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Impact of bacterial biofilm on the treatment of prosthetic joint infections

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Microbial biofilm contributes to chronic infection and is involved in the pathogenesis of prosthetic joint infections. Biofilms are structurally complex and should be considered a dynamic system able to protect the bacteria from host defence mechanisms and from antibacterial agents. Despite the use of antibiotics recognized as effective against acute infections, prosthetic joint infections require long-term suppressive treatment acting on adherent bacteria. Conventional *in vitro* susceptibility testing methods are not suitable for biofilm-associated infections given that these tests do not take into account the physiological parameters of bacterial cells *in vivo*. Most anti-staphylococcal drugs are able to inhibit *in vitro* the adhesion of bacteria to a surface, considered to be the first step in biofilm formation. Recent studies suggest that the lack of activity of antibiotics against biofilm-embedded bacteria seems to be more related to the decreased effect of the drug on the pathogen than to the poor penetration of the drug into the biofilm. Eradication of biofilm-embedded bacteria is a very difficult task and combination therapy is required in the treatment of persistent infections involving biofilm. Although several combinations demonstrate potent efficacy, rifampicin is the most common partner drug of effective combinations against staphylococcal biofilms. Considering the complexity of biofilm-related infections, further studies are needed to assess the activity of new therapeutic agents in combination with antibiotics (quorum-sensing inhibitors, biofilm disruptors and specific anti-biofilm molecules).

Keywords: joint infections, biofilms, osteomyelitis, adhesion, antibiotic resistance

Introduction

Prosthetic joint infection (PJI) following prosthetic joint implantation is a serious complication requiring surgical intervention and aggressive antimicrobial treatment. Infection occurs if bacteria coming into contact with the prosthesis adhere to and colonize the implant and trigger an inflammatory and immune response by the host. The underlying pathogenesis of PJI involves the formation of a bacterial biofilm that protects the pathogen from both the host immune response and antibiotics, making it difficult to eradicate such infections.[1,2] Biofilm formation reflects one of two ways by which bacteria adapt to environmental conditions, the other being to exist as free planktonic cells. Bacteria in biofilms are surrounded by a matrix of bacterial exopolysaccharides and exogenous substances (polysaccharides, proteins, mineral crystals, extracellular DNA). Such biofilms can be formed by both virulent bacteria such as *Staphylococcus aureus* and by opportunistic pathogens such as *Staphylococcus epidermidis*.[3] Planktonic bacteria are susceptible to the action of both specific (antibodies) and non-specific (phagocytes) host defence mechanisms, and are easily eradicated by antibiotics. In contrast, bacteria within biofilms are protected from the host's immune defences as the extracellular material forms a barrier that is relatively impervious to antibodies and phagocytes.[4] Furthermore, phagocytic cells not only penetrate into the biofilm with difficulty but may undergo deactivation.[5] Bacteria in biofilms are also less susceptible to the action of antibiotics, which may contribute to the development of chronic infections and relapses. The diagnosis of biofilm-related infection is not simple and few diagnostic criteria have been proposed.[6]

Conventional *in vitro* susceptibility testing methods are not suitable for biofilm-associated infections

The decreased susceptibility of bacteria in biofilms to antibiotics is a consequence of the penetration barrier that biofilms present to antimicrobial agents.[7] However, many additional factors are involved in this process, including slow rates of bacterial growth, heterogeneity within the biofilm, general stress responses, quorum sensing and induction of a biofilm phenotype. Considering this, the laboratory methods usually used for the determination of bacterial susceptibility to antibiotics (MICs, MBCs) are not appropriate in biofilm-related infections, such as PJI.[8] However, no standard methods are currently approved by CLSI or EUCAST for the evaluation of the efficacy of antibacterials against biofilm.

Defining the problem

Considering that biofilm-embedded bacteria [slow- or non-growing (stationary) state] are 100–1000 times less susceptible to antibiotics than are planktonic bacteria,[9] the treatment of PJIs involving biofilm-forming staphylococci could appear to be

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futile. Indeed, the minimum biofilm-eliminating concentrations (MBECs) of antibiotics commonly used as standard therapeutic options against staphylococci, such as linezolid, vancomycin, daptomycin, teicoplanin and ciprofloxacin, are between 10- and 8000-fold higher than the MICs.[10,11] The ideal anti-biofilm antibiotic should display the following desirable characteristics: bactericidal mode of action, activity against bacteria in stationary phase, no inoculum effect, efficacy against biofilm-producing pathogens and ability to penetrate within slime. Given that no ideal antimicrobials currently exist, the choice of the appropriate treatment for PJIs should be based on available experimental data.

