Biofilms: prevention and treatment

Biofilms are a major aetiological factor in many infections. Bacteria growing within a biofilm are extremely resilient to standard antimicrobials; biofilm-associated infections are thus challenging to treat. Understanding how and why biofilms form can improve prevention and management of biofilm infections.

Biofilms are sessile microbial communities embedded in an extracellular matrix of their own synthesis, attached to a surface (Costerton et al, 1999). Historically bacteria were thought to exist as solitary, free-floating, 'planktonic' organisms. A paradigmatic shift towards a biofilm-centric microbial world occurred in the 1970s as we began to appreciate the ubiquity of bacterial biofilms in nature and their fundamental importance to bacterial survival. Much of the pioneering work on bacterial biofilms originated from the field of dental microbiology. Biofilms are now understood to be the predominant form of microbial growth (Donlan and Costerton, 2002). The ability of bacteria to form biofilms is an ancient evolutionary adaptation to provide homeostasis in the face of fluctuating and harsh environmental conditions (Hall-Stoodley et al, 2004).

Biofilms are of major medical importance; they occur commonly on medical devices and can form on living tissue (Costerton et al, 1999). The US Centers for Disease Control and Prevention estimates that 65% of human bacterial infections involve biofilms (Potera, 1999). Biofilms represent a protected mode of growth, and the resilience of bacteria sequestered in biofilms presents a significant challenge for treating biofilm infections as they are inherently more tolerant to antimicrobial drugs and the host immune response, so are often extremely difficult to eradicate. Biofilms can also cause problems for industry – they form readily in water distribution systems and can lead to contaminated drinking water. The industrial aspects of biofilm formation will not be discussed here further.

This review outlines the biology of biofilms, infections associated with biofilms and explains why bacteria sequestered within a biofilm are so much more resilient to antimicrobials than planktonic bacteria. Strategies to combat and prevent biofilm-associated infections are also discussed.

Biofilm formation

Biofilms are structurally and developmentally complex. The formation of a microbial biofilm is a dynamic process that takes place over several stages (*Figure 1*). The first stage in biofilm formation is attachment: this is a complex process dependent on several factors, including the bacterial cell itself, and the characteristics of the surface and surrounding environment. The properties of the surface influence the extent and rate of microbial attachment; microorganisms attach more readily to surfaces that are rough and non-polar, such as Teflon and other plastics, than to hydrophilic materials such as glass and metal (Donlan, 2002). The finish of the surface also influences the extent of biofilm formation; electropolished stainless steel has lower bacterial attachment than rougher, sandblasted stainless steel (Arnold and Bailey, 2000).

The second stage in development occurs as bacterial cells attached to the surface undergo cell division and start to form micro-colonies. The bacterial cells produce extracellular matrix; this consists primarily of polysaccharides, is highly hydrated and bound tightly to the underlying surface. The extracellular matrix is a defining feature of the biofilm and acts as the structural scaffold holding the biofilm together (Sutherland, 2001). As the biofilm matures, multiple layers of cells build up on the surface and more matrix is produced. This leads to the formation of a complex and heterogeneous three-dimensional architectural structure. Matrix-enclosed micro-colonies are interspersed with open-water channels which facilitate transport of essential nutrients and oxygen to cells growing within the biofilm (Donlan, 2001). The final stage of biofilm life occurs when bacterial cells detach and disperse to spread and colonize new niches.

Within the biofilm

Bacteria living within biofilms can be thought of as living together in complex, interdependent communities (Watnick and Kolter, 2000). In these communities, bacterial cells can communicate with one another using secreted chemical signals. Cell-to-cell communication allows bacteria to sense

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Figure 1. Stages in biofilm formation.

