

Review Article

Animal Models for Fracture-Related Infections: A Systematic Review

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Abstract

Background: Fracture-Related Infections (FRI) are among the most challenging complications in orthopaedics. The incidence of these infections is high, particularly in complex, open fractures. FRI management typically involves irrigation and debridement of the fracture site, implant exchange and prolonged antibiotic therapy. This regimen is often ineffective resulting in poor patient outcomes and inefficient use of healthcare resources. As such, improved diagnostic, preventative and therapeutic interventions are needed. To effectively address these gaps, valid preclinical animal models that accurately replicate clinical FRI are required. The purpose of this systematic review was to synthesize the relevant peer-reviewed literature related to FRI animal models to analyze their translational rigor and potential.

Methods: An online database search was conducted using PubMed in which 77 articles were eligible for inclusion in this review.

Results: Data extraction revealed a wide spectrum of animal species, methods for bone defect creation and fixation, bacterial inoculum deliveries and doses and intervention time points among the studies. Further, clinical, radiographic, microbiologic and histologic outcomes of infected control groups were assessed to determine validity of each model. Importantly, FRI-defining features such as bacterial biofilms and delayed fracture union were only reported in 20.8% and 29.9% of models, respectively.

Conclusion: While it is challenging to incorporate and validate all clinically relevant components of FRI into an animal model, many of the gaps identified in this systematic review can and should be addressed to improve the efficacy of preclinical evidence aimed at advancing FRI management.

Keywords: Complex Open Fractures; Fracture-Related Infections; Fracture Management; Pre-Clinical Models; Animal Models

Introduction

Fracture-related Infections (FRI) present a major challenge in orthopaedics based on the associated burdens and costs for patients, healthcare teams and the healthcare system. In the United States, the reported incidence of FRI ranges from 1% in closed fractures to up to 30% in complex open fractures [1,2]. Of these, there is a subsequent treatment failure rate of up to 11% [3]. FRI most frequently involve *Staphylococcus aureus*. However, many other organisms can be noted in mono- or polymicrobial infections, including Streptococci, Pseudomonas and Enterobacteriaceae species [4-6]. A defining feature of these pathogens in FRI is their capability to produce biofilms on fracture fixation implants. Biofilms cause microbes to enter a stationary and nongrowing state, rendering them up to 1,000 times more resistant to antibiotic agents than in their planktonic form [7,8]. As such, biofilm production in FRI is a key determinant for the likelihood of infection resolution and retention of fracture fixation implants.

In characterizing FRI, the time point of infection is classified as early (<2 weeks), delayed (2-10 weeks) or late (>10 weeks) [4]. Diagnostic criteria are categorized as confirmatory or suggestive by an international FRI consensus group (Table 1) [9]. Treatment of FRI typically requires additional surgeries involving Irrigation and Debridement (I&D) along with fracture-fixation hardware removal and exchange accompanied by antibiotic therapy. Typically, fracture fixation implants are retained or exchanged during

acute management to maintain fracture stability for achieving bone union. Successful treatment of FRIs is most often defined by fracture union with control of symptomatic infection. Despite being the clinical standard-of-care, this treatment regimen is often ineffective, especially when biofilms are established. Current evidence suggests that successful union can be achieved in 68% to 71% of infected fractures when implants are retained [10]. However, recurrence of infection after union occurs in 40% to 58% of these patients, requiring further treatment including late implant removal. Taken together, the data indicate that with standard-of-care treatment approximately 30% of infected fractures fail to heal and only 34% of all acute FRIs can be expected to retain implants long-term. A recent systematic review focusing on chronic or late-onset FRIs reported a 6% to 9% rate of infection recurrence after initial resolution, which resulted in amputation of the respective limb in 3% to 5% of patients [11]. As such, associated healthcare costs per patient are six to seven times higher when compared to uninfected fractures, which is greatly exacerbated by the burden the patient feels by repeat hospitalizations and surgeries, as well as the indirect costs associated with lost productivity and wages, among other factors [12,13].

Due to the unique challenges and profound impacts associated with FRIs, it is vital to improve diagnostic, prophylactic and therapeutic strategies toward optimizing patient outcomes. To ethically and effectively address these gaps in evidence, valid preclinical animal model studies are needed. To assess animal models for effective translational application to clinical FRIs, a systematic review of the best current evidence is required. Therefore, the purpose of this review was to analyze and synthesize the relevant peer-reviewed literature related to animal models of FRI in addressing three important questions:

1. Are the methods used to create the long-bone trauma biologically and biomechanically relevant for FRI?
2. Are the pathogens and inoculation methods clinically relevant for FRI?
3. Are the studies' interventional time points clinically realistic?

Confirmatory Criteria	Suggestive Criteria
<i>Clinical</i> : fistula, sinus tract, wound breakdown, purulent drainage, or presence of pus	<i>Clinical</i> : local redness or fever, new onset wound drainage
<i>Microbiological</i> : phenotypically identical pathogens identified from at least two separate deep tissue/implant specimens.	<i>Radiographic</i> : imaging consistent with bone healing complications or implant failure
<i>Histological</i> : presence of microorganisms in deep tissue specimens, presence of >5 PMNs/HPF in chronic/late-onset cases	<i>Laboratory</i> : markers consistent with infection (i.e., WBC, ESR, CRP)

Table 1: Confirmatory and suggestive criteria for fracture-related infection diagnosis.

Methodology

Search Strategy

Using Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, a PubMed search was performed to include peer-reviewed articles from inception (1977) to 2023 to identify eligible studies for systematic review of preclinical animal model studies that investigated FRI [14]. Using the advanced search feature, keywords of (1) "animal model OR *in-vivo*", (2) "infection OR osteomyelitis" and (3) "fracture" were combined for the search. All titles and abstracts were screened, eliminating articles irrelevant to this review. Following this, a full-text review of the remaining articles was performed based on inclusion and exclusion criteria.

Inclusion and Exclusion Criteria

Articles included in this review required the use of (1) a preclinical animal model, (2) a defect of a long bone, (3) the use of fracture fixation implants and (4) local bacterial inoculation. Articles excluded in this review included (1) review articles, (2) inaccessibility to full-text literature and (3) studies unavailable in English. Eligible articles were further analyzed and underwent data extraction.

Data Extraction

Self-identified study classifications were categorized based on the primary focus of the research, as follows:

- Characterization - focused on advancing scientific understanding of the bacteriological, immunological, biomechanical, histological and/or pathomechanistic aspects of FRI

- Model development - described the development and/or validation of an animal model for FRI
- Prevention - tested prophylactic interventions for FRI
- Treatment - tested therapeutic interventions for FRI

For each study, the following data were extracted and compiled:

- Animal species
- Bacteria species
- Inoculation method
- Inoculum dose [Colony-Forming Unit (CFU) count]
- Anatomical location of the defect
- Method of defect creation
- Fixation method
- Fixation implant
- Fixation timepoint
- Intervention details
- Key findings regarding the clinical, radiographic, microbiological and histologic validations of FRI. (For studies incorporating therapeutic or preventative interventions, validations were based on infected control groups only)

References	Animal Species	Bacteria Species	Inoculation Method	Inoculum Dose	Fracture Site	Bone Defect Method	Fixation Method and Implant	Fracture Fixation Time Point	Systemic Antibiotic Time Point	Secondary Surgery Time Point	Other Interventions	Model Validation: Clinical	Model Validation: Radiographic	Model Validation: Microbiology	Model Validation: Histologic
Characterization															
Curtis et al. ¹⁵	Goats	<i>S. aureus</i>	The inoculum was placed at the fracture site on an absorbable gelatin sponge.	1x10 ³ CFU/mL	Tibial diaphysis	A chevron osteotomy, simulating an open tibial fracture, was created with a power saw.	External fixator device with two 5 mm diameter cortical pins proximal and two distal to the fracture or intramedullary nails	Immediately post-trauma	N/A	N/A	During primary surgery, the treatment group receiving intramedullary nail implants underwent reaming of the medullary cavity.	For the first 2 days, temperatures were elevated. Animals were lame on fractured limb post-operatively. Soft tissue swelling at fracture sites was observed.	At week 2, 4/5 animals in the nailing and reaming group had signs of infection with periosteal reaction and local soft tissue swelling. 1/5 fixed with nailing without reaming and 0/5 of those fixed with the external fixator showed signs of infection; all in these two groups showed early callus formation.	At week 2, light to heavy amounts of <i>S. aureus</i> could be cultured from all tibias in the nailing and reaming group. Light to heavy amounts of <i>S. aureus</i> could be cultured from 10/15 tibias in the nailing without reaming group. Light amounts of <i>S. aureus</i> could be cultured from 4/15 tibias in the external fixator group.	At week 2, animals treated by nailing and reaming showed cortical necrosis, loss of normal marrow contents, and a fibrous layer adjacent to the implant. In the other groups, partial necrosis, some cortical infection, and callus formation was observed.
Khodaparast et al. ¹⁶	Canines	<i>S. aureus</i>	1 mL of bacterial suspension was injected into the medullary canal proximal and distal	1x10 ⁶ CFUs	Tibial diaphysis	To create the open fracture, the captive bolt device was	Interlocking intramedullary nail	Immediately post-trauma	N/A	N/A	At primary surgery, rotational gastrocnemius muscle flaps were used for the	Not specified	Not specified	Not specified	Not specified

			to the fracture and was allowed to flow freely into the surrounding soft tissue.			loaded with a no. 13 cartridge and fired to deliver 6800 N to the proximal tibia.					treatment group.				
Brown et al. ¹⁷	Canines	<i>S. aureus</i>	1 mL of bacterial suspension was placed into the medullary canal proximal and distal to the fracture. Bacterial suspension was also placed under the rotational myoplasty and allowed to flow freely into the surrounding soft tissue.	1x10 ⁶ CFUs	Tibial diaphysis	The captive-bolt device was loaded with a no. 13 cartridge and fired, delivering 6800 N to the proximal tibia, and creating an open fracture.	Intramedullary nail with distal interlocking cortical screws	Immediately post-trauma	N/A	N/A	At primary surgery, rotational gastrocnemius muscle flaps were used for the treatment group.	Throughout the study, the injured limbs were edematous.	Not specified	Not specified	Not specified
Shiels et al. ¹⁸	Sprague–Dawley rats	<i>S. aureus</i>	Collagen was presoaked with bacterial suspension and placed into the fracture site.	1x10 ⁴ CFUs	Femoral diaphysis	A 6 mm section of bone was removed by reciprocating saw under copious saline.	Polyacetyl plate affixed to the anterior surface with six stainless steel threaded Kirschner wires	Pre-trauma	Postoperative	6 hours	After secondary surgery, FK506 was administered intraperitoneally once daily for 14 days for the treatment group.	At week 2, body weights had decreased.	Not specified	At week 2, <i>S. aureus</i> could be cultured from 2/10 femurs and 0/10 implants.	Not specified
Bilgili et al. ¹⁹	Sprague–Dawley rats	<i>S. aureus</i>	Not specified	1x10 ⁸ CFU/mL	Femoral diaphysis	One-third of the diaphyseal diameter was drilled, and the remaining bone was broken manually.	Kirschner wires (0.71–1.25 mm diameter)	Immediately post-trauma	N/A	N/A	N/A	Not specified	At week 3, unbridged callus formation was detected. At 6 weeks, the initial stages of the bony union were detected.	At weeks 3 and 6, <i>S. aureus</i> could be cultured from the femur, implant, and soft tissue. The proportion of animals with positive cultures is not specified. At 6 weeks, <i>S. aureus</i> could be cultured from blood samples.	At week 6, there were concentrated areas of polymorphonuclear leukocytes and abscesses in bone.
Hamza et al. ²⁰	Sprague–Dawley rats	<i>S. aureus</i>	100 µL of treatment solution with bacterial suspension was pipetted into the fracture site without contacting the surgical	20 CFUs of extracellular bacteria, 100 or 1x10 ⁶ CFUs of intracellular bacteria, or a combination	Femoral diaphysis	A fracture was created using a custom compression device.	Kirschner wires	Immediately post-trauma	N/A	N/A	N/A	At week 3, a significant loss in body weights were observed in groups receiving an inoculation dose of 10 ⁶ CFUs. At day	At week 3, severe osteolysis was observed in groups receiving an inoculation greater than 10 ² CFU.	At week 3, <i>S. aureus</i> could be cultured from all animals inoculated with at least 10 ² CFU of intracellular <i>S. aureus</i> .	Not specified