Inhibition of biofilm formation

Bacterial attachment to a surface can be divided into two distinct phases: a primary and reversible adhesion followed by an irreversible adhesion.[12] Based on the immobilization of magnetic beads embedded in bacterial aggregates following biofilm formation in microplates, the Biofilm Ring Test method allows the adhesion ability of bacteria, which is essential for formation of the biofilm complex (Figure 1), to be measured.[13,14] This method has been applied to assess the adhesion ability of *S. aureus* isolates with or without addition of antibacterials at different concentrations. The results indicated that linezolid, vancomycin and daptomycin prevented the initiation of biofilm formation at concentrations close to the MIC when added simultaneously, suggesting a strong inhibition of the initial bacterial adhesion. However, no effect on the entrapment of the beads was observed when the antibacterials were added to a pre-formed biofilm, even with concentrations up to 100 times the MIC. This confirms that inhibition of the early stages of biofilm formation may be an easier target than eradication of bacteria in mature biofilms.

Are antimicrobial agents unable to penetrate biofilms?

The ability of antibiotics to penetrate the biofilm (i.e. the proportion of drug reaching the bacteria) is commonly regarded as a major characteristic correlated with anti-biofilm activity. Using fluorescently labelled daptomycin, the time course for penetration of daptomycin into large, dense clusters of staphylococcal biofilm was evaluated. Daptomycin could readily penetrate thick *S. epidermidis* biofilms with an estimated diffusion coefficient of 28% of its value in pure water.[15] Dunne *et al.*[16] also demonstrated that therapeutic levels of vancomycin and rifampicin can penetrate an artificial staphylococcal biofilm. The same observation was made using an *in vitro* model of prosthesis-related infection in which vancomycin levels (measured by fluorescent polarization immunoassay) exceeded the MIC/MBC for the isolate.[17] These observations are inconsistent with the theory that the biofilm layer constitutes a major physical barrier to the penetration of antibiotics. It is interesting to note that despite effective concentrations of drugs being measured within biofilms, bacterial growth was unaffected and no eradication of bacteria embedded in biofilm was seen, even with high antibiotic concentrations.[16–18] Thus, the lack of activity of antibiotics against biofilm-embedded bacteria seems to be more related to the decreased effect of the drug on the pathogen than to the poor penetration of the drug into the biofilm.

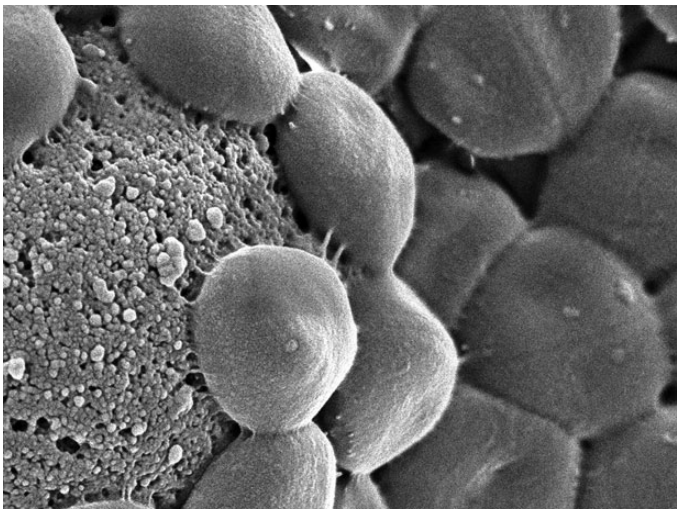

Figure 1. Scanning electron micrograph of *Staphylococcus aureus* cells surrounding a magnetic bead in the Biofilm Ring Test after 6 h of incubation (magnification ×45 000).

Activity of daptomycin and vancomycin in fibrin clots

Evaluation of the antibacterial activity of antibiotics by *in vitro* methods cannot predict the complex interactions observed within the biofilm. Experimental models are not easy to develop. Also, other models have been developed, such as fibrin clots, which represent a complex environment, in which bacteria are able to adhere and form biofilm. The efficacies of daptomycin and vancomycin were evaluated against methicillin-resistant *S. epidermidis* (MRSE) strains in fibrin clots using low and high concentrations (50 and 200 times the MIC).[19] After 24 h of incubation, surviving bacteria were counted and were significantly reduced in all fibrin clots treated with daptomycin, whatever the concentration used. In addition, high concentrations of daptomycin were significantly more effective, as expected with a concentration-dependent drug.

Are combinations of drugs required to achieve antibacterial efficacy in mature biofilms?

