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Implants coating strategies for antibacterial treatment in fracture and defect models: A systematic review of animal studies

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ABSTRACT

Objective: Fracture-related infection (FRI) remains a major concern in orthopaedic trauma. Functionalizing implants with antibacterial coatings are a promising strategy in mitigating FRI. Numerous implant coatings have been reported but the preventive and therapeutic effects vary. This systematic review aimed to provide a comprehensive overview of current implant coating strategies to prevent and treat FRI in animal fracture and bone defect models.

Methods: A literature search was performed in three databases: PubMed, Web of Science and Embase, with predetermined keywords and criteria up to 28 February 2023. Preclinical studies on implant coatings in animal fracture or defect models that assessed antibacterial and bone healing effects were included.

Results: A total of 14 studies were included in this systematic review, seven of which used fracture models and seven used defect models. Passive coatings with bacteria adhesion resistance were investigated in two studies. Active coatings with bactericidal effects were investigated in 12 studies, four of which used metal ions including Ag^{+} and Cu^{2+}; five studies used antibiotics including chlorhexidine, tigecycline, vancomycin, and gentamicin sulfate; and the other three studies used natural antibacterial materials including chitosan, antimicrobial peptides, and lysostaphin. Overall, these implant coatings exhibited promising efficacy in antibacterial effects and bone formation.

Conclusion: Antibacterial coating strategies reduced bacterial infections in animal models and favored bone healing *in vivo*. Future studies of implant coatings should focus on optimal biocompatibility, antibacterial effects against multi-drug resistant bacteria and polymicrobial infections, and osseointegration and osteogenesis promotion especially in osteoporotic bone by constructing multi-functional coatings for FRI therapy.

The translational potential of this paper: The clinical treatment of FRI is complex and challenging. This review summarizes novel orthopaedic implant coating strategies applied to FRI in preclinical studies, and offers a perspective on the future development of orthopaedic implant coatings, which can potentially contribute to alternative strategies in clinical practice.

1. Introduction

Infection remains a challenging complication in orthopaedic trauma, which is often complicated with complex fractures and defects from high-energy injuries. Due to deficiency in soft tissues and blood supply, there is a high risk of bacterial colonization in these regions [1], which

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can significantly impair the bone healing process resulting in non-union, permanent loss of function, and even amputation [2]. This is particularly alarming for elderlies who are more prone to fragility fractures and may have less reserve for infections [3,4]. Furthermore, recent studies have also shown an increasing incidence of open fractures in elderly patients [5]. Although fracture-related infection (FRI) occurs in only 1–2% of closed fractures after internal fixation, the incidence can reach up to 30% in open fractures [6,7] as exogenous contamination can occur when the skin is breached [8]. Another source of FRI is during surgical operations, in which pathogens can be introduced *via* the surgical site along with implants [9]. Implant fixation is often performed to stabilize the fractured bones, while large-segment defects may require bone grafting in the future to induce bone repair [10]. Conventional materials including stainless steel and titanium often do not possess any antibacterial properties and can be prone to be contaminated by bacteria. To address this potential problem, surface functionalization for antibacterial purposes have been intensively explored. The benefit of this would be decreasing the susceptibility to bacterial infections, thereby limiting severe clinical complications, including implant loosening, osteomyelitis and even mortality [11]

Currently, one of the leading causative pathogens of FRI is *Staphylococcus aureus* (*S. aureus*), accounting for 18.4-37.4% of cases [26–28], followed by *Staphylococcus epidermidis* and Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [27,29]. Furthermore, multidrug-resistant (MDR) strains have been increasingly isolated at surgical sites [30], which often lead to high risks of recurrence, longer hospitalization, and increased mortality [31]. Methicillin-resistant *S. aureus* (MRSA) [30], which is resistant to multiple commonly used antibiotics [32], is one of the major isolates. After colonizing on an implant surface, MRSA forms a biofilm composed of extracellular polymeric substances, including bacterial exopolysaccharides and extracellular DNA of host and bacterial origin [33]. Moreover, there is complex cell-to-cell communication between bacteria, which is known as quorum sensing (QS) [34]. Bacteria can transmit information by secreting and receiving signaling molecules, thereby regulating gene expression to cope with the dynamic environment [35]. QS contributes to bacterial virulence, biofilm formation and antimicrobial resistance, making the organism particularly challenging to eradicate [36]. A common practice for preventing FRI is the prophylactic administration of systemic antibiotics [37], but with the growing prevalence of MDR bacteria, antibiotics have become increasingly ineffective in preventing and managing post-operative infections. Therefore, it is important to develop alternate strategies for orthopaedic implants capable to prevent infections.

Implants coating is now considered as a promising strategy to tackle FRIs [38], especially on targeting biofilm-related infections [39]. Currently, coatings against bacterial infections can be broadly categorized as: (1) passive coatings for preventing bacteria attachment and (2) active coatings for bacteria killing and growth inhibition [40]. Passive coatings prevent bacterial infection at the initial stage by changing the surface wettability properties, surface energy, and surface topography of implants [41]. Common materials used for passive coatings are polyethylene glycol (PEG), hyaluronic acid (HA) and hierarchical nanostructured superhydrophobic material, etc. [42–44]. Active coating utilizes antibacterial agents, such as metal ions, antibiotics, and antimicrobial peptides, to functionalize the implant surface and eradicates bacteria *via* contact killing or antibacterials released into surrounding tissues [45–47]. Additionally, other biomaterials promoting osseointegration, angiogenesis and immunomodulation can be co-coated along with antibacterial materials [48,49].

Various coatings designed for orthopaedic applications have been reported, although most are *in vitro* studies. Some novel coatings addressing bacterial infections have been investigated in animal models with bone tissue injury to verify their bactericidal effects and the effects on bone healing *in vivo*. With the increasing number of recent publications, the aim of this study was to provide a comprehensive overview of current implant coating strategies that prevent and treat infections in animal fracture and bone defect models.

2. Methods

2.1. Search strategy

This systematic review was conducted in three databases: PubMed, Web of Science, and Embase. The search keywords were: "(orthopaedic implant) AND (infection OR bacteri* OR antibacterial) AND (coating OR surface modifi* OR functional*) AND (fracture OR defect)". Additional studies were identified manually when relevant. The search of eligible studies was up to 28 February 2023. Duplicated articles were excluded, and studies were further selected based on inclusion and exclusion criteria. Study selection was conducted by two independent reviewers.

2.2. Criteria for eligibility

Studies were included based on the following criteria: 1) orthopaedic implant coatings; 2) fracture or defect models in animal; 3) assessed the antibacterial effect *in vivo*; 4) assessed bone healing *in vivo*. The exclusion criteria were: 1) clinical studies; 2) *in vitro* studies; 3) no antibacterial coatings; 4) no bacterial infections; 5) no bone healing assessments; 6) inadequate sample size; 7) reviews, expert opinion articles, conference papers, and case reports.

2.3. Data extraction

Data from eligible studies were extracted and listed. The following information were collected: authors, year, animal species and models, bacteria strains and inoculation methods, type of implants, antibacterial agents, coating strategies, implant characterization methods, assessments of biocompatibility, infection and bone healing outcomes.

3. Results

A total of 1280 records were identified. After removing duplicate records, 1127 studies were under further selection based on inclusion and exclusion criteria. Upon screening titles and abstracts, 92 articles with full text were reviewed. 14 studies were included in the systematic review. Details of the selection are shown in Fig. 1.

3.1. Animal models

Amongst the 14 studies, seven were defect models [13,14,18,20,22–24] and seven were fracture models [12,15–17,19,21,25]. The defect models included cylindrical bone defects and segmental defects, which were established in New Zealand white rabbits (four studies) [14,24,13,23], Sprague Dawley (SD) rats (three studies) [22,20,23], C57BL/6 mice (one study) [18]. One study used both New Zealand white rabbits and SD rats for defect models [23]. For the fracture models, six of them were open fractures conducted with saws/blades and one did not mention the details [25]. The fracture surgeries were performed on New Zealand white rabbits [12,15,25], ewes [19,16], SD rats [17], and C57BL/6 mice [21] (refer to Table 1).

3.2. Bacteria inoculation

S. aureus was used in all 14 studies [12–25]. *S. aureus* was directly injected into the medullary cavity or surgical sites in nine studies with a bacterial load ranged from 10^2 to 10^8 colony-forming units (CFUs) [14,18,20,22,24,15,17,25,16]. Whilst in the other five studies, *S. aureus* was pre-incubated with implants [12,13,23,19,21]. (refer to Table 1)

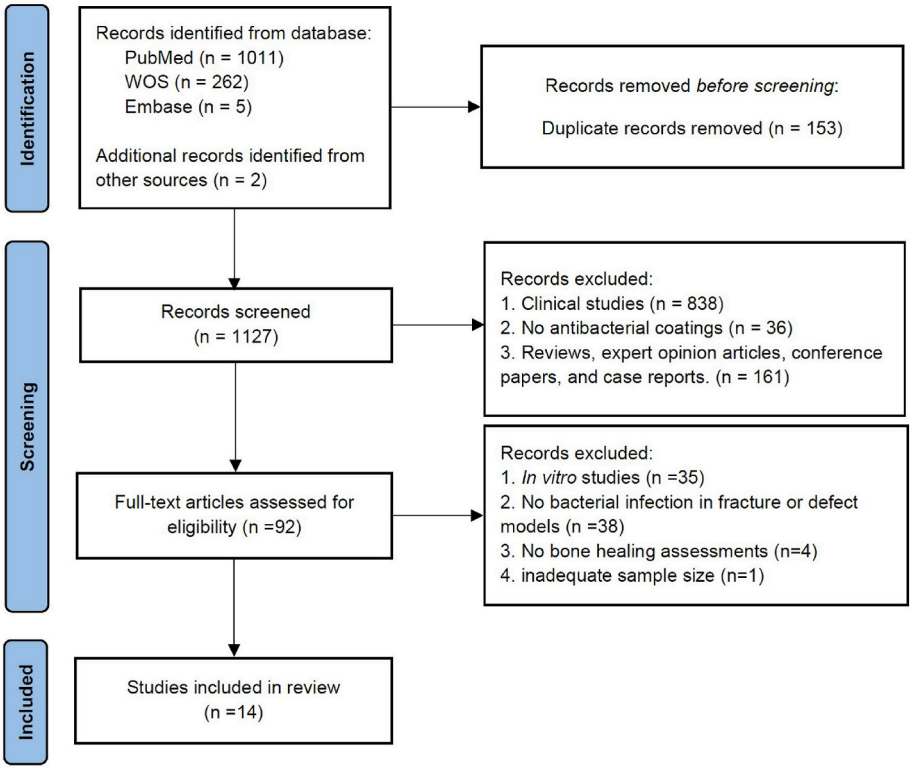

Fig. 1. Flow diagram of the literature selection.