			instruments.									21, neutrophils were significantly elevated in groups receiving an inoculation dose greater than 10 ² CFU.			
Büren et al. ²¹	BALB/c-mice	<i>S. aureus</i>	1 µL of PBS containing bacterial suspension was placed in the osteotomy gap.	1x10 ³ CFUs	Femoral diaphysis	An osteotomy was created using a Gigli saw (diam. 0.22 mm).	Four-hole plate (length 7.75 mm, width 1.5 mm, thickness 0.7 mm) with two self-cutting screws at the proximal and distal fragments.	Immediately post-trauma	N/A	N/A	N/A	Not specified	Not specified	Not specified	Animals showed progressive destruction of the fracture gap and cortical bone with nonunion. >10 bacterial colonies were quantified on histopathological slices.
Oezel et al. ²²	BALB/c-mice	<i>S. aureus</i>	The fracture gap was injected with 1 µL of bacterial suspension.	1.35x10 ⁵ CFUs	Femoral diaphysis	An osteotomy was created using a Gigli saw (diam. 0.22 mm).	Six-hole titanium locking plate with locking self-tapping micro-screws	Pre-trauma	N/A	1 or 6 weeks	During primary surgery, CAS/HA was packed into the fracture gap of treatment groups.	At weeks 1 and 6, neutrophil counts were elevated.	At weeks 1 and 6, fracture nonunion, bone lysis, and bone destruction was detected.	At weeks 1 and 6, <i>S. aureus</i> could be cultured from all lavages.	Not specified
Sabaté - Brescó et al. ²³	C57BL/6, BALB/c, C57BL/6 IL-17A KO mice	<i>S. aureus</i> , <i>S. epidermidis</i> (monomicrobial)	2.5 µL of bacterial suspension was injected in the osteotomy site.	1x10 ⁴ CFUs	Femoral diaphysis	Not specified	Titanium 4-hole rigid and titanium 4-hole flexible plate implants, and Titanium Aluminum Niobium alloy screws	Pre-trauma	N/A	N/A	N/A	Not specified	Not specified	At day 14 and 30, <i>S. epidermidis</i> could be cultured 6/8 and 5/8 bones, soft tissues samples, or implants, respectively.	At day 14 and 30, the osteotomy gap was filled with new bone. Osteolytic regions around the screws and granulocyte infiltrate were observed.
Rochford et al. ²⁴	C57BL/6 and BALB/c mice	<i>S. aureus</i>	The inoculum was introduced to the mice on pre-contaminated Mousefix plates.	9x10 ⁵ CFUs per implant	Femoral diaphysis	A 0.44 mm defect osteotomy was performed using a jig and a Gigli wire.	Titanium or PEEK plate implants	Pre-trauma	N/A	N/A	N/A	Not specified	Not specified	At days 3 and 7, <i>S. aureus</i> could be cultured from all femurs, soft tissue samples, and implants. Bacterial loads were highest in the soft tissue for C57BL/6 mice and on the implant for BALB/c mice.	At day 3, soft tissue revealed inflammatory cell invasion consisting of polymorphonuclear cells. At day 7, the tissue overlying the plate was necrotic with cellular debris. Bacteria were observed at both time points.
Baertl et al. ²⁵	C57BL/6N mice	<i>S. aureus</i>	An inoculum of 1 µL was pipetted into the fracture gap.	1x10 ⁴ CFUs	Femoral diaphysis	A 0.44 mm osteotomy was performed using the MouseFix Drill-	Titanium four-hole locking plate fixated with four self-cutting, angular	Pre-trauma	N/A	N/A	N/A	At day 4 and 14, body weights had decreased.	At day 4, no signs of bone healing or infection-related changes	At days 4 and 14, <i>S. aureus</i> could be cultured from all soft tissue samples. 24/36 organs	At day 4, no soft callus or woven bone formation was present in either group. At day 14, osteonecrosis

						&Saw guide and a Gigli hand saw.	stable screws						were observed. At day 14, erodent fracture ends and osteolysis is adjacent to the screws and plates were detected in the high-virulence <i>S. aureus</i> group. Early signs of callus formation at the fracture site were visible in the low-virulence <i>S. aureus</i> group.	(liver, kidney, and spleen) contained bacteria in the high-virulence <i>S. aureus</i> group. 5/36 organs contained bacteria in the low-virulence <i>S. aureus</i> group.	s and fibrinous tissue were observed at the fracture gap in the high-virulence <i>S. aureus</i> group, whereas bony callus formation was present in the low-virulence <i>S. aureus</i> group. At days 4 and 14, bacterial colonies were found below the plate and bone marrow in both groups.
Hofstede et al. ²⁶	C57Bl/6N mice	<i>S. aureus</i>	1 µL of bacterial suspension was pipetted on top of the bone marrow of the segment.	1×10 ⁴ CFUs	Femoral diaphysis	The 2-inner screw-holes of the fixation plate were left empty and used to generate two 2 mm osteotomies with the MouseFix Drill&Saw guide and a Gigli hand saw.	Titanium six-hole locking plates and four-outermost screws	Pre-trauma	N/A	N/A	N/A	At week 4, animals had lost an average of 6.5% of body weight.	At week 4, nonunion and osteolysis is at osteotomy ends were detected.	At week 3, <i>S. aureus</i> could be cultured from all femurs, bone marrow samples, soft tissue samples and implants. Some biofilm growth was detected on screws and implants.	At week 1, lymphocytes and macrophages began infiltrating into the bone and soft tissue. At day 28, bacterial aggregates and osteonecrosis were observed in bone segment.
Rochford et al. ²⁷	C57bl/6 mice	<i>S. aureus</i>	The fixation plates were completely submerged in the bacterial suspension and incubated statically at room temperature for 20 minutes. The implants were then air dried sterilely for 5 minutes.	9×10 ⁵ (± standard deviation 2.6 × 10 ⁵) CFUs per implant	Femoral diaphysis	A 0.44 mm defect osteotomy was performed using a jig and a Gigli wire.	Titanium 4-hole rigid and titanium 4-hole flexible plate implants, and Titanium Aluminum Niobium alloy screws	Pre-trauma	N/A	N/A	N/A	Not specified	At week 5, nonunion and significant osteolysis is was detected.	At each endpoint, <i>S. aureus</i> could be cultured from all femurs, soft tissue, and implants. Bacterial loads peaked at day 1 and 3.	At day 3, a moderate degree of inflammatory cell infiltration was observed. At day 7, massive myocytic necrosis and fibroblast proliferation was observed. Bacterial colonies and thin biofilms were present.
Model Development															
Hill et al. ²⁸	SuKolk-cross sheep	<i>S. aureus</i>	An inoculum was introduced to the fracture	3×10 ⁸ CFUs	Tibial diaphysis	A chevron osteotomy was created using a	Intramedullary humeral nail (20 cm in length and	6 hours post-trauma	Postoperative	6 hours	N/A	At week 1, surgical sites showed early	At week 6, diffuse periosteal reaction	At secondary surgery, <i>S. aureus</i> could be cultured	At week 6, interlocking screws were loose and purulent material

			site on bovine type I collagen.			Gigli saw under saline irrigation.	8 mm diameter) and interlocking screw holes placed both proximally and distally					signs of infection, with soft tissue swelling. At week 2, wound breakdown was observed.	and osteolysis is around hardware were detected. An involucrum had developed in 3 animals.	from all reamers, all proximal screw holes, and 5/6 distal screw holes. At week 6, <i>S. aureus</i> could be cultured from all proximal nails, proximal screws, osteotomy sites, distal screws, and distal nails.	was present throughout the medullary cavity.
Zhang et al. ²⁹	New Zealand White rabbits	<i>S. aureus</i>	5 mL of bacterial suspension and a steel plate were placed into a 15 mL centrifuge tube. The tube was incubated for 48 h at 37°C with constant shaking. The plate was taken out from the tube and washed 3 times with PBS to remove bacteria on the surface of plate.	1x10 ⁶ CFU/ml	Femoral diaphysis	A fracture was created with a 1 mm diameter wire saw at the area between the second and third screws of fixation plate.	316L stainless steel five-hole plates (35 mm in length, 6.5 mm in width and 2.0 mm in thickness) with four screws (10 mm in length and 2.4 mm in diameter)	Pre-trauma	N/A	N/A	N/A	At week 3, all animals showed signs of soft tissue and bone infection with significant swelling, pus formation and local tissue destruction.	On week 2, periosteal reaction was detected. At week 3, significant osteolysis appeared around the implants with severe periosteal reaction away from the fracture. Unbridged callus formation was present.	At week 3, biofilm growth was detected on implants.	At week 3, cell-free sequestrum, inflammatory cell infiltration, and signs of 'moth eaten' bone were observed.
Arens et al. ³⁰	New Zealand White rabbits	<i>S. aureus</i>	34 µL of the bacterial suspension was pipetted directly over the osteotomy site.	Plate study: 6x10 ³ CFUs to 6x10 ⁶ CFUs Nail study: 6x10 ² CFUs to 6x10 ⁶ CFUs	Humeral diaphysis	A full osteotomy was created using a 0.45 mm Gigli	Plate study: 49 mm, seven-hole, locking compression plates fixated with six 2 mm steel locking screws. Nail study: stainless steel intramedullary nail (55 mm in length with four interlocking bolt holes)	Pre-trauma	N/A	N/A	N/A	At week 4, all animals had lost weight. CRP levels were persistently elevated in the nail study.	At week 4, nonunion and periosteal reaction is detected.	At 4 weeks, <i>S. aureus</i> could be cultured from 14/21 animals in the nail study and 11/17 animals in the plate study. As inoculation dose increased, the proportion of infected animals increased.	At week 4, an absence of osteotomy closure, active inflammation, necrotizing inflammation were observed.
Helbig et al. ³¹	Sprague-Dawley rats	<i>S. aureus</i>	10 µL of bacterial suspension was injected into the medullary cavity.	1x10 ³ CFUs	Femoral diaphysis	A 5 mm osteotomy was performed using a diamond disk.	Primary surgery: Stainless steel Kirschner wires (1.2–1.6 mm in diameter)	Immediately post-trauma	N/A	5 weeks	N/A	At day 5, body weights had decreased.	At week 13, nonunion, hypertrophic callus formation, change of the trabecular	At week 13, <i>S. aureus</i> could be cultured from all soft tissue samples and implants.	At week 13, nonunion, hypertrophic callus formation, a reduction in bone density, and change of the trabecular structures

						Second surgery: angle-stable plate and six angle-stable screws							structures and peri-implant loosening were detected.		were visible.
Helbig et al. ³²	Sprague-Dawley rats	<i>S. aureus</i>	10 µL of bacterial suspension was injected into the medullary cavity.	1x10 ³ CFU/10 µL	Tibial and fibular diaphysis	A weight (650 g) was fixed on a bolt with a removable pin at a height of 15 cm. Using this, an impulse of p = 1.12 Ns generated a closed transverse fracture.	Titanium Kirschner wires (0.8 mm in diameter)	Immediately post-trauma	N/A	N/A	N/A	At week 2, the leucocyte counts were elevated. Body weights decreased initially following primary surgery. Body temperatures remained stable throughout the study.	At week 5, nonunion, osteolysis, periosseal new bone formation, and sequestered bone were detected.	At week 5, <i>S. aureus</i> could be cultured from all implants.	Not specified
Penn-Barwell et al. ³³	Sprague-Dawley rats	<i>S. aureus</i>	The defect was contaminated with 30 mg of sterile bovine collagen soaked with bacterial suspension in 0.5 ml of saline.	1x10 ¹ CFUs to 1x10 ⁵ CFUs, in order of single magnitudes.	Femoral diaphysis	A 6-mm defect was made using a reciprocating saw.	Bespoke polyoxymethylene plates fixated with six threaded Kirschner wires	Pre-trauma	Postoperative	6 hours	N/A	Not specified	Not specified	At week 2, <i>S. aureus</i> could not be cultured from femurs and implants at an inoculation dose of 10 ¹ CFUs. <i>S. aureus</i> could be cultured from 4/10 femurs and 7/10 implants at an inoculation dose of 10 ² CFUs. <i>S. aureus</i> could be cultured from all femurs and implants at an inoculation dose of 10 ³ CFUs or greater.	Not specified
Robinson et al. ³⁴	Sprague-Dawley rats	<i>S. aureus</i>	50 µL of bacterial suspension was injected into the medullary cavity via an 18-gauge polypropylene catheter that was left in place for 2-min following inoculation.	1x10 ⁴ CFUs	Femoral diaphysis	A fracture was created by a blunt guillotine device driven by a dropped weight.	316L stainless steel intramedullary pins (1.4 mm in diameter and 26 mm in length)	Pre-trauma	Postoperative	N/A	N/A	Throughout the study, body weights had increased in all animals.	At week 3, nonunion, severe periosteal reaction, and osteolytic areas extending from the fracture site were detected.	At week 3, <i>S. aureus</i> could be cultured from all femurs. <i>S. aureus</i> could not be cultured from 8/10 stifles. Blood cultures were negative for all animals.	At week 3, inflammation of the periosteum, extensive myelofibrosis, unbridged callus, loss of cortical bone, and necrotic fragments were observed.

Alt et al. ³⁵	Sprague–Dawley rats	<i>S. aureus</i>	The osteotomy site was contaminated with 20 µL of the bacteria suspension applied in BHI/20% glycerol.	1x10 ⁴ CFUs	Tibial diaphysis	An osteotomy was created with an oscillating saw.	21 G intramedullary microlance needle	Immediately post-trauma	Postoperative	N/A	N/A	At week 6, no systemic signs of infection were observed. Clear signs of soft tissue and bone infection were observed with pretibial swelling, pus formation, and local tissue destruction.	At week 6, nonunion, chronic bone inflammation, sequester formation, cortical lysis, and thickening of the periosteum were detected in 10/11 animals.	At week 6, <i>S. aureus</i> could be cultured from 10/11 implants. Biofilm growth was detected on implants.	At week 6, bacterial colonies with extracellular matrix formation, inflammatory cells, sequesters, cortical lysis were observed.
Chen et al. ³⁶	Sprague–Dawley rats	<i>S. aureus</i>	Collagen moistened with the bacterial suspension was packed into the defect.	1x10 ³ , 1x10 ⁴ , 1x10 ⁵ , or 1x10 ⁶ CFUs	Femoral diaphysis	A 6 mm full-thickness defect was created with a mini-driver and small oscillating saw blade.	Polyacetyl plates (length 25 mm, width 4 mm and height 4 mm) containing six predrilled holes for threaded Kirschner wires	Pre-trauma	Postoperative	2 weeks	N/A	Not specified	At weeks 1, 2, 3, and 4, bone implants remained intact. The median number of lysis sites increased over the 4 weeks, reaching peak levels of 2-3.5 sites at 4 weeks. The higher inoculation dose, the greater number of lysis sites.	At 1, 2, 3, and 4 weeks, <i>S. aureus</i> could be cultured from all femurs. The mean CFUs of recovered bacteria for each inoculum peaked at week 1, then declined over the following 3 weeks.	Not specified
Lovati et al. ³⁷	Wistar rats	<i>S. epidermidis</i>	30 µL of the bacterial suspension was injected into the femoral defect and allowed to spread throughout the medullary canal.	1x10 ³ , 1x10 ⁵ or 1x10 ⁸ CFUs	Femoral diaphysis	A 1 mm non-critical, full-thickness defect was created after a localized periosteal elevation with an electric circular saw under continuous sterile saline irrigation.	A compression stainless steel four-hole-mini-plate (length 20mm, width 4mm, height 1mm) fixed using four 1.5mm bicortical screws	Immediately post-trauma	Preoperative	N/A	N/A	At week 1, body weights had decreased, but then increased steadily. At week 2, all animals exhibited an increase in neutrophil counts above basal levels. Neutrophil counts returned to basal levels by week 8.	At week 8, 67%, 83%, and 100% of the animals showed signs of altered bone healing in the 10 ³ , 10 ⁵ , and 10 ⁸ CFU groups, respectively. Altered bone healing included nonunion, osteolysis, resorption of the cortex, cortical bone thickening, and/or	At week 8, <i>S. epidermidis</i> could be cultured from 3/5 implants in the 10 ³ CFU group and from all implants in the remaining groups. Biofilm growth was detected in greater amounts on implant surfaces as the inoculum increased.	At week 8, animals with detectable infection in the 10 ³ CFU group had incomplete bone healing with fibrovascular tissue, inflammatory cells, and gram-positive cocci. The 10 ⁵ and 10 ⁸ CFU groups had nonunion, myeloid hyperplasia, bone sequestra, polymorphonuclear cells, and a massive presence of gram-positive cocci.

