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SYSTEMATIC REVIEW

Current therapeutic interventions combating biofilm-related infections in orthopaedics

A SYSTEMATIC REVIEW OF IN VIVO ANIMAL STUDIES

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Aims

Biofilm-related infection is a major complication that occurs in orthopaedic surgery. Various treatments are available but efficacy to eradicate infections varies significantly. A systematic review was performed to evaluate therapeutic interventions combating biofilm-related infections on in vivo animal models.

Methods

Literature research was performed on PubMed and Embase databases. Keywords used for search criteria were "bone AND biofilm". Information on the species of the animal model, bacterial strain, evaluation of biofilm and bone infection, complications, key findings on observations, prevention, and treatment of biofilm were extracted.

Results

A total of 43 studies were included. Animal models used included fracture-related infections (ten studies), periprosthetic joint infections (five studies), spinal infections (three studies), other implant-associated infections, and osteomyelitis. The most common bacteria were Staphylococcus species. Biofilm was most often observed with scanning electron microscopy. The natural history of biofilm revealed that the process of bacteria attachment, proliferation, maturation, and dispersal would take 14 days. For systemic mono-antibiotic therapy, only two of six studies using vancomycin reported significant biofilm reduction, and none reported eradication. Ten studies showed that combined systemic and topical antibiotics are needed to achieve higher biofilm reduction or eradication, and the effect is decreased with delayed treatment. Overall, 13 studies showed promising therapeutic potential with surface coating and antibiotic loading techniques.

Conclusion

Combined topical and systemic application of antimicrobial agents effectively reduces biofilm at early stages. Future studies with sustained release of antimicrobial and biofilm-dispersing agents tailored to specific pathogens are warranted to achieve biofilm eradication.

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Keywords: Biofilm, Fracture-related infection, Periprosthetic joint infection, Orthopaedic infection

Article focus

- In this review, we analyzed current animal models, interventions, and outcome measures of biofilm-related bone infections in vivo.
- We elucidated the research gap in effective methods and therapies for the eradication of biofilm-related bone infections, and provided insight for future experimental designs targeting biofilm in animal studies.

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Key messages

- Recent findings on the dynamic evolution from biofilm formation, and maturation to distant repopulations, will facilitate the design of targeted therapy.
- Given the limited effects of systemic mono-antibiotics, a combined local and systemic therapy is recommended to achieve effective biofilm reduction.
- For established biofilms, a tailored release with matrix-dispersing, antimicrobial, and bone-forming agents loaded on biodegradable hydrogels would potentially eradicate infection and facilitate bone regeneration.

Strengths and limitations

- The current study provides a comprehensive overview of animal model development and biofilm characterizations in different orthopaedic scenarios, current therapeutic interventions, and effectiveness for biofilm-related bone infection research.
- Due to the heterogeneity of the interventions and outcome measures, only qualitative analysis was performed.

Introduction

Device-related infections (DRIs) are a major concern in orthopaedic surgery. Despite tremendous efforts to reduce the risk, these events still occur. The rate of periprosthetic joint infection (PJI) is present in 1% to 2% of all cases.[1] The increasing number of open fractures in aged patients also adds to the risk of fracture-related infection (FRI),[2,3] which occurs in 4% to 52% of Gustilo Anderson grade III[4] fractures with concomitant soft-tissue damage.[5] Based on different diagnostic techniques and heterogeneous patient populations, the risk for implant-associated spinal infection ranged from 0.5% to 10%.[6] One of the critical issues in the clinical treatment of DRI is biofilm formation, which leads to antibiotic tolerance, infection recurrence,[1,7] and poor clinical outcomes. Biofilm-related infections often cause prolonged disability, recurrent hospital admissions, and even patient mortality, triggering a huge care burden. The estimated cost per patient reaches USD $17,000 to $150,000.[8]

Biofilm is defined as clusters of microorganisms that are adhered to biological or non-biological surfaces, often encased in an outer polymer layer.[9] Mediated by quorum-sensing signalling systems, bacteria cells undergo orchestrated biofilm formation, maturation, release of virulent factors, and dispersal in a population-based manner.[10,11] In a mature biofilm, the non-growing bacteria cells and high-density extracellular polymeric substances (EPSs) cause antibiotic tolerance and persistent infection.[12] In clinical settings, the success rate of debridement, antibiotics, and implant retention (DAIR) for acute PJI reaches 92.3%, but only 44.4% for late-onset infections.[13] Comparatively, the success rate of DAIR is 86% to 100% for acute FRI, but only 67% for late-onset FRI.[14] The major difficulty is biofilm formation, which often requires debridement, implant exchange/cement spacer for PJI,[15] and external fixation for FRI.[3] Despite two-stage strategies, reinfection rates reach 8.4% and 16.2% for hip and knee arthroplasties, respectively.[16] Given the progressive nature of biofilm-related infection and low success rate of treatment at late stages, one study has suggested that the best possible treatment is to inhibit bacteria attachment and prevent biofilm maturation at the beginning.[17]

Therefore, research and development of biomaterials targeting biofilm-related infection have been of great interest in the recent decade. Various techniques to prevent DRI, including titanium and copper alloy implants, antimicrobial surface coatings, antimicrobial agent(s) loaded scaffold, or hydrogel have been reported.[18–20] However, emerging cases of antibiotic-resistant strains, polymicrobial infections, and biofilms pose new challenges.[21,22] To our knowledge, numerous studies have reported successful eradication of biofilm in vitro, however there is a lack of validation on clinically relevant animal models before translation.[23–25] The purpose of this study was to summarize relevant animal models, bacterial strains, evaluation of biofilm and bone infection, complications, key findings, observations, prevention and treatment of biofilm to provide information for development of novel treatments, and future clinical translation.

Methods

Search strategy. PubMed and Embase (date last accessed 1 February 2022) were searched. Keywords used for search criteria were “bone AND biofilm”.

The inclusion criteria were: 1) preclinical studies; 2) use of animal models; and 3) study on biofilm-related DRI. The exclusion criteria were: 1) lack of analysis or evaluation of biofilm ex vivo; 2) in vitro study; 3) review article; 4) abstract or conference paper; and 5) non-English article.

Selection of studies was based on the evaluation of biofilm.[9] Eligible studies must include the confirmation of in vivo biofilm formation by visualization or imaging techniques, and the quantification of biofilm mass and colony-forming unit (CFU) load. Two independent reviewers (JL, RMYW) screened all titles and abstracts, and performed the selection from the search results based on inclusion and exclusion criteria. Each article was reviewed with any disagreement resolved by discussion until consensus was met.

Data extraction and analysis. For eligible studies, the two reviewers extracted information on: 1) species and strains of the animal; 2) bacteria strain; 3) animal model characteristics; 4) evaluation of biofilm and bone infection; 5) complications; and 6) key findings on observational study, prevention of biofilm formation, and treatment of biofilm. Due to the large heterogeneity in

animal models and methodology, a qualitative review was performed.

Results

Characteristics of the papers. A total of 1,681 and 2,122 studies were identified from Embase and PubMed, respectively. After removal of duplicated entries, 2,071 records remained. Each title and abstract were reviewed, and 1,969 records were excluded based on criteria. Upon detailed review of the full text, an additional 59 studies were excluded: in vitro study of antibiotics, bone substitutes, biomaterials, and other agents (n = 39); lack of explicit biofilm evaluation (n = 18); and studies on the dental bone (n = 2). Finally, a total of 43 studies published from 1998 to 2021 (Table I and Table II) were included in our systematic review (Figure 1).[11,26–67]

Animals. Mice were used in 15 studies including eight with C57BL6,[11,26–32] eight with BALC/c,[11,30,33–37,65] and CD 1 mice and NOD/ShiLtJ mice in one study.[38] Rats were used in 16 studies, including ten using Sprague-Dawley rats,[39–47,67] five using Wistar rats,[48–51,66] and one with no specified strain.[52] The other models were developed with New Zealand rabbits in six studies,[53–58] pigs in four studies,[59–62] and sheep in two studies.[63,64]

Bacteria strains. *Staphylococcus aureus* was used in 25 studies, methicillin-resistant *S. aureus* (MRSA) in ten studies, and methicillin-resistant *Staphylococcus epidermidis* (MRSE) in two studies. Other staphylococcus strains used included *Staphylococcus lugdunensis*[55] and *S. epidermidis RP62A.*[37] Bioluminescently engineered strains including *S. aureus Xen 29*, *Xen 31*, and *Xen36* were applied to allow continuous monitoring of bacteria load.[11,26,30,31,33,34,40,46] An additional gram-positive species was *Propionibacterium acnes*,[55,65] which is now reported as *Cutibacterium acnes*. Gram-negative strains included *Pseudomonas aeruginosa*,[31,52] and various strains of *Escherichia coli*,[28,31,47] among which one study developed a polymicrobial infection model with *S. aureus* and *E. coli.*[28]

Bacteria isolated from a patient with PJI were used in one study, and strain characterization based on polymerase chain reaction (PCR) was performed to identify the *C. acnes* strain *LED2* and *S. lugdunensis 010729.*[55] One study reported genetic confirmation of *C. acnes* isolated from an implant, showing that the bacteria strain could survive for over six months.[65] Additionally, one study used *agr* gene mutant *S. aureus (UAMS-1)*Δ agr,[11] and showed that biofilm formation was *agr*-dependent. Another study found that MRSA with antisense yycG overexpression had reduced biofilm formation and pathogenicity in a rat osteomyelitis model.[45]

Animal models. Animal models were developed to mimic FRI in ten studies,[29,32–34,36,47,50,56,63,64] PJI in five studies,[31,38,46,51,68] implant-associated spinal infection model (IASI) in three studies,[53,57,60] and other implant-associated bone infections. The healing in FRI was monitored by radiograph analysis,[32,33,56,64] micro-CT,[32–34,50,56,63,64] and bone histology.[32,36,47,50,56] The anatomical location of infection included the femur in 20 studies, tibia in 15 studies, spine in three studies,[53,57,60] and both the knee and hip joint in five studies.[31,38,46,51,68] A total of 41 studies used implants, including intramedullary nails, screws, or pins in 14 studies,[26,28,29,32,35,39,40,44,47,49,54,55,59,62] plates and screw fixation,[33,34,36,41,50,56,63,64] joint prostheses,[27,31,46,51,68] pedicle screws or rods,[53,57,60] transcortical pins,[30] and other implants.[11,37,38,42,43,48,52,65–67] Among the studies, 33 used planktonic bacteria solution for inoculation in bone tissue or implant, and ten used precultured bacteria on the implant, three of which showing biofilm formation on the implant or collagen sheet.[33,43,56] In another two studies, infections were performed by injection of *S. aureus* into the femoral artery or a hole in the tibia.[45,61]

Evaluation of biofilm and bone infection. A total of 36 studies performed quantification of CFU load on tissue or implant. Biofilm was visualized by scanning electron microscopy (SEM) in 31 studies, confocal laser scanning microscopy (CLSM) in six studies,[30,41,43,45,54,56] and light microscope with crystal violet staining in another six studies.[29,39,42,50,54,55] Other imaging techniques included radioactive emission CT (ECT) for quantifying bacterial load through measuring the concentrated ^{99m}Tc radioactivity,[39] FDG-PET/CT imaging,[57] and fluorescent microscope for monitoring bioluminescent-engineered bacteria strains. Radiological assessments to monitor bone lysis included radiograph analysis in ten studies,[26,27,31–33,39,51,53,56,64] micro-CT in 18, and MRI in one.[46] Staining methods for bacteria identification included Giemsa stain[36,54] and gram stain.[32,34,37,38,40,45,49,50] Commonly used histological assessments were haematoxylin and eosin (H&E) to observe inflammation in 21 studies, and tartrate-resistant acid phosphatase (TRAP) staining to evaluate osteoclastogenesis in three studies.[34,37,40] Serum and local inflammatory markers include white blood cell count,[38,50] CRP,[38] amyloid,[27] interleukin-10 (IL-10), tumour necrosis factor-α, IL-1α, and IL-1β.[32,37,43] Local inflammation was also observed with positron emission tomography (PET) imaging and flow cytometry cell sorting.[31,36]

Complications. Complications were documented in five studies.[32,38,43,46,53] One study reported one dislocation and one loose implant among eight animals with hip PJI infection.[46] Zhu et al[43] documented that three of 60 rats with implant-associated tibia infection died from swelling and white purulent secretion at the wound. Hazer et al[53] showed that four of 14 rats with IASI had pus formation localized in the fascia plane. Cahill et al[32] reported two mice with FRI dead within 24 hours post-surgery, with no cause specified. Lovati et al[38] showed that two out of 32 animals with diabetic PJI demonstrated severe signs of infection, including joint abscess and fistulae.

