Journal of Orthopaedic Translation 23 (2020) 8–20

Contents lists available at ScienceDirect

Journal of Orthopaedic Translation

journal homepage: www.journals.elsevier.com/journal-of-orthopaedic-translation

Review Article

A systematic review on current osteosynthesis-associated infection animal fracture models

Ronald M.Y. Wong [a], Tsz-kiu Li [a], Jie Li [a], Wing-Tung Ho [a], Simon K.-H. Chow [a], Sharon S.Y. Leung [b], Wing-Hoi Cheung [a,*], Margaret Ip [c]

[a] Department of Orthopaedics and Traumatology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong
[b] School of Pharmacy, The Chinese University of Hong Kong, Hong Kong
[c] Department of Microbiology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong

ARTICLE INFO

Keywords:
Animal models
Fracture
Infection models
Osteosynthesis-associated infection
Systematic review

ABSTRACT

Objective: Osteosynthesis-associated infection is a challenging complication post fracture fixation, burdening the patients and the orthopaedic surgeons alike. A clinically relevant animal model is critical in devising new therapeutic strategies. Our aim was to perform a systematic review to evaluate existing preclinical models and identify their applications in aspects of animal selection, bacterial induction, fracture fixation and complications.

Methods: A systematic literature research was conducted in PubMed and Embase up to February 2020. A total of 31 studies were included. Information on the animal, bacterial induction, fracture fixation, healing result and complications were extracted.

Results: Animals selected included murine (23), rabbit (6), ewe (1) and goat (1). Larger animals had enabled the use of human-sized implant, however small animals were more economical and easier in handling. *Staphylococcus aureus (S. aureus)* was the most frequently chosen bacteria for induction. Bacterial inoculation dose ranged from 10^{2-8} CFU. Consistent and replicable infections were observed from 10^4 CFU in general. Methods of inoculation included injections of bacterial suspension (20), placement of foreign objects (8) and pretreatment of implants with established biofilm (3). Intramedullary implants (13), plates and screws (18) were used in most models. Radiological (29) and histological evaluations (24) in osseous healing were performed. Complications such as instability of fracture fixation (7), unexpected surgical death (5), sepsis (1) and persistent lameness (1) were encountered.

Conclusion: The most common animal model is the *S. aureus* infected open fracture internally fixated. Replicable infections were mainly from 10^4 CFU of bacteria. However, with the increase in antibiotic resistance, future directions should explore polymicrobial and antibiotic resistant strains, as these will no doubt play a major role in bone infection. Currently, there is also a lack of osteoporotic bone infection models and the pathophysiology is unexplored, which would be important with our aging population.

The translational potential of this article: This systematic review provides an updated overview and compares the currently available animal models of osteosynthesis-associated infections. A discussion on future research directions and suggestion of animal model settings were made, which is expected to advance the research in this field.

Introduction

Infections after fracture fixation is one of the most challenging complications in trauma surgery, which often leads to delayed healing, permanent function loss and even amputation of the affected limb [1]. In open fractures, wound contamination with microorganisms can reach as high as 65%, with infection rates after osteosynthesis up to 30%, resulting in prolonged recovery periods and hospitalisation [2]. *Staphylococcus aureus* is the most common virulent microorganism causing osteosynthesis-associated infection and often forms a biofilm making the treatment difficult [3]. Recent studies show costs exceeding USD 108,000 per patient with reported treatment success rates ranging from 70% to

* Corresponding author. Department of Orthopaedics and Traumatology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.
E-mail address: louischeung@cuhk.edu.hk (W.-H. Cheung).

https://doi.org/10.1016/j.jot.2020.03.002
Received 23 December 2019; Received in revised form 18 February 2020; Accepted 2 March 2020
Available online 30 March 2020

90% [4]. The current goal of treatment is therefore to eradicate infection, allow fracture healing and preserve function.

With the increasingly frequent use of internal fixation and high morbidity of osteosynthesis-associated infections, it is imperative to further investigate factors that affect bone stability during fracture healing and potential interventions. Major factors that play a role include the bacterial type and size of inoculum, fracture and fixation method, and wound size. With the heterogeneity in clinical settings, the use of an animal model would allow reproducible investigations that simulate these important clinical situations. Furthermore, it saves research costs [5] and most importantly, intervention must be well-validated in animal models before conducting clinical trials.

With current existing models, clinicians need to be aware which is most suitable for their use. The purpose of this systematic review was to identify and character

rise the strengths and limitations of current fracture models for infection. An accurate simulation of the clinical setting would be crucial for the development of new therapeutics that would significantly benefit the patient and decrease healthcare costs.

Materials and methods

Search strategy

The PubMed and Embase databases (date last accessed 16 February 2020) were searched. The keywords used for the search criteria were "fracture*" AND "animal*" AND "infection*".

Search criteria

The inclusion criteria were: (1) preclinical studies; (2) use of animal model; (3) fractures performed with fixation; and (4) study on fracture healing. The exclusion criteria were: (1) review papers; (2) conference/ abstracts; (3) no analysis on fracture healing; (4) no fracture fixation performed; (5) no full-text literature: and (6) not in English language.

Selection of studies

Two independent reviewers performed the selection process on two databases. Each reviewer screened the titles and abstracts of each published study. Articles were selected based on the inclusion and exclusion criteria. A third reviewer resolved any disagreement upon group discussion.

