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Orthopaedic device-related infection: current and future interventions for improved prevention and treatment

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- Orthopaedic and trauma device-related infection (ODRI) remains one of the major complications in modern trauma and orthopaedic surgery.
- Despite best practice in medical and surgical management, neither prophylaxis nor treatment of ODRI is effective in all cases, leading to infections that negatively impact clinical outcome and significantly increase healthcare expenditure.
- The following review summarises the microbiological profile of modern ODRI, the impact antibiotic resistance has on treatment outcomes, and some of the principles and weaknesses of the current systemic and local antibiotic delivery strategies.
- The emerging novel strategies aimed at preventing or treating ODRI will be reviewed. Particular attention will be paid to the potential for clinical impact in the coming decades, when such interventions are likely to be critically important.
- The review focuses on this problem from an interdisciplinary perspective, including basic science innovations and best practice in infectious disease.

Keywords: orthopaedic implant infections; osteomyelitis; biofilm; treatment; novel antimicrobials; immunisation; anti-biofilm agents

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Introduction

Orthopaedic and trauma device-related infection (ODRI) remains a major complication in modern trauma and orthopaedic surgery.[1] Despite best practice in medical and surgical management, neither prophylaxis nor treatment of ODRI is effective in all cases, and can lead to infections that negatively impact clinical outcome and significantly increase healthcare expenditure.[2] Pre-operative and correctly-timed prophylactic antibiotic intervention is mandatory for a majority of orthopaedic procedures. However, despite this, the incidence of infection following elective orthopaedic surgery is in the range of 0.7% to 4.2%,[3-7] while the incidence can be much higher in trauma cases where infection rates range from approximately 1% after operative fixation of closed low-energy fractures, to more than 30% in complex open tibia fractures.[8,9] Treatment success rates vary, with between 57% and 88% often reported.[10-12] Current curative approaches (radical debridement, revision surgery and prolonged antibiotic therapy) often result in significant socioeconomic costs, not to mention the risk of life-long functional impairment for the patient. Against this background, and with the increasing issue of antibiotic-resistant bacteria, the problem of ODRI is set to continue to pose a challenge for practising clinicians in the coming decades.

The clinical and microbiological challenges of modern device-related infections

The most prevalent species in ODRIs are Staphylococci.[13-17] *Staphylococcus (S.) aureus* accounts for between 20% and 30% of cases of infection after fracture fixation and prosthetic joint infections (PJI), with coagulase-negative staphylococci (CoNS) accounting for 20%–40% of cases,[13-16] including small colony variants.[18] Other Gram-positive cocci including Streptococci (1%–10%) and Enterococci (3%–7%) are less frequently encountered. Infections caused by Gram-negative bacilli, including *Pseudomonas aeruginosa* and Enterobacteriaceae account for approximately 6%–17%,[13-17] and anaerobes (including Propionibacteria and Peptostreptococci) are comparatively rare at approximately 4%–5%.[13-17] Shoulder ODRIs, however, may have higher *Propionibacterium (P.) acnes* prevalence, at up to 38%.[19]

Table 1. Biggest challenges in the diagnosis and treatment of ODRI

1.Proof of infection and detection of disease-causing pathogen
A considerable number of infections are 'culture-negative' despite being clinically apparent.[20,21] In some cases, the causative bacteria are difficult to grow because they exist in a metabolically less active state as a biofilm. Bacteria may also be difficult to culture when the patient has been empirically treated with antibiotics.[21] In such cases, cessation of all antibiotic therapy for at least two weeks, followed by open biopsy of tissue and sonication of the device, may offer additional opportunity to culture the organism.[21]
This raises the question: can we do better with diagnosis? Establishing the correct diagnosis with a new test would represent a major breakthrough in the field. Similarly, rapid, non-invasive diagnostics and those offering pre-operative diagnostics have the potential to change medical and surgical treatment without requiring invasive biopsies.
2. Antimicrobial resistance
Multi drug-resistant organisms are becoming increasingly challenging to treat over time. Many reports now exist of pan drug-resistant organisms and extensively drug-resistant pathogens such as vancomycin-resistant *S. aureus* and *Enterococcus* spp.
3. Persistence and recurrence of infection
One of the major challenges with treatment of a device-associated infection is the reimplantation of the device, which in most cases is required for the function of the patient. The issue is that organisms frequently reside in a biofilm state that is not usually completely eradicated or resected during the explantation phase. The biofilm tends to be harboured on tiny fragments of necrotic bone known as sequestra, and may also reside within the cortical bone itself. During reimplantation, the biofilm-residing bacteria may be liberated and re-enter their planktonic growth phase, resulting in reinfection. This remains one of the great challenges in infection surgery.