Basic observational study findings. A total of 12 studies were observational without interventions. Two studies showed atypical implant-associated infection at a lower grade and lack of osteolysis caused by *C. acnes* and *S. epidermidis RP62A.*[37,55] In the study by Nishitani et al,[11] the natural history of biofilm maturation was revealed

Table I. Summary of the study characteristics.

Study	Animal	Model features	Bacteria strain and inoculation methods	Clinical scenarios	Intervention	Systemic antibiotics	Treatment duration	Biofilm evaluation
Li et al[39]	SD rats	Osteomyelitis (femur Intramedullary nail with infection)	*MRSA* pre-cultured on the implant	Implant associated infection	Magnesium implant	None	None	1. CV staining 2. FESEM 3. RTPCR: icaA, agr RNAIII expression
Hazer et al[53]	14 New Zealand rabbit	Pedicel screw related infection in the lumbar spine	Planktonic *MRSA* at 10^6	Implant associated spinal infection	Plyethylene glycol grafted, Polypropylene-base silver nanoparticles	None	None	1. CFU counting 2. SEM
Yao et al[67]	50 SD rat	Titanium rod inserted into femur	Planktonic *S. aureus ATCC25923* at 10^6	Implant associated infection	enoxacin -loaded mesoporous silica nanoparticles	None	None	1. SEM 2. live and dead staining 3. CFU counting
Li et al[54]	56 New Zealand white rabbits	Intramedullary nail inserted in tibia	Planktonic *MRSA* at 10^6	Implant associated infection	Mg-Cu with 0.25% Cu implant	None	None	1. CFU plating 2. CLSM 3. FESEM 4. CV staining 5. RT-PCR: biofilm-associated genes including atlE, clfA,
Min et al[40]	Adult SD rats	Implant inserted at tibia	Planktonic *S. aureus Xen 29*. at 10^5	Implant associated infection	Implant coating with sequential release of gentamicin and BMP-2	None	None	1. SEM 2. CFU counting
Gracia et al[66]	36 Wistar male rats	Metal inserted into femur	*S. aureus strain ATCC 29213 biofilm* at 10^9 pre-cultured on implant	Implant associated infection	None	21 day systemic antibiotic treatment using cefuroxime, vancomycin, or tobramycin	None	CFU counting in blood, bone tissue, and implant
Tran et al[41]	SD rats	Plate and screw fixation at femur	Planktonic *MRSA* and *MRSE* at 10^5	Implant associated infection	Selenium nanoparticle coating	None	None	1. CFU counting 2. CLSM (*in vivo*)
Ashbaugh et al[26]	C57BL/6 mice	Intramedullary K-wire fixation at femur	Planktonic *S. aureus Xen36* at 10^3	Implant associated infection	Electrospinning of PLGA and PCL with Vancomycin, Rifampin, linezolid and daptomycin.	None	None	1. SEM 2. bioluminescent monitoring of S. aureus. 3. CFU counting
Carli et al[27]	20 C57BL/6 mice	Tibia-implant insertion (arthrotomy)	Planktonic *S. aureus Xen36*	PJI	Vancomycin-loaded polymethylmethacrylate spacers	None	None	1. SEM
Inzana et al[33]	13 to 15 weeks BALB/cJ mice	6-hole polyether ether ketone plate with titanium coating at femur	*S. aureus Xen36* pre-cultured on a Collagen sheet at 10^4	FRI	Rifampin and Vancomycin laden calcium phosphate scaffolds (CPS)	None	None	1. SEM 2. CFU counting
van der Horst et al[42]	85 Female SD rats	A titanium wire wrapped high-density polyethylene and chrome cobalt at femur	Planktonic *S. aureus 25923* at 10^6	Implant associated infection	None	Systemic ceftriaxone for 5 or 10 days	Tobramycin, gentamycin, rifampin, vancomycin	1. CV staining 2. CFU counting
Greimel et al[48]	61 Male Wistar rats	Intravenous catheter implanted into femur	Planktonic *S. aureus 29213 at 10^8*	Implant associated infection	None	Rifampin (Rif); flucloxacillin (Flu); flu+ Rif; moxifloxacin (Mox); Mox+ Rif	2 weeks antibiotics treatment starts from day 7	CFU counting
Zhu et al[43]	60 Adult SD rats	A titanium rod inserted into the tibia	*S. aureus* 25,923 biofilm cultured on implant at 20 h	Implant associated infection	Human β-defensin 3	None	None	1. Live and dead bacterial viability assay assessed by CLSM
Jensen et al[59]	25 Pigs	A k-wire inserted at the proximal tibia	Planktonic *S. aureus S54F9 spa type t1333* at 10^4	Implant associated infection	None	None	Single dosage in 2X 160, to160,000 times MIC Gentamycin injected locally	1. Immunohistochemical staining 2. CFU counting
Boles et al[28]	C57BL/6 mice	K-wire inoculated with bacterial inserted into femur	Planktonic *S. aureus* (UAMS-1) and *E. Coli (ATCC 25922)* at 10^4	Implant associated infection	Chitosan sponge loaded with amikacin and vancomycin	None	None	1. CFU counting
Singh et al[60]	5 Yorkshire pig	Surgical implantation of a titanium rods in spinous process	Planktonic *MRSA* solution at 10^6	Implant associated spinal infection	Negative pressure wound therapy with antiseptic instillation	None	None	1. Tissue culture 2. SEM
Kandemir et al[52]	26 Rats	A silicone drain inserted in the medullary canal	Planktonic *Pseudomonas aeruginosa* solution at 10^8	Implant associated infection	None	Subcutaneous injection of Ceftazidime and Clarithromycin	None	1. CFU counting 2. SEM
Inzana et al[34]	Female BALB/cJ mice	Transverse osteotomy at femur fixed with polyether ether ketone (PEEK) plate	*S. aureus Xen36* pre-cultured on implant overnight	FRI	Polymethyl methacrylate spacer loaded with vancomycin	Vancomycin subcutaneous injection	None	1. SEM 2. CFU counting 3. Gram staining
Fang et al[49]	60 Wistar rats	18 G needle inserted into the femur bone marrow cavity	Planktonic *S. aureus (MSSA BCRC10451)*	Implant associated infection	Nano-particle-induced hyperthermia therapy combined with vancomycin therapy	Systemic vancomycin for 40 days	Vancomycin injection into the cavity for 40 days	1. SEM 2. CFU counting 3. Cango red staining 4. Gram staining (biofilm in bone)
Tomizawa et al[35]	125 BALB/c rats	Stainless-steel implant inserted at the tibia	*S. aureus (UAMS-1)* pre-cultured on implant overnight	Implant associated infection	None	cefazolin, gentamycin, and vancomycin with or without rifampin for 14 days started from 0, 3, 7 days	None	1. SEM 2. CFU counting
Lindsay et al[29]	58 Male C57BL/6 j mice	gauge needle fixation at femur with osteotomy	Planktonic *S. aureus 29,213*	FRI	None	Oral antibiotics (cephalexin) for 14 days	Metalloporphyrin Antioxidant (MnTE-2-PyP) injection start one-day post-surgery	1. CFU counts