													periosteal reaction		
Gilbert et al. ³⁸	Brown Norway rats	<i>S. aureus</i> , <i>A. baumannii</i> (polymicrobial)	10 µL of a saline dilution prepared from an actively growing overnight culture containing <i>A. baumannii</i> followed by a second 10 µL saline dilution containing <i>S. aureus</i> was injected into the fracture site.	<i>A. baumannii</i> : 1×10^5 CFUs <i>S. aureus</i> : 1×10^4 CFUs	Femoral diaphysis	A drop-weight apparatus with a crushing arm was used to create a midshaft, comminuted fracture by dropping a 500-g weight from a height of 25 cm. A rotary saw was used to create an 8-mm gap.	1.6-mm Kirschner wire	Immediately post-trauma	N/A	N/A	N/A	At weeks 1, 2, and 4, muscle atrophy was observed.	At weeks 2 and 4, osteolysis is, reactive bone, loss of fixation, and increases in blood vessel volume was detected.	At weeks 1, 2, and 4, <i>S. aureus</i> could be cultured from all femurs. At weeks 1 and 2, <i>A. baumannii</i> could only be cultured in less than half of the femurs. All animals had positive blood cultures for <i>A. baumannii</i> , but negative blood cultures for <i>S. aureus</i> .	Not specified
Inzana et al. ³⁹	BALB/c mice	<i>S. aureus</i>	A 1 mm radius semi-circle of fibrillar collagen sheet was soaked for at least 2 hours in an overnight culture of bacteria then placed into the defect.	$8 \pm 2.9 \times 10^4$ CFUs	Femoral diaphysis	A 0.7 mm transverse osteotomy was made using a 0.67 mm wire Gigli saw and a cutting guide.	Six-hole radiolucent PEEK plate with a 40 nm titanium coating fixed using four titanium screws	Pre-trauma	Postoperative	1 week	At secondary surgery, a vancomycin-PMMA spacer was tied into the defects of the treatment group.	At week 4, substantial subcutaneous and peri-implant abscesses had formed. None of the animals lost a significant amount of weight.	At week 4, severe osteolysis is distal to the defect site, resorbed bone, and implant loosening was detected.	At week 4, <i>S. aureus</i> could be cultured from all femurs, soft tissue samples, PMMA spacers, and implants. Biofilm growth was detected on implants.	At week 4, bacterial colonization within bones was observed in gram-stained sections. Active osteoclast bone resorption in the vicinity of the screws was observed.
Windolf et al. ⁴⁰	BALB/c mice	<i>S. aureus</i>	1 µL of bacterial suspension in phosphate buffered BactoTryptic Soy Broth was injected with a micropipette into the fracture gap.	2×10^3 , 5×10^4 , 1×10^6 , or 5×10^6 CFUs	Femoral diaphysis	An osteotomy was created using a Gigli saw (diam. 0.22 mm).	Titanium locking plates	Pre-trauma	N/A	1 or 2 weeks	N/A	At an inoculum of 10^6 CFU or greater, severe clinical signs of systemic infection were observed.	At week 4, nonunion, osteolysis, and an increased fracture gap was detected.	At weeks 1, 2, and 4, <i>S. aureus</i> could be cultured from surgical lavages. At week 4, <i>S. aureus</i> could be cultured from all femurs. Blood cultures were negative. Biofilm growth was detected on implants.	At week 4, bacteria were observed in the bone adjacent to drill holes.
Prevention															
Tran et al. ⁴¹	Goats	<i>S. aureus</i>	1 mL of bacterial suspension was injected into the medullary canal at the osteotomy site.	2×10^4 CFU/mL	Tibial diaphysis	A simulated open fracture was created with a sagittal saw cooled by saline	Stainless steel alloy intramedullary nail with proximal and distal interlocking screws	Immediately post-trauma	N/A	N/A	Treatment group's implants were coated with titanium oxide and siloxane polymer doped	At week 5, animals had lost an average of 8.4% of body weight and were lame on	At week 5, periosteal reactions, bone lysis, and necrosis were observed.	At week 5, <i>S. aureus</i> could be cultured from all distal screw sites, proximal screw sites, and	At week 5, multifocal bacterial colonies, necrotic bone, disorganized periosteal callus, small sequestra, and nonunion

						irrigation.					with silver.	the injured limb. The neutrophil percentages were elevated after primary surgery but decreased by week 5.	Implant loosening and sequestrum were not detected.	fracture sites. At weeks 0, 1, 2, 4, and 5, blood cultures were negative for bacterial growth. Limited biofilm growth was detected heterogeneously on implants.	union with a large component of fibrovascular connective tissue were observed.
Stewart et al. ⁴²	Dorset sheep	<i>S. aureus</i>	The plate was inoculated with 2.5 mL of saline solution containing bacterial suspension via a indwelling catheter.	1x10 ⁶ CFU/mL	Tibial diaphysis	The osteotomy was created under constant copious irrigation with use of a Synthes Large Battery Drive fitted with an oscillating saw and blade (50 × 27 × 0.6 mm) to yield an osteotomy gap of 0.6 mm.	Titanium locking compression plates and titanium alloy locking head screws	Immediately post-trauma	Preoperative	N/A	Treatment group's implants were coated with vancomycin.	Following primary surgery, animals exhibited lameness in the injured limb. The surgical wounds healed without visible signs of infection or the formation of draining tracts.	At week 12, nonunion, cortical thinning, widening of the medullary canal, periosteal reaction, and osteolysis were detected.	At week 12, <i>S. aureus</i> could be cultured from 3/4 fracture-site swabs. Biofilm growth on 60-80% of implants was detected.	At week 12, disorganization of callus, minimal remodeling, osteolysis, and necrotic bone was observed. Large clusters of bacteria within canaliculi and haversian canals were present.
Schaer et al. ⁴³	Dorset-sheep	<i>S. aureus</i>	2.5 mL of bacterial suspension was injected via a silastic catheter directed onto the osteotomy site.	2.5x10 ⁶ CFUs	Tibial diaphysis	A unilateral transverse tibial osteotomy was performed using a 0.6-mm oscillating bone saw.	Safety study: 4.5-mm eight-hole or nine-hole cpTi plates Efficacy study: stainless steel narrow locking compression plates	Immediately post-trauma	Preoperative	N/A	Treatment group's implants were coated with N, N-dodecyl, methyl-PEI.	Following primary surgery, limb lameness, soft tissue swelling, heat, and pain were observed.	At day 30, cortical thinning, periosteal reaction, and osteolysis were detected.	At day 30, <i>S. aureus</i> could be cultured from all fracture-site swabs. Biofilm growth was detected on implants.	At day 30, lytic and disorganized callus architecture, large aggregates of necrotic neutrophils, and fibrous tissue was observed.
Zhang et al. ⁴⁴	New Zealand White rabbits	<i>S. aureus</i>	50 µL of bacterial suspension was injected into the proximal and distal stumps, respectively (total: 100 µL)	1x10 ⁵ CFU/mL	Tibial diaphysis	Not specified	Ti6Al4V Kirschner wires	Pre-trauma	Postoperative	N/A	Treatment group's implants were coated with copper.	At day 7, rectal temperatures, WBCs, and CRPs were elevated.	At week 4, nonunion and bone resorption was observed.	Not specified	At week 4, the fracture gap was visible and infiltrated by fibrous tissues. Extramembranous osteogenesis was observed.
Diaz et al. ⁴⁵	New Zealand white rabbits	<i>S. aureus</i>	100 µL of the bacterial suspension was applied over a 2 mm drill hole and throughout the wound margins.	1-2x10 ⁶ CFU	Humeral diaphysis	A full osteotomy was created using a 0.45-mm Gigli saw through the inoculation drill hole created in the	Seven-hole stainless steel locking plate	4 hours post-trauma	Postoperative	4 hours	15 minutes post-trauma, a treatment group received gentamicin-loaded hydrogel into the wound which was removed during	Throughout the study, no animal presented with clinical signs of infection.	Not specified	At secondary surgery, <i>S. aureus</i> could be cultured from all lavages. At week 1, <i>S. aureus</i> could be cultured from all humeri, soft tissue samples,	Not specified

						first surgery.					secondary surgery.			and implants.	
Puetzler et al. ⁴⁶	New Zealand White rabbits	<i>S. aureus</i>	Three 34 µL injections of a freshly prepared bacterial suspension were pipetted onto the central screw hole overlying the osteotomy and to the adjacent proximal and distal screw holes.	2×10 ⁶ CFUs	Humeral diaphysis	An osteotomy was created with a 0.44-mm Gigli saw.	Seven-hole locking compression plates and locking screws made of electropolished stainless steel	Pre-trauma	Preoperative and postoperative	N/A	N/A	Throughout the study, CRP levels were elevated with peak levels at day 3. WBC counts were initially elevated, then decreased to normal levels. Body temperatures were within normal ranges. An average of 10% weight loss was observed. 2/6 animals had abscesses at fracture sites.	At week 2, no signs of healing were observed.	At week 2, <i>S. aureus</i> could be cultured from all humeri, soft tissue, and implants.	Not specified
Ter Boo et al. ⁴⁷	New Zealand White rabbits	<i>S. aureus</i>	Three 34 µL injections of a freshly prepared bacterial suspension were pipetted onto the central screw hole overlying the osteotomy and to the adjacent proximal and distal screw holes.	2×10 ⁶ CFUs	Humeral diaphysis	An osteotomy was created using a Gigli saw (diam. 0.22 mm).	Seven-hole locking plate and screws	Not specified	N/A	N/A	During primary surgery, 800 µL of the gentamicin loaded hydrogel or gentamicin loaded collagen fleece was injected over the implant in treatment groups.	After surgery, CRP levels were elevated and peaked at day 2. WBC counts were elevated and increased steadily over the study period.	Not specified	At week 1, <i>S. aureus</i> could be cultured from all humeri, soft tissue samples, and implants.	At 1 week, suppurative inflammation surrounding the implant, coccoid bacterial microcolonies, and polymorphonuclear granulocytes were observed.
Xie et al. ⁴⁸	New Zealand White rabbits	<i>S. aureus</i>	A 1-mL tuberculin syringe was placed through the incision adjacent to the tibial tubercle and into the intramedullary canal. A 0.1 mL inoculum was injected through the syringe into the site of the fracture. Another syringe containing 0.1 mL of a 0.9% solution	1×10 ⁷ CFU/mL	Tibial diaphysis	The tibia was fractured 0.5 cm distal to the tibial tubercle with a hand saw and a 0.5 cm saw blade.	Six-hole 2.0-mm plates and six 2.0-mm screws	Immediately post-trauma	Preoperative	N/A	During primary surgery, 300 mg of particulate bioglass was packed into the treatment group's defects.	Throughout the study, 3 animals died from septic complications. Swelling and redness on the injured limb was observed in all animals. Dehiscence of the wound was observed in 4 animals.	At week 6, periosteal reaction, involucrum formation, widening of the bone shaft, architectural deformation, sclerosis, and osteolysis around the implant was observed in infected animals.	At week 6, <i>S. aureus</i> could be cultured from 6/10 fracture-site swabs.	At day 30, infected animals had infiltrated polymorphonuclear leukocytes, fibrosis with proliferative lymphocytes, fat necrosis, and necrotic debris.