Recently, more attention has been focussed upon polymicrobial infections, which may account for 10%-20% of cases.[13,14,17] Furthermore, studies using molecular diagnostic techniques indicate that, in addition, there is a significant proportion (5%-34%) of culture-negative infections.[13,20,21]

Antibiotic resistance

Infections caused by antibiotic-resistant pathogens are a major public health concern, and their treatment can be challenging.[22] With reference to ODRI, bacteria resistant to the few antibiotics with proven anti-biofilm activity (Rifampicin-resistant staphylococci and ciprofloxacin-resistant Gram-negatives) are among the most difficult pathogens to treat. Methicillin-resistant *S. aureus* (MRSA) has also emerged as a significant threat in both the hospital and community environment.[23] Within the healthcare setting alone, MRSA infections are estimated to affect more than 150000 patients annually in the European Union (EU), resulting in additional in-hospital costs of EUR 380 million for EU healthcare systems.[24] Between 25% and 32% of infections after fracture fixation in the United States are caused by MRSA,[25,26] but this is highly dependent on the local epidemiology, with lower rates also observed. With limited treatment options, MRSA infections are associated with a higher mortality and increased financial costs relative to sensitive equivalents.[10,27-30] However, this has not been a universal finding.[31] Recent publications on PJIs stated that treatment decisions should focus more on the identified pathogen, and not merely on its methicillin resistance.[32]

The rise of antimicrobial resistance is one of the major challenges in the treatment of ODRIs; however, there are also many other challenges (Table 1).

State-of-the-art treatment for orthopaedic device-related infection

Systemic antibiotic therapy

The goal of any medical strategy for the treatment of ODRI should consist of the long-term elimination of pain, restoration of function of the affected joint and, in trauma cases, consolidation of the fracture with prevention of osteomyelitis. Usually this includes a therapeutic approach aiming for definite eradication of the micro-organisms causing infection, but in some circumstances can entail long-term suppressive antibiotic therapy. Hence, each treatment must be tailored to the needs and the medical conditions of the individual patient.

To date, a curative therapy always includes surgery, since antibiotics alone are not capable of eradicating biofilm infections. The surgical approach varies from debridement with retention of the prosthesis to one-stage or two-stage exchange procedures. In fracture care, the chosen operative intervention often depends on the grade of fracture healing. An algorithm for choosing the optimal procedure has been proposed,[6,33] but there are still substantial differences in procedural preferences between countries and institutions. Nevertheless, the therapeutic approach should always be decided by an interdisciplinary team comprised of orthopaedic surgeons and infectious disease specialists and/or microbiologists.

High-quality evidence on the choice of antibiotics is scarce. Therefore, therapeutic decisions are often based on retrospective data, on pharmacokinetic/pharmacodynamic principles and on results from animal models. The optimal antibiotic should reach high bactericidal concentrations in the organic and inorganic bone tissue, on the surface of the device and in intracellular compartments. It should be active against slow-growing biofilms and against the metabolically quiescent small colony variants. It should have a low propensity to induce bacterial resistance and low toxicity towards the patient. In each case, it is essential to know which bacteria are responsible for the infection. Hence, antibiotics should be withheld until appropriate diagnostics have been performed. Mounting evidence shows that routine susceptibility tests that determine the minimal inhibitory concentration (MIC) do not reflect the real-life susceptibility of the biofilm-embedded bacteria on the surface of the device; antibiotic susceptibility in biofilms can be reduced a thousand-fold.[34] Therefore, even when bacteria are reported as sensitive to an antibiotic, clinicians should