Continued

Table I. Continued

Study	Animal	Model features	Bacteria strain and inoculation methods	Clinical scenarios	Intervention	Systemic antibiotics	Treatment duration	Biofilm evaluation
Jørgensen et al[30]	65 Female C57B6 mice	A pin inserted transcortically in the tibia metaphysis	*S. aureus Xen29* and *S. aureus Xen 31* pre-cultured on implant	Implant associated infection	None	Systemic injection of Vancomycin (110 to 180 mg/kg) for 14 days	None	1. SEM 2. Live/dead staining; CLSM 3. CFU counting 4. bioluminescent monitoring
Shiono et al[65]	18 BALB/c mice	A hole drilled at distal femur with or without an implant	Planktonic *Propionibacterium acnes* at 10^6	Implant associated infection	None	None	None	1. SEM imaging 2. Fluorescent bacterial probe detection
Thompson et al[31]	Male C57BL/6	K-wire inserted into the femur	Planktonic *Pseudomonas aeruginosa Xen 41*, *E. coli Xen 14*, *E. coli ATCC25922*, *E. coli ATCC K12* at 10^5	PJI	Bispecific antibody MEDI3902 targeting Biofilm related antigens PcrV and Psl	None	None	1. bioluminescent imaging 2. SEM
Nishitani et al[11]	C57BL/6 and BALB/c mice	L shaped implant inserted to the tibia	*S. aureus (UAMS-1, Xen 40, agr gene mutant UAMS-1Δagr)* pre-cultured on implant	Implant associated infection	None	None	None	1. SEM 2. bioluminescent imaging 3. CFU counting and PCR for bacteria load quantification
Gahukamble et al[55]	New Zealand white rabbits	Intramedullary nail fixation	Planktonic *Propionibacterium acnes LED2* (from a clinical isolate) and *Staphylococcus lugdunensis* at 10^7	Implant associated infection	None	None	None	1. bacteria molecular analysis 2. SEM (bacteria in medullary canal) 3. CFU counting
Lovati et al[50]	24 Wistar rats	Osteotomy at the femur fixed with plate and screw	Planktonic *MRSE GOI1153754-03-14* at 10^3 10^5 10^8	FRI	None	None	None	1. CFU counting 2. Gram staining 3. SEM
Johansen et al[61]	7 Yorkshire-Landrace-cross pig	Femur artery injection	Planktonic *S. aureus S54F9*, *S. aureus NCTC-8325 to 4*, *S. aureus UAMS-1* at 10^4	Hematogenous osteomyelitis	None	None	None	1. CFU counting 2. IHC staining for S. aureus 3. Fluorescent *in situ* hybridization
Zhang et al[56]	24 New Zealand rabbits	Plate and screw fixation with osteotomy	*Staphylococcus. aureus 25,923* biofilm pre-cultured on implant	FRI	None	None	None	1. SEM of the plate 2. CLSM
Cahill et al[32]	C57BL/6 j mice	Tibia fracture fixed with pin	Planktonic *MRSA USA 300* at 10^6	FRI	None	Systemic injection of vancomycin and rifampin for 3 days started on day 0	local injection rifampin in hydrogel	1. CFU counting 2. Gram staining
Marston et al[44]	SD rat	Intramedullary pin within distal femur	Planktonic *S. aureus* at 10^4	Implant associated infection	None	Systemic ceftriaxone treatment for 4 weeks	tobramycin or doxycycline powder placed on pin and soft tissue	1. CFU counting 2. SEM
Wei et al[51]	32 Wistar rat	A screw inserted at the knee	Planktonic *MRSA* (*ATCC BAA-1026*) at 10^8	PJI model	None	Vancomycin with IP injection for 14 days	intra-articular injection for 14 days	1. CFU counting 2. SEM
Lovati et al[38]	32 CD 1 mice and NOD/ShiLtJ mice	Gauge inserted into femur	Planktonic *S. aureus ATCC 25923* at 10^5	PJI in diabetic patients	None	None	None	1. SEM 2. CFU counting
Hu et al[47]	12 SD rat	Intramedullary Neil inserted into femur	*MSSA ATCC 25923*, *Escherichia coli ATCC 25922* pre-cultured on the implant	Fracture related infection	TaON-Ag Nanocomposite coated titanium	None	None	1. SEM imaging
Stewart et al[64]	9 Sheep	Tibia osteotomy fixed with a titanium plate	Planktonic *S. aureus at 10^6*	Fracture related infection	Vancomycin modified AEEA-AEEA-APTS-Ti surface	None	None	1. SEM imaging 2. CFU counting 3. Live and dead staining
Tomizawa et al[37]	49 BALB/c female mice	L shaped rod inserted into the tibia	Planktonic *S. aureus USAA300 LAC* and *S. epidermidis RP62A* at 10^5	Implant related infection	None	None	None	1. CFU counting 2. SEM 3. Gram staining
Schaer et al[63]	16 Sheep	Compression plate fixation after tibia osteotomy	Planktonic *S. aureus ATCC 25923* at 10^6, 10^8,10^{11}	Fracture related infection	hydrophobic polycation N, N- dodecyl,methyl-PEI (PEI 1⁄4 polyethylenimine) coated surface	None	None	1. SEM 2. CFU counting
Gordon et al[57]	14 Rabbit	Pedicel screw and titanium plate	Planktonic Community-acquired *MRSA strain SAP231* at 10^4,10^5,10^6	Implant associated spinal infection (IASI)	None	None	None	1. SEM 2. bioluminescent imaging 3. CFU counting
Hadden et al[46]	8 Sprague-Dawley rats	cemented hemiarthroplasty	Planktonic *Staphylococcus aureus Xen36* at 10^8	PJI related infection	None	None	None	1. Field emission scanning electron microscopy 2. In vivo luminescent imaging 3. Tissue culture
Windolf et al[36]	Balb/c mice	Osteotomy at the femur with plate fixation	Planktonic *Staphylococcus aureus ATCC 29213*	FRI	None	None	None	1. SEM 2. CFU counting 3. Giemsa staining
Wu et al[45]	10 Sprague Dawley rats	A hole in tibia	Planktonic *MRSA* ASyycG over-expression, and *MRSA ATCC29213*	Osteomyelitis	None	None	None	1. 647-labelled dextran conjugate, and SYTO9 labeling; CLSM 2. SEM 3. Gram staining 4. FISH
Hovis et al[58]	18 New Zealand rabbits	Plate and screw fixation at the tibia	Planktonic *MRSA* at 10^8	Implant associated infection	None	None	125 mg vancomycin powder applied direct to the implant	1. CFU counting 2. SEM imaging

Continued

Table I. Continued

Study	Animal	Model features	Bacteria strain and inoculation methods	Clinical scenarios	Intervention	Systemic antibiotics	Treatment duration	Biofilm evaluation
Blirup-Plum et al[62]	9 pigs	A K-wire insert into tibia	Planktonic *S. aureus S54F9* at 10^4	Implant associated infection	Injectable ceramic bone graft substitute loaded with gentamycin and debridement surgery	Intramuscular injection of gentamycin	None	1. Immunohistochemistry staining for biofilm 2. CFU counting

CFU, colony-forming unit; CLSM, confocal laser scanning microscope; CV staining, crystal violet staining; ELISA, enzyme-linked immunosorbent assay; FESEM, field emission scanning electron microscopy; FISH, fluorescence in situ hybridization; FRI, fracture-related infection; IHC, immunohistochemistry; LC/MS, liquid chromatography-mass spectrometry; MRSA, methicillin-resistant *Staphylococcus aureus*; PEI, polyethyleneimine; PJI, periprosthetic joint infection; PLGA, poly(lactic-co-glycolic acid); PMMA, polymethyl methacrylate; ROS, reactive oxygen species.

by SEM, including an initial attachment on day 1, robust proliferation on day 3, maturation with increased matrix on day 7, and dispersal after day 14 (Figure 2). The authors also showed that bacteria migration from biofilm was *agr*-dependent. Johansen et al[61] developed a haematogenous osteomyelitis model by injecting *S. aureus* into the femoral artery and found biofilm formation in the bone tissue.

Prevention effects on biofilm formation. A total of 26 papers studied the preventive effects of various techniques on biofilm formation (Figure 2). Antibiotic treatment for durations of three,[32] five,[42] ten,[42] 14,[29,30,35] 21,[66] 28,[44] and 40 days[49] were used in different studies for the prevention of biofilm formation. Accordingly, serum concentrations of vancomycin or tobramycin were measured in three studies,[44,51,58] including one by liquid chromatography-mass spectrometry (LC-MS).[44] Only one study quantified the concentration of gentamycin in bone tissue.[62] Biomaterials and antibiotic-loaded carriers were applied in 16 studies, of which only one study applied systemic antibiotic treatment as a control group.[34]

Tomizawa et al[35] found that gentamycin and vancomycin could reduce the bacteria load of *S. aureus* when starting on day 0, which was enhanced in combination with rifampin. Cahill et al[32] showed that a higher dosage of topical rifampin reduced MRSA biofilm load, which was enhanced with systemic rifampin application. By loading amikacin and vancomycin on a chitosan sponge, one study found synergistic bactericidal effects against polymicrobial biofilm infections caused by *S. aureus* and *E. coli*.[28] Jensen et al[59] showed that a single dose of gentamycin, at least 1,600 × minimum inhibition concentration (MIC) value, was required to prevent *S. aureus* attachment to bone implants. van der Horst et al[42] showed that topical aminoglycoside and systemic ceftriaxone could eradicate *S. aureus* biofilm. Hovis et al[58] showed successful infection prevention in all nine rabbits with implant-associated MRSA infection by the local spreading of vancomycin. One study showed more significant CFU reduction of *S. aureus* effects of topical tobramycin than doxycycline.[44] Clarithromycin was found to enhance the bactericidal ability of ceftazidime in reducing the biofilm caused by *P. aeruginosa*.[52] Among the ten studies on the effects of systemic mono-antibiotic therapy, only two of six using vancomycin showed significant biofilm reduction,[30,32,35,49,51,66] and one of two studies using tobramycin showed significant biofilm reduction.[66] None of these studies reported eradication. Two studies showed a significant reduction effect with ceftriaxone on implant, but one found no effects on CFU on bone.[42,44] For the three studies on topical mono-antibiotic therapy, including vancomycin, tobramycin, and doxycycline, only one showed eradication,[58] while the other two studies showed no significant reduction.[42,44]

Inzana et al[33] showed that 3D-printed rifampin and vancomycin-laden calcium phosphate scaffold (CPS) reduced culture-positive rate to 50%. An electrospun composite coating composed of poly (lactic-co-glycolic acid) PLGA nanofibres and poly-ɛ-caprolactone (PCL) was shown to deliver multiple antibiotics, including vancomycin, rifampin, linezolid, and daptomycin to eradicate MRSA biofilm in vivo.[26] Stewart et al[64] first showed the vancomycin-modified aminoethoxyethoxyacetate (AEEA)-AEEA-aminopropyltriethoxysilane (APTS)-Ti surface technique in a commercially available titanium plate, and found significant biofilm inhibition effects in a sheep model with FRI. Another study also showed that N, N-dodecyl, methyl-PEI coating prevented biofilm formation, and supported healing in a sheep FRI model.[63] Li et al[39] found that magnesium implant reduced biofilm formation through downregulation of the transcription levels of icaA, agr ribonucleic acid (RNA) III, and other virulence and antibiotics-associated genes.[54] Min et al[40] showed that sequential release of gentamicin and BMP-2 eradicated biofilm and promoted bone healing. Additionally, silver and selenium nanoparticles were shown to inhibit biofilm on pedicle screws and femur implants.[41,53]

For the specific pathogens, antibody MEDI3902 targeting *P. aeruginosa* PcrV and Psl exopolysaccharide were found to decrease biofilm by ten-fold load.[31] A novel antibiotic agent, human *β*-defensin, was shown to reduce MRSA biofilm in vivo by regulating inflammation and immune responses.[43] Singh et al[60] showed that negative-pressure wound therapy decreased CFU and biofilm formation. Fang et al[49] showed that magnet nanoparticles-induced hyperthermia enhanced the biofilm eradication rate compared with systemic vancomycin treatment. Lindsay et al[29] showed that reactive oxygen species (ROS) scavenger treatment improved oral antibiotic treatment on bacteria clearance.

Treatment effects on established biofilm. Six articles showed that treatment effects on established biofilms started at three to seven days after planktonic inoculation or revision surgery (Figure 2).[27,34,35,48,51,62] Notably, Tomizawa et al[35] found that gentamycin reduced biofilm load on day 3, and none of the antibiotics groups showed

Table II. Key findings of each study.