In vitro models evaluating the activity of monotherapy showed that eradication of biofilm-embedded bacteria is a very difficult task, especially in mature biofilms. Indeed, bacteria in ageing biofilms have been shown to be less susceptible to antimicrobial agents than those in younger biofilms. Optimal treatment for PJI requires antibiotic combinations, ideally including an agent acting on adhering stationary-phase isolates.[20] Using an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model of biofilm formation to assess antimicrobial activities, Parra-Ruiz *et al.*[21] found that neither moxifloxacin (400 mg every 24 h) nor high-dose daptomycin (10 mg/kg every 24 h) alone exhibited bactericidal activity against staphylococcal biofilms. However, the combination of daptomycin or moxifloxacin with the macrolide antibiotic clarithromycin significantly increased the bacterial killing effect against biofilms produced by staphylococci. This study suggests potent activity for moxifloxacin, as highlighted by work evaluating

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the activity of telavancin and four comparators (vancomycin, teicoplanin, linezolid and moxifloxacin) at concentrations achievable in humans.[22] Moxifloxacin produced the greatest reduction in biofilm cells among all the antibiotics tested against glycopeptide-susceptible isolates (both *S. aureus* and *S. epidermidis*), followed by telavancin against glycopeptide-intermediate *S. aureus* strains. Combination therapy seems to be the only way to achieve eradication of bacteria. Although clarithromycin, cefazolin and vancomycin individually were not able to eradicate *S. aureus* biofilm, destruction was observed with clarithromycin combined with cefazolin or vancomycin.[23] In the same way, the combination of linezolid and daptomycin was superior to each agent alone, suggesting another therapeutic option.[24]

Finally, many studies support rifampicin as a powerful partner agent against staphylococcal biofilm (Table 1). Rifampicin was the most common constituent of antibiotic combinations active against staphylococcal biofilms in an *in vitro* study assessing the activity of many antimicrobials (linezolid, cefazolin, oxacillin, vancomycin, gentamicin, azithromycin, ciprofloxacin and fusidic acid).[8] The same results were observed against *S. epidermidis* biofilms.[25]

In vivo evaluation of biofilm-related infections

Most of the molecular mechanisms involved in the formation of biofilm were elucidated using *in vitro* biofilm models, which are highly valuable for improving our understanding of this complex phenomenon. However, the correlation between *in vitro* and *in vivo* biofilm formation remains poor and *in vivo* models are often necessary to validate the *in vitro* observations.

In vivo studies are scarce compared with *in vitro* studies assessing the activity of antibiotics against staphylococcal biofilms. One explanation is that animal experimental models are difficult to handle, time-consuming and expensive. One interesting approach is a rat model of foreign-body osteomyelitis induced by a methicillin-resistant *Staphylococcus aureus* (MRSA) isolate. Using a titanium wire acting as a foreign body, the investigators showed that neither linezolid nor vancomycin had significant activity against bacteria in the bone surrounding the implant or on the implant itself.[26] Combination therapy was evaluated using the same model with rifampicin as the partner drug for each antibiotic, and treatment initiated 4 weeks after establishing infection and maintained for 21 days was effective.[27] The authors concluded that the combination of either linezolid plus rifampicin or vancomycin plus rifampicin is effective in a rat model of MRSA chronic osteomyelitis. In addition, a rabbit experimental model mimicking a prosthesis infection in humans was developed by Saleh-Mghir *et al.*[28] After 7 days of treatment, both vancomycin and daptomycin were effective in combination with rifampicin, confirming the crucial role played by rifampicin in the treatment of biofilm-associated infections. These *in vivo* data confirm that combination therapy is beneficial in the treatment of infections involving biofilms, such as PJI (Table 1).

Table 1. Major characteristics of antibiotics active against staphylococcal biofilm

Antibiotics	Inhibition of biofilm formation (adhesion)	Biofilm penetration	Bactericidal activity in biofilm
Vancomycin	+	++[16,17]	+[16,17]
Linezolid	+	++[24,29]	+[24]
Daptomycin	+	+++[15]	++[21,24]
Rifampicin	+	+++[8,16,18]	++[16,30]
Moxifloxacin	+	++[31]	++[21,31]
Rifampicin + daptomycin	+	+++[2,30]	+++[28,30]
Rifampicin + vancomycin	+	++[16,18]	++[16,27,32]
Rifampicin + linezolid	+	+++[16,29]	+++[27,32]

Conclusions

Problems with PJI are associated with the presence of biofilm, which plays a central role in the pathogenesis of the infection. Current *in vitro* susceptibility tests fail to effectively assess the ability of antibiotics to kill bacteria embedded in a complex structure such as biofilm. To date, standardized laboratory tests and well-defined parameters are lacking to predict the failure or success of therapy. Given that bacteria growing in biofilms are more tolerant to antimicrobial agents than planktonic cells, the use of effective combination therapies is necessary to eradicate biofilm-producing bacteria. Alternative therapeutic strategies, such as quorum-sensing inhibitors, bacteriophages, interspecies interaction, biofilm disruptors and specific anti-biofilm molecules, should help to fight biofilm-related infections in combination with more conventional drugs, i.e. antibiotics.

Transparency declarations

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