Data extraction

For eligible studies, the two reviewers extracted information on: (1) animal used; (2) bacteria species, inoculum size and method of inoculation; (3) location and type of fracture, and fixation method; (4) radiological and/or histological evidence of fracture healing; (5) interventions used in current models; (6) additional parameters assessed; and (7) complications.

Data analysis

Due to the data heterogeneity in animal models and methodology, a qualitative review was performed.

Results

Results of the search

A total of 1142 and 1459 studies were identified from PubMed and Embase respectively (date last accessed 16 February 2020). All duplicate entries were removed, leaving 1895 records. Each title and abstract was reviewed and 1837 records were excluded based on inclusion and exclusion criteria. Upon detailed review of each study in full text, an additional 27 were excluded. 6 of these studies did not employ fracture fixation [6–11]; 10 did not perform a fracture [11–20]; 4 were not related to fracture healing [21–24] and 7 did not have analysis of fracture healing [25–31]. Our results show a total of 31 studies for our systematic review (Figure 1).

Characteristics of the papers

The 31 studies were published from 2002 to 2020. All studies were preclinical experiments performed in the mouse (9 studies) [32–38], rat (14 studies) [39–52], rabbit (6 studies) [53–58], goat (1 study) [59] and ewes (1 study) [60]. Fracture and fixation were performed and bacteria were inoculated in each study. Please refer to Table 1 for details.

Bacteria species, load and method of inoculation

All studies used *S. aureus* as the inoculum species, except for a rat model infected with *Staphylococcus epidermidis* [39]. The inoculum load ranged from 10^2 to 10^{10} CFU. In 19 models, bacteria suspensions were induced with the aid of injectable devices to the fracture site [34,35,38, 41,43,47,53,54,56,60–62] or throughout the medullary canal [39,44, 47–49,51,52,59]. 3 studies carried out inoculation by immersing the fixation plates in bacterial suspension prior to fracture fixation [33,37, 55]. 8 studies utilised a foreign carrier, including fibrillar collagen [32, 40,45,46,50], colloidal clay [58] or hydrogel [36], which was placed into the bone defect for inoculation. 1 induced the bacteria through subcutaneous injection 48 h post-operatively [57]. Please refer to Table 1.

Location, type of fracture and fixation

A variety of fracture models were utilised. Twenty studies performed fractures at the femoral shaft [32–41,44–47,50,52,55,57]; 7 at the tibial shaft [42,43,48,49,54,59,60]; and 2 at the humerus [53,56]. In 1 case, the fracture was created at the medial femoral condyle [58] and another included groups of different fracture location in their model design [51]. Twenty-seven studies adopted an open fracture model [32–35,37–41,43, 45–48,50–60] while four were closed fractures [36,42,44,49]. Thirteen models used an intramedullary device [36,40–44,48–51,54,56,59], and 18 models used plate and screws [32–35,37–39,45–47,52,53,55–57,60] for fracture fixation. In 2 models, cerclage wires were used to stabilise the bone for additional support [47,57]. Please refer to Table 1.

Interventions and utilisation of the model

One frequent application of the bacterial-induced fracture was the investigation of therapeutic treatment. Thirteen studies used an antibiotic or antimicrobial material [32,34,36,37,44,48,50,52–54,59,60,62]. Two assessed the efficacy of osteoinductive agents [47,49]. One employed a lavage irrigation remedy [58], One applied a hyperbaric oxygen therapy [38] and another used adenoviral gene therapy [57]. 1 study compared two fixation techniques in fracture healing with infection [56]. 5 studies adopted two or more therapeutic strategies in their investigation [40,45,46,51,62]. The remaining 8 articles reported findings on pathophysiology and did not involve any therapeutic intervention [33,35,39,41–43,55,61]. Please refer to Table 2.

Evidence of osseous healing

Of the 22 studies that exploit a therapeutic intervention, 13 reported both radiological and histological findings of osseous healing [32,36, 44–50,52,53,57,59]. 8 did not include histological evaluation [34,38,40, 51,54,58,60,62] and 2 without radiological assessment [37,61]. Based on these two assessments, it is shown that a number of treatments were effective in reversing osteolysis from bone infection, and promoting fracture healing. In a rabbit infection model, Ter Boo, G. demonstrated

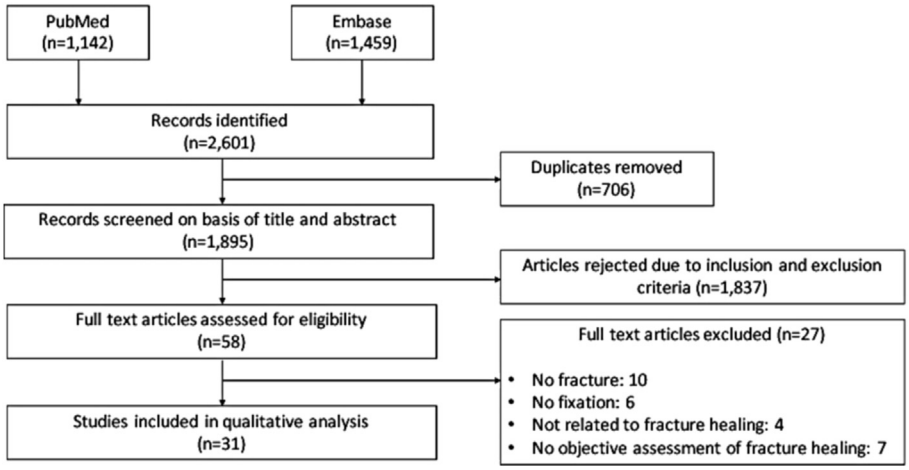

Figure 1. Flowchart of study selection.