Study	Key findings	Other assessments conducted
Li et al[39]	1) Mg was highly effective against MRSA-induced osteomyelitis and improved the peri-implant bone formation. 2) The antibiofilm effects of Mg were achieved by reducing bacterial icaA and agr RNAIII transcription levels.	1. X-ray99mTc radioactivity emission CT 2. Micro-CT 3. CFU plating
Hazer et al[53]	1) PP-g-PEG-A g grafted pedicle screw showed antimicrobial effect and inhibit biofilm formation. 2) Complications: 4 of 14 rats had pus formation localized in the fascia plane.	1. Radiograph 2. H&E staining of muscle tissue
Yao et al[67]	The current study provides a novel biomaterial in preventing *Staphylococcus aureus* related implantation infections and bone loss.	1. Micro-CT 2. H&E and TRAP staining 3. Drug release study 4. In vitro antimicrobial study
Li et al[54]	The Mg-Cu alloy showed antibacterial ability demonstrated by microbiological and biofilm formation assays with reduced expression of biofilm, virulence, and antibiotic-resistant genes.	1. Radiograph 2. Bone histology: H&E and Giemsa staining
Min et al[40]	The rapid release of antibiotics and sustained release of BMP-2 successfully eradicated the biofilm and accelerated bone tissue formation.	1. Bioluminescent monitoring 2. Micro-CT 3. Pull-out tensile test 4. Histology: H&E, Masson's trichome, TRAP, and gram staining
Gracia et al[66]	Cefuroxime significantly reduced the bacteria load on bone and K-wire, which was consistent with the antimicrobial effects of 48 hours biofilm in vitro.	Serum antibodies against *S. aureus*
Tran et al[41]	The nanoparticles coating strongly inhibited biofilm formation on the implant and reduced the number of CFU in the surrounding tissue.	1. In vitro characterization of nanoparticles 2. In vitro antimicrobial properties 3. Biocompatibility test
Ashbaugh et al[26]	The polymeric coating can be applied to deliver various antibiotics to prevent biofilm-associated orthopaedic infection by varying the PLGA versus PCL ratios.	1. Radiograph 2. Micro-CT 3. Sanderson's and acid fuchsin counterstaining
Carli et al[27]	The antimicrobial effects of PMMA spacers fail to eradicate periprosthetic joint infection in the clinically representative mouse model.	1. Radiograph 2. Serum amyloid A
Inzana et al 2015[33]	Co-delivery of rifamycin and vancomycin from 3D-printed CPS significantly reduced the bacteria burden but cannot fully eradicate the biofilm on implant.	1. Bioluminescent imaging 2. Radiograph and micro-CT
van der Horst et al[42]	1) 5 days daily injection significantly reduced CFU but cannot eradicate. 2) 10 days of systemic ceftriaxone and local gentamicin showed complete clearance.	None
Greimel et al[48]	1) Only mono rifampin can significantly reduce the biofilm on the implant but not the bone and soft tissue. 2) Mox plus rif or Flu plus rifamycin showed significant reduction in CFU in bone, soft-tissue and biofilm, and Mox + rif showed eradication of biofilm on implant, but not on bone tissue.	None
Zhu et al[43]	β-defensin 3 inhibits the bacterial growth by regulating inflammatory and immune response in the MRSA-induced implant biofilm infection. Complications: 3 rats died with swelling and white purulent secretion on the wound.	1. ELISA test: IL-10, TNF-α, IL-1α, and interferon-γ 2. IHC staining of NF-κB and TLR-4
Jensen et al[59]	1,600 times of MIC is required to prevent the bacteria attachment, indicating that susceptibility in intro may not reflect in vivo susceptibility.	1. Bone 2. H&E staining
Boles et al[28]	Chitosan loaded vancomycin and amikacin (5 mg/ ml) showed higher percentage of clearance rate, which can be further augmented by double the serum concentration.	None
Singh et al[60]	NPWTi therapy is associated with decreased bacterial load and biofilm formation compared to wet-to-dry wound dressing.	None
Kandemir et al[52]	Clarithromycin enhanced the activity of concomitantly used bactericidal agents by destroying the biofilm formation.	None
Inzana et al 2015[34]	PMMA-loaded vancomycin only showed significant effect of decreasing the bacterial burden and osteolysis when combined with systemic antibiotics in a revision model.	1. Bioluminescence imaging 2. Histology: ABH/Orange G, TRAP 3. Micro-CT
Fang et al[49]	1) Systemic application of vancomycin did not eradicate the biofilm infection. 2) Magnet nanoparticles combined with local administration of vancomycin enhance the eradication of bacteria in the biofilm-based colony.	1. H&E staining 2. Micro-CT
Tomizawa et al[35]	Combination with rifampin is recommended to inhibit implant associated osteomyelitis, due to the limited effects of monotherapy, especially cefazolin.	None

Continued

Table II. Continued

Study	Key findings	Other assessments conducted
Lindsay et al[29]	The *S. aureus* biofilm is redox-sensitive and ROS scavenger treatment improved the efficacy of antibiotic treatment on bacteria clearance.	1. Crystal violet staining 2. Nitroblue tetrazolium assays
Jørgensen et al[30]	1) This model is suitable for testing antimicrobial agent treatment as both biofilm and CFU can be assessed. 2) 14 days vancomycin injection was unable to eradicate biofilm infection. 3) Key research gap: despite clear dosage, the serum vancomycin levels cannot be monitored.	None
Shiono et al [65]	The presence of an implant is essential for the development of delayed surgical site infection model.	1. Myeloperoxidase activity 2. Bone histology 3. Genetic confirmation of *C. acnes* by PCR
Thompson et al[31]	1) In vitro biofilm-producing activity was associated with the in vivo gram-negative bone infection characterized by bacteria infection, biofilm formation reactive bone changes and inflammatory cells infiltration. 2) Biospecific antibody-targeting *Pseudomonas aeruginosa* virulence factors reduced the bacteria burden in vivo.	1. Radiograph imaging 2. Bone histology 3. PET imaging 4. Flow cytometry
Nishitani et al[11]	1) This study showed the *S. aureus* attachment, proliferation and maturation from day 0 to day 7. 2) Biofilm dispersal was achieved by *S. aureus* migration in an agrin-dependent way, as presented with empty lacunae and retention of few culture-negative, RNA-positive residual bacteria.	None
Gahukamble et al[55]	1) *C. acnes* and *S. lugdunensis* infection model caused different clinical presentations, including low-grade infection in *C. acnes* and acute infection in *S. lugdunensis*. 2) This model is relevant to the periprosthetic joint infection and nonunion fracture fixation, which may be reported as aseptic failure.	1. H&E staining 2. Modified Brown and Brenn staining
Lovati et al[50]	1) The severity of osteomyelitis signs and nonunion rate was dosage-dependent. 2) This study provides a relevant preclinical model for subclinical infections in orthopaedic trauma.	1. WBC count 2. Micro-CT 3. H&E staining 4. CV staining
Johansen et al[61]	Bacteria embedded in the opaque biofilm matrix was demonstrated by FISH in a haematogenously spread osteomyelitis model.	1. Blood culture for bacteremia 2. Micro-CT 3. H&E staining
Zhang et al[56]	This study provides a novel rabbit model of infection following internal fixation with biofilm formation.	1. Radiograph and micro-CT 2. H&E staining
Cahill et al[32]	Local application of high-dosage rifampin-loaded hydrogel reduced the bacteria load, which was further enhanced when combined with systemic rifampin application. Complications: two rats died within 24 hours postoperatively with no specified course.	1. Radiograph and micro-CT 2. H&E staining 3. Immunohistochemistry for IL-1β, p-p65, Sox9, Runx2
Marston et al[44]	Local tobramycin showed more significant CFU reduction than doxycycline in the synovium, supporting the current evidence of local application of antibiotics	1. Monitoring the serum antibiotics concentrations by LC/MS 2. Bone histology
Wei et al[51]	IA injection is superior to systemic injection, whereas the combined treatment can eradicate the infection in the two-week course after revision surgery.	1. Serological analysis of vancomycin 2. Radiograph and micro-CT 3. H&E staining
Lovati et al[38]	Diabetic mice challenged with a single inoculum of *S. aureus* displayed severe osteomyelitis changes and biofilm formation on implant. Complications: two mice had severe signs of infection including joint abscess and fistulae.	1. Serum white blood cell and CRP 2. Micro-CT analysis 3. H&E staining 4. Gram staining
Hu et al[47]	TaON-Ag nanocomposite coated titanium inhibited pathogen adhesion and biofilm formation in both *S. aureus* and *Escherichia coli* in vivo.	1. H&E staining 2. Radiograph 3. In vitro antibacterial assay

Continued

Table II. Continued

Study	Key findings	Other assessments conducted
Stewart et al[64]	Vancomycin-derivatized plate surfaces inhibited implant colonization with *S. aureus* and supported bone healing in an infected large animal model.	1. Calcified tissue staining 2. Radiograph 3. Micro-CT
Tomizawa et al[37]	Biofilm-producing *S. epidermidis* RP62A does not cause prominent osteolysis, reactive bone formation, but persists in biofilm, stimulates a low-grade proinflammatory environment, and inhibits osseous integration.	1. Micro-CT: bone healing 2. H&E staining 3. TRAP staining 4. Proinflammatory cytokines transcriptome in tibia
Schaer et al[63]	The presence of a N, Ndodecyl,methyl PEI coating on the surface of a metal implant was effective in eliminating the clinical signs of infection, preventing biofilm formation and support bone healing.	1. Von Kossa staining 2. Safranin O staining 3. Micro-CT
Gordon et al[57]	This rabbit model could serve a valuable preclinical model of IASI to study the pathogenesis and novel diagnostic and therapeutic methods, which allows for real-time monitoring of bacteria burden and inflammation.	1. FDG-PET/CT imaging 2. Micro-CT analysis
Hadden et al[46]	The study showed a new, high-fidelity model of in vivo PJI using cemented hip hemiarthroplasty in rats. Complications: One rat with prosthesis dislocation and one with implant loosening.	1. Gait analysis 2. MRI 3. Micro-CT
Windolf et al[36]	Implant-associated localized osteitis in murine femur fracture by biofilm-forming *S. aureus* was established, with increased leucocyte count and IL-6 levels	1. Lavage: cell sorting by flow cytometry, IL-6 expression 2. H&E staining
Wu et al[45]	The overexpression of ASyycG leads to a reduction in biofilm formation and bacterial pathogenicity in vivo.	1. qPCR: inflammatory markers and biofilm-related genes 2. H&E staining 3. Micro-CT
Hovis et al[58]	Vancomycin spreading at the infection site successfully prevents infection of the bone and implant in all cases.	1. H&E staining 2. Serum vancomycin measurement
Blirup-Plum et al[62]	The injectable ceramic bone graft substitute loaded with gentamycin cannot be used as a standalone alternative to extensive debridement or be used without the addition of systemic antibiotics.	1. CT scanning 2. FISH 3. Bone histology: H&E and Masson-trichome staining 4. Gentamycin concentrations

ABH, alcian blue/haematoxylin; BMP-2, bone morphogenetic protein 2; CFU, colony-forming unit; CPS, calcium phosphate scaffold; CV, crystal violet; ELISA, enzyme-linked immunosorbent assay; FDG, fluorodeoxyglucose; FISH, fluorescence in situ hybridization; H&E, haematoxylin and eosin; IA, intra-articular; IASI, implant-associated spinal infection; IHC, immunohistochemistry; IL, interleukin; K-wire, Kirschner-wire; LC/MS, liquid chromatography-mass spectrometry; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; NF-κB, nuclear factor kappa B; NPWTi, negative pressure wound therapy with instillation; PCL, poly-ϵ-caprolactone; PCR, polymerase chain reaction; PEI, polyethyleneimine; PET, positron emission tomography; PJI, periprosthetic joint infection; PLGA, poly(lactic-co-glycolic acid); PMMA, polymethyl methacrylate; ROS, reactive oxygen species; TLR-4, toll-like receptor 4; TNF-α, tumour necrosis factor-alpha; TRAP, tartrate-resistant acid phosphatase.

any effects when starting on day 7. Inzana et al[34] showed that vancomycin loaded on polymethyl methacrylate (PMMA) spacers only showed positive effects when combined with systemic vancomycin in a revision model for FRI. Similarly, Carli et al[27] showed that vancomycin-loaded PMMA failed to decrease bacterial load. However, it prevented biofilm formation on the implant, reduced the inflammatory response, and preserved the tibial bone in a PJI revision model. An injectable composite of ceramic bone graft substitute loaded with gentamycin was ineffective in reducing *S. aureus* biofilm formation without extensive debridement and systemic antibiotic treatment in a pig osteomyelitis model.[62] Greimel et al[48] tested different combinations of moxifloxacin, flucloxacillin, and rifampin, and found that moxifloxacin combined with rifampin was most effective in reducing CFU of *S. aureus* and biofilm in vivo. Wei et al[51] showed that intra-articular injection of vancomycin showed superior antibiofilm efficacy than systemic application in a PJI model caused by MRSA.