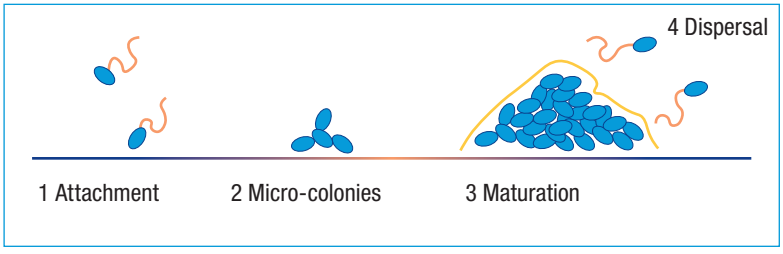

Table 1. Microorganisms associated with biofilms of indwelling medical devices

Medical device	Principal microorganisms
Central venous catheter	Coagulase-negative staphylococci, *Staphylococcus aureus*, enterococci, *Candida* spp
Prosthetic heart valve	Alpha-haemolytic streptococci, coagulase-negative staphylococci, enterococci, *S. aureus*
Urinary catheter	*Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, enterococci
Prosthetic joint	Coagulase-negative staphylococci, *S. aureus*, streptococci, enterococci, *E. coli*, *P. mirabilis*
Endotracheal tubes	Enteric Gram-negative organisms

adapted from Donlan (2001)

Table 2. Microorganisms associated with human tissue infections involving biofilms

Infectious disease	Micro-organisms
Otitis media	*Haemophilus influenzae*
Dental caries	Gram-positive anaerobic species, Gram-negative anaerobic oral bacteria
Cystic fibrosis pneumonia	*Pseudomonas aeruginosa*, *Burkholderia cepacia*
Endocarditis	Alpha-haemolytic streptococci
Prostatitis	*Escherichia coli* and other enteric Gram-negative organisms
Diabetic foot infection	*Corynebacterium* spp and anaerobes
Chronic sinusitis	*Staphylococcus aureus* and coagulase-negative staphylococci
Osteomyelitis	*S. aureus*
Chronic wounds	*S. aureus*, *P. aeruginosa*

adapted from Dufour et al (2012)

and phenotypically respond to environmental conditions. Through a process known as 'quorum sensing' bacterial cells can sense and respond to changing levels of population density. Bacteria growing within a biofilm secrete signalling molecules known as autoinducers – as the population density increases the concentration of autoinducer molecules rises. This is detected by the bacteria which can then respond to changing population density by altering gene expression (Miller and Bassler, 2001). Bacterial cells can thus modify their behaviour to perform functions that are only effective when many cells act together in conjunction. There is evidence in some bacterial species that quorum sensing has a role in the regulation of biofilm formation and is important for processes such as bacterial surface attachment, maturation of the biofilm and dispersal of cells from the biofilm (Parsek and Greenburg, 2005).

The gene expression of bacterial cells growing in a biofilm is different to that of planktonic bacteria; up to 10% of genes are observed to be differentially expressed during biofilm *vs* planktonic growth. The altered transcriptional profiles are associated with phenotypic changes influencing factors including the production of extracellular products, cell adhesion, motility and metabolism (Nadell et al, 2009).

Bacterial communities within the biofilm are often polymicrobial; even within a single species biofilm, cells from the same bacterial species often exhibit different phenotypes and form distinct subpopulations.

Biofilm infections

Biofilms play a major role in a wide range of human infections. They can form readily on a wide range of medically important surfaces such as indwelling medical devices and living tissues such as heart valves, tooth enamel, bone, airways of the lung, and mucosa of the middle ear.

Biofilms grow slowly and are associated with chronic infections. Antibiotics and host immune responses typically fail to eradicate the biofilm; biofilm infections therefore typically recrudesce after courses of antibiotic therapy and persist until the biofilm is physically removed from the body.