3.3. Implants and coating strategies

Plates and screws [12,16,19,21], and Kirschner wires (K-wires) [15, 17,25] were used for fracture fixation. K-wires [18], rods [13] and scaffolds made from polycaprolactone [14], poly(lactic-co-glycolic acid) (PLGA)/HA [23], polyether ether ketone (PEEK) [22,20] and coral hydroxyapatite (CHA) [24] were applied in the bone defect models. (refer to Table 1)

Two studies investigated the application of passive coatings in fracture. Chae et al. fabricated a superhydrophobic coating on the micro/nanostructured surface with a perfluoropolyether lubricant layer, which presented a long-lasting antibiofouling effect against cells, proteins, calcium, and bacteria [12]. Schaer et al. synthesized the N,N-dodecyl, methyl-PEI to prepare a hydrophobic coating by repeated immersion, in which the coating prevented colonization of *S. aureus* and biofilm formation [16].

The other 12 studies reported active coatings with antibacterial agents including metal ions, antibiotics, chitosan, peptides, and proteins. Four studies used metal ions, including Ag^{+} [14,24] and Cu^{2+} [15, 25] to establish antibacterial effects. Li et al. utilized PDA coating on polycaprolactone scaffolds and coated Ag^{+} by immersing the scaffolds in $AgNO_3$ solutions [14]. Zhang et al. also prepared silver-coated coral hydroxyapatite (CHA) scaffolds by $AgNO_3$ solutions immersion [24]. Prinz et al. fabricated copper coated nails by Cu^{2+} galvanically deposition [15]. Zhang et al. loaded $CuCl_2$ into polylactic acid (PLA) and then coated them on the surface of titanium K-wires [25].

Five studies used antibiotics, including chlorhexidine (CHX) [17], tigecycline [18], vancomycin [18,19], gentamicin sulfate (GS) [22,20]. Shiels et al. used dip-coating to load chlorhexidine onto the Ti K-wires and covered N-(3-Sulphopropyl)-N methacryloxyethyl-N, N-dimethylammonium betaine (SBMA) by immersion for better anti-biofouling properties [17]. Stavrakis et al. manufactured the Ti K-wires with tigecycline or vancomycin encapsulated PEG-PPS polymers to achieve local control-releasing of the antibiotics. The K-wires were pretreated with oxygen plasma treatment followed by submersion with antibiotics-loaded PEG-PPS [18]. Stewart et al. modified vancomycin which was bonded to the Ti surface treated with nitrohydrofluoric acid and anodized with gold Ti 101 *via* aminoethoxyethoxyacetate (AEEA)-AEEA- aminopropyltriethoxysilane (APTS) mediated covalent coupling [19]. PEEK implants with CaP containing GS were fabricated by Xue et al. through layer-by-layer self-assembly technology [22]. Similarly, Sun et al. modified porous PEEK through co-deposition of dopamine and GS, thereby forming a GS loaded polydopamine (PDA) coating [20].

Three studies applied natural antibacterial materials such as chitosan, peptides, and enzymes for surface functionalization of the implants. Yang et al. developed a 3D printed scaffold based on PLGA and HA, and then coated hydroxypropyltrimethyl ammonium chloride chitosan (HACC) which is a chitosan derivative with improved water solubility and antibacterial effect through covalently grafting [23]. Chen et al. designed a fused peptide with antibacterial peptide HHC36 and vascular endothelial growth factor (VEGF)-mimetic peptide QK, APTS was also used for surface modification firstly and the fused peptides were linked to the modified Ti surface *via* click reaction in CuAAC-SB solutions [13]. Windolf et al. loaded lysostaphin into a poly(D,L)-lactide (PDLLA) matrix and then coated it onto Ti plates [21].

3.4. Outcome assessments of biocompatibility

Six studies investigated the biocompatibility of the coatings *in vitro* with peripheral blood mononuclear cells (PBMCs) [14], rabbit bone marrow derived mesenchymal stem cells (BMSCs) [25], MC3T3-E1 cells [24,20], RAW264.7 cells [20], human umbilical vein endothelial cells (HUVECs) and human BMSCs [13], or MG-63 cells [22]. Cell proliferation and viability was assessed by cell counting kit-8 (CCK-8) assay [14, 13,20,22,25], live/dead assay [14]. Six studies reported positive effect of the coatings on cell proliferation and viability [14,25]. However, coatings using excess concentration of Cu^{2+} [25], Ag^{+} [24] and GS [22] hindered cell proliferation and viability. One study using SPEEK-PDA-GS showed a negative effect on viability of MC3T3-E1 and

Table 1
Summary of the study characteristics.

Study	Animals	Model	Postoperative endpoint	Bacteria strain	Inoculation methods	Implant	Antibacterial agents	Coating strategy	Implant characterization
Chae et al. (2020) [12]	24 male New Zealand white rabbits	Mid-diaphyseal osteotomy in femur using saw blade	Week 4, Week 6	*S. aureus* (ATCC 25923)	Implants pre-incubation with 10^6 CFU/ml MRSA for 12 h	Stainless steel plates	Perfluoropolyether lubricant	1. Acids etching 2. POTS coating 3. Perfluoropolyether lubricants coating	SEM AFM XPS CA and SA Mechanical test
Chen et al. (2021) [13]	35 New Zealand white rabbits	2 defects on the femur (d = 2 mm)	Day 7, 14, 60	*S. aureus* (ATCC 6538P)	Implants pre-inoculation (10^8 CFU/mL, 15 μL)	Titanium rods	Fusion peptide	1. APTS modification 2. Immersion into click solution (CuAAC-SB) and fusion peptides	XPS Fluorescence intensity for fusion peptide
Li et al. (2019) [14]	20 female New Zealand white rabbits	Cylindrical defect in tibia	Week 8	*S. aureus* (ATCC 25923)	Injection into the marrow cavity, 100 μL, 10^5 CFU/mL	PCL scaffold	Ag^+	1. Polydopamine coating 2. Implant immersion with $AgNO_3$	SEM EDS WCA WAR ICP-MS
Prinz et al. (2017) [15]	12 female New Zealand white rabbits	Mid-diaphyseal osteotomy in tibia using a micro saw	Week 4	*S. aureus* (ATCC 25923)	Injection into the medullary cavity, 100 μL, 10^5 CFU/mL	Titanium K-wires	Cu^{2+}	1. Plasma electrolytic oxidation 2. Cu^{2+} deposition 3. Cu/Ti oxide composite layer blasting	SEM EDS Cu^{2+} releasing measurement
Schaer et al. (2012) [16]	12 Dorset-cross ewes	Mid-diaphyseal osteotomy in unilateral tibia using oscillating saw	Week 4 (efficacy study); Week 12 (safety study)	*S. aureus* (ATCC 25923)	Inoculation in fracture site, 2.5 mL, 1×10^6 CFU/mL	Stainless steel plate	N,N-dodecyl, methyl-PEI	1. Linear PEI N-alkylation with dodecyl and methyl moieties 2. Implant immersion in N,N-dodecyl,methyl-PEI	None
Shiels et al. (2018) [17]	54 SD rats	Osteotomy in the tibia (12 mm distal to the tibial plateau)	Week 4	*S. aureus* (UAMS-1, ATCC 49230)	Injection into the medullary cavity, 10 μL, $5.9 \times 10^2 \pm 30.8_{SEM}$ CFUs	Titanium K-wires	CHX	1. Implant dip-coating with CHX 2. Immersion into polymerisation initiator 3. Immersion into 10 % SBMA	MS
Stavrakis et al. (2019) [18]	18 male C57BL/6 mice	Segmental defect in the midshaft femur	Week 6	*S. aureus* (Xen36)	Inoculation into the fracture site, 2 μL, 1×10^8 CFUs	Titanium K-wires	Tigecycline and vancomycin	1. Oxygen plasma treatment 2. Reaction with 1% (3-mercaptopropyl) trimethoxysilane 3. Immersion in antibiotics-encapsulated PEG-PPS solution.	SEM X-ray Microanalysis
Stewart et al. (2012) [19]	9 mature Dorset-cross ewes	Mid-diaphyseal osteotomy in tibia using oscillating bone saw	Week 4, 12	*S. aureus* (ATCC 25923)	Implants pre-inoculation, 2.5 mL, 1×10^6 CFU/mL	Titanium plates	Vancomycin	1. Hydrothermal aging passivation 2. Aminopropylation using 5% APTS. 3. FMOC-AEEA linkers coupling 4. Vancomycin coupling	CLSM SEM
Sun et al. (2021) [20]	12 SD rats	Defect on the lateral side of the vertical femur (d = 2 mm)	Week 6	*S. aureus* (ATCC 6538)	Injection into femoral cavity, 30 μL 10^4 CFU/ml	PEEK implants	GS	1. Acid etching 2. Immersion into dopamine and GS solutions	SEM CA FTIR
Windolf et al. (2014) [21]	40 female Balb/c mice	Mid-diaphyseal osteotomy in femur using a Gigly saw	Week 4	*S. aureus* (ATCC 29213)	Inoculation into the fracture site, 1 μL, 1.94×10^3 CFU/μL	Titanium plates	Lysostaphin	1. Lysostaphin loading to PDLLA 2. PDLLA-lysostaphin coating	None
Xue et al. (2020) [22]	15 adult SD rats	Cylinder defect on the outside of the vertical femur (d = 2 mm, h = 5 mm)	Week 4	*S. aureus* (ATCC 6538)	Injection into femoral cavity, 30 μL, 10^4 CFU/ml	PEEK implants	GS	1. PEI modification 2. PSS assembly 3. $Ca(NO_3)_2{\cdot}4(H_2O)$ and GS coating by layer by layer reaction	SEM XRD FTIR GS releasing measurement

(*continued on next page*)

Table 1 (*continued*)

Study	Animals	Model	Postoperative endpoint	Bacteria strain	Inoculation methods	Implant	Antibacterial agents	Coating strategy	Implant characterization
Yang et al. (2018) [23]	80 female SD rats; 36 mature female New Zealand white rabbits	Segmental defect in the femoral mid-shaft (6 mm) in rats; cylinder defect (6 mm*4 mm) perpendicular to the femoral shaft in rabbits	Week 2, 4, 8 (rats); Week 8 (rabbits)	*S. aureus* (ATCC 25923)	Implants pre-inoculation (10^6 CFU/mL) for 10 min	PLGA/HA scaffold	HACC	1. PLGA/HA scaffolds manufacture 2. HACC covalently grafting	Micro-CT Mechanical test
Zhang et al. (2014) [24]	36 New Zealand white rabbits	15-mm segmental defect in upper 1/3 of the radius	Week 4	*S. aureus* (ATCC 25923)	Dispersion into the operation wound for 15 min	CHA scaffold	Ag^+	1. CHA scaffold immersion with $AgNO_3$	Ag^+ releasing measurement XRD SEM
Zhang et al. (2022) [25]	24 male New Zealand rabbits	Mid-diaphyseal osteotomy in tibia	Week 2, 6, 10	*S. aureus* (BNCC186335)	Injection into the proximal and distal stumps, 50 μL, 1×10^5 CFU/mL	Titanium K-wires	Cu^{2+}	1. Acids etching 2. $CuCl_2$ loading to PDLLA 3. PDLLA- Cu^{2+} coating	SEM MS CA CLSM

