Biofilm and orthopaedic implant infection

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Biofilm is a colony of microorganisms suspended within a self-produced matrix, the extracellular polymeric substance (EPS). Estimates report that 99% of bacteria can exist within a biofilm state. The likely organisms responsible in approximately 75% of biofilm-associated infection include S.epidermidis, S.aureus and Pseudomonas aeruginosa. 

Biofilm formation

A surgical skin incision exposes otherwise harmless bacteria to a change in environment leading to an opportunistic change in behaviour. The adhesion of a planktonic bacterium to an inert surface leads to the formation of a biofilm in phases: adhesion - aggregation - mutation - dispersal. The process is dependent upon local conditions such as the hydrophobicity and acidity, oxygen concentration, presence of inert material (and its surface area) and the ability of the bacterium to initiate contact via pili/flagella. Secretion of positively charged homopolymers such as polysaccharide intercellular adhesin (PIA) leads to the creation of a protective extracellular matrix that acts as a physical and electrostatic barrier. Recruitment, proliferation and maturation follow, resulting in the adhesion and incorporation of other microbes. In its early phase of formation, the structure of the biofilm is susceptible to the host immune system and antibiotic penetration but, within one hour, DNA transcription changes alter gene expression. Proliferation and phenotypic modulation within the biofilm culminates in maturation and dissociation/disaggregation with dispersal of bacterial cells.
Biofilm function

The ultimate function is to create a protective environment within which bacteria can sustain a state of existence without overwhelming the host. Secretion of virulence factors conveys a unique environment for the bacteria to continue to evolve and mature allowing evasion of innate and adaptive immune responses and development of tolerance to antimicrobial agents.

The structure of biofilm is designed such that the high cell density allows cell-to-cell communication through quorum sensing (QS). Bacteria can modify gene transcription based on cell density and population, resulting in areas of differentiated metabolic activity that create subpopulations of phenotypically distinct bacteria. Surface bacteria are more metabolically active and demonstrate some susceptibility to antibiotic penetration yet the sub-lethal exposure encountered by centrally located dormant cells often leads to expression of virulence factors with up to a 1000 fold increase in resistance to systemic therapy.

Biofilm prevention

Anti-adhesins

The attachment of a planktonic bacterium is a key phase in formation. Surface proteins known as adhesins expressed from the planktonic cell initiate cell attachment. Within each microbial species there are hundreds of different adhesins, such as the sortase family in gram positive bacteria. Many of these membrane enzymes have proven to be universal virulence factors for gram positives and offer a potential drug target for anti-adhesive activity. In vitro studies demonstrate promising results leading to the blockage of bacterial adherence to fibronectin coated surfaces.

Quorum-sensing inhibitors

Cells within a biofilm behave as communities rather than individuals, using the communication pathway referred to as ‘quorum sensing’. Cell density and population influence gene expression within the biofilm resulting in adaptive behaviour. This reduces metabolic activity in the central cells of the biofilm which modulate and mutate enhancing the biofilm’s ability to evade the host immune system. Numerous quorum sensing pathways exist in gram positive and negative species with two major classes - furanones and RNAIII-inhibiting peptides (RIP). RIP has proven to be most effective against staphylococcal species including MRSA and S. epidermidis. Further work has suggested that the use of QSIs in combination with conventional antimicrobial agents enhanced the susceptibility of the biofilm to standard antibiotics but further research into the product stability and toxicity is needed.

Biofilm prevention

Biosurfactant/Antimicrobial peptides/NSAIDs

Biosurfactant and macromolecule (heparin/albumin) coating of prosthetic surfaces reduces the adhesion of microbes. Once attachment is successful, the microbes can modify the coated surface to break down the protective layer and create further adhesions/aggregates. Further research is ongoing to produce more stable inhibition.

Antimicrobial peptides (AMPs) such as melanine and citropin are effective against biofilm formation due to their ability to bind to negatively charged structural molecules on the microbial membrane. AMPs combine with certain antibiotics to enhance their effects. However, these peptides are susceptible to protease digestion so research is now focused on developing synthetic and selectively targeted second and third generation AMPs.

In vitro studies have proven that non-steroidal anti-inflammatory drugs can influence the synthesis of bacterial and fungal prostaglandins by targeting the cyclooxygenase dependant pathways thereby inhibiting biofilm formation.

Antibiotics

Systemic antimicrobials continue to be effective against bacteria in their planktonic state yet cannot target biofilm bacteria.