that a gentamicin grafted hyaluronic acid hydrogel could encourage periosteal callus formation at 28 days [53]. Another study used a lysostaphin loaded hydrogel [36]. The treatment resulted in significant higher bone and callus volume around the fracture site. Co-delivery of local and systematic vancomycin were shown to significantly reduce bone resorption in Inzana's study [32]. Schear T.P. explored the possibility of N, N-dodecyl, methyl-polyethleneimine (PEI) derivatised coating on fixations [60]. More bridging callus were observed at one-month between the treatment and control groups. Complete fracture consolidation was achieved in 28 days using a lysostaphin-coated titanium implant on mice [34]. In a rat model that applied recombinant human bone morphogenetic protein-2 and systemic ceftriaxone, the bone defect healed securely at 12 weeks [45]. Furthermore, in a mice study investigating the effect of gentamicin or vancomycin infused calcium sulfate/hydroxyapatite (CAS/HA) insets, the mice with infection did not show a healing fracture gap [62]. Table 2 summarises the radiological and histological findings for all 31 studies.

Additional parameters

Clinical evaluation was included in 22 studies [32,37,39–43,45–57,59,60]. Parameters of body temperature, body weight, evaluations for well-being and signs of infection, i.e. the presence of abscesses, purulent discharge and swelling, lameness and weight bearing were monitored clinically. Samples of soft tissue, synovial, bone samples, lavage and implants were used for bacteriology and documented in 22 studies [32,33,35–39,41–44,48,50,52–58,60,62]. Scanning electron microscopy (SEM) [32,35,39,43,55,60], confocal laser scanning microscopy [43,55,59] and crystal violet staining[39] were applied to visualise dislodged bacteria and biofilm formation. One study employed a newer imaging technology where spatial signals of the bioluminescent bacteria on live animals could be captured over the observation period [32]. haematology was investigated in 12 studies [34,36,38,39,42,51–54,56,59,62]. C-reactive protein (CRP) level and white blood cell (WBC) count were common parameters of assessment. The elevation of these blood markers is associated with systemic immune response to inflammation and serves as indicators for progression of bone infection in these models. One study applied a local therapeutic treatment and incorporated blood test for renal function to investigate on systemic toxicity [54]. Apart from histology and radiology, mechanical testing was included in 10 studies for additional assessment on fracture healing [36,41,42,45,46,48,49,53,56,59].

Complications

Twenty studies [32,38–45,47–49,53,55–60,62] reported adverse events and complications on animals, including loosening of fixation, persistent lameness, signs of septic infection and unexpected fracture due to extensive osteolysis. Animal deaths and failures during surgical operation are also common [39,42,45,48,49,53,62]. Premature euthanasia was required in some occasions. In a 16-week study, 19 of 64 rabbits were euthanised for humane reasons [57]. Another study showed that 21 of 72 mice died during experimental procedures, among which 9 were early euthanised because of non-weight bearing of the operated extremity in 3 mice, and non-controllable wound defects in 6 mice [62]. Only one rat and one rabbit model reported no complications where all animals tolerated well from the operations and intervention till end-point [46,54].

Discussion

Implant infection during the setting of fracture repair, is a challenging complication in orthopaedic trauma. Prolonged hospitalisation, repeated operations, and the demand of a multidisciplinary approach in management are all contributing factors to encumber patients and health workforce, along with high socio-economic burden [2]. Despite prophylactic administration of antibiotics, the incidence of fracture-related infection is still commonly encountered [63]. A recent review on preclinical models of fracture-related infection highlighted that only 6.7% of the studies included in their review combined the key features of having the presence of a fracture, delay before treatment and soft tissue damage [64]. A clinically relevant animal model is thus important to simulate these scenarios for establishing translational therapeutic strategies.

Different animal models have been described in the literature to study bone infection associated with fracture healing. In a previous study by Schaer et al., a sheep model on OAI was selected to investigate the bactericidal properties of internal fixation coated with N, N-dodecyl,methyl-polyethylene imine [65]. An existing locking compression plate was used to stabilize the mid-diaphyseal fracture. In the infection group, 100% of the control animals had non-union of the fracture combined with consistent results in radiographic signs, soft tissue destruction, implant colonization and delayed fracture healing. This study highlighted the fundamental advantages of using large animals for OAI, since a larger bone size could facilitate both surgical fixation and evaluation with actual implants. A goat model was adopted by Tran et al. to evaluate the antimicrobial efficacy of silver-doped Ti/Siloxane coating on intramedullary nails [59]. The larger medullary canal and bone size of the animal had allowed the application of human scale implants in which modification of implants were not necessary. A higher antibiotic tolerance is another advantage of using large animals in infection studies [66]. However, only two goats were used in the study. Cost and ease of handling could be limitations of a larger sample size. On the other hand, rabbits, which are less expensive and

Table 1
Summary of the study characteristics.