POTS, 1H,1H,2H,2Hperfluorooctyltriethoxysilane; SEM, scanning electron microscope; AFM, atomic force microscopy; XPS, X-ray photoelectron spectroscopy; CA, contact angle; SA, sliding angle; PEI, polyethylenimine; PCL, polycaprolactone; EDS, energy dispersive spectrometer; WCA, Water contact angle; WAR, water absorption rate; ICP-MS, inductively coupled plasma mass spectrometry; CHA, coral hydroxyapatite; XRD, X-ray Diffraction; PDLLA, poly(D,L)-lactide; CLSM, confocal laser scanning microscope; CHX, chlorhexidine; SBMA, N-(3-Sulphopropyl)-Nmethacryloxyethyl-N,N-dimethylammonium betaine; PEG, poly(ethylene glycol); PPS, poly(propylene sulfide); APTS, aminopropyltriethoxysilane; FMOC-AEEA, fluorenylmethyloxycarbonyl chloride-aminoethoxyethoxyacetate; PEEK, polyetheretherketone; GS, gentamicin sulfate; FTIR, Fourier-transform infrared spectroscopy; CuAAC-SB, Cu(I)-catalyzed azide-alkyne cycloaddition; PLGA, poly(lactic-co-glycolic acid); HA, hualuronic acid; HACC, hydroxypropyltrimethyl ammonium chloride chitosan

RAW264.7 cells [20]. Cell differentiation activity was also evaluated by alkaline phosphatase (ALP) activity test, RT-PCR, immunofluorescence, or western blot in five studies [24,13,20,22,25]. Ti implants coated with fusion proteins promoted angiogenic activity and osteogenic activity of HUVECs and hBMSCs [13]. Other coatings with Cu^{2+}, Ag^+ and GS also increased the cell differentiation level *in vitro*, but high level of Cu^{2+}, Ag^+ and GS were reported to have negative effects on cell osteogenic activity [24,22,25]. (refer to Table 2)

The other eight studies evaluated the biocompatibility of the implant coatings *in vivo*. To investigate the effects of the coatings on natural healing process, non-infected fracture models were established in two studies. Schaer et al. reported that N,N-dodecyl,methyl-PEI coated LCPs showed equal effects in radiographic and histologic assessments with the uncoated LCPs, indicating that N,N-dodecyl,methyl-PEI coatings did not impair fracture healing during 3-months implantation. However, Shields et al. found that the CHX-loaded coatings decreased bone formation and implant integration with surrounding bone tissues. One study reported unexplained death of two animals with bacterial infection treated with bare nails and copper coated nails, respectively [15]. HE staining of liver and kidney sections was performed in two studies to evaluate the systemic toxicity. No obvious structure abnormality or inflammatory response was reported. Other studies found no obvious systemic toxicity or side effects.

3.5. Antibacterial effect of the coatings in vivo

Histological assessments were conducted in 12 studies, including hematoxylin-eosin (HE) staining for bone tissues histological analysis and infection evaluation, Masson's trichrome staining, immunohistochemistry (IHC) for interleukin-10 (IL-10), IL-6, IL-4, and tumor necrosis factor-α (TNF-α), Gram staining, Giemsa staining, Brown and Brenn staining [14,24,12,13,15,20,22,23,25,16,19,21]. Seven studies examined the bacterial burden in bone or on implant surface by CFUs counting, bacteria culture, or bioluminescence imaging [18,13,23,15,17,25,16]. Local inflammation was also assessed by flow cytometry (FCM) for neutrophils and enzyme-linked immunosorbent assay (ELISA) for IL-6 in surgical site lavage fluids in one study [21]. Levels of systemic inflammation was evaluated in four studies through blood cell counting, C-reactive protein (CRP), or procalcitonin (PCT) detection [20,15,25]. Four studies used confocal laser scanning microscopy (CLSM) or scanning electron microscope (SEM) to visualize the biofilms on bones or implant surfaces [23,19,16]. X-ray imaging was applied in three studies to monitor bone infection [24,22,23]. (refer to Table 2)

Chae et al. found no infection in the tissues surrounding fracture site in all rabbits treated with lubricated orthopedic implant surface (LOIS), and the level of local immune response was reduced [12]. Schaer et al. confirmed that N,N-dodecyl,methyl-PEI-coating was effective in preventing biofilm formation on plate implants by SEM examination [16].

Li et al. reported that silver-coated polycaprolactone (PCL)/PDA scaffolds reduced the infection rate in the rabbit defect model [14]. Zhang et al. showed that silver-coated CHA scaffolds controlled bone infection as shown in X-ray imaging [24]. Prinz et al. examined the reversibly attached or biofilm-forming bacteria on the nail surface and found no bacteria on copper coated nails, demonstrating the antimicrobial effect of copper released from the nail surface [15]. Zhang et al. reported that no bacteria colony was found in the implants coated with copper, which also alleviated systemic inflammation [25].

Shiels et al. reported reduced bacteria and neutrophils in the bone and callus with SBMA+CHX coated K-wires. They also revealed that the addition of systemic cefazolin improved antibacterial efficacy of SBMA+CHX [17]. Vancomycin or tigecycline coatings on K-wires developed by Stavrakis et al. significantly reduced bacteria burden on Day 14 which lasted throughout the 42-day study period [18]. CaP-and-GS modified PEEK implants controlled bone infection as shown in X-ray imaging [22]. Vancomycin modified titanium plates decreased clinical and histological signs of infection and prevented biofilm formation [19]. Sun et al. showed that SPEEK–PDA–GS could weaken the expression of proinflammatory factors (TNF-α, IL-6 and chemokine receptor-7) but enhance expression of anti-inflammatory factors (IL-4, IL-10 and CD206), and prevent purulent osteomyelitis [20].

Yang et al. constructed a HACC-grafted PLGA/HA scaffold which not only reduced the amount of attached bacteria on the fixation devices but also inhibited the formation of biofilms, and the bacterial burden in femoral shaft and condyle was remarkably decreased [23]. Chen et al.

Table 2
Assessment of biocompatibility, infection, and bone healing in each study.

Study	Biocompatibility	Assessments of infection	Assessments of bone healing	Outcomes
Chae et al. (2020)[12]	*In vivo*	HE staining MT staining IHC	X-ray Micro-CT TRAP staining	No bacterial infection and reduced levels of local immune response in the rabbits implanted with LOIS. LOIS promoted callus formation and osteoclast activity. Infected group using LOIS showing same degree of bone healing as that of the noninfected group with bare surface.
Chen et al. (2021)[13]	*In vitro* test with HUVECs and hBMSCs: CCK-8, cell morphology, qPCR, IF, WB	CFUs counting HE staining	methylene blue & basic fuchsin staining HE staining	99% *S. aureus* on implants was killed by FP coating and AMP coating Ti–125FP and Ti-125AMP reduced inflammatory cells in around tissues. Ti–125FP promoted vascularization in early stage and osseointegration between the implant and the bone.
Li et al. (2019) [14]	*In vitro* test with PBMCs: CCK-8, live/dead assay	HE staining	Micro-CT HE staining Safranin-O/Fast Green staining	PCL/PDA/AgNPs enhanced callus mineralization and osseointegration, increasing BV/TV and Tb.N in group implanted with PCL/PDA/AgNPs scaffolds. PCL/PDA/AgNPs scaffolds promoted bone cells attachment and proliferation.
Prinz et al. (2017)[15]	*In vivo*	CFUs counting CRP HE staining	X-ray HE staining	No attachment of reversibly attached or biofilm-forming bacteria on copper-coated implants Copper coated nails increased callus formation compared in both infected and non-infected model but no complete fracture healing.
Schaer et al. (2012)[16]	*In vivo*	Bacteria culture SEM HE staining Brown and Brenn staining	X-ray Micro-CT HE staining Toluidine blue staining	N,N-dodecyl,methyl-PEI coating eliminated the clinical signs of infection and completely prevented biofilm formation. N,N-dodecyl,methyl-PEI coating enhanced osteotomy bridging, hyaline cartilage formation, and the following ossification and remodeling in treatment group.
Shiels et al. (2018)[17]	*In vivo*	CFU counting Gram staining	X-ray Micro-CT Mechanical test HE staining	SBMA+CHX coating reduced bacteria and neutrophils in the bone and callus. SBMA+CHX coating improved bone formation and increased BV/TV. Effect of SBMA+CHX coating was improved combining with systemic cefazolin.
Stavrakis et al. (2019)[18]	*In vivo*	Bioluminescence imaging CFUs counting	X-ray	Vancomycin and tigecycline coatings reduced bacteria burden and on the implant surface, surrounding bone and soft tissue. Vancomycin and tigecycline coatings prevented osteolysis and bony destruction.
Stewart et al. (2012)[19]	*In vivo*	SEM Brown and Brenn staining	X-ray micro-CT HE staining Van Kossa staining Toluidine blue staining	Vancomycin-loading coating decreased clinical and histological signs of infection and prevented biofilm formation on plates. Vancomycin-loading coating improved callus formation and promoted callus remodeling and bridging.
Sun et al. (2021)[20]	*In vitro* test with MC3T3-E1 and RAW264.7 cells: CCK-8, qPCR, SEM *In vivo* test with HE staining of liver and kidney	IHC WBC counting HE staining	X-ray micro-CT HE staining Toluidine blue staining	No imaging signs of osteomyelitis in SPEEK-PDA-GS coating treated rats. SPEEK-PDA-GS lowered the local inflammation, anti-inflammatory factors (IL-4 and IL-10) were increased, proinflammatory factors (IL-6 and TNF-a) and neutrophils were decreased in SPEEK-PDA-GS group. SPEEK-PDA-GS promoted trabecular bone formation around the implant, which were continuous and tightly combined with implant.
Windolf et al. (2014)[21]	*In vivo*	CFUs counting Flow cytometry ELISA	X-ray	Lysostaphin-coated plates reduced bacteria loads and inflammatory level by decreasing the number of neutrophils and IL-6 levels in bones throughout 28-days. lysostaphin-coated group showed signs of fracture healing by Day 14 and complete fracture consolidation by Day 28 while no healing sign in control groups.
Xue et al. (2020)[22]	*In vitro* test with MG-63 cells: CCK-8, ALP enzyme activity, SEM; *In vivo* test with HE staining of liver and kidney	X-ray HE staining	X-ray Micro-CT HE staining Toluidine blue staining	CaP-GS*6 and CaP-GS*9 coating decreased the number of neutrophils and controlled bone infection. CaP-GS*6 and CaP-GS*9, but not CaP-GS*3 favored the new bone formation around implants and enhanced implant integration with new bone tissue.
Yang et al. (2018)[23]	*In vivo*	X-ray Bacteria culture CLSM SEM Giemsa staining HE staining	X-ray micro-CT HE staining Stevenel's blue and Van Gieson staining Masson's trichrome staining TRAP staining Calcein staining	PLGA/HA/HACC composite scaffold reduced the attached bacteria and inhibited biofilm formation on implant, decreased bacterial burden in femur. PLGA/HA/HACC scaffold promoted new bone formation and reduced osteoclasts, relatively complete bone morphology was found in cortical bone defect in rats and cancellous bone defect in rabbits. PLGA/HA/HACC scaffold significantly increased cortical bone mineral density and increased trabecular bone formation.
Zhang et al. (2014)[24]	*In vitro* test with MC3T3-E1 cells: ALP enzyme activity	X-ray HE staining	X-ray HE staining Masson staining	Silver-loaded coral hydroxyapatite (SLCHA) controlled bone infection. The 15 mm segmental defect showed relatively complete reconstrction when treated with silver-loaded CHA scaffold after 10 weeks, but no difference in bone regeneration was found between sliver-loaded CHA and bare CHA.
Zhang et al. (2022)[25]	*In vitro* test of BMSCs: CCK-8, qPCR	WBC count CRP PCT CFU counting HE staining	X-ray HE staining	No visible bacteria colonies from copper-loaded PLA coated implant. Copper-PLA coated implant alleviated systemic inflammation. Copper-PLA coating accelerated fracture-end union and callus formation under both infection and non-infection circumstances.