Table 2. Summary of targets required for improvement of treatment outcomes in ODRI

Systemic antibiotic therapy	Local antibiotic therapy
• Improved diagnostic methods to predict bactericidal activity against biofilm-embedded bacteria	• Introduction of guidelines for local delivery (antibiotic agent selection made on a species and resistance status)
• Evidence for timing of antibiotic switching (parenteral *vs* oral) and duration of treatment	• Establishment of pharmacodynamic principles applicable to local delivery
• New antibiotics with increased anti-biofilm activity	• Design of local delivery vehicles that attain pharmacodynamic principles
• Better oral formulations and drugs with less toxicity	• Biomaterials that can accommodate a wider range of antibiotics

be aware that this does not reflect the ability of the antibiotic to kill the same bacteria when growing in a biofilm.

The best evidence for antibiotic selection is available for staphylococci. For other bacteria (such as streptococci, enterococci, Gram-negatives) the evidence for antibiotic selection is less clear. Rifampicin is of critical importance in the treatment of staphylococci as an anti-staphylococcal biofilm antibiotic, and has been associated with a higher rate of treatment success.[35-37] Rifampicin should never be administered by itself due to rapid development of resistance. The initial partner antibiotic most often consists of a beta-lactam and later switched to a quinolone (historically ciprofloxacin, nowadays often levofloxacin).[38] In case of quinolone resistance, various other antibiotic partners have been used such as fusidic acid,[39,40] cotrimoxazole,[41] linezolid,[42] clindamycin or minocycline.[31,43] In the case of rifampicin resistance, alternative antibiotics are chosen, with one study showing good results with moxifloxacin monotherapy.[44] Alternatives to beta-lactams, for example in the case of methicillin-resistant staphylococci, are vancomycin or daptomycin,[45] both of which are generally well-tolerated.

Great variability in total duration and the time point of the switch[46] from intravenous to oral antibiotics exists between different countries and hospitals. Guidelines recommend between two and six weeks of initial intravenous therapy, according to the circumstances.[33] An early switch to oral antibiotics does not seem to be associated with a worse outcome.[47] The total duration of therapy is usually between three and six months. Nevertheless, a duration of six weeks may be sufficient.[48-50] Long-term suppression therapy is used alternatively in cases of inoperable patients, multi drug-resistant bacteria,[51] but also in specific fracture cases where consolidation of the fracture has not yet occurred and the surgical treatment consistent of debridement with implant retention.[52] On the other hand, successful experiences from single centres with a very short duration of systemic antibiotic therapy of less than one week, or solely intra-articular application of antibiotics, have been reported.[46,53]

There are still a lot of open questions to be answered (Table 2) and high-level evidence studies are urgently needed to overcome these gaps in knowledge.

Local antibiotic delivery

The use of biomaterials as carriers, or vehicles, for the delivery of antibiotic agents to the site of infection has become a regular adjunct in the treatment of ODRIs.[54,55] Local delivery has numerous theoretical advantages over systemic delivery, which can offer the potential for significant supportive antimicrobial action. Since the antibiotic is placed directly at the site of interest, an intact vascular system is not required to reach the surgical site, which may be particularly beneficial in trauma patients. Local delivery can also achieve local concentrations exceeding those achievable systemically, while requiring a lower total drug amount, thereby not only improving the local concentration, but simultaneously reducing the risk of systemic toxicity. Interestingly, the local application of antibiotics has even been shown in preclinical *in vivo* studies to offer protection against bacteria that are resistant to the applied antibiotic,[56] indicating that local delivery may offer some hope for further improvements in antibiotic therapy in the face of bacteria resistant to conventional, systemic dosing regimens.