Discussion

Device-related infection is a major complication that occurs in orthopaedic surgery. The major obstacle in clinical treatment is the eradication of bacteria biofilm. To address this, research and development of new techniques for prevention and treatment, including implant surface modification,[69,70] bone allografts,[71] controlled

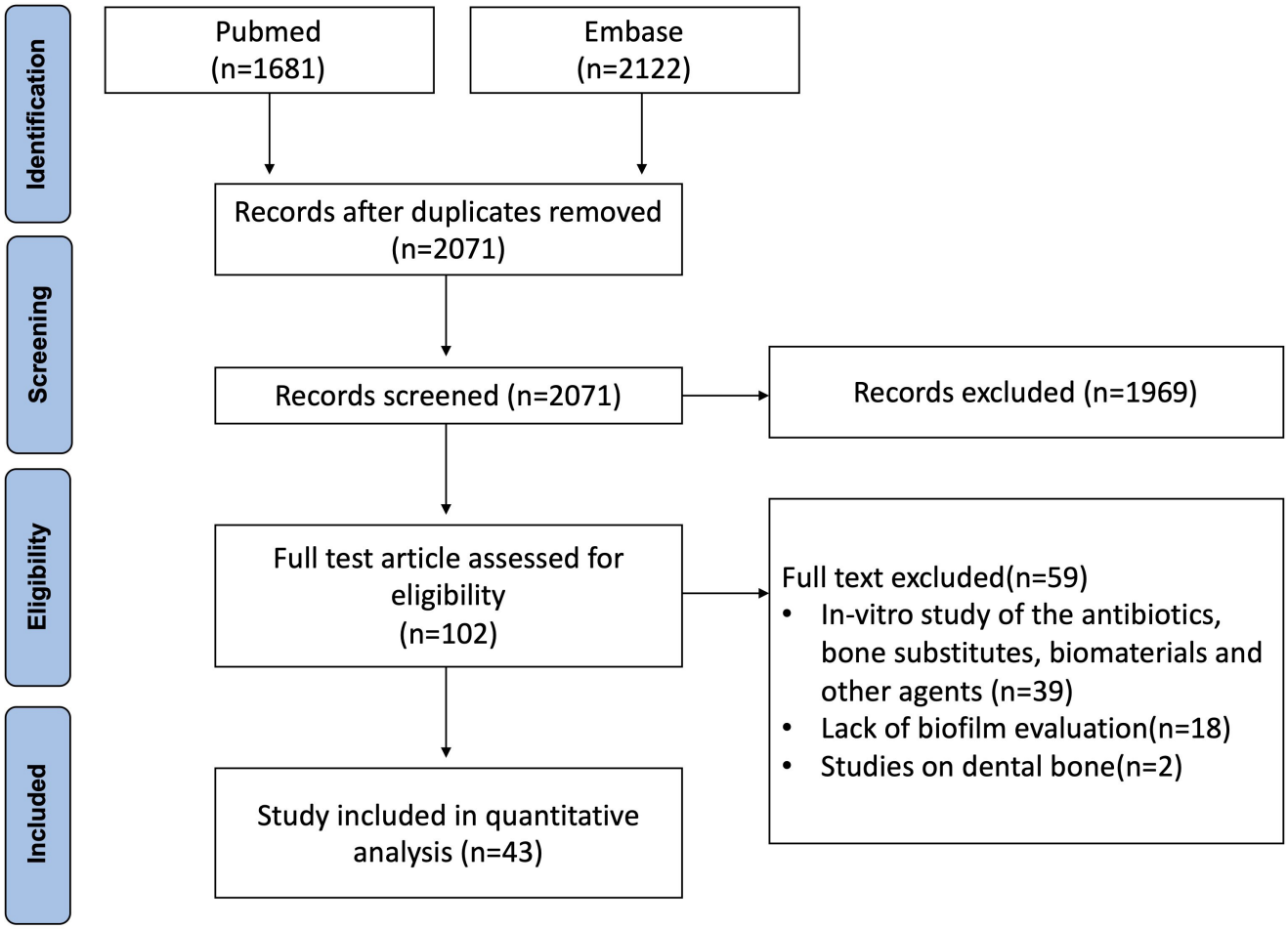

Fig. 1

Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flowchart of study selection.

release of antibiotics,[72] and novel bactericidal agents have shown promising potential.[53] In this review, we summarized the current preclinical evidence in animal studies, the advancement of recent therapeutic interventions, and the clinical translational value of the in vivo findings.

The principles of developing a biofilm-related bone infection animal model include the presence of a foreign implant,[65] and biofilm that was cultured in advance.[43] Large animals, including rabbits or sheep, are suggested to have better translational potential due to size, and a phylogenetically closer immune response to humans.[73] Lovati et al[50] identified dosage-dependent osteolysis and histological changes with increased bacterial load. In their study, low-grade infection and impaired healing caused by low-dosage CFU of *S. epidermidis* matched the observations in subclinical infections without prominent infection signs. Nishitani et al[11] showed the natural history of biofilm attachment, proliferation, maturation, and dispersal. This explains the decrease of therapeutic effects when antibiotic treatment was started at a later period post-infection, and the high recurrence rate of implant retention.[11] In another study by Windolf et al,[36] local but not systemic elevation of leucocyte and IL-6 was observed with the formation of biofilm in the FRI model, where the unfavourable environment created by local inflammation and necrosis was found to be associated with poor healing. Among the 42 studies with implants, 15 used actual prosthesis or fracture devices customized to the animal size, including 3D-printed knee joint prostheses and spacers,[27,68] cemented hip hemiarthroplasty implant,[46] and commercially available plate fixation.[64] Functional implants that provide better tolerance to surgery and mechanical stability enhance clinical relevance, because they enable functional outcome analyses like gait analysis for the PJI models and load-bearing in the FRI models.

Senneville et al[74] showed a significantly higher rate of coagulase-negative strains from bone biopsy samples than from swab culture. Unlike the classic infection induced by *S. lugdunensis*, asymptomatic biofilm infection caused by *C. acnes* underscores the importance of long-term observation and the possibility of latent *C. acnes* infection in aseptic prosthetic loosening.[55] Asymptomatic biofilm formation and low-grade inflammatory cytokines without prominent bone lysis were also observed in *S. epidermidis* infections.[37] Meanwhile, a

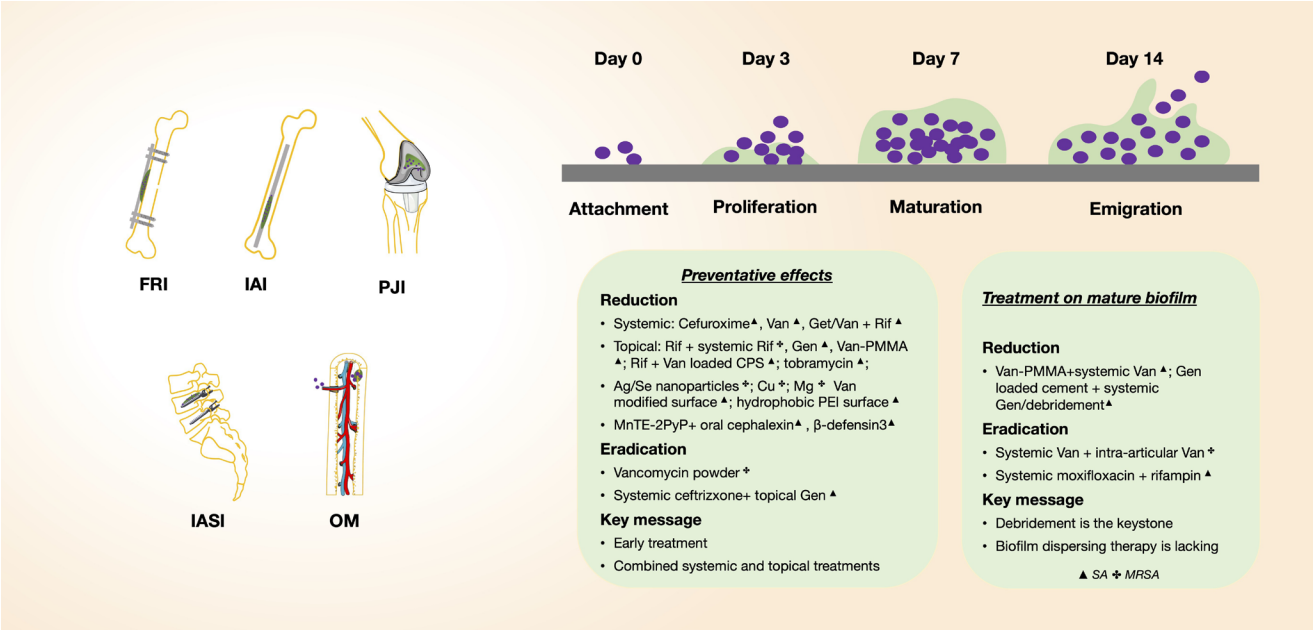

Fig. 2

A summary of the animal models, biofilm development, and therapeutic effects against *Staphylococcus aureus* (SA) and methicillin-resistant *S. aureus* (MRSA). CPS, calcium phosphate scaffold; FRI, fracture-related infection; Gen, gentamicin; IASI, implant-associated spinal infection model; OM, osteomyelitis; PJI, periprosthetic joint infection; PMMA, polymethyl methacrylate; Rif, rifampin; Van, vancomycin.

model of gram-negative PJI, which accounts for 3% to 6% of total cases, has been developed.[31] In the gram-negative (GN)-PJI model developed by Thompson et al,[31] the different infection rates among different strains revealed a chronic inflammatory response in *P. aeruginosa* infection compared to *E. coli* infection. These species-specific pathological patterns were suggested to cause different clinical outcomes.[55] Given the high comorbidities in elderly patients, further investigation with osteoporotic or diabetic models is needed to elucidate disease-specific pathology of biofilm-related infections.[38]

Various imaging tools have been applied to observe biofilm ex vivo, but each has its advantages and limitations. Crystal violet staining is the most feasible method for biofilm observation and quantification,[29,39,42,50,54,55] but the morphological observation lacks detail. Other techniques include peptide nucleic acid fluorescence in situ hybridization (FISH) to detect biofilm formation in bone tissue,[45,61,62] immunohistochemical staining,[59,61,62] and fluorescent bacteria detection probe.[65] Positively stained bacteria cells and biofilm can be visualized under the light microscope, but magnification is limited. The most common method with SEM can provide images with a high range of magnifications and complex shapes,[11,75] but the potential limitations are a long sample preparation time and lack of vertical resolution. Another frequently used technique is the CLSM that can visualize 3D images at single-cell resolution, offer discrimination of bacteria and biofilm polysaccharide matrix, distinguish live or dead cells, and provide quantification by the integrated optical density.[41,43,45,56,64] However, the biofilm structure and properties may interfere with the fluorescence probe. Other imaging tools, such as radioactive CT or fluorescent microscope of bioluminescent bacterial strains, allow for non-invasive real-time monitoring of the infection development and inflammation in vivo, but are not specific to biofilm formation.[11,30,46,57] For future studies, we recommend using SEM or CLSM, combined with CV staining and CFU counting, for better visualization and quantification.