HE, hematoxylin-eosin; MT, Masson's trichrome; IHC, immunohistochemistry; TRAP, tartrate resistant acid phosphatase; LOIS SEM, scanning electron microscope; PBMC, peripheral blood mononuclear cell; CCK-8, cell counting kit-8; BV, bone volume; TV, total volume; Tb.N, trabecular number; PCL, polycaprolactone; PDA,

polydopamine; NP, nanoparticle; ALP, alkaline phosphatase; CHA, coral hydroxyapatite; WST-1, water-soluble tetrazolium salt-1; WBC, white blood cell; CFU, colony-forming units; CRP, C-reactive protein; BMSC, bone marrow derived mesenchymal stem cell; PCT, procalcitonin; BMA, N-(3-Sulphopropyl)-Nmethacryloxyethyl-N,N-dimethylammonium betaine; CHX, chlorhexidine; SEM, scanning electron microscope; PEEK, polyetheretherketone; GS, Gentamicin sulfate; qPCR, quantitative polymerase chain reaction; IHC, immunohistochemistry; SPEEK, sulfuric acid treated polyetheretherketone; PDA, polydopamine; IL, interleukin; TNF, tumor necrosis factor; HUVEC, human umbilical vein endothelial cell; FP, fusion protein; AMP, antimicrobial peptide; ELISA, enzyme linked immunosorbent assay; PLGA, poly(lactic-co-glycolic acid); HA, hualuronic acid; HACC, Hydroxypropyltrimethyl ammonium chloride chitosan.

demonstrated that AMP coating on Ti surface exhibited excellent antimicrobial activity, killing more than 99% of *S. aureus* on implants therefore reducing the inflammation in bone tissues [13]. Windolf et al. significantly lowered the bacteria quantity in fracture site and inflammation response of mice using lysostaphin-coated plates throughout the 28-days study period [21].

3.6. Bone healing effect of the coatings in vivo

A total of 12 studies conducted X-ray imaging to examine bone healing [18,20,22,24,12,15–17,19,21,23,25] and nine studies used microcomputed tomography (micro-CT) to evaluate bone formation [14,22,20,23,12,17,19,16]. Histological assessments including HE staining, safranin-O/fast green staining, tartrate resistant acid phosphatase (TRAP) staining, Stevenel's blue and Van Gieson staining, Masson's trichrome staining, calcein staining, toluidine blue staining and methylene blue & basic fuchsin staining were conducted in 13 studies [14,24,12,13,15–17,19–23,25]. One study performed mechanical tests [17]. (refer to Table 2)

Chae et al. proved that fractures with *S. aureus* infection showed poor healing results compared to contamination-free ones. Little callus formation and osteoblasts were found around the contaminated fracture sites when using plain plates. While plates with LOIS remarkably improved callus formation and activity of osteoclasts, LOIS demonstrated the same degree of bone healing compared to that of the non-infected group with control plates in week 6 [12]. Sheep implanted with N,N-dodecyl,methyl-PEI-derivatized LCPs showed less signs of acute postoperative infection in clinical observation, consistently with enhanced osteotomy bridging and hyaline cartilage formation observed by digital radiography and histological examination [16].

Scaffolds with silver coating increased the bone volume (BV)/total volume (TV) ratio and trabecular number (Tb.N) in a rabbit defect model, and with the presence of PDA, PCL/PDA/AgNPs scaffolds exhibited better healing effect compared to PCL/AgNPs scaffolds [14]. Silver-loaded coral hydroxyapatite bone scaffolds had similar effects in bone healing with CHA, displaying a comparable level of bone density increase, cell ingrowth, new trabeculae formation, scaffold degradation and lamellar bone appearance [24]. Prinz et al. confirmed the bone-forming effect of Cu^{2+} *in vivo* by showing the increased callus formation in animals using a copper nail with or without bacterial inoculation [15]. Copper-loaded PLA coating on wires significantly enhanced the callus formation in fracture model with *S. aureus* infection, which also promoted healing on fracture without bacterial infection compared to bare PLA coatings [25].

Shiels et al. found that bones implanted with an unmodified wire showed signs of periosteal reaction, osteolysis and non-union of the osteotomy even when treated with systemic cefazolin (1 mg/kg) for 72 h, whilst implantation with a SBMA+CHX coated wire led to bone quality improvement and signs of union. SBMA+CHX coatings also increased the BV/TV ratio without systemic cefazolin treatment [17]. Local delivery of vancomycin or tigecycline by PEG-PPS coating implants prevented osteolysis and bony destruction in an open fracture model [18]. Endosteal callus was present in vancomycin-modified implant treated group but not in the group treated with control implants [19]. PEEK/CaP-GS coatings with different layers of GS showed different treatment efficacies in rat femoral defects. In PEEK/CaP-GS*6 and PEEK/CaP-GS*9 groups, trabecular bone formation and surrounding bone tissue attachment were improved compared to PEEK/CaP-GS*3 and PEEK [22]. More trabecular bone and continuous new bone were found around the PDA and GS layer-modified porous PEEK (SPEEK–PDA–GS) implants in infected bone defect model, suggesting the enhanced osseointegration ability of the PDA–GS coatings [20].

HACC-grafted 3D-printed PLGA/HA composite scaffolds promoted new bone formation in both infected femoral shaft defects in rats and femoral condyle defects in rabbits within 8 weeks [23]. Ti implant coated with fusion peptide of QK and AMP showed promoted osteogenesis and osseointegration because of the angiogenic activity and vascularization of the VEGF-mimetic peptide QK [13]. Mice femoral defects fixed with lysostaphin-loaded PDLLA coated Ti plates showed signs of fracture healing on Day 14 and complete fracture consolidation on Day 28 while no healing sign were observed in groups using control plates [21].

4. Discussion

In this review, we summarized the current landscape of orthopaedic implant coatings with antibacterial activity in preclinical studies with animals. We hope to offer a perspective on an optimal design of orthopaedic implant coating strategies for future research and treatment of FRI.

4.1. MRSA-associated FRI

Although being a commensal bacterium, *S. aureus* has high virulence and invasive ability to cause severe osteomyelitis, osteolysis and even sepsis [50]. Current orthopaedic infection studies mainly focused on *S. aureus* [51]. It is worth noting that *S. aureus* is capable of quickly evolving and developing resistance to antibiotics used in clinical treatments [52]. Moreover, the treatment of MRSA infections is much more complicated. Therefore, *in vivo* investigations that develop novel strategies to combat MRSA infections are essential. However, none of the studies included in this systematic review investigated the effectiveness of implant coatings with the presence of MRSA *in vivo*. Future studies should be conducted to evaluate effectiveness of coatings in MRSA infection prevention and treatment.

Moreover, the focus on *S. aureus* is certainly a limitation, as enterobacterales and nonfermenters are playing a substantial role as infectious agents and the infections caused by these pathogens are different from *S. aureus* and require different treatment strategies [53].

4.2. Osteoporosis-associated FRI

Studies have shown that osteoporotic patients are at higher risk of infections [54]. Osteoporotic bones with FRI also show more severe infection and delayed healing. Systemic antibiotics therapy was found ineffective in FRI model in rats with osteoporosis, possibly due to the bacterial colonization in the implant and cortical porosities [55]. More investigations are needed to develop alternative therapies targeting FRI in osteoporotic bones. Implant coatings that can achieve bacterial adhesion resistance, local control releases of antibacterial agents and bone formation acceleration may show promising effects in favoring osteoporotic bone healing [56].

4.3. Antibacterial coating strategies

4.3.1. Passive coatings for anti-bacterial adhesion

According to the action mode, strategies of antibacterial implant coatings can be categorized as passive coatings and active coatings [40].