The local application of antibiotics in orthopaedic medicine has been described since the 1970s, when gentamicin-loaded bone cement was first tested in humans.[57] Bone cement was a convenient vehicle for antibiotic delivery, as it was routinely applied in cemented arthroplasties. Gentamicin was identified as a suitable antibiotic due to the fact that it was found to withstand the elevated temperatures of curing bone cement, and was considered to offer an acceptable profile against the most common pathogens associated with ODRI. Antibiotic-loaded bone cements have been shown to improve ODRI outcomes.[58,59] Bone cement, however, was not designed in the first instance as an antibiotic delivery vehicle. Therefore, the usual pharmacodynamic principles governing systemic antibiotic therapy were not part of the equation in the advent of antibiotic-loaded bone cements. Unfortunately, despite the passage of more than four decades since the first use of antibiotic-loaded bone cements, pharmacodynamic principles are still not established specifically for use in this way. Therefore, it is perhaps not surprising that resistance against gentamicin has emerged secondary to gentamicin use in local delivery vehicles.[60,61] The reason for the development of resistance is probably the prolonged release of antibiotics at sub-therapeutic levels from local delivery vehicles, which is in direct opposition to ideal release kinetics for a concentration dependent antibiotic such as gentamicin.[62]

There are antimicrobial-loaded device surfaces and coatings which have passed through the regulatory approval process, have been described in clinical studies,[63-65] and may be expected to emerge in greater

Table 3. Outline status of novel interventional strategies targeting ODRI

Ionic silver	Active and passive immunisation for *S. aureus*	Antimicrobial peptides and immunomodulatory peptides	Quorum sensing inhibitors and biofilm degrading enzymes
Research status and gaps in the knowledge			
• Widely-studied antimicrobial, particularly in the experimental preclinical phase • Comparative studies against conventional antibiotic agents are required • A full understanding of the risk factors for the emergence of silver resistance is required	• Has been the focus of industrial research strategies for decades • Needs full understanding of the immune response against *S. aureus* to make real progress • Needs full understanding of the nature of the *S. aureus* antigen(s) and antibody response • Needs to identify appropriate patient populations for evaluating vaccine efficacy	• Thousands of peptides described from a wide range of sources • Potential target for antibiotic-resistant infections • Toxicity at high concentrations a concern	• Require advancement through the preclinical translational research pathway • *In vitro* and early *in vivo* studies show promise • Debate over whether resistance against these compounds can develop is still ongoing
Current clinical application and future outlook			
• Currently available for limited number of orthopaedic devices • Clinical data promising, with specific application in the most challenging cases • **Outlook**: Role in antibiotic-resistant cases, or for coverage of multiple species	• Numerous clinical trials ongoing • To date, no trial shown efficacy in terms of reduced incidence • **Outlook**: Great potential for treating most challenging cases, where even optimal antimicrobial strategies have high failure rate	• Not yet applied in orthopaedic setting • Currently only available for topical application • **Outlook**: Future role for antibiotic-resistant isolates	• Not yet clinically applied • Still early stage of translation • **Outlook**: Potential novel approach, particularly important for resistant biofilm infections

numbers in future. However, a number of critical issues must be resolved prior to achieving the maximum benefit of local antibiotic delivery vehicles (Table 2).

New approaches for prevention and treatment

Active and passive vaccines

Based on its cost-effectiveness, which is unparalleled by any other medical intervention, vaccination is an obvious approach to prevent, treat and potentially eradicate ODRIs. Unfortunately, all efforts to develop an effective vaccine against *S. aureus*, the primary pathogen involved in ODRI, have failed for a number of reasons (Table 3).[66-68] The most prominent reason is that, in contrast to successful bacterial vaccines, which to date have exclusively been against transient flora, *S. aureus* has co-evolved with mammalian hosts to become a human commensal. Thus, all patients have some level of acquired immunity against *S. aureus* prior to surgery. However, the protective *versus* susceptible nature of an individual's immune response against *S. aureus* at this time is virtually unknown. Therefore, a major research focus in targeting the immune response is understanding the functional role of specific T cells (cellular immunity) and antibodies (humoral immunity) in *S. aureus* infections. To this end of vaccine development, several groups have described anti-*S. aureus* immune responses in physiological and pathological situations,[69-75] in order to elucidate the immune proteome of *S. aureus*.[76] Recently, a multiplex immunoassay for characterising a patient's immune response was developed against 14 known *S. aureus* antigens, which was then used to determine if certain antigens dominate humoral immunity in a pilot study of patients with osteomyelitis versus uninfected controls.[75] Measurement of the immune response against *S. aureus* may help guide future prophylaxis and therapy in an era of personalised medicine, and follow-up research is ongoing.