			of saline was used to flush the inoculum into the site of the fracture.												
Darouiche et al. ⁴⁹	New Zealand White rabbits	<i>S. aureus</i>	0.1 mL of bacterial suspension was injected through the tuberculin syringe into the intramedullary canal adjacent to the fracture-fixation device.	1x10 ⁷ CFUs	Tibial diaphysis	The tibia was fractured 0.5 cm distal to the tibial tubercle with a handset sagittal saw and a 0.5-centimeter saw blade.	2.8-by-100-mm stainless-steel intramedullary nails	Pre-trauma	Preoperative	N/A	Treatment group's implants were coated with a combination of chlorhexidine and chloroxylenol.	Not specified	Not specified	At week 6, <i>S. aureus</i> could be cultured from 13/21 tibias and/or implants. Blood cultures were negative for all animals.	At week 6, foreign body cells and granulomas were observed. Bacteria was observed in bone sample in 1/15 animals with infection.
Chai et al. ⁵⁰	Japanese White rabbits	<i>S. aureus</i> , <i>E. coli</i> (polymicrobial)	The inoculum was incubated with metal fixation materials at 37°C for 6, 12, 24, or 48 h.	2x10 ⁶ CFU/mL	Femoral diaphysis	A 2.5-mm hole was drilled into the femur using a hand-held drill.	Austenitic stainless steel or a titanium alloy implant screws (3 mm in diameter by 10 mm in length)	Immediately post-trauma	N/A	N/A	Treatment group's implants contained 4.5% copper.	Not specified	Not specified	At day 5 and 14, <i>S. aureus</i> and <i>E. coli</i> could be cultured from 9/10 soft tissue samples. Biofilm growth on implants was observed.	Not specified
Wei et al. ⁵¹	Japanese White rabbits	<i>S. aureus</i>	Pieces of gelatin sponge were used to absorb 1 mL of bacterial suspension, which was introduced into the defects.	1x10 ⁸ CFU/mL	Femoral diaphysis	A wire saw was used to cut out a 10 mm section.	External fixators	Pre-trauma	Postoperative	N/A	During primary surgery, resorbable polycaprolactone electrospun fiber membranes containing vancomycin were wrapped around both ends of treatment group's fractured bones.	By week 1, 5 animals died from severe infection. Throughout the study, large amounts of pus and leakage from wounds was observed. By week 12, wounds had healed.	At week 6, 8, and 12, a fracture healing rate of 50%, 83%, and 100% was detected, respectively. Irregular and deformed healing was observed.	At week 12, <i>S. aureus</i> could be cultured from all soft tissue samples.	At week 12, inflammatory cell infiltration and new bone formation was observed.
Li et al. ⁵²	Sprague-Dawley rats	<i>S. aureus</i> , <i>P. aeruginosa</i> (polymicrobial)	0.5 mL of bacterial suspension was administered by dripping it onto the surface of the muscle and embrocating with a sterile bacterial inoculation needle.	<i>S. aureus</i> : 1x10 ⁸ CFUs <i>P. aeruginosa</i> : 1x10 ⁶ CFUs	Femoral diaphysis	A bone saw was used to create a femur shaft transverse fracture.	Kirschner wires (1 mm in length)	Immediately post-trauma	N/A	N/A	During primary surgery, gentamicin loaded sponges were placed into the treatment group's defects.	Wound suppuration was observed for all animals.	Not specified	Not specified	Not specified
Li et al. ⁵³	Sprague-Dawley rats	<i>S. aureus</i>	Briefly, the plate head was soaked in overnight cultured bacteria in a 6-well plate	1.07x10 ⁶ CFU per plate	Femoral metaphysis	A 0.35 mm wide osteotomy was made with an	Six-hole T-shaped mini-plates	Pre-trauma	N/A	N/A	During primary surgery, 100 µL of DNase I and/or liposomal Vancomycin	Not specified	At weeks 2 and 6, nonunion, bone lysis, and periosteal	At weeks 2 and 6, <i>S. aureus</i> could be cultured from all bones and implants. Biofilm	At week 2, inflammatory cell infiltrations and disorganized marrow matrix was observed.

			for 48 hours then washed with sterilized PBS 3 times to remove the unbound bacteria. The implant was then sonicated.			oscillating saw.					cin loaded on a thermosensitive hydrogel were injected onto the treatment group's fracture sites and implants.		reaction was observed.	growth was on implants was observed.	At week 6, inflammatory necrosis, fibrous tissue, nonunion was achieved.
Hu et al. ⁵⁴	Sprague-Dawley rats	<i>S. aureus</i> , <i>E. coli</i> (polymicrobial)	Not specified	5×10 ⁵ CFUs	Femoral diaphysis	A closed, transverse fracture was generated in the mid-shaft by impact loading in a three-point configuration.	1-mm-diameter Ti intramedullary needles	Pre-trauma	N/A	N/A	Treatment group's implants were coated with TaON-Ag.	Not specified	At week 9, bone union was observed.	At week 2, biofilm growth was observed.	At week 9, newly generated callus and bone was present and immune infiltration were observed.
Gao et al. ⁵⁵	Sprague-Dawley rats	<i>S. aureus</i>	50 µL of tryptic soy broth containing bacterial suspension was injected into one medullary cavity of the femur.	1×10 ⁵ CFUs/mL	Femoral diaphysis	A hole with a diameter of 2 mm was drilled along the ankle line and through the cortex.	Kirschner wires	Pre-trauma	N/A	N/A	Treatment group's implants contained micro-level concentrations of Ga and or Sr.	Not specified	Not specified	At day 5, <i>S. aureus</i> could be cultured from all implants.	At day 5, implants were surrounded by many mature bone tissues with some fibrous tissue.
Shiels et al. ⁵⁶	Sprague-Dawley rats	<i>S. aureus</i>	10 µL of bacteria was delivered to the medullary canal and then incubated for 2 minutes prior to placement of the fixation hardware.	1x10 ² CFUs	Tibial diaphysis	A 1 mm osteotomy was made using an ultrasonic dental tool, fitted with a serrated bone cutting insert.	Partially threaded titanium Kirschner wires (1.25 mm)	Immediately post-trauma	Postoperative	N/A	Treatment group's implants were coated with chlorhexidine.	Not specified	At week 2, nonunion and osteolysis were detected in 8/9 and 7/9 animals, respectively.	At week 4, <i>S. aureus</i> could be cultured from all tibias and implants.	At week 4, callus contained many spindle and inflammatory cells. Bacteria was present throughout the medullary canal, cortical bone, and callus.
Li et al. ⁵⁷	Sprague-Dawley rats	<i>S. aureus</i>	100 µL of bacterial suspension was pipetted with a sterile pipette directly onto the fracture site.	1x10 ² CFU/0.1mL	Femoral diaphysis	A custom designed compression device.	Kirschner wires	1-hour post-trauma	N/A	N/A	Treatment group's implants were coated with MCP-1 and/or IL-12 p70.	At week 3, mean body weights had decreased.	Not specified	At week 3, <i>S. aureus</i> could be cultured from 9/10 femurs.	Not specified
Bottagisio et al. ⁵⁸	Wistar rats	<i>S. epidermidis</i>	The femoral defect was injected with 30 µL of bacterial suspension.	1x10 ⁵ CFU/30 µL	Femoral diaphysis	Using the distal screw as a pivot, the plate was diverted from the femur and a 1 mm non-critical, full-	Stainless steel plates and bicortical screws	Immediately post-trauma	N/A	N/A	During primary surgery, 6 mg of peptide-enriched silk fibroin sponges with vancomycin-loaded nanoparticles was placed into the treatment	After surgery, 2/8 animals showed local swelling around the fracture site. At weeks 1 and 2, neutrophil counts were elevated	Not specified	At week 8, half of the animals were evaluated. <i>S. aureus</i> could be cultured from 2/4 femurs.	At week 8, 4/5 animals showed a complete disorganization of bone structure, nonunion, abundant fibrovascular tissue, chronic abscesses, necrotic tissue, periosteal inflammation, and the

						thickne ss defect was created after a localize d perioste al elevatio n.					group's defects.	, but decreas ed to normal levels by week 6. At weeks 1 and 3, complet e load bearing was observe d. Body weights increas ed steadily over the study period.			presence of macrophage s and granulocyte s.
Kobata et al. ⁵⁹	Wistar rats	<i>S. aureus</i>	Not specified	2.6x10 ⁶ CF Us	Femor al diaph ysis	A transve rse fracture was created using a guilloti ne device.	Titanium Kirschner wire implants (1.5 mm in diameter and 60 mm in length)	1-hour post- trauma	Postope rative	N/A	Treatment groups' implants were coated with chitosan and different concentrat ions of ciprofloxa cin.	By week 4, suppurati on was observe d in 3/11 and 0/8 animals in the untreate d control and systemic antibiotic control groups, respecti vely.	At week 4, negligible callus formati on was detected .	Not specified	At week 4, inflammatory cells, angiogenesis, fibroplasia were observed in all animals.
Stewart et al. ⁶⁰	Brown Norway rats	<i>S. aureus</i> , <i>E. coli</i> (polymicro bial)	10 µL of bacterial suspension was administered through a sterile pipette at the fracture site and into the medullary canals of the femur.	<i>S. aureus</i> : 1x10 ⁶ CFU/mL <i>E. coli</i> : 1x10 ⁴ CFU/mL	Femor al diaph ysis	A 500- g weight was dropped from a height of 35 cm to create a comminuted fracture 5 mm in length.	Threaded 1.6-mm Kirschner wire	Immedi ately post- trauma	N/A	N/A	During primary surgery, a scaffold loaded with 10 µg BMP- 2 and either 10 mg or 20 mg of gentamicin was placed into treatment groups' defects.	At day 2, 1 animal was found dead due to sepsis.	At week 12, nonunion was observe d at most fracture sites. 11% showed complete bridging of callus formati on.	At week 12, <i>E. coli</i> could not be cultured. <i>S. aureus</i> could be cultured from all femurs.	At week 12, fibrous tissue formation, a lack of osseous bridging, and gram- positive bacteria at the fracture site and within medullary canals was observed.
Johnson et al. ⁶¹	C57/B6 mice	<i>S. aureus</i>	Not specified	ATCC 49230: 1.55 ± 0.51 × 10 ⁸ CFU/mL ATCC BAA- 1556: 3.43×10 ⁸ CFU/mL	Femor al diaph ysis	The femur was fractured with a custom- made three- point bender.	25-gauge intramedul lary needles	Immedi ately post- trauma	Postope rative	N/A	During primary surgery, 5 µL of lysostaphin containin g hydrogel was pipetted over treatment group's defects.	Not specifie d	At week 5, no callus formati on and active bone resorpti on were detected .	At week 1, <i>S. aureus</i> could be cultured from femurs, soft tissue, and implants. The proportion of animals with positive cultures is not specified.	At week 1, leukocyte infiltration, poor collagen staining, and gram- positive bacteria were observed.
Guarcho Pérez et al. ⁶²	C57BL/6J RecHsd mice	<i>S. aureus</i>	1 µL of bacterial suspension was pipetted into the defect hole.	1x10 ⁴ CFUs	Femor al diaph ysis	A hole was drilled in the center of the femur until the medulla r cavity was reached , using a drill bit of	PCL-HA- HNT fixation plates	Pre- trauma	N/A	N/A	Treatment group's implants were coated with a composite of poly-e- caprolacto ne, hydroxya patite and halloysite nanotubes loaded with	At days 1 and 3, body weights had decreas ed.	Not specifie d	At day 3, <i>S. aureus</i> could be cultured from all femurs, soft tissue, and implants.	Not specified

						0.5 mm.					gentamicin sulphate.				
Stavrikis et al. ⁶³	C57BL/6 mice	<i>S. aureus</i>	Bacterial suspension in 2 µL of saline was injected into the fracture site.	1×10 ⁸ CFUs	Femoral diaphysis	Not specified	Titanium Kirschner-wires (diameter 0.6 mm)	Pre-trauma	N/A	N/A	Treatment group's implants were coated with a polymer containing vancomycin or tigecycline.	Not specified	At week 6, osteolysis, bony destruction, involucrum, and implant loosening was observed.	At week 1, bacterial burden peaked as measured by bioluminescence. At week 6, <i>S. aureus</i> could be cultured from the femurs, soft tissue, and implants. The proportion of animals with positive cultures is not specified.	Not specified
Treatment															
Southwood et al. ⁶⁴	New Zealand White rabbits	<i>S. aureus</i>	0.5 mL of inoculum was injected percutaneously 48 hours after surgery.	0.5×10 ⁷ CFU/0.5 mL	Femoral diaphysis	A 10 mm defect was surgically created using a side-cutting carbide burr.	Two stacked 2.0-mm cuttable bone plates and eight 2.0 mm cortical screws	Immediately post-trauma	Preoperative	N/A	During primary surgery, Ad-BMP-2 was administered by percutaneous injection into the treatment group's defects.	Not specified	At 4, 8, 12, and 16 weeks, bridging callus formation, bone lysis, and defect ossification was observed. 7 rabbits had fracture plates bent adjacent to the defects and did not have callus formation.	Not specified	At 16 weeks, inflammation, necrosis, and necrotic bone was observed.
Onsea et al. ⁶⁵	New Zealand White rabbits	<i>S. aureus</i>	34 mL of bacterial suspension was pipetted onto the central screw hole overlying the osteotomy and onto the adjacent proximal and distal screw holes.	2×10 ⁶ CFUs	Humeral diaphysis	An osteotomy was created using a Gigli saw (diam. 0.22 mm).	Seven-hole locking plate and six 2 mm locking screws	Immediately post-trauma	Postoperative	2 weeks	Treatment groups either received phage-loaded hydrogel during secondary surgery or 1 mL injections of phage suspended in normal saline (10 ⁸ PFU/mL) through a subcutaneous access tube for 7 days.	Throughout the study, WBC counts were within normal ranges. Body weights had decreased.	Not specified	At week 4, <i>S. aureus</i> could be cultured from 1/7 soft tissue samples and from all humeri and implants.	Not specified
Yan et al. ⁶⁶	New Zealand White rabbits	<i>S. aureus</i>	Bacterial suspension in 2 mL of saline was pipetted into the femoral space containing	1×10 ⁵ CFU/mL	Humeral diaphysis	Not specified	Intramedullary rod	Immediately post-trauma	Postoperative	N/A	During primary surgery, the medullary canal was reamed. After surgery, the	At day 2, CRP levels were elevated and peaked.	Not specified	At week 1, <i>S. aureus</i> could be cultured from implants. The proportion of	Not specified