Source	Animal	Bacteria species and concentration (CFU)	Method of inoculation	Site of Fracture	Year	Osteotomy type (open/closed; bone defect/fracture)	Fixation technique
Buren et al.	BALB/c-mice	*Staphylococcus aureus*, 1×10^6/mL	Bacteria solution was inoculated to the osteotomy gap	Femoral diaphysis	2019	Open fracture	4-hole plate and screw combination
Oezel et al.	BALB/c mice	*S. aureus*, 1.35×10^8/mL	Inoculation of the fracture gap with bacteria solution	Femoral diaphysis	2019	Open fracture	6-hole titanium locking plate with locking self-tapping microscrews
Buren et al.	BALB/c mice	*S. aureus*, 1.94×10^3	Bacterial solution is induced to the fracture gap	Femoral diaphysis	2018	Open, fracture (0.22 mm)	4-hole titanium locking plate with locking self-tapping microscrews
Cui et al.	Sprague–Dawley rats	*S. aureus*, concentration not specified	Injection of bacterial solution through the intramedullary needle	Tibia fracture, femoral fracture, humerus fracture, ulnar and radial fractures, diaphysis fracture and metaphyseal fracture	2018	Open, fracture	7 G needle
Helbig, L. et al.	Sprague–Dawley rats	*S. aureus*, 10^3	Injected to the medullar cavity of the tibiae with a microlitre syringe	Fibula and tibia diaphysis	2018	Closed, fracture	Kirschner wires
Johnson et al.	C57/B6 mice	*S. aureus*, $1.55 \pm 0.51 \times 10^8$/mL (UAMS-1); 3.43×10^8/mL (USA300)	Bacteria is mixed with the hydrogel components and polymerised *in situ* over the fracture	Femoral diaphysis	2018	Closed, fracture	25 G needle
Mills, R. et al.	Wistar rats	*Staphylococcus aureus*, methicillin-resistant *S. aureus*; methicillin-resistant *Staphylococcus epidermidis*, 10^4	Collagen sponge loaded with bacteria placed to the fracture site	Femoral diaphysis	2018	Open, fracture	Kirschner wires
Rochford, E. T. J. et al.	C57BL/6 and BALB/c mice	*S. aureus*, 9×10^5	Immersion of implant plates into bacteria suspension for 20 min and air-dried for 5 min	Femoral diaphysis	2018	Open, fracture	Surface polished titanium and oxygen plasma-treated polyetheretherketone (PEEK) plate and screws
Shiels et al.	Sprague–Dawley rats	*S. aureus*, 10^2	Bacteria solution is applied to the intramedullary (IM) canal and incubated for 2 min prior to placement of the experimental K-wire.	Tibial diaphysis	2018	Open, fracture	Kirschner wires
Shiels et al.	Sprague–Dawley rats	*S. aureus*, 10^5	Via a collagen prewetted with bacterial solution placed into the defect	Femoral diaphysis	2018	Open, bone defect (2.58+-0.005 mm)	radiolucent plate, affixed with K-wires
Ter Boo, G. J. et al.	New Zealand white rabbits	*S. aureus*, 2.0×10^6	Injections of bacterial suspension into the empty screw hole overlying the osteotomy and on the head of adjacent proximal and distal screws	Humeral diaphysis	2018	Open, fracture	locking plate
Lv Zhou et al.	New Zealand white rabbits	*S. aureus*, 10^6/mL	Inoculated into the medullary cavity of the fracture edges	Tibial diaphysis	2017	Open, fracture	Kirschner wires
Zhang, X. et al.	New Zealand White rabbits	*S. aureus*, 10^6/mL	Steel implant was placed with 5 mL of bacterial solution and incubated for 48 h at 37 °C. The plate was then rinsed and taken for surgery.	Femoral diaphysis	2017	Open, fracture	Stainless steel plate
Lovati, A. B. et al.	Wistar rats	*S. epidermidis*, 10^3, 10^5, 10^8	The bacterial suspension was injected into the femoral defect and the suspension was allowed to spread throughout the medullary canal.	Femoral diaphysis	2016	Open, bone defect (1 mm)	Stainless steel plate
Arens, D. et al.	New Zealand White rabbits	*S. aureus*, $6 \times 10^{2-6}$	Injections of bacterial suspension onto the central screw hole overlying the osteotomy and to the	Humeral diaphysis	2015	Open, fracture (0.45 mm)	Locked plate/a custom designed interlocked intramedullary nail

(*continued on next page*)

Table 1 (*continued*)