Many nosocomial infections are related to the presence of indwelling medical devices and are associated with biofilm (Percival et al, 2015). *Table 1* lists indwelling medical devices on which biofilms can develop and the organisms associated with biofilm infections at these sites. Catheter-associated urinary tract infection is the most common device-associated biofilm infection; the presence of a urinary catheter undermines the usual host defences at this site, and virtually all long-term urinary catheters will eventually become colonised with bacteria (Stickler, 2014). Central line-associated bloodstream infection and ventilator-associated pneumonia are other significant and commonly encountered device-associated biofilm infections. Prosthetic joint infection is another important and costly device-associated biofilm infection, with an estimated cost of infection per patient of $50 000–100 000 and an incidence of approximately 0.5–2% (Romling et al, 2014).

As well as forming on prosthetic devices biofilms can also form on living tissue and lead to chronic infections (*Table 2*). Pseudomonal lung infection in patients with cystic fibrosis was one of the first chronic infections to be linked to the formation of biofilms (Lam et al, 1980). Biofilm formation has since been implicated in the mediation of numerous other chronic tissue infections including chronic

wound infections, chronic otitis media, osteomyelitis, endocarditis and chronic urinary tract infections.

Biofilms are often difficult to diagnose; conventional culture techniques frequently fail to adequately isolate bacteria growing in biofilms because of the difficulty in detaching and isolating bacteria from the biofilm surface. Numerous studies, for example in infections such as chronic otitis media and infective endocarditis, have demonstrated discrepancy between standard culture techniques and molecular methods, with greater sensitivity found using molecular methods for diagnosis (Hall-Stoodley et al, 2012). Molecular methods may therefore be helpful in detecting and diagnosing bacteria when there is suspicion of biofilm infection. Common molecular techniques in use include polymerase chain reaction and 16S ribosomal probes.

A number of diagnostic criteria have been proposed to help diagnose biofilm infections (Parsek and Singh, 2003; Hall-Stoodley et al, 2012) and include the following features:

1. Positive culture or molecular identification of microbes known to be associated with biofilm formation
2. Microscopic evidence of microbial aggregates and biofilm structure
3. History of condition which predisposes to development of biofilm, e.g. presence of implanted device, cystic fibrosis
4. Recurrence of infection
5. Evidence of antibiotic failure despite adequate choice of antimicrobial.

Biofilms and antimicrobial recalcitrance

Bacteria living within a biofilm have the capacity to withstand and persist in the presence of higher concentrations of antibiotics than free living planktonic bacteria – this property is called 'recalcitrance'. The minimum inhibitory concentration of antibiotics which will affect bacteria growing within a biofilm may be up to 100–1000-fold greater compared with planktonic bacteria (Høiby et al, 2010). The minimum inhibitory concentration is the lowest concentration of antibiotic to inhibit bacterial growth, so bacteria with higher minimum inhibitory concentrations are more resistant than bacteria with lower minimum inhibitory concentrations. This high tolerance to antimicrobials means that chronic tissue-related and device-related biofilm-associated infections are difficult to treat successfully and frequently relapse when antimicrobials are stopped.

One of the first mechanisms proposed to account for the phenomenon of antimicrobial recalcitrance is the presence of the extracellular matrix. The extracellular matrix acts as a mechanical barrier and impairs the penetration and diffusion of some antibiotics. Not all antibiotics are equally affected by this; some agents have been observed to penetrate well through the matrix. In vitro studies have demonstrated that vancomycin and rifampicin penetrate through staphylococcal biofilm, achieving bactericidal levels at the surface of an infected implant (Dunne et al, 1993). Ciprofloxacin readily penetrates *Klebsiella pneumoniae* biofilm, and has been shown to achieve therapeutic levels (Anderl et al, 2000). Studies indicate that the extent of antimicrobial penetration is specific to the agent and organism involved. Despite the observed good penetration of agents such as ciprofloxacin and rifampicin, complete bacterial eradication is not achieved, suggesting further mechanisms must also exist to account for antimicrobial recalcitrance (Lebeaux et al, 2014).