			g the cut end of the implant.								treatment group received injections of a cationic peptide, LL-37.			animals with positive cultures is not specified. Biofilm growth was detected on implants.	
Xu et al. ⁶⁷	New Zealand White rabbits	<i>S. aureus</i>	Bacterial suspension in 2 mL of saline was pipetted into the femoral space containing the cut end of the implant.	3×10 ⁵ CFU/mL	Femoral diaphysis	The bone fracture was made using a drill with a diameter of 1 mm.	Mini-titanium plates (0.5 mm in diameter)	Immediately post-trauma	Postoperative	N/A	After primary surgery, treatment groups received a local injection of tea polyphenols and bacteriocins, cefradine and bacteriocins, or cefradine and tea polyphenols.	Not specified	Not specified	At day 2, biofilm growth was detected on implants.	Not specified
Caprice et al. ⁶⁸	New Zealand White rabbits	<i>S. aureus</i>	Dry clay mix was combined with bacterial suspension to form a 3-mL slurry that was placed into a syringe for injection into the wound.	5×10 ⁶ CFU/mL	Femoral metaphysis	The medial femoral condyle was osteotomized using a ¼ inch osteotome placed on the distal end of the femoral condyle to create a sagittal plane fracture.	Singular 2.7 mm screws	Immediately post-trauma	N/A	N/A	During primary surgery, treatment groups received either unpressurized lavage or a high-pressure pulsatile lavage.	At week 2, purulence was observed in all knees.	At week 2, nonunion was detected in 77% of osteotomies. Calcified new bone was detected.	At week 2, <i>S. aureus</i> could be cultured from all fracture-site swabs.	Not specified
Penn-Barwell et al. ⁶⁹	Sprague-Dawley rats	<i>S. aureus</i>	The defect was contaminated with 30 mg of bovine collagen soaked with bacterial suspension in 0.5 mL of saline.	1×10 ⁵ CFUs	Femoral diaphysis	A 6-mm defect was created with a reticulating saw, cooled with saline.	Bespoke polyoxymethylene plates fixated with six threaded Kirschner wires	Pre-trauma	N/A	6 hours	During secondary surgery, either PMMA beads, PMMA beads and antibiotic gel, or antibiotic gel was placed into treatment groups' defects.	At week 2, body weight had decreased.	Not specified	At week 2, <i>S. aureus</i> could be cultured from all femurs and implants.	Not specified
Sanchez et al. ⁷⁰	Sprague-Dawley rats	<i>S. aureus</i>	The defects were implanted with 30 mg of type I bovine collagen wetted with one of the bacterial suspensions.	1×10 ² CFU	Femoral diaphysis	A 6 mm segmental defect was created using a small reciprocating saw blade.	Polyacetyl plates (length 25 mm, width 4 mm and height 4 mm) fixed using threaded Kirschner wires	Immediately post-trauma	N/A	6 hours	During secondary surgery, polyurethane scaffolds with 1%, 5%, or 10% d-amino acids were placed into treatment groups' defects.	Not specified	Not specified	At week 2, <i>S. aureus</i> could be cultured from 5/10 femurs. Extensive biofilm growth was detected on polyurethane scaffolds.	Not specified
Li et al. ⁷¹	Sprague-Dawley rats	<i>S. aureus</i>	The defects were implanted with 30 mg of	1×10 ⁵ CFUs	Femoral diaphysis	A 6 mm full-thickness defect was	Polyacetyl plate (length 25 mm, width 4 mm and height 4	Immediately post-trauma	N/A	6 hours	During secondary surgery, vancomycin-releasing	Not specified	Not specified	At week 4, <i>S. aureus</i> could be cultured from	Not specified

			type I bovine collagen that was ethanol sterilized and subsequently wetted with bacteria suspended in 0.1 mL of saline.			created with a small reciprocating saw blade under continuous irrigation with sterile saline.	mm) fixed to the surface of the femur using six threaded Kirschner wires				polyurethane scaffolds were packed into treatment group's defects.			femurs. The proportion of animals with positive cultures is not specified.	
Brick et al. ⁷²	Sprague-Dawley rats	<i>S. aureus</i>	0.1 mL of saline containing bacterial suspension was added to a type I bovine collagen sponge, and the wetted sponge was packed into the defect.	5×10 ⁵ CFUs	Femoral diaphysis	A full-thickness 6-mm defect was created with a small pneumatic-powered oscillating saw.	Polyacetyl plates fixed with six Kirschner wires	Pre-trauma	N/A	N/A	During primary surgery, type I bovine collagen sponge wetted with 200 µg rhBMP-2 was placed into the treatment group's defects.	Not specified	Not specified	At weeks 1, 2, and 4, <i>S. aureus</i> could be cultured from all swab samples.	Not specified
Chen et al. ⁷³	Sprague-Dawley rats	<i>S. aureus</i>	Type I bovine collagen sponge was wetted with 0.1 mL of the bacterial suspension in sterile saline and placed in the defect.	1x10 ⁴ CFUs	Femoral diaphysis	A 6 mm full-thickness defect was created with a mini-driver and small oscillating saw blade.	Polyacetyl plate and six Kirschner wires	Immediately post-trauma	Postoperative	2 weeks	During secondary surgery, 20 or 200 µg of rhBMP-2 in a type I bovine collagen sponge was placed into treatment group's defects.	Throughout the study, none of the animals exhibited lameness, a draining sinus, or clinical symptoms indicative of systemic infection.	At weeks 4 and 12, bone lysis was detected	At weeks 4 and 12, <i>S. aureus</i> could be cultured from all femurs.	At weeks 4 and 12, minimal new bone and fibrous tissue filled defects. A layer of periosteal bone appeared alongside the femoral cortex.
Chen et al. ⁷⁴	Sprague-Dawley rats	<i>S. aureus</i>	All defects were contaminated with bacterial suspension in 0.1 mL of normal saline solution mixed with 60 mg of lyophilized type-I bovine collagen.	1x10 ⁴ CFUs	Femoral diaphysis	A 6 mm full-thickness defect was created with a small pneumatic-powered oscillating saw blade.	Polyacetyl plate and six Kirschner wires	Pre-trauma	Postoperative	2 weeks	During secondary surgery, 20 or 200 µg of rhOP-1 dissolved in buffer was placed into treatment group's defects.	After primary and secondary surgeries, animals resumed a normal activity level and gained weight. None of the animals exhibited signs of lameness, a draining sinus, or clinical symptoms indicative of systemic infection.	Not specified	At week 12, <i>S. aureus</i> could be cultured from all femurs.	At week 12, there was minimal newly mineralized callus within or bridging around the defect.
Helbig et al. ⁷⁵	Sprague-Dawley rats	<i>S. aureus</i>	10 µL of bacterial suspension was injected into the	1x10 ³ CFUs	Tibial and fibular diaphysis	A weight of 600 g was fixated 15 cm above	0.8 mm Kirschner wires	Pre-trauma	N/A	5 weeks	During secondary surgery, 30 µg of rhBMP-7 or 25 µg of	Throughout the study, body weights had decreased	At week 10, unbridged callus formation and	Not specified	At week 10, fibroblasts and cartilage were observed in the fracture

			medullar cavity.			the leg with a removable pin. Removal of the pin resulted in a sudden free fall of the weight and a transverse fracture of tibia and fibula.					rhBMP-2 was injected into the treatment group's intramedullary cavities.	ed, then increased steadily. Body temperatures were normal.	nonunion was detected.		region. Gram staining showed <i>S. aureus</i> in the cortex and cancellous bone.
Caroom et al. ⁷⁶	Sprague-Dawley rats	<i>S. aureus</i>	The defect was then inoculated with 30 mg of sterile bovine collagen soaked with bacterial suspension in 0.5 mL of saline.	1×10 ⁵ CFUs	Femoral diaphysis	A 6-mm defect was made using a reciprocating saw.	Bespoke polyoxymethylene plates fixated with six threaded Kirschner wires	Pre-trauma	N/A	6 hours	During secondary surgery, PMMA beads containing antibiotic powders or 10 mg of antibiotics powders were placed in the treatment group's defects.	Not specified	Not specified	At week 2, <i>S. aureus</i> could be cultured from all femurs and implants.	Not specified
Penn-Barwell et al. ⁷⁷	Sprague-Dawley rats	<i>S. aureus</i>	The defect was contaminated with 30 mg of sterile bovine collagen soaked with bacterial suspension in 0.5 mL of saline.	1×10 ⁵ CFUs	Femoral diaphysis	A 6-mm defect was created with a reticulating saw, cooled with saline.	Bespoke polyoxymethylene plate secured with six threaded Kirschner-wires	Pre-trauma	Postoperative	6 hours	Immediately after secondary surgery, a single dose of bismuth thiols suspended in hydrogel were administered to the treatment group's wounds.	Throughout the study, local toxicity with wound breakdown was observed.	Not specified	At week 2, <i>S. aureus</i> could be cultured from 5/10 femurs and 6/10 implants.	Not specified
Rand et al. ⁷⁸	Sprague-Dawley rats	<i>S. aureus</i>	The defect was then packed with 30 mg of rehydrated, sterilized bovine type I collagen mixed with the bacterial suspension.	1×10 ⁵ CFUs	Femoral diaphysis	A 6-mm defect was created with a reticulating saw, cooled with saline.	A non-absorbable polyoxymethylene plastic plate fixed using threaded 0.9 mm Kirschner wires	Pre-trauma	Postoperative	6 hours	During secondary surgery, antibiotic gel or PMMA beads were placed into the treatment group's defects.	Not specified	Not specified	At week 2, <i>S. aureus</i> could be cultured from femurs and implants. The proportion of animals with positive cultures is not specified.	Not specified
Penn-Barwell et al. ⁷⁹	Sprague-Dawley rats	<i>S. aureus</i>	The defect was contaminated with 30 mg of sterile bovine collagen soaked with bacterial suspension in 0.5 mL of saline.	1×10 ⁵ CFUs	Femoral diaphysis	A 6 mm defect was created by an oscillating saw, cooled with saline.	Bespoke polyoxymethylene plate, secured to the bone with six threaded Kirschner wires	Pre-trauma	Postoperative	2, 6, or 24 hours	N/A	Not specified	Not specified	At week 2, <i>S. aureus</i> could not be cultured from animals receiving I&D and antibiotics at the 2-hour time point. In animals receiving I&D and antibiotics at the 24-hour time-point, <i>S. aureus</i> could be	Not specified

														cultured from all femurs and implants.	
Lindsey et al. ⁸⁰	Sprague-Dawley rats	<i>S. aureus</i>	0.1 mL of bacterial suspension was placed directly into the wound after both ends of the fracture were exposed	1x10 ² CFUs	Femoral diaphysis	A defect was created by dropping a weight of 0.94 kg from 15.3 cm, which impacted the blunted blade delivering a force of 104.80 N.	Kirschner wires	1-hour post-trauma	N/A	N/A	After primary surgery, daily intraperitoneal injections of 200 ng of IL-12 for a total of 10 doses were given to the treatment group.	At week 3, macrophage activation, WBC counts, neutrophil counts, and platelet counts were elevated.	At week 3, sequestrum formation and destruction of bone was detected.	At week 3, moderate amounts of <i>S. aureus</i> could not be cultured from femurs. The proportion of animals with positive cultures is not specified.	At week 3, signs of infections and improper bone healing were observed.
Whitely et al. ⁸¹	Lewis rats	<i>S. aureus</i>	The defect was packed with 100 mg of sterile collagen wetted with bacterial suspension.	1x10 ⁵ CFUs	Femoral diaphysis	A 6-mm defect was created with a reticulating saw, cooled with saline.	Radiolucent plates fixed using six 0.9 mm diameter threaded Kirschner wires	Pre-trauma	Postoperative	6 hours	During secondary surgery, 2 mL Bactisure™ Wound Lavage was used, and/or 50 mg of vancomycin powder was placed in defects of treatment groups.	Not specified	Not specified	At week 2, <i>S. aureus</i> could be cultured from all femurs and implants.	Not specified
Shiels et al. ⁸²	Lewis rats	<i>S. aureus</i>	A 30 mg collagen matrix was prewetted with bacterial suspension then placed in the wound.	7.62x10 ⁵ ± 1.23x10 ⁵ CFUs	Femoral diaphysis	A 6-mm defect was created with a reticulating saw, cooled with saline.	Six 0.9 mm threaded Kirschner wires	Pre-trauma	Postoperative	1 day	During secondary surgery, 50 mg rifampin, vancomycin, and/or daptomycin powder was placed in defect of treatment groups.	At week 2, redness, swelling, and purulence were observed in all animals.	Not specified	At week 2, <i>S. aureus</i> could be cultured from all femurs and implants.	Not specified
Shiels et al. ⁸³	Lewis rats	<i>S. aureus</i>	An absorbable collagen matrix wetted with bacterial suspension was placed in the fracture site.	3.59x10 ⁵ ± 2.00x10 ⁴ CFUs	Femoral diaphysis	A 6 mm segment of bone was removed with a reciprocating saw under copious saline irrigation.	24-mm radiolucent polyacetyl plate affixed to the femur with six 0.9 mm threaded stainless steel Kirschner-wires	Immediately post-trauma	N/A	6 or 24 hours	During secondary surgery, either PMMA beads containing rifampin and vancomycin or only rifampin was placed in defects of treatment groups.	Throughout the study, body weights decreased steadily.	Not specified	At week 2, <i>S. aureus</i> could be cultured from 9/10 femurs and all implants.	Not specified
Tennent et al. ⁸⁴	Lewis rats	<i>S. aureus</i>	A bovine collagen matrix wetted with bacterial suspension was placed in the fracture site.	1.2x10 ⁶ ± 1.9x10 ⁵ CFUs	Femoral diaphysis	A 6 mm segment of bone was removed with a saw under saline irrigation.	24 mm polyacetyl plates fixed with six 0.9 mm threaded Kirschner wires	Pre-trauma	Postoperative	6 or 24 hours	During secondary surgery, either PMMA beads containing vancomycin or vancomycin powder was placed into defects of treatment groups.	Not specified	Not specified	At 6 and 24 hours, <i>S. aureus</i> could be cultured from all femurs and implants.	Not specified