Passive coatings are designed to prevent infection by resisting pathogens adhesion instead of killing them directly. This strategy usually focuses on modifying implant surface structure, roughness, and wettability [41]. The engineering of nanoscale microstructure on implant surfaces and increased surface roughness showed beneficial effects in preventing bacterial attachment [57,58]. Interestingly, both superhydrophilic and superhydrophobic surfaces were proved to be effective in reducing bacterial adhesion [59]. Polymers with high hydrophilicity such as PEG, HA, and zwitterionic polymers have been utilized to construct the superhydrophilic surfaces [60,61]. While superhydrophobic surfaces were fabricated with low surface energy polymers, such as fluorinated polymers and polydimethylsiloxane, which can achieve anti-biofouling effect against bacteria and proteins [62,63]. However, this anti-adhesion property of the implant surface will not only prevent the attachment of bacteria but also the cells responsible for osteogenesis and osseointegration. It may result in inadequate integration between the implant and the surrounding tissues, which will lead to implant loosening and poor bone healing [64]. Therefore, simple passive coatings may not be the ideal strategy for implants applied in fracture and defect. To address this problem, antiadhesive passive coatings often combine with bactericidal agents to construct active coatings on implants [65]. (refer to Fig. 2)

4.3.2. *Active coatings for bactericidal effects*

Active coatings are endowed with direct antimicrobial effects *via* utilizing bactericidal substances, including metal ions, antibiotics, AMPs, and enzymes [40]. Metal ions including Ag^{+}, Cu^{2+}, Zn^{2+}, and Mg^{2+}, which are strong broad-spectrum antibacterial agents [66]. Cu^{2+}, Zn^{2+}, and Mg^{2+} were reported to have additional biological activities helping osteogenesis and vascularization, as well as the low cytotoxicity, which make them good candidates for antibacterial coatings [51,67,68]. But it should be cautious that high level of metal ions will cause surrounding tissue injuries and systemic toxicity [69]. Implant coatings with antibiotics can realize the local delivery of antibiotics to the fracture sites with effective concentration. Strategies to achieve the controlled release of antibiotics and avoid toxicity are the main concerns of *in vivo* application for antibiotics-loaded coatings [70]. Furthermore, the application of antibiotics-loaded coatings is restricted by the prevalence of drug-resistant bacteria and infections caused by multi-organisms. Natural antibacterial agents such as AMPs and antibacterial enzymes are promising alternatives of traditional antibiotics, they are broad-spectrum antibacterial agents with good

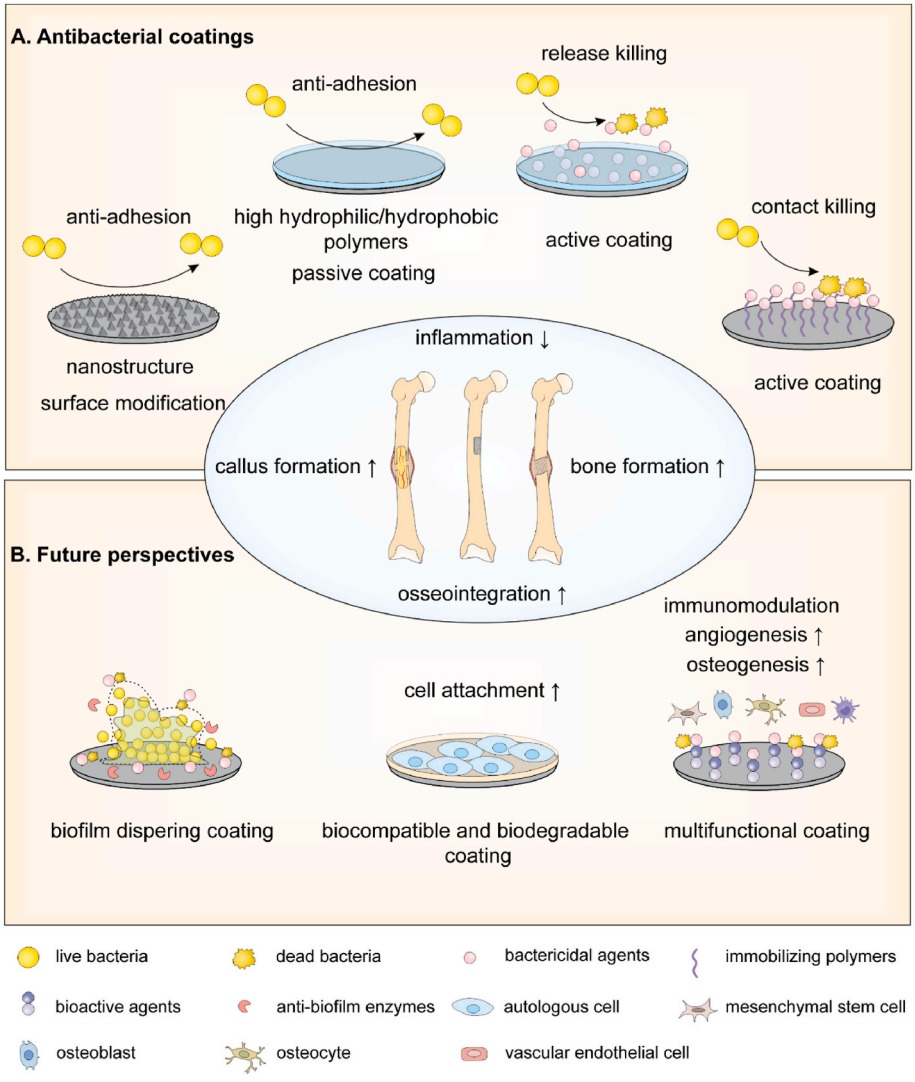

Fig. 2. Schematic diagram of antibacterial implant coatings and their effects on bone repair in the presence of bacterial infection. The application of implants with antibacterial coatings effectively prevents or treats bacterial infection, allowing bone formation and osseointegration of implants in fracture and defect models. (A) The mechanism of antibacterial coating can be categorized as passive and active. Surface modification is often performed to create nano-structures in advance. (B) Future perspectives on implant coating development.

biocompatibility and low drug resistance [21,71,72]. More importantly, enzymes with antibiofilm activity can prevent the biofilm formation and even remove the established biofilms by degrading the bacterial exopolysaccharides and extracellular DNA, or by quorum quenching [73]. Coatings with antibiofilm enzymes can be utilized to tackle the biofilm-related FRI which is difficult to treat by conventional antibiotics.

The combination of anti-adhesion coatings and bactericidal coatings allows to kill adhered bacteria or surrounding bacteria by releasing bactericidal substances, meanwhile the passive function helps to prevent bacteria attachment and remove the dead bacteria from implant surface, preventing biofilm formation and ensuring long-lasting antibacterial effect [74]. All implant coatings reported in the studies exhibited certain level of antibacterial effects and bone formation improvement under the situation of fracture or defect related infections. These coatings decreased the levels of inflammation in fracture sites through the reduction or elimination of bacterial infections, and subsequently improved the bone formation and fracture union [20,13,12,19,21]. (refer to Fig. 2)

4.4. Multifunctional coating strategies

Apart from antibacterial activity, coating strategy can further provide implants with multiple functions such as excellent biocompatibility, osteogenesis and osseointegration promotion, as well as immunomodulating ability [75]. In the cases of Cu^{2+}-TiO_2 coating and Cu^{2+}-PLA coating, Cu^{2+} was found to promote bone formation with/-without bacterial infection [15,25]. Coating using fusion protein of antimicrobial peptides and VEGF-mimetic peptides was also capable of improving osseointegration and osteogenesis by promoting vascularization around the implants and modulating osteoblasts activity [13]. Porous PEEK implant coated with PDA and gentamicin had the advantage of osseointegration promotion which may benefit from the enhanced cell attachment on PDA coating [20]. Coatings with additional functions such as osteogenesis and osseointegration activity were able to directly enhance the bone healing [24,20,13,25]. The promotion of osteogenesis and osseointegration will in turn help to reduce bacterial adhesion and proliferation [76]. However, bone regeneration is depressed under the circumstances of bacterial infection, necrotic bones, and vascularization deficiency, which will continually lead to persistent infection. In view of this, future studies should investigate how to effectively restrain the infection and strengthen bone regeneration process with multifunctional coatings. The combination of high-efficiency bactericidal effects and bacteria attachment resistance, good biocompatibility, osseointegration and osteogenesis promotion would be a promising direction for the development of novel implant coatings. (refer to Fig. 2)

4.5. The effects of antibacterial coatings on bone repair

When fracture fragments are anatomically reduced with stable fixation, the fracture will undergo primary bone healing with cutting cones. Intramembranous ossification occurs through Haversian remodeling. The other type of healing is secondary bone healing [77]. In this process, initially a hematoma forms around the injury site. Inflammatory cells, including neutrophils, macrophages, and lymphocytes are found infiltrating within the hematoma, inducing acute inflammation. This will contribute to mesenchymal stem cells (MSCs) differentiation, fibrocartilaginous callus formation which bridge the bone gap, as well as vascularization [78]. Subsequently, fibrocartilaginous callus is calcified and substituted by bony callus, followed by the remodeling *via* interplay between osteoblasts and osteoclasts [79].

Bacterial infection negatively impacts the healing process [80]. Firstly, the acute inflammation is pronounced and can develop into chronic inflammation in the presence of invading bacteria, which reduces the osteogenic differentiation and migration of MSCs [81]. Secondly, excessive inflammation can interfere with normal angiogenesis and revascularization [82]. Thirdly, bacterial infection enhances the activities of osteoclasts by proinflammatory cytokines [8], which inhibit osteogenesis by inducing osteoblasts apoptosis [83]. The dysregulation between osteoclasts and osteoblasts leads to the formation of structurally compromised bone [80]. Lastly, biofilm on implants can act as barrier, impairing implant osseointegration and causing implant loosening [84].

The elimination of destructive bacteria by antibacterial implant coatings help to provide suitable conditions for the series of biological activities of bone repair. This contributes to a balanced osteoclast and osteoblast activity that allows normal fracture healing to occur [12,17,19,21]. Critical-sized and large-fragment defects are more complicated conditions in bone repair, which requires large quantity of bone regeneration. In these situations, three-dimensional-printed scaffolds have been applied in defect sites, providing porous structures for tissue regeneration [85]. Similarly, new bone formation was also promoted in porous scaffolds supplemented with antibacterial properties [14,24,23]. Various metals and biomaterials are occasionally used as bone graft substitutes to fill defect sites to provide mechanical support. Since bacterial infection often interferes with bone attachment onto implant surface, ensuring optimal biocompatibility and integration with surrounding bone tissues under bacterial infection has become a challenge [86]. Implants with antibacterial coatings were found to have better osseointegration, enhancing the trabecular bone formation and bridging between implant and bone [13,20,22]. Interestingly, antibacterial coatings combined with bioactivities promote bone healing show promising therapeutic effects in infected bone repair. Vascular regeneration and reconstruction are the essential parts, which will provide oxygen, nutrients, and cells for bone repair. Invading bacteria may contribute to vascular blockage and necrosis, and can alter the inflammatory response to modulate the vascularization. Chen et al. found that the vascularization was severely impaired in the circumstance of infections [13], while Gilbert et al. reported that open fractures with bacterial infection had greater perfusion and vascularity compared to non-infected fractures, which maybe results from the inflammation caused by bacterial infection [87]. It has been reported that inflammatory cytokines such as TNF-α and IL-1β can induce expression of VEGF and enhance angiogenesis, and immunocytes including macrophages, neutrophils, and lymphocytes also play important roles in inducing angiogenesis-related pathway activation [88–90]. However, the immuno-vascular interaction in infected bone remains poorly understood, and future studies should investigate the underlying mechanisms. (refer to Fig. 2)

4.6. Biocompatibility of the implant coatings

Implant coatings are required to have good biocompatibility, more specifically, they should exhibit desired functions without causing local and systemic toxicity or disturbing natural biological response [91]. Biocompatibility tests are mandatory for novel coatings before their application in clinical practice. Common biocompatibility tests evaluating the cytocompatibility of the biomaterials include MTT test, CCK-8 test, WST test, live/dead assay, cell morphology and adhesion observation, which mainly reflect the effects of the materials on cell proliferation and viability [92]. ALP activity is usually conducted to examine osteogenic cells activity [93]. *In vivo* biocompatibility is mainly evaluated by histological analysis of implant surrounding tissues, liver and kidney, animal behavior, body weight and survival [75]. Additionally, it is recommended to carry out hemolysis and coagulation test and ensure the hemocompatibility of coating materials, since there may be risks of thrombosis and hemolysis when implants are exposed to *in vivo* [94].

