.[4] Bacteria within biofilms are significantly less susceptible to antibiotics and host defences than the planktonic (free-swimming) forms of the same organisms[5,6] making them difficult to treat, often necessitating the removal of the infected implant.[7,8] In this review we describe the process of biofilm formation, quorum sensing, and how biofilm infection impacts on healthcare with particular reference to implantable medical devices.

Formation of biofilms

Immediately upon insertion of a medical device into the patient, macromolecules such as fibrinogen and immunoglobulins are deposited on the implant's surface.[2] This is called the conditioning film and generally makes it easier for bacteria to attach to the implant. Several proteins produced by Staphylococcaceae produce proteins that bind specifically to host factors in the conditioning film.[9]

Bacteria can randomly come in contact with implant surfaces by sedimentation and Brownian motion while motile microbial cells may actively seek the implant surface. Initial bacterial adhesion occurs due to van der Waals forces and

Implications

- Bacteria live attached to surfaces in structured communities called biofilms.
- Biofilms colonising surfaces have increased resistance to removal by detergents and disinfectants.
- Biofilms colonising implantable medical devices are resistant to antibiotic treatment and the host immune response.
- It is imperative that methods to prevent biofilm colonisation are developed.

surface structures such as fibrils or polymers create a link between the substrate and individual bacteria. Once attached, the bacteria produce diffusible signalling molecules in a process called quorum sensing which, among other things, induces the bacteria to secrete EPS. It is the EPS that cement the bacteria to each other and the surface and provides mechanical stability for the three-dimensional development of the biofilm. The biofilm structure itself is dynamic with redistribution of reversibly-attached cells, recruitment of cells from the surrounding fluid, replication of attached cells and dispersal of cells back into the surrounding areas.[1,2]

Quorum sensing

Quorum sensing (QS) allows communication between bacteria, synchronising alteration in genetic expression of the whole bacterial population, thus coordinating activities such as biofilm formation and the production of virulence factors.[10,11] Each bacterial species produces its own QS chemical signal.

Gram-negative bacteria use N-acylhomoserine lactones (AHL) as QS molecules. These consist of a conserved homoserine lactone ring attached to an acyl side chain. Species specificity is provided by various chemical modifications including the length of the acyl side chain (4 to 18 carbons). Generally, AHLs are synthetised by homologues of *Vibrio fischeri* Lux1 protein.[10] Short-chain AHLs diffuse passively, in and out of the bacterial cell, along a concentration gradient, whilst long-chain AHLs may be actively transported. Each bacterial cell makes small amounts of AHLs, but with increasing numbers of bacteria the concentration of AHL increases in the local environment. Once a critical concentration is reached, AHL binds to a cytoplasmic transcription factor (homologous to LuxR proteins of *V. fischeri*), which then bind directly to bacterial DNA and regulate genetic transcription.[10]

Gram-positive organisms use small peptides of 5 to 17 amino acids in length as QS molecules. Species specificity is determined by modification of the peptide's side chains, for example QS molecules of Staphylococcaceae spp. contain a thiolactone ring.[12] The peptides are synthetised in the cytoplasm, modified and then excreted. Gram-positive organisms have two component QS detection systems, including sensor and regulator proteins.[13] High concentrations of QS peptides are detected by the membrane-bound sensor protein, which activates the cytoplasmic regulator protein which in turn regulates genetic transcription.

In addition to intra-species communication, bacteria can communicate with other species of bacteria. Auto-inducer 2 has been found in over 50 different species of bacteria may be the QS signal for universal communication.[10]

Antibiotic resistance

Biofilm bacteria can survive up to 1500, typically 100 to 250 times, the amount of an antibiotic needed to kill the same bacteria growing in liquid culture. This increased antibiotic resistance is due to the biofilm lifestyle, as when biofilm bacteria are dispersed they once again become susceptible to antibiotics.[14] The mechanism of increased resistance of biofilm bacteria is multifactoral and may be different for different bacteria;[5,6] also resistance increases as the biofilm ages.[14]

The diffusion of both nutrients and oxygen has been shown to be limited in the deeper layers of the biofilm, which affects bacterial phenotypic expression, resulting in bacteria growing very slowly or not at all. One consequence of this decreased metabolism is antibiotic resistance e.g. an ampicillin-resistant biofilm phenotype in cells genotypically sensitive to ampicillin.[15] These metabolically-inactive cells are also very difficult to culture and thus make diagnosis of implant infection very difficult.[5] Other mechanisms of antibiotic resistance include binding and inactivation by the EPS – as occurs with some antibiotics such as tobramycin[16] – and induction of a distinct biofilm phenotype or 'dormant resistant cell.'[6]

Biofilms in the healthcare setting

Healthcare environment

Environmental biofilms are generally found in wet areas such as water pipes.[17] Indeed in healthcare settings, due to organisms being protected in biofilms, HAI outbreaks have centred around drains[18] and showers. For example, a hand hygiene sink drain infected with a biofilm composed of multidrug-resistant *Pseudomonas aeruginosa*, resulted in bacterial contamination of adjacent medication and the sterile dressing preparation area, due to splashing when the faucet was turned on. Disinfection of the sink failed to remove the biofilm and the outbreak was only terminated when the sinks were renovated to prevent splashing.[18]

Contamination of the inanimate environment around patients in the hospital setting constitutes an important reservoir of bacteria[19] and the risk of obtaining a HAI is increased on average by 74% if the previous patient occupying that room had a multi-antibiotic resistant organism (MRO), as reviewed by Carling.[20] We propose that bacteria exist on these dry surfaces as biofilms and are protected from desiccation and moreover have increased resistance to removal by detergents[21] and increased resistance to disinfectants.[6] A more