Mills et al. ⁸⁵	Wistar rats	<i>S. aureus</i>	An absorbable collagen sponge was dosed with bacterial suspension immediately before implantation. The sponge was wrapped circumferentially around the fracture.	1x10 ⁴ CFUs	Femoral diaphysis	An Einhorn drop-weight apparatus.	1.1-mm Kirschner wires	Pre-trauma	N/A	1 or 5 days	During secondary surgery, 500 µg of CSA-90 was placed into the fracture site of treatment groups.	Not specified	At week 3, nonunion was detected.	At week 3, <i>S. aureus</i> could be cultured from most deep tissue swabs. The proportion of animals with positive cultures is not specified.	At week 3, nonunion and inflammatory debris was observed.
Lovati et al. ⁸⁶	Wistar rats	<i>S. epidermidis</i>	30 µL of bacterial suspension was injected into the femoral defect.	1×10 ⁵ CFU	Femoral diaphysis	Not specified	Stainless steel plates and bicortical screws	Immediately post-trauma	Preoperative and postoperative	N/A	Either immediately after or 24 hours following primary surgery, treatment groups received allogeneic rat bone marrow MSCs.	From days 3 to 7, animals showed a partial load bearing on the operated limb without any clinical evidence of infection. At week 2, a significant neutrophil increase was observed compared to basal levels.	At week 6, 5/6 animals displayed nonunion with <75% bony bridging.	At week 6, <i>S. aureus</i> could be cultured from all implants and femurs.	At week 6, polymorphonuclear cells, disorganized bone architecture, fibrovascular tissues, and nonunion were observed. Gram-staining depicted bacteria clustered in bone and periosteal tissue.
Roukoz et al. ⁸⁷	Wistar rats	<i>S. aureus</i>	0.5 mL of bacterial suspension was injected over fracture site.	1x10 ⁸ CFUs	Femoral diaphysis	A fracture is made with Liston bone cutting forceps.	Kirschner wires (5 mm in length)	Immediately post-trauma	N/A	6 weeks	During secondary surgery, bone cement with 3 g of vancomycin were used for the treatment group.	Throughout the study, body weights increased steadily.	At week 6, nonunion was detected in all animals.	At week 12, <i>S. aureus</i> could be cultured from all femurs and induced membranes.	At week 12, granulomatous reaction, abscess, and nonspecific inflammatory reaction is observed.
Şener et al. ⁸⁸	Wistar rats	<i>S. aureus</i>	PBS containing bacterial suspension was injected into the medullary cavity at the fracture site.	1x10 ³ CFU/10 µL	Tibial diaphysis	A transverse fracture was created via a mini osteotomy.	Kirschner wires (0.8 mm in diameter)	Immediately post-trauma	Postoperative	3 weeks	During secondary surgery, either teicoplanin embedded PMMA beads or teicoplanin embedded autogenous bone grafts were placed into defects of treatment groups.	At week 6, purulent drainage and abscess formation was observed in all animals.	At week 6, nonunion was observed in 6/8 animals.	At week 6, <i>S. aureus</i> could be cultured from all tibias.	Not specified
Büren et al. ⁸⁹	BALB/c-mice	<i>S. aureus</i>	1 µL of bacterial solution was injected into the	1.94x10 ³ CFU/µL	Femoral diaphysis	An osteotomy was created using a Gigli saw (diam.	Four-hole titanium locking plate with locking self-tapping	Pre-trauma	N/A	1, 2, 4, and 8 weeks	From day 7 to 21, treatment groups received HBO therapy	Not specified	At week 1, 2, 4, and 8, nonunion was detected.	At week 1, 2, 4, and 8, <i>S. aureus</i> could be cultured from lavages.	Not specified

			fracture gap.			0.22 mm).	micro-screws				for 90 minutes.			The proportion of animals with positive cultures is not specified.	
Yu et al. ⁹⁰	C57BL/6J mice	<i>S. aureus</i>	5 µL of bacterial suspension was applied to the fracture site. Ten minutes later, sterile gauze was used to absorb the fluid in the fracture site.	1×10 ⁶ CFUs	Femoral diaphysis	A femoral shaft transverse fracture was performed with a water-port clamp.	0.6 mm intramedullary needles	Immediately post-trauma	N/A	N/A	After primary surgery, hyaluronic acid-based hydrogel loaded with antagonism R-708-5p was administered to the treatment group once daily for 3 days.	Not specified	At week 3, nonunion was detected.	Not specified	Not specified
Sumral et al. ⁹¹	C57BL/6 mice	<i>S. aureus</i>	Not specified	1×10 ⁴ CFUs	Femoral diaphysis	Not specified	4-hole titanium plate	Immediately post-trauma	Postoperative	5 days	During secondary surgery, 50 µL equimolar enzymatic combination (M23/GH15/DA7 at 1 mg/mL) and/or local antibiotics.	Throughout the study, body weights decreased steadily.	Not specified	At day 13, <i>S. aureus</i> could be cultured from all femurs, soft tissue, and implants.	At day 13, soft tissue adjacent to the osteotomy contained positive staining for <i>S. aureus</i> .

Table 2: Summary of study characteristics. Clinical, radiographic, bacteriologic, and histologic validations of the models are based on infected control groups. Information not provided is denoted by “Not specified”. Interventions not incorporated into the model are denoted by “N/A”. BHI: brain heart infusion broth,

BMP: bone morphogenetic protein, CAS/HA: calcium sulfate/hydroxyapatite, CFU: colony-forming unit, CRP: C-reactive protein, CSA: cationic steroid antibiotic, HBO: hyperbaric oxygen, I&D: irrigation & debridement, IL: interleukin, MCP: monocyte chemoattractant protein, MSCs: mesenchymal stem cells, OP: osteogenic protein, PBS: phosphate-buffered saline, PEEK: polyetheretherketone, PMMA: Poly(methyl methacrylate), PFU: plaque-forming units, WBCs: white blood cells.

Results

Search Results

From the initial search, 615 articles were identified. Following screening for title and abstract relevance, 474 studies were excluded. The remaining 141 studies were assessed for eligibility; 77 met the criteria for inclusion and data extraction (Fig. 1).

Study Classifications

Among these 77 articles, 13 (16.9%) were classified as characterization studies, 13 (16.9%) as model development studies, 23 (29.9%) as prevention studies and 28 (36.4%) as treatment studies.

Model Animals

Seven articles (9.1%) used a large animal model of FRI; 3 used sheep, 2 used goats and 2 used dogs. The remaining 70 articles (90.9%) used small animal models of FRI; 40 used rats, 15 used rabbits and 15 used mice.

Bone Defects

The most common anatomic location for bone defect creation was the femoral diaphysis with 54 (70.1%) studies using this site. Other sites included the tibial diaphysis (n=13, 16.9%) humeral diaphysis (n=6, 7.8%), femoral metaphysis (n=2, 2.6%) or both tibial and fibular diaphysis (n=2, 2.6%).

For articles specifying a method of defect creation (n=71), 46 (64.8%) used oscillating or Gigli wire saws to create an osteotomy, while the remaining articles described fracture induction via drop-weight apparatus (n=8, 11.3%), drill (n=5, 7%), compression device (n=5, 7%), osteotome (n=2, 2.8%), captive bolt gun (n=2, 2.8%), manual force (n=2, 2.8%) or bone-cutting forceps (n=1, 1.4%).

Long bone defects were predominantly stabilized by bone plates and screws. Among the included articles, 42 (54.5%) used plate-and-screw fixation, 18 (23.4%) used Kirschner wires, 11 (14.3%) used an intramedullary device, 2 (2.6%) used screws alone and 1 (1.3%) used external skeletal fixators. For the remaining articles, 1 (1.3%) study compared plate to intramedullary fixation, 1 (1.3%) compared external to intramedullary fixation and 1 (1.3%) study exchanged Kirschner wires for bone plates at a secondary surgery.

Defect fixation occurred before defect creation in 36 studies (47.4%) and immediately following defect creation in 35 studies (46.1%). In 5 studies (6.6%), fixation was delayed for 1 hour (n=3), 4 hours (n=1) or 6 hours (n=1) after defect creation. One study did not specify fixation timing.

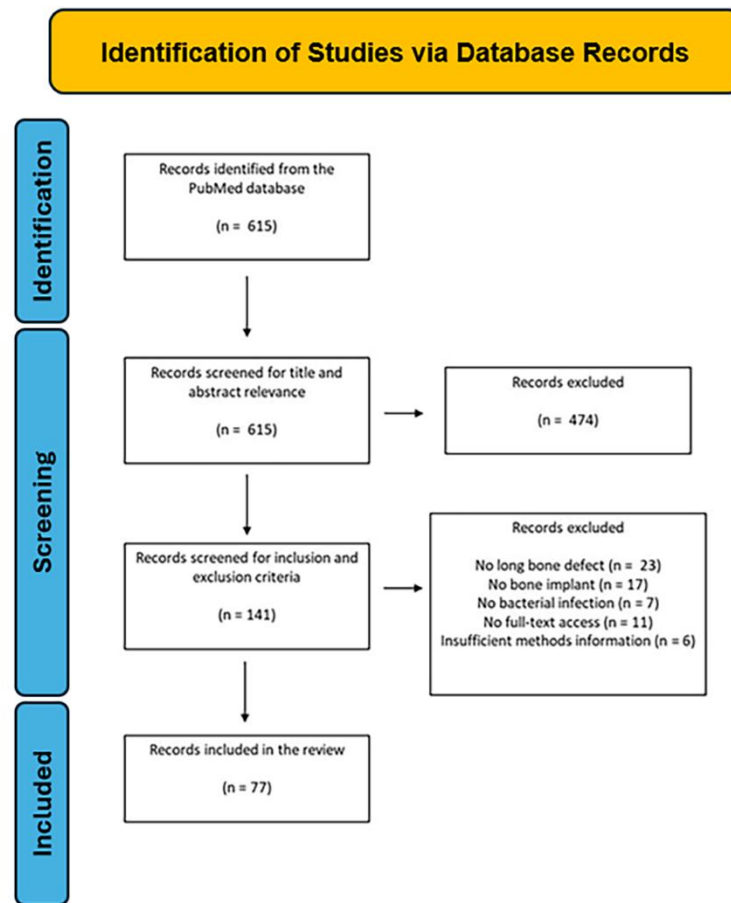


Figure 1: Outline of the literature search process for study inclusion and exclusion.

Bacterial Inoculations

For the 77 articles reviewed, 74 studies (96.1%) induced FRI using *Staphylococcus aureus*, including 5 studies (6.5%) that established a polymicrobial infection by also including *Escherichia coli* (n=3), *Pseudomonas aeruginosa* (n=1) or *Acinetobacter baumannii* (n=1). Four studies (5.2%) induced FRI using *Staphylococcus epidermidis*. Inoculum doses ranged from 20 CFUs to 3×10^8 CFUs with the most common dose being 1×10^5 CFUs. Methods for bacterial inoculation included application of a planktonic bacterial suspension directly to the fracture site (i.e., via pipetting or indwelling catheter) (n=44, 61.1%), placement of a bacteria-soaked carrier into the fracture site (i.e., via collagen sponge) (n=23, 31.9%) or fracture-fixation implant incubation in bacterial suspension prior to implantation (n=5, 6.9%). Five studies did not specify a method of bacterial inoculation.

Interventions

Systemic antibiotics were used in 34 studies (44.2%). Administration occurred preoperatively (n=6, 17.6%), postoperatively (n=26, 76.5%) or both preoperatively and postoperatively (n=2, 5.9%). Secondary surgeries involving I&D were used in 29 studies (37.7%). These occurred within 6 hours of inoculation (n=11, 37.9%) or were delayed for 24 hours (n=2), 5 days (n=2), 1 week (n=3), 2 weeks (n=6), 3 weeks (n=1), 4 weeks (n=1), 5 weeks (n=2), 6 weeks (n=2) and/or 8 weeks (n=1). Three studies compared

early and delayed I&D time points. A variety of other interventions were incorporated into the included studies such as local antibiotic therapy (i.e., via hydrogel, implant coating or other) (n=25), bone regeneration therapies (i.e., via bone morphogenetic proteins, mesenchymal stem cells or other) (n=9) or metal ion implant coatings (n=5).

Model Validations

Forty-five articles (58.4%) reported clinical validation of FRI in their infected control groups with 17 (37.8%) specifying signs of local infection. Signs of local FRI included fracture site swelling, redness, edema, purulent drainage, abscess formation and/or wound breakdown. FRI was validated radiographically in 43 articles (55.8%). Of these, fracture nonunion or delayed union was reported in 23 studies (53.5%) at their respective endpoints. Other common radiographic signs of FRI included osteolysis (n=23, 53.5%), periosteal reaction (n=12, 27.9%) and/or sequestrum formation (n=4, 9.3%). Local microbiological validation of FRI from culturing bone, soft tissue, implants, I&D lavage and/or fracture-site swabs was performed in 65 articles (84.4%). In infected control groups, a positive culture rate of 100% in at least one type of culture was reported in 44 studies (67.7%), whereas a rate of less than 100% was reported in 12 studies (18.5%). Nine studies (13.8%) did not specify the proportion of positive cultures. Using a variety of methods, bacterial biofilm growth was directly detected in 16 studies (20.8%). Lastly, histological validation of FRI was performed in 42 studies (54.5%). Common histological signs of FRI included the identification of necrotic bone and tissue, disorganized bone and callus architecture, inflammatory cell infiltration and/or bacteria aggregates. Overall, 76 of the 77 included articles reported at least one clinical, radiographic, microbiologic or histologic outcome measure to validate at least one component of FRI in the model.

Literature Synthesis

Are the methods used to create the long-bone trauma biologically and biomechanically relevant for FRI?