Source	Animal	Bacteria species and concentration (CFU)	Method of inoculation	Site of Fracture	Year	Osteotomy type (open/closed; bone defect/fracture)	Fixation technique
			adjacent proximal and distal screws holes with a pipet				
Bilgili, F. et al.	Sprague–Dawley rats	*S. aureus*, 1×10^8/mL	As opaque solution, injected into fracture area *(according to an established model)	Femoral diaphysis	2015	Open, fracture	Kirschner wire
Helbig, L. et al.	Sprague–Dawley rats	*S. aureus*, 10^3	By injection into the medullary cavity with a microsyringe	Tibial diaphysis	2015	Closed, fracture	Titanium Kirschner wire
Inzana, J. A. et al.	BALB/cJ mice	*S. aureus*, $8.0 \pm 2.9 \times 10^4$ per fibrillar collagen sheet	Loaded on a fibrillar collagen sheet placed into the bone defect	Femoral diaphysis	2015	Open, bone defect (0.7 mm)	Titanium coated- polyether ether ketone plate, titanium screws
Rochford, E. T. J. et al.	C57BL/6 mice	*S. aureus*, 9×10^5 per implant	Immersion of implant plates into bacteria suspension for 20 min and air-dried for 5 min	Femoral diaphysis	2015	Open, bone defect (0.44 mm)	Titanium plates and screws
Schindeler, A. et al.	Wistar rats	*S. aureus*, 10^4	The bacterial suspension was loaded in a collagen carrier and packed into the defect.*	Femoral diaphysis	2015	Open, fracture	Kirschner wire
Windolf, C. D. et al.	BALB/c mice	*S. aureus*, 1.94×10^3/mL	Inoculated into the fracture gap	Femoral diaphysis	2014	Open, bone defect (0.22 mm)	Titanium locking plate
Tran, N. et al.	Goat	*S. aureus*, 2×10^4/mL	Injection into the medullary canal at the fracture site	Tibial diaphysis	2013	Open, fracture	Stainless steel alloy intramedullary nail with interlocking screws
Windolf, C. D. et al.	BALB/c mice	*S. aureus*, 10^4	Injection into the fracture gap with a micropipette	Femoral diaphysis	2013	Open, bone defect (0.22 mm)	Titanium locking plate
Alt, V. et al.	Sprague–Dawley rats	*S. aureus*, 10^4	In the form of bacteria suspension inoculated at the osteotomy site	Tibial diaphysis	2011	Open, fracture	Kirschner wire
Schaer, T. P. et al.	Dorset-cross ewes	*S. aureus*, 10^6, 10^8, 10^{10}/mL	Via a temporary indwelling silastic catheter inserted into the osteotomy site	Tibial diaphysis	2011	Open, fracture (0.6 mm)	locking compression plate
Robinson, D. A. et al.	Sprague–Dawley rats	*S. aureus*, 10^4	injected into the medullary cavity via a polypropylene catheter	Femoral diaphysis	2010	Closed, fracture	Stainless steel intramedullary pins
Chen, X. et al.	Sprague–Dawley rats	*S. aureus*, 10^4	A collagen sponge was wetted with the bacterial suspension and placed within the bone defect.	Femoral diaphysis	2007	Open, bone defect (6 mm)	Polyacetyl plate, Kirschner wires
Chen, X. et al.	Sprague–Dawley rats	*S. aureus*, 10^4	The bacterial suspension was loaded in a collagen carrier and packed into the defect.	Femoral diaphysis	2006	Open, bone defect (6 mm)	Polyacetyl plate, Kirschner wires
Southwood, L. L. et al.	New Zealand White rabbits	*S. aureus*, 10^7/mL	Percutaneous injection (48 h after surgery)	Femoral diaphysis	2003	Open, bone defect (10 mm)	Stacked-cuttable bone plates, cortical screws, cerclage wire
Caprise, P. A., Jr. et al.	New Zealand White rabbits	*S. aureus*, 5×10^6/mL	Via a colloidal clay in a syringe injected into the fracture site	Medial femoral condyle	2002	Open, fracture	Single screw
Chen, X. et al.	Sprague–Dawley rats	*S. aureus*, 10^5	Injected into the opening of the medullary canal on both ends of the defect with a syringe needle	Femoral diaphysis	2002	Open, bone defect (6 mm)	Polyacetyl plate, Kirschner wires, cerclage wire

Table 2
Summary of the study characteristics.