The major difference between prevention and intervention is the decreased therapeutic effect of antibiotics against mature biofilms due to antibiotic tolerance and protection from the host's immune response. When planktonic bacteria were inoculated, systemic or topical antibiotics,[42] coating techniques with antibiotic or nanoparticles,[26,47,53] and hydrogel delivery of antibiotics[28] showed significant biofilm reduction. Eradication of biofilm was observed when topical and systemic antibiotics were applied together.[32,42,48] Therapies applied to prevent biofilm adhesion from overnight precultured bacteria also showed preventative effects[33,35,39,66] as biofilm maturation often occurs three days after attachment. Despite numerous preventative methods proposed, only three studies had intervention on mature biofilms,[35,48,62] and another three studies had intervention on established biofilm in revision surgeries.[27,34,51] More often, established biofilm was found to be resistant to combined topical treatment with vancomycin and rifampin when the treatment was started after biofilm maturation.[35] Greimel et al[48] found that systemic moxifloxacin and rifampin treatment, starting on day 7 post-infection, eradicated the biofilm on implant but failed to clear the bacteria in the knee joint and bone tissue. In

cases of revision surgery, Wei et al[51] showed that systemic and intra-articular injection of vancomycin eradicated the infection. Other studies found a reduction, but not eradication, of biofilm treated with vancomycin loaded on PMMA,[27] even when combined with systemic application.[34] We would encourage future investigations on the treatment effects of novel EPS dispersing agents in conjunction with antimicrobials as an alternative for current methods.

Systemic antibiotic treatment is the conventional treatment method for biofilm infections, albeit with limited effectiveness.[30,35,42] For methicillin-sensitive *S. aureus* (MSSA) biofilm, first-line antibiotic cefazolin showed no effects of biofilm reduction even when it was started at day 0, and eradication was only achieved through a combination of topical aminoglycoside and systemic ceftriaxone.[42] For MRSA and MRSE infections, vancomycin remains the primary course of treatment.[43,51] However, treatment failure by three or 40 days of systemic vancomycin therapy for biofilm reduction caused by *S. aureus* or MRSA was noted.[32,49] Three studies suggested that the lack of sufficient local concentrations is the cause of the failure, while none measured tissue concentration.[30,35,42] Furthermore, some strains even showed resistance to single vancomycin treatment in vivo at increased MIC to 4 ug/ml.[76] Rifampin has been recognized as a cornerstone for treating biofilm in PJI, yet the monotherapy of rifampin is no longer recommended due to antibiotic tolerance.[48] The effects of systemic rifampin have been tested in three studies.[32,35,48] These studies suggested that systemic rifampin combined with high-dosage topical rifampin or moxifloxacin enhanced the effects against MRSA or MRSE.[32,35,48] Quinolones including moxifloxacin can be a favourable option for combination with rifampin, due to the advantages of higher bioavailability and broad-spectrum bactericidal activity.[48]

Given that a sufficient local concentration of antibiotics is the prerequisite for successful bacteria eradication, topical application of antibiotics has been widely studied.[32,42,44,51,59] Topical spreading of vancomycin successfully prevented biofilm infection of the bone in all nine rabbits with implant-associated infection caused by MRSA. Similarly, topical tobramycin powder spreading showed more significant biofilm reduction compared to four weeks of systemic ceftriaxone, suggesting similar prophylactic effects of topical application compared to systemic antibiotics.[44] However, current clinical evidence supporting antibiotic powder in orthopaedic trauma and infection is sparse.[77] Increased MRSA biofilm reduction or eradication was achieved by intra-articular injection of vancomycin combined with systemic application,[51] or high-dosage topical rifampin combined with systemic rifampin.[32] The major advantage of this application method was a higher concentration at the target site. However, only one study in our review evaluated antibiotics concentration in bone tissue.[62] Importantly, vancomycin has nephrotoxicity, and rifampin has toxicity on osteoblasts and antagonistic effects with gentamicin,[33,42,51] warranting more attention for application. Despite some evidence reporting positive results of topical antibiotic treatment, direct injection is flawed by its drug distribution and leakage problems, which warrants drug encapsulation and delivery with biomedical carriers.[42]

Biocompatibility, degradability, and sustained drug release are the fundamental characteristics of successful biomaterials targeting bone infection.[78] As a clinically used carrier, PMMA loaded with vancomycin shows bacterial load reduction but cannot eradicate the biofilm.[27,34] However, the primary concern is the elution from the depot to the surrounding tissue, which may fail to reach the optimal concentration.[34] The non-degradable nature and low compatibility of rifampin with PMMA also limit the application of this combination.[79] Alternative carriers with good biocompatibility and biodegradability have been studied.[28,33,62] Inzana et al[33] successfully incorporated rifampin with vancomycin-loaded calcium phosphate scaffold that is not feasible in PMMA. Compared to PMMA, their findings confirmed the advantage of co-delivery on CFU reduction on bone and implant, but the observation of persistent biofilm indicated limited effects of this combination.[33] Chitosan loaded with amikacin and vancomycin showed complete clearance against polymicrobial infection caused by *S. aureus* and *P. aeruginosa*.[28]

Surface modification or coating with nanoparticles or hydrogels represents another direction of intervention. Ashbaugh et al[26] developed a polymeric nanofibre coating with tunable combinatorial antibiotic delivery that prevented biofilm formation in vivo. Metal particles including Ag coating techniques have also been shown to reduce biofilm, but concerns include the durability of antibiotic activity and low cytotoxicity of Ag.[47,53] Other proposed therapies including selenium nanoparticles or magnet nanoparticle-induced hyperthermia therapy show biofilm reduction effects at pilot stages.[41,49] Notably, mesoporous silica nanoparticles loaded with enoxacin inhibited osteoclast activation, thereby mitigating bone loss and reducing biofilm formation.[67] Biodegradable magnesium-based implants have shown promising potential in preventing biofilm attachment.[39,54] The major advantage is that local degradation of Mg will reduce bacteria adhesion and biofilm formation, and stimulate bone formation. Interestingly, one study that applied a dual therapy by sequentially delivering gentamicin and BMP-2 showed biofilm eradication effects and promoted healing.[40] These findings inspire novel therapies bridging the gap between degeneration and regeneration profiles of single treatments, highlighting the promising potential of a layered release strategy with antibiotic and bone-forming agents to achieve pathogen clearance and subsequent bone regeneration.

The strength of the current review is that we have presented existing knowledge gaps of research, novel findings regarding biofilm development within bone tissue, and the benefits and pitfalls of new treatment options. For this purpose, we had stringent inclusion and

exclusion criteria to allow for an in-depth and accurate assessment of the treatment against biofilm. However, our study is limited by a lack of meta-analysis due to the large heterogeneity of bacteria species and strains, animal models, and treatment protocols. Another limitation is that many studies that did not explicitly evaluate biofilm were excluded. Additionally, newly identified intestinal MRSA carried by neutrophils and intracellular *S. aureus* in phagocytes are also suggested to be associated with PJI and recurrent infections on FRI models.[80,81] Further studies are required to confirm if there is any interaction between these intracellular bacteria and biofilm pathology. With improved detection methods and growing use of permanent implants, similar challenges for biofilm eradication have been encountered in other devices including dental implants, catheters, and shunts.[82,83] Treatment success rates for device-related biofilm infections range from 32% to 70%.[84,85] Despite different pathologies of infections in different scenarios, surface modification techniques including anti-adhesive and antibacterial coating may serve as promising directions for future implant design in orthopaedics. However, these therapies require substantial modifications for orthopaedic applications, due to the disparities of the pathogens, wound environment, and the function of the implant.

In this review, we summarized the development of preclinical models on biofilm-related bone infections, in vivo characterization of biofilm, and advances in therapeutic interventions and outcomes. A comprehensive understanding of the matrix complex components, homeostasis, and molecular pathways in the local biofilm environment is the prerequisite for developing novel therapies. Given that the biofilm natural history and biological behaviour varies greatly between each pathogen and animal model, experimental designs should match the particular clinical scenario. Despite robust knowledge of the molecular pathways of biofilm formation in vitro, studies looking into the pathology of genetically modified strains are warranted to elucidate functional roles of the target genes in vivo.[45]

Current antibiotic treatment regimens show significant biofilm reduction effects when applied at early stages, and these effects can be further enhanced by a combination of rifampin with topical vancomycin that is already used in clinical application.[86] However, only three studies that used systemic moxifloxacin plus topical rifampin, topical vancomycin powder spreading,[58] or systemic plus topical vancomycin showed eradication.[48] Given the difficulty of achieving eradication, surgical debridement is still the keystone in infection control. For clinical translation, data from animal studies will illustrate the mechanisms underlying persistent and recurrent infections. The findings from the current review revealed novel targets for future study and aid clinical decision-making on the optimal timing and combination of therapies. Emerging biodegradable and biocompatible biomaterials that act as drug carriers showed promising therapeutic potential when applied locally. Further validations of these novel treatments designated to individual clinical scenarios will allow for continuous eradication rates and safety improvement.

So far, there is still a major knowledge gap on the combination, dosage, delivery, and pharmacokinetics of current interventions. To address this, novel therapies should be tested in a valid animal model under a clear design for prevention or treatment for biofilm-related infections in orthopaedics. Evaluating the pharmacokinetics of antibiotics is necessary to determine the optimal dosage and concentration and avoid systemic toxicity. Measurements of antibiotic concentrations at tissue levels will justify the claimed drug release profile and further confirm the superiorities of topical application. The development of novel antimicrobial materials or agents is warranted to improve therapeutic effects and mitigate the tolerance of conventional antibiotics. For biofilm prevention, surface coating techniques of implants with nanoparticles or hydrogels showed the most promising potential, as the integration of antimicrobial properties with functional devices can facilitate recovery and improve clinical outcomes. We recommend a tailored release with EPS-dispersing, antimicrobial, and bone-forming agents loaded on biodegradable hydrogels for established biofilms. These therapies can be applied adjunctly with surgical debridement to promote subsequent healing and bone regeneration. With the ageing population, future directions, including the susceptibility, pathology, and treatments for biofilm-related bone infection with concurrent osteoporosis will provide therapeutic targets and improve outcomes in elderly patients.