It should be noted that high level of metal ions and antibiotics may show negative effects on cell proliferation and viability [95,96]. *In vitro* cytotoxicity from implants releasing silver, copper or GS has been reported by four studies in this review [24,22,25]. Since the metal ions and antibiotics mainly function in a releasing way, concentrations in blood,

organs and local tissues around the implants should also be monitored to ensure the safety *in vivo*. Furthermore, due to the differences in loading efficacy and releasing proportion of various coating strategies, it is of significant importance to investigate cytotoxic effect of the coatings prepared with different concentrations of antibacterial agents to determine their safety threshold, thereby avoiding side effects. Components with good biocompatibility and biodegradability such as PDA, PEG, PLA, polycaprolactone (PCL), and HA are recommended to include in the coating formulation, in order to ensure the implant biocompatibility and therefore improve osteointegration of the implants [51,97].

4.7. Recommendations on antibacterial assessments

The assessments of orthopaedic infections caused by bacteria can be broadly categorized into three aspects: (1) clinical observations, hematology analysis, and radiological observations to evaluate whether there is systemic and local infection; (2) microbiological analysis of bone tissues or implants; (3) histological analysis of bone tissues and surrounding soft tissues to evaluate local inflammation. Most studies in this systematic review conducted infection assessment in all three aspects. X-ray imaging and HE staining were the most used methods to examine fracture sites infection or osteomyelitis, which can show bone structure changes caused by infections. However, several studies only carried out these two assessments to evaluate the anti-infection efficacy of implant coatings, which was insufficient [14,24,22]. X-ray radiography shows soft tissue swelling, periosteal reaction, bone lysis, and loss of trabecular architecture [98], whilst HE staining can show these changes at the histological level. X-ray radiography often does not show bone abnormalities at the early onset of infection [99]. Besides, *S. aureus* can directly recognize and invade osteoblasts, osteocytes, and macrophages, induce their apoptosis and necrosis, subsequently causing exaggerated inflammatory response and delaying bone formation [100–102]. Some bacteria that escape from eradication by antibacterial coatings may hide within the macrophages or osteocyte lacuno-canalicular network (OLCN), becoming a potential risk for next outbreak of infections [102,103]. Thus, microbiological examination is indispensable for the evaluation of antibacterial efficacy. Bacteria on the surface of implants or bones can be visualized by SEM or quantified roughly by culture of the bone and implant sample or their sonication fluids. Bioluminescence imaging is also a direct semi-quantitative method to evaluate the local bacteria burden, but it requires the inoculation of bioluminescent bacteria strains. While the bacteria in deep tissues need to be detected tissue sections staining, such as with Gram staining, Giemsa staining, Brown and Brenn staining, which can display the distribution of the target bacteria. The bone healing process is closely related to the immune response, and excessive inflammation caused by bacterial infection will dampen the bone healing [104]. Assessments of inflammation in fracture sites and implant surrounding tissues will provide corroborative evidence for infection diagnosis as well as the improvement of bone healing. HE staining of infiltrated immunocytes such as macrophages and neutrophils is the basic histological analysis of inflammation. IHC analysis of proinflammatory cytokines (TNF-α, IL-6, interferon-γ) and anti-inflammatory cytokines (IL-4, IL-10, transforming growth factor-β) can better characterize the local inflammatory response [105,106].

4.8. The strength and limitation of this systematic review

Several systematic reviews have been conducted to summarize the antibacterial efficacy of orthopaedic implant coatings with antibacterial property using specific substances but were mainly focused on the assessment of antibacterial effects in implant-related infection [107–109]. Only two systemic reviews analyzed the treatment efficacy of gentamicin-coated implant in clinical fracture treatment [110,111], but no review investigated the antibacterial and osteogenic effects of the current novel implant coatings in preclinical studies. The strength of this systemic review is that we provided a comprehensive summary of antibacterial coatings on implants used in fracture and defect models, where both antibacterial efficacy and their effects on osteogenesis were assessed. This helps to provide novel strategies for the development of multifunctional orthopaedic implants. Coating strategies for antibacterial and osteogenesis studied only by *in vitro* methods were not included. As the function of coated implants may be interfered by the complex tissue environment, purely *in vitro* investigations may not be sufficient to prove their efficacy. The limitation of this review is that the comparison of the efficacies of different coating strategies was not conducted due to the differences of the infection models (animals, bacteria strains, inoculation way and quantity, devices).

5. Conclusion

In conclusion, the antibacterial coating strategies can reduce bacterial infections in animal models and favor bone healing *in vivo*. *S. aureus* is the main pathogenic bacteria in orthopaedic infection studies, at the same time, infections caused by MDR bacteria such as MRSA require more attention for efficient treatments. To cope with the complex clinical challenges of FRI, implant coatings could be endowed with multifunction combining good biocompatibility, excellent antibacterial effects, as well as osseointegration and osteogenesis promotion.

Author contribution

Conception and design of study: R.M.Y. Wong, S.S.Y. Leung, W.H. Cheung, M.Ip, P. Thebault, acquisition of data: B. Li, R.M.Y. Wong, analysis and/or interpretation of data: B. Li, R.M.Y. Wong, Drafting the manuscript: B. Li, B. Labat, G. Ladam, V. Alt, M. Rupp, C. Brochausen, N. Zhang, revising the manuscript critically for important intellectual content: B. Li, P. Thebault, B. Labat, G. Ladam, V. Alt, M. Rupp, C. Brochausen, J. Jantsch, M. Ip, N. Zhang, W.H. Cheung, S.S.Y. Leung, R. M.Y. Wong, Approval of the version of the manuscript to be published (the names of all authors must be listed): B. Li, P. Thebault, B. Labat, G. Ladam, V. Alt, M. Rupp, C. Brochausen, M. Ip, J. Jantsch, N. Zhang, W.H. Cheung, S.S.Y. Leung, R.M.Y. Wong.

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Declaration of competing interest

A conflict of interest occurs when an individual's objectivity is potentially compromised by a desire for financial gain, prominence, professional advancement or a successful outcome. The Editors of the *Journal of Orthopaedic Translation* strive to ensure that what is published in the Journal is as balanced, objective and evidence-based as possible. Since it can be difficult to distinguish between an actual conflict of interest and a perceived conflict of interest, the Journal requires authors to disclose all and any potential conflicts of interest.

References

[1] Nair MB, Kretlow JD, Mikos AG, Kasper FK. Infection and tissue engineering in segmental bone defects - a mini review. Curr Opin Biotechnol 2011;22(5):721–5.
[2] Metsemakers WJ, Morgenstern M, McNally MA, Moriarty TF, McFadyen I, Scarborough M, et al. Fracture-related infection: a consensus on definition from an international expert group. Injury 2018;49(3):505–10.
[3] Wu A-M, Bisignano C, James SL, Abady GG, Abedi A, Abu-Gharbieh E, et al. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. Lancet Healthy Longev 2021;2(9):e580–92.