Deep within the biofilm, pockets of altered microenvironments, including anaerobic and nutrient sparse conditions, are frequently encountered. The presence of such environments may adversely affect the action of antimicrobials. For example, gentamicin is less effective in anaerobic conditions than in aerobic. In nutrient deplete areas bacteria may also be less metabolically active, thus limiting the action of agents such as β-lactams which are only effective against actively dividing bacteria (Stewart and Costerton, 2001). However, this cannot explain the recalcitrance towards antibiotics which are also active against non-dividing bacteria.

A further mechanism, thought to be the major factor accounting for antimicrobial recalcitrance, is the existence of small, phenotypically distinct sub-populations of highly tolerant cells within the biofilm known as 'persister' cells. Persisters are cells that have entered a dormant state and can survive the effects of any bactericidal antibiotic. Antibiotics require active targets to kill bacteria, thus explaining the tolerance of persisters. The formation of persisters is thought to result from a combination of stochastic and deterministic events (Lewis, 2010). Persisters are believed to represent a survival mechanism in the face of adverse environmental conditions. Following the cessation of antibiotic treatment small numbers of surviving persisters can resume growth and repopulate the biofilm, leading to recurrence of infection (Lewis, 2007).

In addition to these mechanisms leading to greater antibiotic tolerance, the biofilm environment also favours the acquisition of antimicrobial resistance genes. Horizontal gene transfer rates are typically higher within the densely populated biofilm, thus favouring the transfer of resistance genes (Madsen et al, 2012). The rate of transfer of an extended-spectrum β-lactamase-encoding plasmid was higher in a *K. pneumoniae* biofilm than in planktonic conditions (Hennequin et al, 2012). Impaired antimicrobial diffusion through the extracellular matrix may also lead to bacterial exposure to sub-inhibitory concentrations of antimicrobials, which could increase the likelihood of selecting out resistant mutants.

Treatment and preventative approaches for biofilm infections

Conventional treatment regimens may successfully treat acute exacerbations of biofilm infections caused by the release of planktonic organisms but, as a consequence of antimicrobial recalcitrance, frequently fail to eradicate the biofilm itself and the infection typically recrudesces.

Conventional approaches may even be detrimental as they can promote the development of further resistance within the bacterial biofilm (Wilkins et al, 2014). The most effective way of successfully treating and eradicating biofilm infection is therefore removal of the infected device or debridement of infected tissue or bone; however, this option is not always feasible and may be costly or associated with complications.

A number of non-specific strategies can be used to help prevent and combat biofilm-associated infections. These strategies act to either:

1. Prevent initial microbial contamination of the device
2. Minimize attachment of microorganisms to the device
3. Enhance penetration into the biofilm to eradicate the bacterial cells within the biofilm
4. Mechanically remove the source of infection (Donlan and Costerton, 2002).

Improving hygiene measures for device insertion and handling can prevent microbial contamination of implanted devices, thus averting the start of biofilm formation. For central venous catheters, for example, guidelines have been developed describing strategies to control the number of device-related infections. These encompass hygiene aspects including appropriate sterile precautions for insertion, topical skin disinfection and limiting the time over which the catheter remains in situ (O'Grady et al, 2011). Delivery of systemic antibiotic prophylaxis immediately before the insertion of long-term prosthetic devices, such as orthopaedic devices, can also reduce the likelihood of the device becoming infected (Alijanipour et al, 2014).

Altering the surface properties of indwelling medical devices to prevent initial microbial colonization and thus avert the formation of biofilm is an area of interest in the prevention of biofilm infections. One approach is to coat the surface of the implanted device with bactericidal or bacteriostatic substances. Strategies that involve coating or impregnating devices with antibiotics, such as vancomycin, and silver particles are being developed, but the clinical effectiveness of such strategies has not yet been clearly established (Chen et al, 2013).