Source	Intervention	Radiological/histological evidence of osseous healing	Parameters assessed	Complication
Büren et al.	NA	(Histology) At 4 weeks postoperatively, the callus formation in infection group was smaller compared with the control group; Mice in infection group showed no complete osteotomy consolidation. In control group, 1 of 16 mice showed complete osteotomy consolidation and 12 showed partial consolidation.	Histology Microbiological analysis	No case fatalities due to surgery or anaesthesia
Oezel et al.	Gentamicin or vancomycin infused calcium sulfate/ hydroxyapatite (CAS/ HA) insets	(X-ray) All mice with infection did not show a healing fracture gap, independently of the application of calcium sulfate/hydroxyapatite (CAS/HA) insets or antibiotics; Mice of the groups infected solo, CAS/HA and CAS/HA-G had similar destruction of the bone, mice of the CAS/HA-V group suggest that vancomycin infused insets show a less distinct bone destruction;	X-ray Microbiological analysis Haematology (polymorphonuclear (PMN) leucocytes, interleukin (IL-6))	21 of 72 mice died during experimental procedures
Buren et al.	Hyperbaric oxygen therapy	(X-ray) All mice in the control and treatment group showed a healing fracture gap. The infection group had the same bone healing score as the controls but showed a greater individual heterogeneity and non-union number.	X-ray Microbiological analysis Haematology (AP, procollagen type I N propeptide (PINP)) Cytokine analysis	18 of 120 mice died during the experimental procedures. Reasons were not specified.
Cui et al.	Masquelet induced membrane therapy: (1) vancomycin, mixed with poly(methyl methacrylate) [PMMA] bone cement, placed locally in bone defect (2) morselised cancellous bone grafting	(X-ray) Primary bone healing was achieved in 50 rats with an average healing time of 15 $\pm$ 1.56 weeks.	X-ray Haematology (tumour necrosis factor alpha (TNF-α), white blood cell (WBC), C-reactive protein (CRP)) Clinical Microbiological analysis (methodology not specified)	Not specified
Helbig, L. et al.	rhBMP-7, rhBMP-2, intramedullary injection five weeks post-fracture	(micro-computed tomography [CT]) The sterile group showed complete bridging of the fracture gap. The infection and treatment groups showed increased hypertrophic callus formation. Callus formation showed no differences between the two treatments. (histology) The sterile group showed good callus formation and progressed bone remodelling with few connective tissues. The infection group showed more fibroblast and cartilage in the fracture region. The treatment group showed partially remodelled fracture.	X-ray, Micro CT Histology Mechanical test Clinical Microbiological analysis	Of the rats, 3 died due to complications with general anaesthesia, 2 were sacrificed due to postoperative infected haematoma; 5 were excluded due to technical problems during preparation of the tibiae
Johnson et al.	Lysostaphin, loaded in hydrogel injected to osteotomy gap	(micro-CT) The infection group shows no callus formation, presence of bone resorption and reactive bone formation around the fracture site. The sterile group showed robust fracture callus. The treatment group showed significant bone healing with a significantly higher bone and callus volume. (histology) Staining of the cartilage also showed no gross differences in healing between sterile control fractures and fractures treated with lysostaphin-delivering hydrogels.	X-ray, Micro CT Histology, Cytokine analysis Haematology (antilysostaphin antibody and liver enzyme test) Mechanical testing Microbiological analysis	Not specified
Mills, R. et al.	BMP-2, CSA-90, loaded in collagen sponge placed to the fracture site	(micro-CT) The methicillin-resistant Staphylococcus aureus (MRSA) infected treatment group showed an increased bone volume around the fracture site compared to the untreated MRSA group. Maximal ectopic bone formation was achieved with 500 mg CSA-90 and 10 mg bone morphogenic protein-2 (BMP-2). (X-ray) 19 of the 20 infected fracture achieved a modified radiographic union scale in tibial fractures (RUST) score consistent with fracture union. No difference was identified between the methicillin-resistant Staphylococcus epidermidis (MRSE) treated and untreated groups with all fractures achieving fracture union. (histology) Inflammatory cell debris and pus are clearly visible around the non-united fractures of the non-treated and delayed treatment groups. Evolving bony architecture was seen in the co-treatment group.	X-ray, micro CT Histology Clinical Microbiological analysis	number not specified
Rochford, E. T. J. et al.	NA	(histology) By Day 7, a minimal to moderate granulocytic to necrotizing myelitis, sometimes	X-ray Histology	Not specified

(*continued on next page*)

Table 2 (*continued*)