[4] Wong RMY, Wong PY, Liu C, Wong HW, Chung YL, Chow SKH, et al. The imminent risk of a fracture-existing worldwide data: a systematic review and meta-analysis. Osteoporos Int 2022;33(12):2453–66.
[5] Lancaster P, Kocialkowski C, Pearce O, Khan U, Riddick A, Kelly M. Open lower limb fractures in the elderly. Injury 2022;53(6):2268–73.
[6] Metsemakers WJ, Kuehl R, Moriarty TF, Richards RG, Verhofstad MHJ, Borens O, et al. Infection after fracture fixation: current surgical and microbiological concepts. Injury 2018;49(3):511–22.
[7] Papakostidis C, Kanakaris NK, Pretel J, Faour O, Morell DJ, Giannoudis PV. Prevalence of complications of open tibial shaft fractures stratified as per the Gustilo-Anderson classification. Injury 2011;42(12):1408–15.
[8] Moriarty TF, Metsemakers W-J, Morgenstern M, Hofstee MI, Vallejo Diaz A, Cassat JE, et al. Fracture-related infection. Nat Rev Dis Prim 2022;8(1):67.
[9] Kennedy DG, O'Mahony AM, Culligan EP, O'Driscoll CM, Ryan KB. Strategies to mitigate and treat orthopaedic device-associated infections. Antibiotics 2022;11 (12):1822.
[10] Vidal L, Kampleitner C, Brennan MA, Hoornaert A, Layrolle P. Reconstruction of large skeletal defects: current clinical therapeutic strategies and future directions using 3D printing. Front Bioeng Biotechnol 2020;8.
[11] Oliver J-aN, Su Y, Lu X, Kuo P-H, Du J, Zhu D. Bioactive glass coatings on metallic implants for biomedical applications. Bioact Mater 2019;4:261–70.
[12] Chae K, Jang WY, Park K, Lee J, Kim H, Lee K, et al. Antibacterial infection and immune-evasive coating for orthopedic implants. Sci Adv 2020;6(44).
[13] Chen J, Hu G, Li T, Chen Y, Gao M, Li Q, et al. Fusion peptide engineered "statically-versatile" titanium implant simultaneously enhancing anti-infection, vascularization and osseointegration. Biomaterials 2021;264:120446.
[14] Li JY, Li LL, Zhou J, Zhou Z, Wu XL, Wang LM, et al. 3D printed dual-functional biomaterial with self-assembly micro-nano surface and enriched nano argentum for antibalctorial and bone regeneration. Appl Mater Today 2019;17:206–15.
[15] Prinz C, Elhensheri M, Rychly J, Neumann HG. Antimicrobial and bone-forming activity of a copper coated implant in a rabbit model. J Biomater Appl 2017;32 (2):139–49.
[16] Schaer TP, Stewart S, Hsu BB, Klibanov AM. Hydrophobic polycationic coatings that inhibit biofilms and support bone healing during infection. Biomaterials 2012;33(5):1245–54.
[17] Shiels SM, Bouchard M, Wang H, Wenke JC. Chlorhexidine-releasing implant coating on intramedullary nail reduces infection in a rat model. Eur Cell Mater 2018;35:178–94.
[18] Stavrakis AI, Zhu S, Loftin AH, Weixian X, Niska J, Hegde V, et al. Controlled release of vancomycin and tigecycline from an orthopaedic implant coating prevents Staphylococcus aureus infection in an open fracture animal model. BioMed Res Int 2019;2019:1638508.
[19] Stewart S, Barr S, Engiles J, Hickok NJ, Shapiro IM, Richardson DW, 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(15):1406–15.
[20] Sun A, Lin X, Xue Z, Huang J, Bai X, Huang L, et al. Facile surface functional polyetheretherketone with antibacterial and immunoregulatory activities for enhanced regeneration toward bacterium-infected bone destruction. Drug Deliv 2021;28(1):1649–63.
[21] Windolf CD, Lögters T, Scholz M, Windolf J, Flohé S. Lysostaphin-coated titan-implants preventing localized osteitis by Staphylococcus aureus in a mouse model. PLoS One 2014;9(12):e115940.
[22] Xue Z, Wang Z, Sun A, Huang J, Wu W, Chen M, et al. Rapid construction of polyetheretherketone (PEEK) biological implants incorporated with brushite (CaHPO(4)·2H(2)O) and antibiotics for anti-infection and enhanced osseointegration. Mater Sci Eng C 2020;111:110782.
[23] Yang Y, Chu L, Yang S, Zhang H, Qin L, Guillaume O, et al. Dual-functional 3D-printed composite scaffold for inhibiting bacterial infection and promoting bone regeneration in infected bone defect models. Acta Biomater 2018;79:265–75.
[24] Zhang Y, Yin QS, Zhou CS, Xia H, Zhang Y, Jiao YP. Osteogenic activity of silver-loaded coral hydroxyapatite and its investigation in vivo. J Mater Sci Mater Med 2014;25(3):801–12.
[25] Zhang C, Li X, Xiao D, Zhao Q, Chen S, Yang F, et al. Cu(2+) release from polylactic acid coating on titanium reduces bone implant-related infection. J Funct Biomater 2022;13(2).
[26] Ma T, Lyu J, Ma J, Huang X, Chen K, Wang S, et al. Comparative analysis of pathogen distribution in patients with fracture-related infection and periprosthetic joint infection: a retrospective study. BMC Muscoskel Disord 2023; 24(1):123.
[27] Rupp M, Baertl S, Walter N, Hitzenbichler F, Ehrenschwender M, Alt V. Is there a difference in microbiological epidemiology and effective empiric antimicrobial therapy comparing fracture-related infection and periprosthetic joint infection? A retrospective comparative study. Antibiotics 2021;10(8):921.
[28] Zhang Z, Liu P, Wang W, Wang S, Li B, Li J, et al. Epidemiology and drug resistance of fracture-related infection of the long bones of the extremities: a retrospective study at the largest trauma center in southwest China. Front Microbiol 2022;13.
[29] Graan D, Balogh ZJ. Microbiology of fracture related infections. J Orthop Surg 2022;30(3):10225536221118512.
[30] Yang X, Guo R, Zhang B, Xie B, Zhou S, Zhang B, et al. Retrospective analysis of drug resistance characteristics and infection related risk factors of multidrug-resistant organisms (MDROs) isolated from the orthopedics department of a tertiary hospital. Sci Rep 2023;13(1):2199.
[31] Weigelt JA, Lipsky BA, Tabak YP, Derby KG, Kim M, Gupta V. Surgical site infections: causative pathogens and associated outcomes. Am J Infect Control 2010;38(2):112–20.
[32] Tran NN, Morrisette T, Jorgensen SCJ, Orench-Benvenutti JM, Kebriaei R. Current therapies and challenges for the treatment of Staphylococcus aureus biofilm-related infections. Pharmacotherapy 2023.
[33] Filipović U, Dahmane RG, Ghannouchi S, Zore A, Bohinc K. Bacterial adhesion on orthopedic implants. Adv Colloid Interface Sci 2020;283:102228.
[34] Miller MB, Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol 2001;55 (1):165–99.
[35] Whiteley M, Diggle SP, Greenberg EP. Progress in and promise of bacterial quorum sensing research. Nature 2017;551(7680):313–20.
[36] Gimza BD, Cassat JE. Mechanisms of antibiotic failure during Staphylococcus aureus osteomyelitis. Front Immunol 2021;12:638085.
[37] Iliadis AD, Shivji F, Debuka E, Trompeter A, Narayan B, Heidari N. Current concepts in the prevention, diagnosis and treatment of fracture-related infection (FRI). Eur J Orthop Surg Traumatol 2021;31(5):957–66.
[38] Vallet-Regí M, Lozano D, González B, Izquierdo-Barba I. Biomaterials against bone infection. Adv Healthcare Mater 2020;9(13):e2000310.
[39] Li J, Cheung WH, Chow SK, Ip M, Leung SYS, Wong RMY. Current therapeutic interventions combating biofilm-related infections in orthopaedics : a systematic review of in vivo animal studies. Bone Joint Res 2022;11(10):700–14.
[40] Chen X, Zhou J, Qian Y, Zhao L. Antibacterial coatings on orthopedic implants. Mater Today Bio 2023;19:100586.
[41] Li W, Thian ES, Wang M, Wang Z, Ren L. Surface design for antibacterial materials: from fundamentals to advanced strategies. Adv Sci (Weinh) 2021;8 (19):e2100368.
[42] Ran B, Jing C, Yang C, Li X, Li Y. Synthesis of efficient bacterial adhesion-resistant coatings by one-step polydopamine-assisted deposition of branched polyethylenimine-g-poly(sulfobetaine methacrylate) copolymers. Appl Surf Sci 2018;450:77–84.
[43] Palumbo FS, Bavuso Volpe A, Cusimano MG, Pitarresi G, Giammona G, Schillaci D. A polycarboxylic/amino functionalized hyaluronic acid derivative for the production of pH sensible hydrogels in the prevention of bacterial adhesion on biomedical surfaces. Int J Pharm 2015;478(1):70–7.
[44] Zhang J, Pei X, Huang J, Ke X, Xu C, Zhao W, et al. Construction of hierarchical micro/nanostructured ZnO/Cu-ZnMOFs@SA superhydrophobic composite coatings with excellent multifunctionality of anticorrosion, blood-repelling, and antimicrobial properties. ACS Appl Mater Interfaces 2023;15(1):265–80.
[45] Kuehl R, Brunetto PS, Woischnig A-K, Varisco M, Rajacic Z, Vosbeck J, et al. Preventing implant-associated infections by silver coating. Antimicrob Agents Chemother 2016;60(4):2467–75.
[46] Pavlukhina SV, Kaplan JB, Xu L, Chang W, Yu X, Madhyastha S, et al. Noneluting enzymatic antibiofilm coatings. ACS Appl Mater Interfaces 2012;4(9):4708–16.
[47] Aydemir T, Liverani L, Pastore JI, Ceré SM, Goldmann WH, Boccaccini AR, et al. Functional behavior of chitosan/gelatin/silica-gentamicin coatings by electrophoretic deposition on surgical grade stainless steel. Mater Sci Eng C 2020; 115:111062.
[48] Ma H, Han H, Zhao X, Ma J, Qu X, Lou X, et al. Engineering multifunctional polyether ether ketone implant: mechanics-adaptability, biominerialization, immunoregulation, anti-infection, osteointegration, and osteogenesis. Adv Healthcare Mater 2023;12(12):e2202799.
[49] Bai Z, Hu K, Shou Z, Yu J, Meng H, Zhou H, et al. Layer-by-layer assembly of procyanidin and collagen promotes mesenchymal stem cell proliferation and osteogenic differentiation in vitro and in vivo. Regen Biomater 2023;10:rbac107.
[50] Cunha BA. Methicillin-resistant Staphylococcus aureus: clinical manifestations and antimicrobial therapy. Clin Microbiol Infect 2005;11:33–42.
[51] Dong JY, Zhong JQ, Hou RX, Hu XD, Chen YJ, Weng HB, et al. Polymer bilayer-Micro arc oxidation surface coating on pure magnesium for bone implantation. J Orthop Translat 2023;40:27–36.
[52] Esposito S, Blasi F, Curtis N, Kaplan S, Lazzarotto T, Meschiari M, et al. New antibiotics for Staphylococcus aureus infection: an update from the world association of infectious diseases and immunological disorders (WAidid) and the Italian society of anti-infective therapy (SITA). Antibiotics 2023;12(4):742.
[53] Depypere M, Sliepen J, Onsea J, Debaveye Y, Govaert GAM, Ffa IJ, et al. The microbiological etiology of fracture-related infection. Front Cell Infect Microbiol 2022;12:934485.
[54] Zhang XW, Man KW, Li GHY, Tan KC, Kung AWC, Cheung CL. Osteoporosis is a novel risk factor of infections and sepsis: a cohort study. Eclinicalmedicine 2022; 49.
[55] Li J, Wong RMY, Chung YL, Leung SSY, Chow SKH, Ip M, et al. Fracture-related infection in osteoporotic bone causes more severe infection and further delays healing. Bone Joint Res 2022;11(2):49–60.
[56] Li J, Leung SSY, Chung YL, Chow SKH, Alt V, Rupp M, et al. Hydrogel delivery of DNase I and liposomal vancomycin to eradicate fracture-related methicillin-resistant staphylococcus aureus infection and support osteoporotic fracture healing. Acta Biomater 2023;164:223–39.
[57] Wu S, Altenried S, Zogg A, Zuber F, Maniura-Weber K, Ren Q. Role of the surface nanoscale roughness of stainless steel on bacterial adhesion and microcolony formation. ACS Omega 2018;3(6):6456–64.
[58] Puckett SD, Taylor E, Raimondo T, Webster TJ. The relationship between the nanostructure of titanium surfaces and bacterial attachment. Biomaterials 2010; 31(4):706–13.
[59] Dou X-Q, Zhang D, Feng C, Jiang L. Bioinspired hierarchical surface structures with tunable wettability for regulating bacteria adhesion. ACS Nano 2015;9(11): 10664–72.