Only 17 articles (23.9%) described models that created fractures using environmental forces while the remaining articles described surgically created fractures. While the surgically induced fracture models allow for a more controlled, reproducible bone defect, they do not comprehensively recapitulate biologic and biomechanical contributors to FRI, including bone fragmentation, cancellous bone impaction or damage to soft tissue and vasculature. These factors exacerbate instability, necrosis and disruption of the host immune system, facilitating microbial proliferation and bone nonunion [92,93]. Ideally, FRI models should mimic the biomechanical and biological trauma of complex fractures while also maintaining a high level of reproducibility. Current best evidence suggests that traumatic fracture creation via captive bolt gun, drop-weight apparatus, compression device or manual force or surgically induced models via gap osteotomy with soft tissue disruption, provide the most clinically relevant methods for FRI.

Are the pathogens and inoculation methods clinically relevant for FRI?

An epidemiologic study on patients with trauma-related osteomyelitis reported that 45% of infections involved *Staphylococcus aureus*, 17% involved *Staphylococcus epidermidis*, 14% involved a *Streptococcus species* and 8% involved a *Pseudomonas species* with 35% being polymicrobial infection [4]. Nearly all the FRI models included in the present systematic review used *Staphylococcus aureus* while only five (6.5%) incorporated a polymicrobial infection. Inoculation was performed predominantly by the direct application of planktonic bacteria to the fracture site following bone defect creation. Five (6.5%) articles pre-inoculated bone implants before fixation, effectively establishing a bacterial biofilm. While the former method is a more standardized approach for inoculating a consistent dose of bacteria, the latter establishes a defining component of FRI that accounts for a key clinical feature. A wide spectrum of inoculum doses, ranging from 20 to 3×10^8 CFU, were used and several models used multiple doses to represent different levels of infection severity. The most common dose used was 1×10^5 CFU while Arens, et al., reported that the minimum CFU dose needed to consistently induce infection in a rabbit humeral osteotomy model was 6×10^6 CFU [30]. While the optimal dose for each preclinical FRI model will vary depending on animal species, pathogen, purpose of the research and other variables, 10^5 to 10^7 CFU appears to be an appropriate range for consistent induction of fracture site infection.

Are the studies' intervention time points clinically realistic?

A study evaluating 17,993 patients who underwent femur fracture fixation at trauma centers in the United States reported a median time to fixation of 15 hours. Delayed fixation (≥ 24 hours) was performed in 26% of patients [94]. Importantly, the timing of fracture fixation has been reported to influence the development of fracture-related infections, wherein delayed treatment is associated with a higher likelihood of FRI [95]. As such, animal models that include delayed fracture fixation provide a more

realistic representation of the clinical scenario. Only 5 (6.6%) articles described FRI animal models that incorporated fixation that was delayed for at least 1 hour after defect creation, whereas all others provided fixation prior to or immediately after defect creation. While ethical care and use of research animals, in conjunction with the study logistics and costs, must govern fixation type and timing, optimal FRI animal models should strive to incorporate a clinically relevant delay in definitive stabilization after defect creation.

Currently, the clinical standard for FRI management includes antibiotics administered systemically and often locally. Antibiotics are typically initiated pre- or intra-operatively and then continued post-operatively with adjustments to type, dose and route of administration as needed based on sensitivity testing and response to treatment [10]. While the majority of articles in this systematic review followed clinically relevant protocols for timing, type, dose and route of antibiotic administration, 8 (23.5%) of the articles incorporated systemic antibiotic administration prior to defect creation and bacterial inoculation, which is clinically unrealistic.

Secondary surgeries are another characteristic component of FRI management. These most commonly involve debridement of necrotic and grossly infected tissue, copious irrigation of infected tissues and implants and removal of grossly infected, loose or ineffective implants. In a study assessing open fractures, the mean time to I&D for fractures that did not become infected was 10.2 hours from primary surgery while the mean time to I&D for fractures that did become FRIs was 13.6 hours [96]. However, other studies have not reported differences in FRI rate between early and delayed I&D [97]. Current clinical guidelines recommend I&D of open fractures within 6 hours of trauma [98]. Among the 29 studies incorporating secondary I&D in this systematic review, 11 (37.9%) performed this intervention within 6 hours and 15 (51.7%) did so greater than 24 hours after defect creation and bacterial inoculation. Three articles compared early and delayed I&D. While modeling an early I&D timepoint aligns with clinical recommendations, delayed I&D allows for further development of infection to model later-onset and more established FRI.

Discussion

This systematic review of the peer-reviewed articles focused on animal models for fracture-related infections; it revealed a wide spectrum of animal species, methods for “fracture” creation, fixation methods, bacterial inoculum delivery and dose and intervention time points, while the bacteria species and antibiotic interventions used were highly consistent among studies. Clinical, radiographic, microbiologic and histologic validation of the models were reported in most articles, particularly in those focused on model development. When considering the translational potential for direct clinical applicability to FRIs, remaining gaps for animal models include:

- Fracture induction method that comprehensively recapitulates biologic and biomechanical contributors to FRI, while maintaining a high level of reproducibility
- Delay in fixation after fracture induction that balances ethical care and use of research animals with clinical relevance
- Bacterial inoculum species, dose, timing and delivery method that consistently result in FRIs with biofilm production
- Inclusion of preventative and/or therapeutic intervention controls that conform to clinical standard-of-care
- Use of large animal models for optimal clinical relevance

The limitations of this systematic review should be considered when interpreting and applying the results. The heterogeneity among the articles in terms of objectives, experimental designs and methodologies limit the generalizability of the findings. In addition, many of the included articles did not provide direct and comprehensive outcomes of model validity. Further, there are not validated methods for scoring study quality or bias for experimental animal model studies. Together, these limitations emphasize the need for a cautious interpretation and application of this systematic review and underscore the necessity for further well-designed research in this field.

Conclusion

Incorporating clinically relevant components of FRIs into a single animal model that is ethically, pragmatically, logistically and financially feasible is challenging, however, many of the gaps identified in this systematic review can and should be addressed to determine and apply evidence-based advances to the management of FRIs. Relative advantages and disadvantages of animal models should be considered and prioritized for selection and experimental design with input from veterinarians and

orthopaedic surgeons who can optimize clinical relevance. Well-designed and executed preclinical animal model studies have strong potential to profoundly enhance the prevention and treatment of this common, devastating and costly fracture complication, improving patient outcomes and optimizing the use of healthcare resources.

Conflict of Interests

The authors declare that they have no conflict of interest in this paper.

Ethical Statement

Institutional Review Board approval was not needed to conduct this review.

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Authors' Contributions

Bryce Rigden: Substantial contributions to research design and article inclusion for review, acquisition and interpretation of data, drafting the paper, revising the paper critically, approval of the submitted and final versions.

Aaron M. Stoker: Substantial contributions to research design, acquisition and interpretation of data, revising the paper critically, approval of the submitted and final versions.

Chantelle C. Bozynski: Substantial contributions to research design, acquisition and interpretation of data, revising the paper critically, approval of the submitted and final versions.

Kyle Schweser: Substantial contributions to analysis and interpretation of data, drafting the paper and revising it critically, approval of the submitted and final versions.

James P. Stannard: Substantial contributions to research design, acquisition and interpretation of data, revising the paper critically, approval of the submitted and final versions.

James L. Cook: Substantial contributions to research design, interpretation of data, revising the paper critically, approval of the submitted and final versions.

References

1. Patzakakis MJ, Wilkins J. Factors influencing infection rate in open fracture wounds. *Clin Orthop Relat Res*. 1989;(243):36-40.
2. Boxma H, Broekhuizen T, Patka P, Oosting H. Randomised controlled trial of single-dose antibiotic prophylaxis in surgical treatment of closed fractures: the Dutch Trauma Trial. *Lancet*. 1996;347(9009):1133-7.
3. Nicholson JA, Makaram N, Simpson A, Keating JF. Fracture nonunion in long bones: A literature review of risk factors and surgical management. *Injury*. 2021;52 Suppl 2:S3-S11.
4. Kremers HM, Nwojo ME, Ransom JE, Wood-Wentz CM, Melton LJ, Huddleston PM. Trends in the epidemiology of osteomyelitis: a population-based study, 1969 to 2009. *J Bone Joint Surg Am*. 2015;97(10):837-45.
5. Senneville E, Morant H, Descamps D. Needle puncture and transcutaneous bone biopsy cultures are inconsistent in patients with diabetes and suspected osteomyelitis of the foot. *Clin Infect Dis*. 2009;48(7):888-93.
6. Zuluaga AF, Galvis W, Jaimes F, Vesga O. Lack of microbiological concordance between bone and non-bone specimens in chronic osteomyelitis: an observational study. *BMC Infect Dis*. 2002;2:8.
7. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis*. 2002;8(9):881-90.
8. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet*. 2001;358(9276):135-8.
9. Govaert GAM, Kuehl R, Atkins BL. Diagnosing fracture-related infection: current concepts and recommendations. *J Orthop Trauma*. 2020;34(1):8-17.
10. Fang C, Wong TM, To KK, Wong SS, Lau TW, Leung F. Infection after fracture osteosynthesis - Part II. *J Orthop Surg (Hong Kong)*. 2017;25(1):2309499017692714.
11. Bezstarosti H, Van Lieshout EMM, Voskamp LW. Insights into treatment and outcome of fracture-related infection: a systematic literature review. *Arch Orthop Trauma Surg*. 2019;139(1):61-72.
12. Metsemakers WJ, Smeets B, Nijs S, Hoekstra H. Infection after fracture fixation of the tibia: Analysis of healthcare utilization and related costs. *Injury*. 2017;48(6):1204-10.
13. Olesen UK, Pedersen NJ, Eckardt H. The cost of infection in severe open tibial fractures treated with a free flap. *Int Orthop*.

- 2017;41(5):1049-55.
14. Page MJ, McKenzie JE, Bossuyt PM. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
 15. Curtis MJ, Brown PR, Dick JD, Jinnah RH. Contaminated fractures of the tibia: a comparison of treatment modalities in an animal model. *J Orthop Res*. 1995;13(2):286-95.
 16. Khodaparast O, Coberly DM, Mathey J, Rohrich RJ, Levin LS, Brown SA. Effect of a transpositional muscle flap on VEGF mRNA expression in a canine fracture model. *Plast Reconstr Surg*. 2003;112(1):171-6.
 17. Brown SA, Mayberry AJ, Mathy JA, Phillips TM, Klitzman B, Levin LS. The effect of muscle flap transposition to the fracture site on TNFalpha levels during fracture healing. *Plast Reconstr Surg*. 2000;105(3):991-8.
 18. Shiels SM, Muire PJ, Wenke JC. FK506 increases susceptibility to musculoskeletal infection in a rodent model. *BMC Musculoskelet Disord*. 2022;23(1):716.
 19. Bilgili F, Balci HI, Karaytug K. Can normal fracture healing be achieved when the implant is retained on the basis of infection? An experimental animal model. *Clin Orthop Relat Res*. 2015;473(10):3190-6.
 20. Hamza T, Dietz M, Pham D, Clovis N, Danley S, Li B. Intra-cellular *Staphylococcus aureus* alone causes infection *in-vivo*. *Eur Cell Mater*. 2013;25:341-50.
 21. Büren C, Hambüchen M, Windolf J, Lögters T, Windolf CD. Histological score for degrees of severity in an implant-associated infection model in mice. *Arch Orthop Trauma Surg*. 2019;139(9):1235-44.
 22. Oezel L, Büren C, Scholz AO, Windolf J, Windolf CD. Effect of antibiotic infused Calcium Sulfate/Hydroxyapatite (CAS/HA) insets on implant-associated osteitis in a femur fracture model in mice. *PloS One*. 2019;14(3):e0213590.
 23. Sabaté-Brescó M, Berset CM, Zeiter S. Fracture biomechanics influence local and systemic immune responses in a murine fracture-related infection model. *Biol Open*. 2021;10(9):bio057315.
 24. Rochford ETJ, Sabaté Brescó M, Poulsson AHC. Infection burden and immunological responses are equivalent for polymeric and metallic implant materials *invitro* and in a murine model of fracture-related infection. *J Biomed Mater Res B Appl Biomater*. 2019;107(4):1095-1106.
 25. Baertl S, Gens L, Nehrbass D. *Staphylococcus aureus* from an acute fracture-related infection displays important bacteriological and histopathologic differences from a chronic equivalent in a murine bone infection model. *Clin Orthop Relat Res*. 2023;481(10):2044-60.
 26. Hofstee MI, Riool M, Gieling F. A murine *Staphylococcus aureus* fracture-related infection model characterised by fracture non-union, staphylococcal abscess communities and myeloid-derived suppressor cells. *Eur Cell Mater*. 2021;41:774-92.
 27. Rochford ETJ, Sabaté Brescó M, Zeiter S. Monitoring immune responses in a mouse model of fracture fixation with and without *Staphylococcus aureus* osteomyelitis. *Bone*. 2016;83:82-92.
 28. Hill PF, Clasper JC, Parker SJ, Watkins PE. Early intramedullary nailing in an animal model of a heavily contaminated fracture of the tibia. *J Orthop Res*. 2002;20(4):648-53.
 29. Zhang X, Ma YF, Wang L. A rabbit model of implant-related osteomyelitis inoculated with biofilm after open femoral fracture. *Exp Ther Med*. 2017;14(5):4995-5001.
 30. Arens D, Wilke M, Calabro L. A rabbit humerus model of plating and nailing osteosynthesis with and without *Staphylococcus aureus* osteomyelitis. *Eur Cell Mater*. 2015;30:148-61.
 31. Helbig L, Guehring T, Titze N. A new sequential animal model for infection-related non-unions with segmental bone defect. *BMC Musculoskelet Disord*. 2020;21(1):329.
 32. Helbig L, Guehring T, Rosenberger S. A new animal model for delayed osseous union secondary to osteitis. *BMC Musculoskelet Disord*. 2015;16:362.
 33. Penn-Barwell JG, Rand BCC, Brown KV, Wenke JC. A versatile model of open-fracture infection: a contaminated segmental rat femur defect. *Bone Jt Res*. 2014;3(6):187-92.
 34. Robinson DA, Bechtold JE, Carlson CS, Evans RB, Conzemius MG. Development of a fracture osteomyelitis model in the rat femur. *J Orthop Res*. 2011;29(1):131-7.
 35. Alt V, Lips KS, Henkenbehrens C. A new animal model for implant-related infected non-unions after intramedullary fixation of the tibia in rats with fluorescent *in-situ* hybridization of bacteria in bone infection. *Bone*. 2011;48(5):1146-53.
 36. Chen X, Tsukayama DT, Kidder LS, Bourgeault CA, Schmidt AH, Lew WD. Characterization of a chronic infection in an internally-stabilized segmental defect in the rat femur. *J Orthop Res*. 2005;23(4):816-23.
 37. Lovati AB, Romanò CL, Bottagisio M. Modeling *Staphylococcus epidermidis*-induced non-unions: Subclinical and clinical evidence in rats. *PloS One*. 2016;11(1):e0147447.