Vaccines

Attempts to produce an antibiofilm vaccine have thus far proved futile. Whilst much of the work has focused on creating an anti-staphylococcal vaccine e.g. StaphVAX - its protective properties at one year post vaccination was less than 30%. Development of a quadrivalent vaccine used in conjunction with antibiotic therapy proved more efficacious suggests that further advances in the field of vaccination may offer future benefits.
Biofilm Disruption

Disrupting an established biofilm is challenging, as a combination of cellular communication and a protective physical barrier provides the biofilm with the ability to evade host immune detection and inhibits the penetration of systemic antibiotics. Agents suitable to target biofilm disruption include proteolytic enzymes, such as deoxyribonuclease I (DNase I) and dispersing B (DspB), and mucolytic agents such as N-acetylcysteine (NAC)\(^1\). NAC has been extensively studied in cystic fibrosis and its mechanism of action as an antioxidant reduces the production of the EPS as well as promoting disaggregation of the mature biofilm. It has a synergistic effect when used in combination with antibiotics, demonstrating biofilm in *S. aureus, S. epidermidis* and gram-negative bacilli\(^1\). Positive results are being seen with NAC in the setting of implant coating of orthopaedic implants\(^2\).

Alternatives proven to disrupt biofilm formation include the use of larval secretions that appear to disrupt biofilm in the digestion of necrotic tissue. Recent studies have also demonstrated that maggot excretions inhibit biofilm formation on biomaterials including polyethylene, titanium and stainless steel\(^3\).

Nanomolar nitric oxide is effective at dispersing motile strains of bacteria and is thought to be useful against gram-negative pathogens, yet its mode of delivery and potential toxicity remains a concern\(^4,7\). An alternative option is to target the ‘persister’ cells whilst they remain in a dormant state whereby activation of cytoplasmic protease by the antibiotic acyldepsipeptide (ADEP4) can initiate autolysis of the cells\(^1\). Development of nanoparticles as drug carriers enable local antibiotic elution, improved drug delivery and avoidance of the burst effect (near complete elution occurring within hours or days, with subsequent sub-inhibitory exposure which theoretically can lead to antibiotic resistance).

It utilises the natural properties of metals such as silver with its antimicrobial capacity against both gram positive and gram negative organisms\(^1,4\). Promising results are seen with core/shell nanoparticles consisting of a bioceramic core and polymeric shell providing controlled drug elution over a period of 30 days\(^4\).

Attempts have also been made to directly tether antibiotics to the surface of prosthetic implants demonstrating potency for up to 48 hours. Alternatively, both biodegradable and non-biodegradable cements can function as local drug delivery systems achieving high local concentrations without the effects of systemic toxicity\(^1,4,8\). This method is however susceptible to the burst effect.

### Implant materials

Research has established the properties of an inert material which influence the formation of biofilm. Advances in orthopaedics have seen modification of implants to improve bearing wear or osseointegration. For example, hydroxyapatite coating creates a rougher surface. This topographical change creates a structurally favourable environment for bacterial adherence\(^2\). Equally, microporous coating is more likely to lead to planktonic attachment than an implant with a smooth polished surface\(^2\). Metallic compounds possess a negative charge thereby attracting positively charged bacterial molecules with high energy surfaces demonstrating increased biofilm\(^2,8\). The hydrophobicity of a material appears to have a parabolic type relationship in terms of biofilm formation with highly hydrophilic properties and superhydrophobic surfaces both associated with a reduction in biofilm adherence\(^2\). Different alloys display different biofilm properties even when they have similar chemical and mechanical characteristics.

It has been demonstrated that titanium materials tend to keep bacteria dispersed on the surface thereby reducing the adherence of bacteria as compared with stainless steel or polymethylmethacrylate\(^7\). Researchers have also found that vanadium free titanium alloys display decreased bacterial adherence as compared to those containing vanadium\(^7\). These titanium alloys are also less biofilm resistant than titanium dioxide, titinate (a mixed titanium and calcium silicate) and medical tantalum.

Silver has long been known to have bactericidal properties by disrupting their folding of proteins and producing toxic reactive oxygen species, harming the DNA and cell membrane. Again, this increases the susceptibility of a bacterium to antibiotics: the combined effect of silver and antibiotics leads to a synergistic response killing between 10 and 1000 times as many bacteria. Silver has already been utilised both as a coating and as nanoparticles which, when combined with titanium dioxide, display further bactericidal properties\(^2,4,7\).
Modification of implant surfaces offers a novel approach to targeting biofilm infection with potential to introduce bactericidal surfaces, bacteriostatic surfaces, anti-adhesive coatings and smart coatings.

Conclusion

The use of prosthetic implants in orthopaedics provides an ideal environment for biofilm formation. Inert material within a hypovascular and hypocellular area of scar tissue creates an environment that is no longer exposed to the routine host immune surveillance and creates additional challenges to the treating clinician. An improved understanding of biofilm within the orthopaedic community will lead to a more streamlined approach in the pre-operative, peri-operative and post-operative periods to optimise treatment and improve patient outcome.

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References can be found online at www.boa.ac.uk/publications/JTO or by scanning the QR Code.

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