[60] Ozcelik B, Ho KKK, Glattauer V, Willcox M, Kumar N, Thissen H. Poly(ethylene glycol)-based coatings combining low-biofouling and quorum-sensing inhibiting properties to reduce bacterial colonization. ACS Biomater Sci Eng 2017;3(1):78–87.
[61] Andrade del Olmo J, Alonso JM, Sáez-Martínez V, Benito-Cid S, Pérez-González R, Vilas-Vilela JL, et al. Hyaluronic acid-based hydrogel coatings on Ti6Al4V implantable biomaterial with multifunctional antibacterial activity. Carbohydr Polym 2023;301:120366.
[62] Li W, Zhang H, Li X, Yu H, Che C, Luan S, et al. Multifunctional antibacterial materials comprising water dispersible random copolymers containing a fluorinated block and their application in catheters. ACS Appl Mater Interfaces 2020;12(6):7617–30.
[63] Zheng W, Jia Y, Chen W, Wang G, Guo X, Jiang X. Universal coating from electrostatic self-assembly to prevent multidrug-resistant bacterial colonization on medical devices and solid surfaces. ACS Appl Mater Interfaces 2017;9(25):21181–9.
[64] Gittens RA, Olivares-Navarrete R, Schwartz Z, Boyan BD. Implant osseointegration and the role of microroughness and nanostructures: lessons for spine implants. Acta Biomater 2014;10(8):3363–71.
[65] Wang M, Tang T. Surface treatment strategies to combat implant-related infection from the beginning. J Orthop Translat 2019;17:42–54.
[66] Claudel M, Schwarte JV, Fromm KM. New antimicrobial strategies based on metal complexes. Chemistry (Easton) 2020;2(4):849–99.
[67] Gao C, Li C, Wang C, Qin Y, Wang Z, Yang F, et al. Advances in the induction of osteogenesis by zinc surface modification based on titanium alloy substrates for medical implants. J Alloys Compd 2017;726:1072–84.
[68] Sen CK, Khanna S, Venojarvi M, Trikha P, Ellison EC, Hunt TK, et al. Copper-induced vascular endothelial growth factor expression and wound healing. Am J Physiol Heart Circ Physiol 2002;282(5):H1821–7.
[69] Zhang E, Zhao X, Hu J, Wang R, Fu S, Qin G. Antibacterial metals and alloys for potential biomedical implants. Bioact Mater 2021;6(8):2569–612.
[70] Liu Y, Li Y, Shi L. Controlled drug delivery systems in eradicating bacterial biofilm-associated infections. J Contr Release 2021;329:1102–16.
[71] Drexelius MG, Neundorf I. Application of antimicrobial peptides on biomedical implants: three ways to pursue peptide coatings. Int J Mol Sci 2021;22(24).
[72] Yeroslavsky G, Girshevitz O, Foster-Frey J, Donovan DM, Rahimipour S. Antibacterial and antibiofilm surfaces through polydopamine-assisted immobilization of lysostaphin as an antibacterial enzyme. Langmuir 2015;31(3):1064–73.
[73] Algburi A, Comito N, Kashtanov D, Dicks LMT, Chikindas ML. Control of biofilm formation: antibiotics and beyond. Appl Environ Microbiol 2017;83(3).
[74] Asha AB, Peng YY, Cheng Q, Ishihara K, Liu Y, Narain R. Dopamine assisted self-cleaning, antifouling, and antibacterial coating via dynamic covalent interactions. ACS Appl Mater Interfaces 2022;14(7):9557–69.
[75] Lu X, Wu Z, Xu K, Wang X, Wang S, Qiu H, et al. Multifunctional coatings of titanium implants toward promoting osseointegration and preventing infection: recent developments. Front Bioeng Biotechnol 2021;9.
[76] Gristina AG. Biomaterial-centered infections - microbial adhesion versus tissue integration. Science 1987;237(4822):1588–95.
[77] Einhorn TA, Gerstenfeld LC. Fracture healing: mechanisms and interventions. Nat Rev Rheumatol 2015;11(1):45–54.
[78] Walters G, Pountos I, Giannoudis PV. The cytokines and micro-environment of fracture haematoma: current evidence. J Tissue Eng Regen Med 2018;12(3):E1662–77.
[79] Kostenuik P, Mirza FM. Fracture healing physiology and the quest for therapies for delayed healing and nonunion. J Orthop Res 2017;35(2):213–23.
[80] Croes M, van der Wal BCH, Vogely HC. Impact of bacterial infections on osteogenesis: evidence from in vivo studies. J Orthop Res 2019;37(10):2067–76.
[81] Li WQ, Liu QQ, Shi JC, Xu X, Xu JY. The role of TNF-α in the fate regulation and functional reprogramming of mesenchymal stem cells in an inflammatory microenvironment. Front Immunol 2023:14.
[82] Schmidt-Bleek K, Kwee BJ, Mooney DJ, Duda GN. Boon and bane of inflammation in bone tissue regeneration and its link with angiogenesis. Tissue Eng Part B 2015;21(4):354–64.
[83] Josse J, Velard F, Gangloff SC. Vs. Osteoblast: relationship and consequences in osteomyelitis. Front Cell Infect Microbiol 2015;5.
[84] Gristina AG, Naylor P, Myrvik Q. Infections from biomaterials and implants - a race for the surface. Med Prog Technol 1989;14(3–4):205–24.
[85] Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C. Bone regeneration strategies: engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. Biomaterials 2018;180:143–62.
[86] Battafarano G, Rossi M, De Martino V, Marampon F, Borro L, Secinaro A, et al. Strategies for bone regeneration: from graft to tissue engineering. Int J Mol Sci 2021;22(3).
[87] Gilbert SR, Camara J, Camara R, Duffy L, Waites K, Kim H, et al. Contaminated open fracture and crush injury: a murine model. Bone Res 2015;3:14050.
[88] Walsh DA, Pearson CI. Angiogenesis in the pathogenesis of inflammatory joint and lung diseases. Arthritis Res 2001;3(3):147–53.
[89] Kreuger J, Phillipson M. Targeting vascular and leukocyte communication in angiogenesis, inflammation and fibrosis. Nat Rev Drug Discov 2016;15(2):125–42.
[90] Arrigoni C, Ostano P, Bersini S, Crippa M, Colombo MV, Gilardi M, et al. Differential angiogenesis of bone and muscle endothelium in aging and inflammatory processes. Commun Biol 2023;6(1):126.
[91] Helmus MN, Gibbons DF, Cebon D. Biocompatibility: meeting a key functional requirement of next-generation medical devices. Toxicol Pathol 2008;36(1):70–80.
[92] Huzum B, Puha B, Necoara RM, Gheorghevici S, Puha G, Filip A, et al. Biocompatibility assessment of biomaterials used in orthopedic devices: an overview (Review). Exp Ther Med 2021;22(5):1315.
[93] Ansari S, Ito K, Hofmann S. Alkaline phosphatase activity of serum affects osteogenic differentiation cultures. ACS Omega 2022;7(15):12724–33.
[94] Nalezinková M. In vitro hemocompatibility testing of medical devices. Thromb Res 2020;195:146–50.
[95] Ning C, Wang X, Li L, Zhu Y, Li M, Yu P, et al. Concentration ranges of antibacterial cations for showing the highest antibacterial efficacy but the least cytotoxicity against mammalian cells: implications for a new antibacterial mechanism. Chem Res Toxicol 2015;28(9):1815–22.
[96] Ferreira MB, Myiagi S, Nogales CG, Campos MS, Lage-Marques JL. Time- and concentration-dependent cytotoxicity of antibiotics used in endodontic therapy. J Appl Oral Sci 2010;18(3):259–63.
[97] Liang JQ, Liu PL, Yang XQ, Liu L, Zhang Y, Wang Q, et al. Biomaterial-based scaffolds in promotion of cartilage regeneration: recent advances and emerging applications. J Orthop Translat 2023;41:54–62.
[98] Lee YJ, Sadigh S, Mankad K, Kapse N, Rajeswaran G. The imaging of osteomyelitis. Quant Imag Med Surg 2016;6(2):184–98.
[99] Pineda C, Espinosa R, Pena A. Radiographic imaging in osteomyelitis: the role of plain radiography, computed tomography, ultrasonography, magnetic resonance imaging, and scintigraphy. Semin Plast Surg 2009;23(2):80–9.
[100] Josse J, Velard F, Gangloff SC. Staphylococcus aureus vs. Osteoblast: relationship and consequences in osteomyelitis. Front Cell Infect Microbiol 2015;5:85.
[101] Yang DQ, Wijenayaka AR, Solomon LB, Pederson SM, Findlay DM, Kidd SP, et al. Novel insights into Staphylococcus aureus deep bone infections: the involvement of osteocytes. mBio 2018;9(2).
[102] Garzoni C, Kelley WL. Return of the Trojan horse: intracellular phenotype switching and immune evasion by Staphylococcus aureus. EMBO Mol Med 2011;3(3):115–7.
[103] Kubica M, Guzik K, Koziel J, Zarebski M, Richter W, Gajkowska B, et al. A potential new pathway for Staphylococcus aureus dissemination: the silent survival of S. aureus phagocytosed by human monocyte-derived macrophages. PLoS One 2008;3(1):e1409.
[104] Baht GS, Vi L, Alman BA. The role of the immune cells in fracture healing. Curr Osteoporos Rep 2018;16(2):138–45.
[105] Brandt SL, Putnam NE, Cassat JE, Serezani CH. Innate immunity to Staphylococcus aureus: evolving paradigms in soft tissue and invasive infections. J Immunol 2018;200(12):3871–80.
[106] Mbalaviele G, Novack DV, Schett G, Teitelbaum SL. Inflammatory osteolysis: a conspiracy against bone. J Clin Invest 2017;127(6):2030–9.
[107] Silva Lima Mendes DT, Leite Matos GR, Stwart de Araújo Souza SA, Souza Silva Macedo MC, Tavares DDS, Resende CX. Does the incorporation of zinc into TiO(2) on titanium surfaces increase bactericidal activity? A systematic review and meta-analysis. J Prosthet Dent 2022.
[108] Sandhu AK, Yang Y, Li WW. In vivo antibacterial efficacy of antimicrobial peptides modified metallic Implants—Systematic review and meta-analysis. ACS Biomater Sci Eng 2022;8(5):1749–62.
[109] Sun Y, Yang Y, Jiang W, Bai H, Liu H, Wang J. In vivo antibacterial efficacy of nanopatterns on titanium implant surface: a systematic review of the literature. Antibiotics (Basel) 2021;10(12).
[110] Kumari S, Tiyyagura HR, Pottathara YB, Sadasivuni KK, Ponnamma D, Douglas TEL, et al. Surface functionalization of chitosan as a coating material for orthopaedic applications: a comprehensive review. Carbohydr Polym 2021;255:117487.
[111] De Meo D, Cannari FM, Petriello L, Persiani P, Villani C. Gentamicin-coated tibia nail in fractures and nonunion to reduce fracture-related infections: a systematic review. Molecules 2020;25(22).