38. Gilbert SR, Camara J, Camara R. Contaminated open fracture and crush injury: a murine model. *Bone Res.* 2015;3:14050.
39. 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;72:128-36.
40. 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):2013-20.
41. Tran N, Tran PA, Jarrell JD. *In-vivo* caprine model for osteomyelitis and evaluation of biofilm-resistant intramedullary nails. *BioMed Res Int.* 2013;2013:674378.
42. Stewart S, Barr S, Engiles J. 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.
43. 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.
44. Zhang C, Li X, Xiao D. Cu²⁺ Release from polylactic acid coating on titanium reduces bone implant-related infection. *J Funct Biomater.* 2022;13(2):78.
45. Vallejo Diaz A, Deimling C, Morgenstern M. Local application of a gentamicin-loaded hydrogel early after injury is superior to perioperative systemic prophylaxis in a rabbit open fracture model. *J Orthop Trauma.* 2020;34(5):231-7.
46. Puetzler J, Metsemakers WJ, Arens D. Antibiotic prophylaxis with cefuroxime: influence of duration on infection rate with staphylococcus aureus in a contaminated open fracture model. *J Orthop Trauma.* 2018;32(4):190-5.
47. Ter Boo GJA, Arens D, Metsemakers WJ. Injectable gentamicin-loaded thermo-responsive hyaluronic acid derivative prevents infection in a rabbit model. *Acta Biomater.* 2016;43:185-94.
48. Xie ZP, Zhang CQ, Yi CQ, Qiu JJ, Wang JQ, Zhou J. *In-vivo* study effect of particulate Bioglass in the prevention of infection in open fracture fixation. *J Biomed Mater Res B Appl Biomater.* 2009;90(1):195-201.
49. Darouiche RO, Farmer J, Chaput C, Mansouri M, Saleh G, Landon GC. Anti-infective efficacy of antiseptic-coated intramedullary nails. *J Bone Joint Surg Am.* 1998;80(9):1336-40.
50. Chai H, Guo L, Wang X. Antibacterial effect of 317L stainless steel contained copper in prevention of implant-related infection in vitro and *in-vivo*. *J Mater Sci Mater Med.* 2011;22(11):2525-35.
51. Wei S, Jian C, Xu F. Vancomycin-impregnated electrospun Polycaprolactone (PCL) membrane for the treatment of infected bone defects: An animal study. *J Biomater Appl.* 2018;32(9):1187-96.
52. Li Y, Zhou J. A Preliminary exploration of the efficacy of gentamicin sponges in the prevention and treatment of wound infections. *Infect Drug Resist.* 2021;14:2633-44.
53. Li J, Leung SSY, Chung YL. 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;164:223-9.
54. Hu CC, Chang CH, Chang Y, Hsieh JH, Ueng SWN. Beneficial effect of TaON-Ag nanocomposite titanium on antibacterial capacity in orthopedic application. *Int J Nanomedicine.* 2020;15:7889-900.
55. Gao Z, Song M, Liu RL. Improving *in-vitro* and *in-vivo* antibacterial functionality of Mg alloys through micro-alloying with Sr and Ga. *Mater Sci Eng C Mater Biol Appl.* 2019;104:109926.
56. 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.
57. Li B, Jiang B, Dietz MJ, Smith ES, Clovis NB, Rao KMK. Evaluation of local MCP-1 and IL-12 nanocoatings for infection prevention in open fractures. *J Orthop Res.* 2010;28(1):48-54.
58. Bottagisio M, Palombella S, Lopa S. Vancomycin-nanofunctionalized peptide-enriched silk fibroin to prevent methicillin-resistant Staphylococcus epidermidis-induced femoral nonunions in rats. *Front Cell Infect Microbiol.* 2022;12:1056912.
59. Kobata SI, Teixeira LEM, Fernandes SOA, Faraco AAG, Vidigal PVT, Araújo ID de. Prevention of bone infection after open fracture using a chitosan with ciprofloxacin implant in animal model. *Acta Cir Bras.* 2020;35(8):e202000803.
60. Stewart RL, Cox JT, Volgas D. The use of a biodegradable, load-bearing scaffold as a carrier for antibiotics in an infected open fracture model. *J Orthop Trauma.* 2010;24(9):587-91.
61. Johnson CT, Wroe JA, Agarwal R. Hydrogel delivery of lysostaphin eliminates orthopedic implant infection by Staphylococcus aureus and supports fracture healing. *Proc Natl Acad Sci USA.* 2018;115(22):E4960-9.
62. Guarch-Pérez C, Shaqour B, Riool M. 3D-printed gentamicin-releasing poly-ε-caprolactone composite prevents fracture-related staphylococcus aureus infection in mice. *Pharmaceutics.* 2022;14(7):1363.

63. Stavrakis AI, Zhu S, Loftin AH. 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.
64. Southwood LL, Frisbie DD, Kawcak CE, Ghivizzani SC, Evans CH, McIlwraith CW. Evaluation of Ad-BMP-2 for enhancing fracture healing in an infected defect fracture rabbit model. *J Orthop Res*. 2004;22(1):66-72.
65. Onsea J, Post V, Buchholz T. Bacteriophage therapy for the prevention and treatment of fracture-related infection caused by *staphylococcus aureus*: a preclinical study. *Microbiol Spectr*. 2021;9(3):e0173621.
66. Yan CY, Liu YZ, Xu ZH, Yang HY, Li J. Comparison of antibacterial effect of cationic peptide II-37 and cefalexin on clinical *staphylococcus aureus*-induced infection after femur fracture fixation. *Orthop Surg*. 2020;12(4):1313-8.
67. Xu Z, Li J, Zhou X. The combined use of tea polyphenols and *lactobacillus plantarum* ST8sh bacteriocin in a rabbit model of infection following femoral fracture with internal fixation. *Med Sci Monit Int Med J Exp Clin Res*. 2019;25:312-7.
68. Caprise PA, Miclau T, Dahners LE, Dirschl DR. High-pressure pulsatile lavage irrigation of contaminated fractures: effects on fracture healing. *J Orthop Res*. 2002;20(6):1205-9.
69. Penn-Barwell JG, Murray CK, Wenke JC. Local antibiotic delivery by a bioabsorbable gel is superior to PMMA bead depot in reducing infection in an open fracture model. *J Orthop Trauma*. 2014;28(6):370-5.
70. Sanchez CJ, Prieto EM, Krueger CA. Effects of local delivery of D-amino acids from biofilm-dispersive scaffolds on infection in contaminated rat segmental defects. *Biomaterials*. 2013;34(30):7533-43.
71. Li B, Brown KV, Wenke JC, Guelcher SA. Sustained release of vancomycin from polyurethane scaffolds inhibits infection of bone wounds in a rat femoral segmental defect model. *J Control Release*. 2010;145(3):221-30.
72. Brick KE, Chen X, Lohr J, Schmidt AH, Kidder LS, Lew WD. rhBMP-2 modulation of gene expression in infected segmental bone defects. *Clin Orthop Relat Res*. 2009;467(12):3096-103.
73. Chen X, Schmidt AH, Mahjouri S, Polly DW, Lew WD. Union of a chronically infected internally stabilized segmental defect in the rat femur after debridement and application of rhBMP-2 and systemic antibiotic. *J Orthop Trauma*. 2007;21(10):693-700.
74. Chen X, Schmidt AH, Tsukayama DT, Bourgeault CA, Lew WD. Recombinant human osteogenic protein-1 induces bone formation in a chronically infected, internally stabilized segmental defect in the rat femur. *J Bone Joint Surg Am*. 2006;88(7):1510-23.
75. Helbig L, Omlor GW, Ivanova A. Bone morphogenetic proteins -7 and -2 in the treatment of delayed osseous union secondary to bacterial osteitis in a rat model. *BMC Musculoskelet Disord*. 2018;19(1):261.
76. Caroom C, Moore D, Mudaliar N. Intrawound vancomycin powder reduces bacterial load in contaminated open fracture model. *J Orthop Trauma*. 2018;32(10):538-41.
77. Penn-Barwell JG, Baker B, Wenke JC. Local bismuth thiols potentiate antibiotics and reduce infection in a contaminated open fracture model. *J Orthop Trauma*. 2015;29(2):e73-8.
78. Rand BCC, Penn-Barwell JG, Wenke JC. Combined local and systemic antibiotic delivery improves eradication of wound contamination: An animal experimental model of contaminated fracture. *Bone Jt J*. 2015;97-B(10):1423-7.
79. Penn-Barwell JG, Murray CK, Wenke JC. Early antibiotics and debridement independently reduce infection in an open fracture model. *J Bone Joint Surg Br*. 2012;94(1):107-12.
80. Lindsey BA, Clovis NB, Smith ES, Salihu S, Hubbard DF. An animal model for open femur fracture and osteomyelitis--Part II: Immunomodulation with systemic IL-12. *J Orthop Res*. 2010;28(1):43-7.
81. Whitely ME, Helms SM, Muire PJ, Lofgren AL, Lopez RA, Wenke JC. Preclinical evaluation of a commercially available biofilm disrupting wound lavage for musculoskeletal trauma. *J Orthop Surg*. 2022;17(1):347.
82. Shiels SM, Tennent DJ, Wenke JC. Topical rifampin powder for orthopedic trauma part I: Rifampin powder reduces recalcitrant infection in a delayed treatment musculoskeletal trauma model. *J Orthop Res*. 2018;36(12):3136-41.
83. Shiels SM, Tennent DJ, Akers KS, Wenke JC. Determining potential of PMMA as a depot for rifampin to treat recalcitrant orthopaedic infections. *Injury*. 2017;48(10):2095-100.
84. Tennent DJ, Shiels SM, Sanchez CJ. Time-dependent effectiveness of locally applied vancomycin powder in a contaminated traumatic orthopaedic wound model. *J Orthop Trauma*. 2016;30(10):531-7.
85. Mills R, Cheng TL, Mikulec K. CSA-90 promotes bone formation and mitigates methicillin-resistant *staphylococcus aureus* infection in a rat open fracture model. *Clin Orthop*. 2018;476(6):1311-23.
86. Lovati AB, Drago L, Bottagisio M. Systemic and local administration of antimicrobial and cell therapies to prevent methicillin-resistant *staphylococcus epidermidis*-induced femoral nonunions in a rat model. *Mediators Inflamm*. 2016;2016:9595706.

87. Roukoz S, El Khoury G, Saghbini E, Saliba I, Khazzaka A, Rizkallah M. Does the induced membrane have antibacterial properties? An experimental rat model of a chronic infected nonunion. *Int Orthop*. 2020;44(2):391-8.
88. Sener M, Kazimoglu C, Karapinar H, Günel I, Afşar I, Karataş Sener AG. Comparison of various surgical methods in the treatment of implant-related infection. *Int Orthop*. 2010;34(3):419-23.
89. Büren C, Lögters T, Oezel L. Effect of hyperbaric oxygen therapy (HBO) on implant-associated osteitis in a femur fracture model in mice. *PloS One*. 2018;13(1):e0191594.
90. Yu C, Chen L, Zhou W. Injectable of miRNA antagonist. *ACS Appl Mater Interfaces*. 2022;14(30):34427-42.
91. Sumrall ET, Hofstee MI, Arens D. An enzymatic regimen for the treatment of methicillin-resistant staphylococcus aureus orthopaedic device-related infection. *Antibiot (Basel)*. 2021;10(10):1186.
92. Perren SM. Evolution of the internal fixation of long bone fractures. The scientific basis of biological internal fixation: choosing a new balance between stability and biology. *J Bone Joint Surg Br*. 2002;84(8):1093-110.
93. Foster AL, Moriarty TF, Zalavras C. The influence of biomechanical stability on bone healing and fracture-related infection: the legacy of Stephan Perren. *Injury*. 2021;52(1):43-52.
94. Byrne JP, Nathens AB, Gomez D, Pincus D, Jenkinson RJ. Timing of femoral shaft fracture fixation following major trauma: A retrospective cohort study of United States trauma centers. *PLoS Med*. 2017;14(7):e1002336.
95. Schepers T, De Vries MR, Van Lieshout EMM, Van der Elst M. The timing of ankle fracture surgery and the effect on infectious complications; a case series and systematic review of the literature. *Int Orthop*. 2013;37(3):489-94.
96. Hull PD, Johnson SC, Stephen DJG, Kreder HJ, Jenkinson RJ. Delayed debridement of severe open fractures is associated with a higher rate of deep infection. *Bone Jt J*. 2014;96-B(3):379-84.
97. Schenker ML, Yannascoli S, Baldwin KD, Ahn J, Mehta S. Does timing to operative debridement affect infectious complications in open long-bone fractures? A systematic review. *J Bone Joint Surg Am*. 2012;94(12):1057-64.
98. Crowley DJ, Kanakaris NK, Giannoudis PV. Debridement and wound closure of open fractures: the impact of the time factor on infection rates. *Injury*. 2007;38(8):879-89.

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