S. aureus is primarily an extracellular pathogen. Thus, its clearance from within mammalian hosts is largely dependent on neutrophils.[77] Importantly for vaccine development, this innate immune mechanism has been modeled by the opsonophagocytic activity assay (OPA), which has been used to quantify *S. aureus* killing *in vitro*.[78] However, antigen-specific T-helper cells are critically involved in antibody responses, and it is known that Th17 cells enhance neutrophil function and bacterial clearance.[79] Thus, although the role of adaptive immunity for protection against *S. aureus* remains controversial, there is a rationale for a human vaccine. For the most part, the molecular targets of *S. aureus* vaccines that have been developed so far have been pathogenic determinants (i.e. clumping factor A, ClfA[80]) and virulence factors (i.e. alpha-toxin[81] and coagulases[82]). Unfortunately, this strategy is limited by great redundancy, as *S. aureus* contains a multitude of factors with similar pathogenic function. Thus, neutralising all of them to decrease pathogenicity seems unlikely. Alternatively, interests have focussed on *S. aureus* autolysin (Atl), which comprises highly conserved aminidase (Amd) and glucosaminidase (Gmd) subunits. Functionally, Atl is known to be essential for cell wall biosynthesis and degradation during binary fission.[83-85]. Atl also functions as an adhesin,[86] a biofilm enzyme,[87] which was identified as a potential molecular target of vancomycin[88] and has been reported to interfere with the production of antibodies in mice.[89] Moreover, Amd and Gmd are immune-dominant antigens in mice and humans,[75,90] and pre-clinical vaccine studies have demonstrated significant efficacy.[91,92]

The most common vaccines involve 'active' immunisation of the host with purified molecular constituents of the

pathogen, and require the host to evolve protective adaptive immunity for this non-virulent challenge. An advantage of active vaccines is the robustness of the resulting immunity, which includes both cellular and humoral immunity, and the potential of life-long immunity from the generation of protective memory T cells and B cells. However, the greatest limitation of active vaccination is its unpredictability in individual patients, particularly immune-compromised individuals from those with established comorbidities (i.e. ageing, autoimmunity, obesity and diabetes).[93-96] Thus, it is not surprising that the two most recent large clinical trials with active *S. aureus* vaccines (StaphVAX (polysaccharide capsular antigens CP5 and CP8),[97] and V710 (IsdB)[98]) failed to meet their primary endpoints. However, what was very surprising was that V710 vaccination was associated with increased sepsis, multi-organ failure and death in patients undergoing heart valve replacement who developed *S. aureus* infections,[98] which is consistent with the finding that high titres of anti-IsdB antibodies are associated with these adverse events in total joint arthroplasty patients.[75] This observation raises a new concern that some anti-*S. aureus* immune responses exacerbate infection and/or its sequelae, and that additional pre-clinical testing is needed to confirm a vaccine's mechanism of action. It also supports transfusion of purified functional anti-*S. aureus* antibodies as a passive immunisation, which is a safer and more predictable vaccine approach. However, it should be noted that passive *S. aureus* vaccines such as Altastaph,[99] Veronate,[100,101] Aurexis,[80,102] Aurograb[103-104] and Pagibaximab,[105,106] have also failed in clinical trials.

Silver

The significant difficulties involved in the treatment of established biofilms prompted research on engineering device surfaces that could resist microbial colonisation. Silver is a potent candidate for coating devices, as it provides a broad spectrum of antibacterial activity against planktonic and sessile, Gram-positive and Gram-negative, and also multi drug-resistant bacteria.[107] Moreover, it demonstrates bactericidal efficacy at a low concentration, with limited toxicity towards human cells. Silver attacks a broad range of bacterial targets by interfering with thiol and amino groups of proteins, with nucleic acids and cell membranes. The disruption of iron-sulphur clusters seems to be particularly detrimental for the affected organism, producing reactive oxygen species and inhibiting the respiratory chain.[108-110]