Source	Intervention	Radiological/histological evidence of osseous healing	Parameters assessed	Complication
		with beginning formation of micro-abscesses, was recorded in two implant groups. (radiographical) only to confirm proper positioning of the implant	Microbiological analysis Atomic force microscopy Cytokine analysis	
Shiels et al.	Cefazolin, systemic; Chlorhexidine, N-(3-Sulfopropyl)-N-methacryloxy ethyl-N,N-dimethyl ammonium betaine, coated on implant	(radiographical) An evident mitigation of osteolysis and increased radiographic union were seen in the intervention group compared to the unmodified control group. (histology) The intervention group showed signs of bone formation, the control group exhibited signs of active bone resorption.	X-ray, micro CT Histology Clinical Microbiological analysis Mechanical test	8 out of 161 were excluded and euthanised (3: uncontrollable oedema; 3: sequestration of the K-wire leading to destabilisation of the operative tibia 1: post-operative torsional destabilisation of the tibia; 1: K-wire rupturing through the posterior cortex of the tibia)
Shiels et al.	Rifampin, vancomycin, topical, powder form	(X-ray) Rifampin-reduced radiographic indications of infection compared to the control empty and vancomycin group. (histology) Presence of bacteria was seen within both the control and vancomycin groups with large abscesses and necrotic bone. The rifampin group showed signs of healthy new bone formation.	Microbiological analysis X-ray Histology Clinical Haematology (WBC count)	Not specified
Ter Boo, G. J. et al.	Gentamicin, loaded in a biodegradable thermo-responsive poly (N-isopropyl acryl amide) grafted hyaluronic acid hydrogel injected to the osteotomy gap and over the locking plate	(X-ray, histology) Group receiving the intervention showed new callus information and no necrotic tissue was observed 28 days post-operative. In the non-inoculated control group, a large amount of periosteal callus was formed around the osteotomy gap at 28 days.	Clinical X-ray Mechanical testing Histology Microbiological analysis Haematology (CRP, WBC count, serum gentamicin level)	5 out of 45 euthanised before end-point due to symptoms of cardiac arrest and a fracture of the operated bone
Lv Zhou et al.	Tobramycin, coated on K-wires with poly(D,L-lactide) [PDLLA]	(X-ray) At day 56, 5 out of 6 in the intervention group and all 6 rabbits in the non-inoculated control group showed healed fractures.	Microbiological analysis Haematology (Blood, urea, nitrogen (BUN), CRP, CR, Hb) Clinical Histology X- ray Urology	All rats recovered well from the operation and survived till end-point.
Zhang, X. et al.	NA	(micro- CT) At day 21, cortical bone in the infected group showed obvious corrosion and absorption, for control group it remained intact. Significant bone callus formation was observed around the fracture site in the control group.	X ray, Micro CT Histology Microbiological analysis Clinical	All rats survived till end-point; clear instability of the plates and screws was observed in the infected group.
Lovati, A. B. et al.	NA	(micro-CT) 103 methicillin-resistant Staphylococcus epidermidis (MRSE) group: 67% showed a fracture healing less than 75% and displayed mainly fibrous non-union; 105 MRSE group: 83% showed a fracture healing less than 75% with absence of bony healing; 108 MRSE group: all samples showed a fracture healing less than 75% and displayed non-union extended across the entire bone. Some animals in the control group showed a well-organised bone callus and remodelling. (histology) Fractures in the control group appeared closed with a great amount of new bone formation in a remodelling phase. 103 MRSE group: incomplete bone healing characterised by a great formation of fibrovascular tissue; 105 MRSE group: missing of cortical bridging with non-union establishment; 108 MRSE group: massive cortical and endosteal osteolysis	Micro CT Haematology (WBC count) Microbiological analysis Histology Clinical	1 was excluded for radiographic evaluation due to mechanical loss of the proximal screws followed by a fracture dislocation from surgical inaccuracy.
Arens, D. et al.	NA	(histology) At day 70, the plate fixation infected group resulted in periosteal osseous new bone formation around the plate; the nail group displayed some endosteal bone formation and bony integration of the implant. In the non-inoculated groups, complete osteotomy closure was observed at 10 weeks. (X- ray) At day 70, the osteotomy gap is still visible in the infected group. All rabbits in the non-inoculated nail group displayed callus bridging between the proximal and distal diaphysis.	X- ray Histology Mechanical testing Haematology (CRP, WBC count) Clinical Microbiological analysis	7 out of 61* were euthanised (6 unexpected fracture, in which 3 had implant failure; 1 persistent lameness post surgery); a small number of rabbits showed proprioceptive deficits on the operated leg due to stress to the radial nerve during surgery,
Bilgili, F. et al.	NA	(X- ray) At 42 days, complete bony union was found in the control group, whereas the infection group showed only initial stages of bony union. (histology) At 42 days, evidence of bone healing was observed by 42 days in the control group but not in the infection group.	Clinical X-ray Histology Mechanical testing Microbiological analysis	One out of 75 died, loosening around the implant was observed in the infection group

(*continued on next page*)

Table 2 (*continued*)

Source	Intervention	Radiological/histological evidence of osseous healing	Parameters assessed	Complication
Helbig, L. et al.	NA	(micro-CT) The infected group showed clearly reduced consolidation of the fractures at day 35. Fracture gap was not bridged for all rats in the group. Fracture in the non-infect group were bridged completely in 9 of 10 animals at day 35.	Micro CT Haematology (blood count, leucocytes count, C-reactive protein (CRP)) Clinical Microbiological analysis Mechanical testing	2 out of 22 died due to anaesthesia immediately after operation
Inzana, J. A. et al.	Vancomycin, IV systemic; local, loaded in a PMMA spacer tied into the defect using a nylon suture	(X-ray, micro-CT) The resorbed bone volume was significantly reduced in groups receiving systemic and local antibiotics on day 14. (histology) A large amount of new reactive bone had formed on the periosteal surface of the control femurs, no osteogenic response was observed on the infected femurs on day 14.	Clinical Histology, X-ray, Micro CT Microbiological analysis (bacterial culture, scanning electron microscopy (SEM), Bioluminescent imaging)	None of the mice died during study, but one infected mouse sustained a fracture between days 10 and 14, which likely resulted from dramatic thinning of the cortex. Implant failure was seen in the placebo group from micro-CT scans.
Rochford, E. T. J. et al.	NA	(X- ray, histology) Osteotomy gap clearly not healed in infected animals on day 35. In non-infected animals, complete healing was observed on day 35.	Clinical X-ray Histology Microbiological analysis Flow cytometry analysis Reverse transcription quantitative polymerase chain reaction (RT-qPCR) Cell stimulation and cytokine quantification	Not specified
Schindeler, A. et al.	Cationic steroid antibiotic and recombinant human bone morphogenetic protein 2, loaded in a collagen sponge disc placed circumferentially around the fracture site	(micro-CT) Increase in callus tissue volume was more pronounced in the rhBMP-2/CSA90 group. (histology) tartrate-resistant acid phosphatase (TRAP) staining revealed no obvious alterations in osteoclasts for the treatment group, although bone nodules were highly heterogeneous, having undergone substantive remodelling.	Clinical X- ray, Micro CT Histology	Did not specify number of unexpected deaths, but mention some were culled due to loss of intramedullary fixation
Windolf, C. D. et al.	Lysostaphin, coated on titanium discs	(X-ray) The intervention group showed clear signs of fracture healing by 14 days and complete fracture consolidation by 28 days. Fracture healing could not be observed at any time point in the group receiving control plates.	X- ray Haematology (PMNs, leukocyte count) IL-6 quantification in lavage Microbiological analysis	Not specified
Tran, N. et al.	Silver, coated on intramedullary nails	(X-ray) Non-union of bone is seen in all groups at 35 days. (histology) Neither animal of the groups formed a bridging callus that filled the osteotomy gap at 35 days.	X-ray, Micro CT Histology Haematology (WBC count, neutrophil and lymphocyte level, silver level) Clinical Mechanical testing Microbiological analysis Silver level testing in organs	Implant loosening was absent in all animals at 35 days
Windolf, C. D. et al.	NA	(X- ray, histology) Infected mice showed a significantly reduced in bony healing at 7, 14 and 28 days, when compared to non-infected mice. All femora from non-infected mice showed early fracture healing by day 7 and complete fracture consolidation by day 28.	Histology X- ray Microbiological analysis Cellular and IL-6 quantification in lavage	Not specified
Alt, V. et al.	NA	(X-ray, micro-CT) All infected animals of the group showed persistence of the osteotomy gap and other signs of infected non-union. Complete bony bridging of the osteotomy gap was observed in the control animals at day 42. (histology) Clear signs of infected non-union without any bony bridging of the fracture site.	Clinical X- ray, Micro CT Histology Microbiological analysis	All animals survived and completed the study, the infection group showed clear instability of the fracture site
Schaer, T. P. et al.	N,N-dodecyl,methyl-polyethyleneimine-derivatised coating on fixations	(micro-CT) The treatment group showed more bridging callus formation at one month post-operative than the control group. (histology) Compared to the control group, there is a significantly lower histology score consistent with improved bone healing and absence of infection in the treated animals.	Clinical X- ray, Micro CT Histology Microbiological analysis	Unstable osteotomy was observed in the control group at the time of explant.
Robinson, D. A. et al.	Ceftriaxone, systemic, subcutaneous injection	(X- ray) The fracture callus did not bridge the fracture site, but a small amount of new bone formation was observed at 21 days with intervention. Images of the control group were characterised by the formation of a normal bridging callus. (histology) Specimen of the intervention group contained a bridging callus that was composed primarily of new bone, similar to the control group.	X- ray Histology Microbiological analysis	2 of 30 were euthanised due to incisional dehiscence and self-mutilation.