References

1. **Masters EA**, **Trombetta RP**, **de Mesy Bentley KL**, **et al**. Evolving concepts in bone infection: redefining "biofilm", "acute vs. chronic osteomyelitis", "the immune proteome" and "local antibiotic therapy." *Bone Res*. 2019;7(1):20.
2. **Romanò CL**, **Tsuchiya H**, **Morelli I**, **Battaglia AG**, **Drago L**. Antibacterial coating of implants: are we missing something? *Bone Joint Res*. 2019;8(5):199–206.
3. **Baertl S**, **Metsemakers W-J**, **Morgenstern M**, **et al**. Fracture-related infection. *Bone Joint Res*. 2021;10(6):351–353.
4. **Kim PH**, **Leopold SS**. In brief: Gustilo-Anderson classification. [corrected]. *Clin Orthop Relat Res*. 2012;470(11):3270–3274.
5. **Fang C**, **Wong T-M**, **Lau T-W**, **To KK**, **Wong SS**, **Leung F**. Infection after fracture osteosynthesis–part I: pathogenesis, diagnosis and classification. *J Orthop Surg (Hong Kong)*. 2017;25(1):2309499017692712.
6. **Köder K**, **Hardt S**, **Gellert MS**, **et al**. Outcome of spinal implant-associated infections treated with or without biofilm-active antibiotics: results from a 10-year cohort study. *Infection*. 2020;48(4):559–568.
7. **Costerton JW**. Biofilm theory can guide the treatment of device-related orthopaedic infections. *Clin Orthop Relat Res*. 2005;amp;NA(437):7–11.
8. **Schwarz EM**, **Parvizi J**, **Gehrke T**, **et al**. 2018 International Consensus Meeting on Musculoskeletal Infection: Research Priorities from the General Assembly Questions. *J Orthop Res*. 2019;37(5):997–1006.
9. **Hall-Stoodley L**, **Costerton JW**, **Stoodley P**. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol*. 2004;2(2):95–108.
10. **Seebach E**, **Kubatzky KF**. Chronic implant-related bone infections-can immune modulation be a therapeutic strategy? *Front Immunol*. 2019;10:1724.
11. **Nishitani K**, **Sutipornpalangkul W**, **de Mesy Bentley KL**, **et al**. Quantifying the natural history of biofilm formation in vivo during the establishment of chronic

implant-associated Staphylococcus aureus osteomyelitis in mice to identify critical pathogen and host factors. *J Orthop Res*. 2015;33(9):1311–1319.
12. **Zapotoczna M**, **O'Neill E**, **O'Gara JP**. Untangling the diverse and redundant mechanisms of staphylococcus aureus biofilm formation. *PLoS Pathog*. 2016;12(7):e1005671.
13. **Kildow BJ**, **Patel SP**, **Otero JE**, **et al**. Results of debridement, antibiotics, and implant retention for periprosthetic knee joint infection supplemented with the use of intraosseous antibiotics. *Bone Joint J*. 2021;103-B(6 Supple A):185–190.
14. **Morgenstern M**, **Kuehl R**, **Zalavras CG**, **et al**. The influence of duration of infection on outcome of debridement and implant retention in fracture-related infection. *Bone Joint J*. 2021;103-B(2):213–221.
15. **Grammatopoulos G**, **Bolduc M-E**, **Atkins BL**, **et al**. Functional outcome of debridement, antibiotics and implant retention in periprosthetic joint infection involving the hip: a case-control study. *Bone Joint J*. 2017;99-B(5):614–622.
16. **Goud AL**, **Harlianto NI**, **Ezzafzafi S**, **Veltman ES**, **Bekkers JEJ**, **van der Wal BCH**. Reinfection rates after one- and two-stage revision surgery for hip and knee arthroplasty: a systematic review and meta-analysis. *Arch Orthop Trauma Surg*. 2021;2021:1–10.
17. **Khatoon Z**, **McTiernan CD**, **Suuronen EJ**, **Mah T-F**, **Alarcon EI**. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon*. 2018;4(12):e01067.
18. **Ramalhete R**, **Brown R**, **Blunn G**, **et al**. A novel antimicrobial coating to prevent periprosthetic joint infection. *Bone Joint Res*. 2020;9(12):848–856.
19. **Zhou J**, **Zhou XG**, **Wang JW**, **Zhou H**, **Dong J**. Treatment of osteomyelitis defects by a vancomycin-loaded gelatin/β-tricalcium phosphate composite scaffold. *Bone Joint Res*. 2018;7(1):46–57.
20. **Liu H**, **Tang Y**, **Zhang S**, **et al**. Anti-infection mechanism of a novel dental implant made of titanium-copper (TiCu) alloy and its mechanism associated with oral microbiology. *Bioact Mater*. 2022;8:381–395.
21. **Park K-H**, **Greenwood-Quaintance KE**, **Patel R**. In vitro activity of ceftaroline against staphylococci from prosthetic joint infection. *Diagn Microbiol Infect Dis*. 2016;84(2):141–143.
22. **Wong RMY**, **Li T-K**, **Li J**, **et al**. A systematic review on current osteosynthesis-associated infection animal fracture models. *J Orthop Translat*. 2020;23:8–20.
23. **Gerits E**, **Kucharíková S**, **Van Dijck P**, **et al**. Antibacterial activity of a new broad-spectrum antibiotic covalently bound to titanium surfaces. *J Orthop Res*. 2016;34(12):2191–2198.
24. **Butini ME**, **Cabric S**, **Trampuz A**, **Di Luca M**. In vitro anti-biofilm activity of a biphasic gentamicin-loaded calcium sulfate/hydroxyapatite bone graft substitute. *Colloids Surf B Biointerfaces*. 2018;161:252–260.
25. **García-Alvarez R**, **Izquierdo-Barba I**, **Vallet-Regí M**. 3D scaffold with effective multidrug sequential release against bacteria biofilm. *Acta Biomater*. 2017;49:113–126.
26. **Ashbaugh AG**, **Jiang X**, **Zheng J**, **et al**. Polymeric nanofiber coating with tunable combinatorial antibiotic delivery prevents biofilm-associated infection in vivo. *Proc Natl Acad Sci U S A*. 2016;113(45):E6919–E6928.
27. **Carli AV**, **Bhimani S**, **Yang X**, **de Mesy Bentley KL**, **Ross FP**, **Bostrom MPG**. Vancomycin-loaded polymethylmethacrylate spacers fail to eradicate periprosthetic joint infection in a clinically representative mouse model. *J Bone Joint Surg Am*. 2018;100-A(11):11.
28. **Boles LR**, **Awais R**, **Beenken KE**, **Smeltzer MS**, **Haggard WO**, **Jessica AJ**. Local delivery of amikacin and vancomycin from chitosan sponges prevent polymicrobial implant-associated biofilm. *Mil Med*. 2018;183(suppl_1):459–465.
29. **Lindsay SE**, **Lindsay HG**, **Kallet J**, **et al**. MnTE-2-PyP disrupts Staphylococcus aureus biofilms in a novel fracture model. *J Orthop Res*. 2021;39(11):2439–2445.
30. **Jørgensen NP**, **Meyer RL**, **Meyer R**, **Dagnæs-Hansen F**, **Fuursted K**, **Petersen E**. A modified chronic infection model for testing treatment of Staphylococcus aureus biofilms on implants. *PLoS One*. 2014;9(10):e103688.
31. **Thompson JM**, **Miller RJ**, **Ashbaugh AG**, **et al**. Mouse model of Gram-negative prosthetic joint infection reveals therapeutic targets. *JCI Insight*. 2018;3(17):17.
32. **Cahill SV**, **Kwon H-K**, **Back J**, **et al**. Locally delivered adjuvant biofilm-penetrating antibiotics rescue impaired endochondral fracture healing caused by MRSA infection. *J Orthop Res*. 2021;39(2):402–414.
33. **Inzana J**, **Trombetta R**, **Schwarz E**, **Kates S**, **Awad H**. 3D printed bioceramics for dual antibiotic delivery to treat implant-associated bone infection. *eCM*. 2015;30:232–247.
34. **Inzana JA**, **Schwarz EM**, **Kates SL**, **Awad HA**. A novel murine model of established Staphylococcal bone infection in the presence of a fracture fixation plate to study therapies utilizing antibiotic-laden spacers after revision surgery. *Bone*. 2015;72:128–136.
35. **Tomizawa T**, **Nishitani K**, **Ito H**, **et al**. The limitations of mono- and combination antibiotic therapies on immature biofilms in a murine model of implant-associated osteomyelitis. *J Orthop Res*. 2021;39(2):449–457.
36. **Windolf CD**, **Meng W**, **Lögters TT**, **MacKenzie CR**, **Windolf J**, **Flohé S**. Implant-associated localized osteitis in murine femur fracture by biofilm forming Staphylococcus aureus: a novel experimental model. *J Orthop Res*. 2013;31(12):2013–2020.
37. **Tomizawa T**, **Ishikawa M**, **Bello-Irizarry SN**, **et al**. Biofilm producing Staphylococcus epidermidis (RP62A strain) inhibits osseous integration without osteolysis and histopathology in a murine septic implant model. *J Orthop Res*. 2020;38(4):852–860.
38. **Lovati AB**, **Drago L**, **Monti L**, **et al**. Diabetic mouse model of orthopaedic implant-related Staphylococcus aureus infection. *PLoS One*. 2013;8(6):e67628.
39. **Li Y**, **Liu G**, **Zhai Z**, **et al**. Antibacterial properties of magnesium in vitro and in an in vivo model of implant-associated methicillin-resistant Staphylococcus aureus infection. *Antimicrob Agents Chemother*. 2014;58(12):7586–7591.
40. **Min J**, **Choi KY**, **Dreaden EC**, **et al**. Designer dual therapy nanolayered implant coatings eradicate biofilms and accelerate bone tissue repair. *ACS Nano*. 2016;10(4):4441–4450.
41. **Tran PA**, **O'Brien-Simpson N**, **Palmer JA**, **et al**. Selenium nanoparticles as anti-infective implant coatings for trauma orthopedics against methicillin-resistant *Staphylococcus aureus* and *epidermidis*: in vitro and in vivo assessment. *Int J Nanomedicine*. 2019;14:4613–4624.
42. **van der Horst AS**, **Medda S**, **Ledbetter E**, **et al**. Combined local and systemic antibiotic treatment is effective against experimental Staphylococcus aureus peri-implant biofilm infection. *J Orthop Res*. 2015;33(9):1320–1326.
43. **Zhu C**, **Bao N-R**, **Chen S**, **Zhao J-N**. The mechanism of human β-defensin 3 in MRSA-induced infection of implant drug-resistant bacteria biofilm in the mouse tibial bone marrow. *Exp Ther Med*. 2017;13(4):1347–1352.
44. **Marston S**, **Mirick Mueller G**, **Sabin A**, **et al**. Systemic versus free antibiotic delivery in preventing acute exogenous implant related infection in a rat model. *J Orthop Res*. 2022;40(2):429–438.
45. **Wu S**, **Liu Y**, **Lei L**, **Zhang H**. Virulence of methicillin-resistant Staphylococcus aureus modulated by the YycFG two-component pathway in a rat model of osteomyelitis. *J Orthop Surg Res*. 2019;14(1):1–10.
46. **Hadden WJ**, **Ibrahim M**, **Taha M**, **et al**. 2021 Frank Stinchfield Award: A novel cemented hip hemiarthroplasty infection model with real-time in vivo imaging in rats: an animal study. *Bone Joint J*. 2021;103-B(7 Supple B):9–16.
47. **Hu C-C**, **Chang C-H**, **Chang Y**, **Hsieh J-H**, **Ueng S-N**. Beneficial effect of TaON-Ag nanocomposite titanium on antibacterial capacity in orthopedic application. *Int J Nanomedicine*. 2020;15:7889–7900.
48. **Greimel F**, **Scheuerer C**, **Gessner A**, **et al**. Efficacy of antibiotic treatment of implant-associated *Staphylococcus aureus* infections with moxifloxacin, flucloxacillin, rifampin, and combination therapy: an animal study. *Drug Des Devel Ther*. 2017;11:1729–1736.
49. **Fang C-H**, **Tsai P-I**, **Huang S-W**, **et al**. Magnetic hyperthermia enhance the treatment efficacy of peri-implant osteomyelitis. *BMC Infect Dis*. 2017;17(1):1–12.
50. **Lovati AB**, **Romanò CL**, **Bottagisio M**, **et al**. Modeling Staphylococcus epidermidis-induced non-unions: subclinical and clinical evidence in rats. *PLoS One*. 2016;11(1):e0147447.
51. **Wei J**, **Wen Y**, **Tong K**, **Wang H**, **Chen L**. Local application of vancomycin in one-stage revision of PJI caused by MRSA in a rat model. *Antimicrob Agents Chemother (Bethesda)*. 2021;00303–00321.
52. **Kandemir Ö**, **Öztuna V**, **Milcan A**, **et al**. Clarithromycin destroys biofilms and enhances bactericidal agents in the treatment of Pseudomonas aeruginosa osteomyelitis. *Clin Orthop Relat Res*. 2005;430:171–175.
53. **Hazer DB**, **Sakar M**, **Dere Y**, **Altinkanat G**, **Ziyal MI**, **Hazer B**. Antimicrobial effect of polymer-based silver nanoparticle coated pedicle screws: experimental research on biofilm inhibition in rabbits. *Spine (Phila Pa 1976)*. 2016;41(6):E323–9.
54. **Li Y**, **Liu L**, **Wan P**, **et al**. Biodegradable Mg-Cu alloy implants with antibacterial activity for the treatment of osteomyelitis: In vitro and in vivo evaluations. *Biomaterials*. 2016;106:250–263.
55. **Gahukamble AD**, **McDowell A**, **Post V**, **et al**. Propionibacterium acnes and Staphylococcus lugdunensis cause pyogenic osteomyelitis in an intramedullary nail model in rabbits. *J Clin Microbiol*. 2014;52(5):1595–1606.
56. **Zhang X**, **Ma Y-F**, **Wang L**, **et al**. A rabbit model of implant-related osteomyelitis inoculated with biofilm after open femoral fracture. *Exp Ther Med*. 2017;14(5):4995–5001.
57. **Gordon O**, **Miller RJ**, **Thompson JM**, **et al**. Rabbit model of *Staphylococcus aureus* implant-associated spinal infection. *Dis Model Mech*. 2020;13(7):dmm045385.