Silver has been used as a disinfectant for many centuries.[111] From the 19th century onwards, silver was employed, among other uses, in the prevention of gonorrhoeal ophthalmia (Crédé prophylaxis), as suture material, or as ointment to treat wound infections.[111,112] Currently, technological advances have created many new formulations of silver, which are either still under development, or already deployed for commercial and medical purposes. Silver is used in its metallic form as a nanoparticle, or silver-containing polymers and composites.[113,114] For orthopaedic applications, silver has been introduced into hydroxyapatite and bone cement, and as a coating for trauma devices.[115] Most formulations exert good antimicrobial properties. Nevertheless, the heterogeneity of materials and methods make direct comparison of the antimicrobial effect difficult. Recently, new compounds called silver oxynitrate ($Ag(Ag_3O_4)_2NO_3$ or Ag_7NO_{11}) showed a better effect against bacterial biofilms than other formulations (Ag_2SO_4, $AgNO_3$, silver sulfadiazine (AgSD), AgO, Ag_2O).[116]

Primary clinical studies are promising, demonstrating a trend in reducing infection with silver-coated central venous catheters,[117] urinary catheters[118] and ventilator endotracheal tubes.[119] Similar results were achieved with silver-coated external fixation pins,[120] proximal femur or tibia megaprostheses[64] and tumour prostheses.[121]

One of the major concerns associated with the use of an antimicrobial substance is the development and spread of resistant mutants. Indeed, development of resistance to silver was reported in relation to *P. aeruginosa* as early as 1966.[122] Thereafter, many publications have demonstrated widespread occurrence of silver resistance in Enterobacteriaceae, but interestingly never in Gram-positive bacteria. These data strengthen the notion that the concerted action against intracellular silver is so far neither known to be inherent nor inducible for Gram-positive bacteria, which makes silver coatings controversial for clinical use.

The toxicity of silver to eukaryotic cells has been another concern.[112] However, the health risk in exposed humans seems to be low, and consists mostly of a discolouration of the skin and eyes due to silver deposition called argyria and argyrosis, respectively.[123-125] Nevertheless, few case reports exist of neural or other systemic toxicity after high exposure to silver.[126,127] In this context, the potent new silver formulations should be tested in solid *in vitro* and *in vivo* toxicity studies. Accordingly, the potential of osseointegration of silver-coated prostheses needs further exploration. However, the available evidence in this respect is encouraging.[128] Finally, one of the biggest hurdles in designing a silver-coated surface is the controlled release of silver. Data on silver release kinetics are mostly lacking, but crucial for defining the optimal clinical application. With further development, knowledge and optimisation of formulations, silver seems a promising addition to our antibacterial arsenal in the fight against device infection.

Antimicrobial and anti-biofilm peptides

Antimicrobial peptides (AMPs) are innate defence molecules of animals, plants and microorganisms, with a broad spectrum of antimicrobial activity and low risk of resistance development in general.[129,130] The low risk of resistance development is due to the fact that AMPs interact with microbial membranes, resulting in membrane depolarisation, destabilisation and/or disruption leading to rapid cell death, or passing of the membrane to reach intracellular targets.[131] Native AMPs have been used as design templates for a large variety of synthetic AMPs, some of which have now reached the stage of phase 2 and 3 clinical trials.[132]

Several AMPs also have the capacity to prevent biofilm formation. A recent study by Mansour et al[133] demonstrated that a synthetic peptide (named 1018) inhibited biofilm formation by *S. aureus* and multiple other species by blocking (p)ppGpp, an important signal in biofilm development, at concentrations that did not affect bacterial growth. A peptide derived from CRAMP (the mouse homologue of the human defence peptide LL-37 (cathelicidin), showed inhibition of biofilm formation of the yeast *Candida albicans*, and also prevented biofilm formation by different bacterial species.[134] Many more examples of AMPs with anti-biofilm activity have recently been listed in the specialised biofilm-active antimicrobial peptides (BaAMPs) database.[135]