Early removal of unnecessary implanted devices can prevent the contamination of the device. This can be an effective strategy for helping to limit biofilm infections associated with short-term devices such as urinary catheters and central venous catheters but is not possible for longer-term devices such as pacemakers and prosthetic joints. In cases of established biofilm infection on such devices, removal may be the only effective strategy. When standard treatment options fail to successfully treat tissue infections associated with biofilm formation, mechanical removal of the biofilm by debridement or excision of infected tissue may, again, be the only successful option for treatment, for example, in cases of infective endocarditis or osteomyelitis failing with standard antimicrobial therapy.

Optimizing the antimicrobial regimen is an important component of any strategy to combat biofilm infection. Adding rifampicin to the antimicrobial regimen significantly improved outcomes in staphylococcal implant-associated infections (Zimmerli et al, 1998). The use of rifampicin in combination with ciprofloxacin is particularly effective for methicillin-susceptible *Staphylococcus aureus* implant-associated infections (Zimmerli and Moser, 2012). Daptomycin used in combination with rifampicin is emerging as a promising treatment option for methicillin-resistant *S. aureus* implant-associated infections (John et al, 2009). For implant-associated Gram-negative infections, fluoroquinolones are the agent of choice (Zimmerli and Moser, 2012). Further to the choice of antimicrobial regimen, prolonged courses and high dosages are required for biofilm-associated infections (Lebeaux et al, 2014).

Novel anti-biofilm strategies

A number of novel approaches for the treatment of biofilm infections are under development and may lead to effective future treatment options.

As previously discussed, quorum sensing is a mechanism by which bacterial cells can respond to changing environmental conditions related to increased population density by the expression of specific genes. Quorum sensing inhibitors and antagonists are currently one of the most promising areas for new therapeutic options for the treatment of biofilm infections (Brackman and Coenye, 2015). In an in vivo *Pseudomonas aeruginosa* foreign-body biofilm model, combination treatment with a quorum sensing inhibitor and tobramycin demonstrated synergistic effects on bacterial killing. Combination treatment, which aims first to disable the quorum sensing system and second to kill the bacteria with an antibiotic, may therefore represent a promising future strategy to combat biofilm infections (Christensen et al, 2012). As quorum sensing is not involved in bacterial growth, quorum sensing inhibitors may have the added benefit of not exerting strong selective pressure towards the development of resistance.

Further potentially useful strategies involve modulation of biofilm metabolic pathways. An example is interference of the intracellular second messenger cyclic di-GMP, which mediates a range of cellular functions including biofilm formation, virulence and dispersal. Low dose nitric oxide signalling has been shown to trigger degradation of cyclic di-GMP, leading to dispersal of *P. aeruginosa* biofilms. Pro-drugs that only release nitric oxide upon contact with a biofilm could allow targeting of antibacterial action while limiting toxic effects to target tissue (Wilkins et al, 2014).

Conclusions

Bacterial biofilms are ubiquitous in nature and are a major cause of human infection. They play a particularly important role in the aetiology of nosocomial infections and represent a significant economic burden. Conventional therapeutic strategies often fail to successfully eradicate biofilm infections, making these infections challenging to treat. Mechanical removal of the biofilm is often the only effective strategy to ensure clearance of the infection. Novel approaches to tackle biofilm infections are being developed

and target specific systems and pathways important in the establishment and continuation of biofilm life. For the present, however, biofilm infections are likely to continue to pose significant difficulties for clinicians. An awareness and recognition of situations where biofilms are likely to play a role in mediating infection can lead to better understanding of the ways to prevent and manage biofilm infections. **BJHM**

Conflict of interest: none.

KEY POINTS

- Bacterial biofilms are ubiquitous in nature and are a major cause of human infection. They have a particularly important role in device-associated and nosocomial infections.
- Bacteria growing within a biofilm are much more tolerant to standard antimicrobial therapies and are therefore very challenging to eradicate.
- The most effective way of eliminating biofilm infections is mechanical removal of the biofilm; however, this is not always feasible.
- There is a need for novel therapeutic strategies to combat chronic biofilm-related infections, new approaches are currently being investigated.

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