58. **Hovis JP**, **Montalvo R**, **Marinos D**, **et al**. Intraoperative vancomycin powder reduces Staphylococcus aureus surgical site infections and biofilm formation on fixation implants in a rabbit model. *J Orthop Trauma*. 2018;32(5):263–268.
59. **Jensen LK**, **Bjarnsholt T**, **Kragh KN**, **et al**. In vivo gentamicin susceptibility test for prevention of bacterial biofilms in bone tissue and on implants. *Antimicrob Agents Chemother*. 2019;63(2):e01889–01818.
60. **Singh DP**, **Gowda AU**, **Chopra K**, **et al**. The effect of negative pressure wound therapy with antiseptic instillation on biofilm formation in a porcine model of infected spinal instrumentation. *Wounds*. 2017;28(6):175–180.
61. **Johansen LK**, **Koch J**, **Frees D**, **et al**. Pathology and biofilm formation in a porcine model of staphylococcal osteomyelitis. *J Comp Pathol*. 2012;147(2–3):343–353.
62. **Blirup-Plum SA**, **Bjarnsholt T**, **Jensen HE**, **et al**. Pathological and microbiological impact of a gentamicin-loaded biocomposite following limited or extensive debridement in a porcine model of osteomyelitis. *Bone Joint Res*. 2020;9(7):394–401.
63. **Schaer TP**, **Stewart S**, **Hsu BB**, **Klibanov AM**. Hydrophobic polycationic coatings that inhibit biofilms and support bone healing during infection. *Biomaterials*. 2012;33(5):1245–1254.
64. **Stewart S**, **Barr S**, **Engiles J**, **et al**. Vancomycin-modified implant surface inhibits biofilm formation and supports bone-healing in an infected osteotomy model in sheep: a proof-of-concept study. *J Bone Joint Surg Am*. 2012;94-A(15):1406–1415.
65. **Shiono Y**, **Ishii K**, **Nagai S**, **et al**. Delayed Propionibacterium acnes surgical site infections occur only in the presence of an implant. *Sci Rep*. 2016;6(1):1–10.
66. **Gracia E**, **Laclériga A**, **Monzón M**, **Leiva J**, **Oteiza C**, **Amorena B**. Application of a rat osteomyelitis model to compare in vivo and in vitro the antibiotic efficacy against bacteria with high capacity to form biofilms. *J Surg Res*. 1998;79(2):146–153.
67. **Yao C**, **Zhu M**, **Han X**, **et al**. A bone-targeting enoxacin delivery system to eradicate Staphylococcus aureus-related implantation infections and bone loss. *Front Bioeng Biotechnol*. 2021;9:749910.
68. **Carli AV**, **Bhimani S**, **Yang X**, **et al**. Quantification of peri-implant bacterial load and in vivo biofilm formation in an innovative, clinically representative mouse model of periprosthetic joint infection. *J Bone Joint Surg Am*. 2017;99-A(6):e25.
69. **Cheng H**, **Yue K**, **Kazemzadeh-Narbat M**, **et al**. Mussel-inspired multifunctional hydrogel coating for prevention of infections and enhanced osteogenesis. *ACS Appl Mater Interfaces*. 2017;9(13):11428–11439.
70. **Drago L**, **Boot W**, **Dimas K**, **et al**. Does implant coating with antibacterial-loaded hydrogel reduce bacterial colonization and biofilm formation in vitro? *Clin Orthop Relat Res*. 2014;472(11):3311–3323.
71. **Dastgheyb SS**, **Toorkey CB**, **Shapiro IM**, **Hickok NJ**. Porphyrin-adsorbed allograft bone: a photoactive, antibiofilm surface. *Clin Orthop Relat Res*. 2015;473(9):2865–2873.
72. **Wan T**, **Stylios GK**, **Giannoudi M**, **Giannoudis PV**. Investigating a new drug delivery nano composite membrane system based on PVA/PCL and PVA/HA(PEG) for the controlled release of biopharmaceuticals for bone infections. *Injury*. 2015;46 Suppl 8:S39–43.
73. **Yagi H**, **Kihara S**, **Mittwede PN**, **et al**. Development of a large animal rabbit model for chronic periprosthetic joint infection. *Bone Joint Res*. 2021;10(3):156–165.
74. **Senneville E**, **Melliez H**, **Beltrand E**, **et al**. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. *Clin Infect Dis*. 2006;42(1):57–62.
75. **Relucenti M**, **Familiari G**, **Donfrancesco O**, **et al**. Microscopy methods for biofilm imaging: focus on SEM and VP-SEM pros and cons. *Biology (Basel)*. 2021;10(1):51.
76. **Park K-H**, **Greenwood-Quaintance KE**, **Schuetz AN**, **Mandrekar JN**, **Patel R**. Activity of tedizolid in methicillin-resistant Staphylococcus epidermidis experimental foreign body-associated osteomyelitis. *Antimicrob Agents Chemother*. 2017;61(2):e01644–01616.
77. **Metsemakers W-J**, **Fragomen AT**, **Moriarty TF**, **et al**. Evidence-based recommendations for local antimicrobial strategies and dead space management in fracture-related infection. *J Orthop Trauma*. 2020;34(1):18–29.
78. **Billings C**, **Anderson DE**. Role of implantable drug delivery devices with dual platform capabilities in the prevention and treatment of bacterial osteomyelitis. *Bioengineering (Basel)*. 2022;9(2):65.
79. **Beeching NJ**, **Thomas MG**, **Roberts S**, **Lang SD**. Comparative in-vitro activity of antibiotics incorporated in acrylic bone cement. *J Antimicrob Chemother*. 1986;17(2):173–184.
80. **Gao T**, **Lin J**, **Zhang C**, **Zhu H**, **Zheng X**. Is intracellular *Staphylococcus aureus* associated with recurrent infection in a rat model of open fracture? *Bone Joint Res*. 2020;9(2):71–76.
81. **Zhu H**, **Jin H**, **Zhang C**, **Yuan T**. Intestinal methicillin-resistant *Staphylococcus aureus* causes prosthetic infection via "Trojan Horse" mechanism: Evidence from a rat model. *Bone Joint Res*. 2020;9(4):152–161.
82. **Douglass M**, **Ghalei S**, **Brisbois E**, **Handa H**. Potent, broad-spectrum antimicrobial effects of S-Nitroso-N-acetylpenicillamine-impregnated nitric oxide-releasing latex urinary catheters. *ACS Appl Bio Mater*. 2022;5(2):700–710.
83. **Liu H**, **Tang Y**, **Zhang S**, **et al**. Anti-infection mechanism of a novel dental implant made of titanium-copper (TiCu) alloy and its mechanism associated with oral microbiology. *Bioact Mater*. 2022;8:381–395.
84. **Donlan RM**. Biofilms on central venous catheters: is eradication possible? *Curr Top Microbiol Immunol*. 2008;322:133–161.
85. **Bunk D**, **Eisenburger M**, **Häckl S**, **Eberhard J**, **Stiesch M**, **Grischke J**. The effect of adjuvant oral irrigation on self-administered oral care in the management of peri-implant mucositis: A randomized controlled clinical trial. *Clin Oral Implants Res*. 2020;31(10):946–958.
86. **Osmon DR**, **Berbari EF**, **Berendt AR**, **et al**. Executive summary: diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2013;56(1):1–10.

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