Application of AMPs to biomaterials

Immobilisation of AMPs on surfaces has been performed with a variety of peptides, and many different chemistries. A good overview of immobilisation strategies has been published by Costa et al.[136] For peptides to be effective after immobilisation, they should retain the structural characteristics important for their antimicrobial activity. Other decisive factors for success are length, flexibility, and kind of spacer connecting the peptide to the surface, the AMP surface density and the orientation of the immobilised peptides.[136] Although peptides are considered to be active through insertion into the microbial membranes, even short surface-attached peptides, which are unlikely to have a free interaction with the membrane, have antimicrobial activity.[137] This activity is thought to be due to destabilisation of the membrane by displacement of positively charged counter-ions, changing bacterial surface electrostatics and activating autolytic enzymes or disrupting the ionic balance.[137]

Chemical procedures of tethering AMPs to surfaces may cause a strong decrease in their antimicrobial activity or even inactivation,[138,139] depending on the combination of peptides and immobilisation technology. A recent, novel approach of attaching peptides to hydrogels used for surface coating is the application of thiol-ene chemistry allowing a fast, single-step immobilisation strategy.[140] Using this strategy, mimics of the HHC-10 peptide[141] with optimal plasma stability were attached to a polymer surface. These surfaces killed inocula of *S. aureus, S taphylococcus epidermidis* and *Escherichia coli* with high efficiency *in vitro*.[140]

Controlled release coatings for orthopaedic and trauma devices, for example, are designed to provide a burst release of an antimicrobial agent during the first days after implantation, preferably followed by a continuous release providing local protective levels during several weeks after implantation. The incorporation of AMPs in such coatings has not yet been extensively developed. In a recent study, a polymer lipid encapsulation matrix (PLEX) coating designed for doxycycline release from a bone filler[142] was tailored to such a preferred release profile. The doxycycline-PLEX coating prevented osteomyelitis caused by *S. aureus* in a rabbit model.[56] Based on these studies, PLEX coatings containing the novel AMPs were recently developed successfully. These coatings show potent antimicrobial activity, prevent biofilm formation and prevent *S. aureus* infection of subcutaneous implants in mice (Zaat et al[143]).

Quorum-sensing inhibitors and biofilm-degrading enzymes

Quorum sensing (QS) is a mechanism that many microorganisms use to coordinate gene expression in populations in response to local conditions, including cell density.[144] The canonical QS system consists of one or more proteins involved in producing and transporting the signalling molecule, the actual signalling molecule, a receptor for the signalling molecule and, in some QS systems, additional regulatory proteins.[144] The most-studied systems are those that use *N*-acyl homoserine lactones (AHL) as signalling molecules (present in many Gram-negative bacteria, including *P. aeruginosa*) and the QS system in *S. aureus* in which auto-inducing peptides (AIP) are used as signalling molecules.[144] In many organisms, biofilm formation is (co-) regulated by QS, making the latter process an interesting target for novel approaches to antimicrobial chemotherapy in biofilm infections such as ODRI.[144,145] In addition, it is well-known from early work in this field that, in at least some microorganisms, QS is involved in tolerance to antimicrobial agents and the immune system.[146,147] These observations suggested that combining a conventional antimicrobial agent with a quorum-sensing inhibitor (QSI) might circumvent the problem of biofilm tolerance.

Experimental evidence for this approach has been provided in several studies in which it was shown that combining antibiotics with QSI increased the success of treatment in different model systems. This was true for various organisms (including *S. aureus* and *P. aeruginosa*) and for different antibiotic/QSI combinations (including the combination vancomycin/hamamelitannin against *S. aureus* and tobramycin/furanone C-30 against *P. aeruginosa*).[148,149] While the QSI described in the literature are extremely diverse in structure, they can be grouped according to their target. A first approach to inhibit QS is the enzymatic degradation of the AHL signalling molecules, by using specific AHL lactonases or acylases produced by bacteria.[145] Also paraoxonases found in human serum and expressed in various cell types can degrade AHLs.[145] A second group of QSIs target the synthesis of the signal molecule. From studies investigating the role of QS-related genes in biofilm formation, we know that mutant strains in which genes involved in the synthesis of the signalling molecule(s) are inactivated, are affected in biofilm formation. This is, for example, the case in *Burkholderia cenocepacia cepI* and *ccil* mutants (both CepI and CciI are AHL-synthases)[150] and in *S. aureus* mutants that are defective in producing AIP.[151] Considering the biosynthesis pathway of AHLs, inhibitors of *S*-adenosylmethionine and fatty acid biosynthesis (including sinefungin and 5-methylthioadenosine) may be used as QSIs.[145] Less is known about QSI targeting AIP synthesis,

although inhibitors of the type-I signal peptidase SpsB that reduce AIP production have been described.[151] Finally, compounds targeting the QS receptors and/or signal analogues can act as QSI. Many AHL analogues (with modifications in the acyl side chain, the central amide moiety, and/or the lactone ring) have been synthesised and tested, and many of these interfere with the process of biofilm formation. For example, application of AHL in which the central amide moiety was replaced by triazolylhydrofuranones resulted both in biofilm inhibition and biofilm eradication in a number of Gram-negative pathogens, including *P. aeruginosa*.[152] One of the most-studied QSIs with activity against *S. aureus* also targets the QS receptor: the RNAIII-inhibiting peptide (RIP), several of its analogues and the non-peptide analogue hamamelitannin are thought to interfere with the RAP/TRAP QS system in *S. aureus*, and by doing so to affect biofilm formation and increase biofilm susceptibility towards antibiotics.[148,151] So far most of the studies on QSIs as anti-biofilm agents have been carried out using *in vitro* model systems, or in simple *in vivo* models.[148,153] In a limited number of studies, QSIs were tested using animal models, for example in a mouse model for pulmonary infection (with *B. cenocepacia*)[148] or for skin infection (with *S. aureus*).[154] However, to our knowledge, testing of QSIs in an appropriate animal model for orthopaedic device-associated biofilm infections has not yet been done, although several foreign body models mimicking biofilm infections on prosthetic devices are available.[155,156]

A second innovative anti-biofilm strategy depends on the use of biofilm-degrading enzymes, and both deoxyribonuclease I (DNase I) and exopolysaccharide-degrading dispersin B (DspB), which could have applications in the prevention or treatment of biofilm infections associated with orthopaedic devices.[157,158] Extracellular DNA (eDNA) is a key component of many microbial biofilms, and the use of DNase I leads to the disruption of pre-existing biofilms in many species, as well as an increased susceptibility to antimicrobial agents.[159] In addition, biofilm formation is inhibited in some species by the presence of DNase I.[159] However, this is not the case for all bacteria tested, and the effect on pre-existing biofilms is also species and biofilm age-dependent.[159] DspB is a β-hexosaminidase capable of degrading poly-(β-1,6)-*N*-acetylglucosamine, an exopolysaccharide that is an important component of the biofilm matrix in various organisms.[157,158] Application of DspB resulted in biofilm dispersal and detachment, and when combined with conventional antimicrobial agent, DspB showed synergism.[157,158,160] In the context of PJIs, it is interesting to see that DspB overall has good activity against staphylococcal biofilms[157,158] and that its activity is maintained *in vivo* (at least in a subcutaneous implant model for *S. aureus* infections in a rabbit).[160] In addition, DspB-loaded coatings were shown to inhibit *S. epidermidis* biofilm formation *in vitro*, without affecting the attachment or growth of cultured human osteoblasts, suggesting that such coatings hold promise for developing medical devices with anti-biofilm properties.[161]

Summary and outlook

ODRI remains one of the most challenging complications in orthopaedics. A wide range of treatment options are available, although the established guidelines and algorithms have improved standardisation and outcomes. However, improvements in preventative and therapeutic strategies are required, as current practices are not completely effective. This is particularly critical considering the increasing challenge of antibiotic-resistant bacteria.

Emerging technologies and interventions may be expected to improve treatment success in the future (Table 3). Crucially, research strategies have focussed on antibiotic resistance and biofilm formation as targets for future interventional strategies. These interventions have the potential to reduce infection rates and improve treatment outcomes, if and when these interventions make it to clinical practice. Few regulatory body-approved antibiotic-functionalised orthopaedic and trauma devices are currently available; however this may yet grow in the coming decades, provided they pass a robust preclinical evaluation and emerge onto the market with a proven ability to improve outcome in the prevention and treatment of ODRIs.

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CONFLICT OF INTEREST

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