(*continued on next page*)

Table 2 (*continued*)

Source	Intervention	Radiological/histological evidence of osseous healing	Parameters assessed	Complication
Chen, X. et al.	Ceftriaxone, systemic, IM injection; recombinant human bone morphogenetic protein-2 (rhBMP-2), local, loaded in a collagen sponge and packed into fracture site	(micro-CT) Greatest amount of new bone formation which consistently and securely connected the ends of the defects occurred in group receiving both interventions at 12 weeks. (histology) Defects in animals treated with the rhBMP-2 and antibiotic exhibited the greatest amount of newly mineralised callus. Only minimal amount of new bone appeared to form within the defect or bridged the outside of the defects in the control group at 12 weeks.	X- ray, Micro CT Mechanical testing Histology Clinical Microbiological analysis	3 out of 127 animals were excluded (2 at the time of anaesthetic administration, and 1 due to a femoral fracture that occurred during the placement of K-wires.
Chen, X. et al.	Ceftriaxone, systemic, IM injection; recombinant human osteogenic protein-1 (rhOP-1), local, loaded in a collagen carrier and packed into fracture site	(micro-CT, histology) Volume and areas of newly mineralised callus within the defect and bridging the outside of the defect increased with time after debridement and was greater with antibiotic treatment than without antibiotic treatment at 12 weeks after debridement in the defects that had been treated with higher dose of rhOP-1.	X- ray, Micro CT Mechanical testing Histology Microbiological analysis Clinical	No complication, the animals tolerate well from the operation and debridement. No signs of lameness, draining sinus or clinical symptoms indicative of systemic infection was observed during the study period.
Southwood, L. L. et al.	Adenoviral transfer of the bone morphogenetic protein-2 (Ad-BMP-2) gene, percutaneous injection	(X- ray) Rabbits in the intervention group had initial and bridging-callus at earlier times than the control group. (histology) There was a trend that the intervention group has a higher grade for new bone at 14 and 28 days than the control group, but not statistically significant.	Microbiological analysis Clinical Histology X- ray	19 out of 64 were euthanised before end-point for humane reason. No complications was mentioned in the paper
Caprise, P. A., Jr. et al.	High-pressure pulsatile lavage irrigation	(micro-radiographs) Statistically significant difference in the amount of new bone formation postoperative day 14 in intervention group than other groups. (histology) No significant difference in new bone formation between the bulb syringe group and HPPL treatment.	Microbiological analysis Histology Micro-radiographs	5 out of 40 died or euthanised before end-point due to symptoms of sepsis
Chen, X. et al.	Osteogenic protein-1 (OP-1), local, mixed with collagen and formed a mixture packed into the fracture site	(X-ray, histology) Bone formation inside and outside the defects with either dose of OP-1 at 63 days, were significantly greater than the untreated groups.	X- ray Histology Microbiological analysis Clinical	Loss of fixation was seen in some infected animals at 14 days. None of the infected animals exhibited a draining sinus, loss of weight commensurate with systemic involvement of infection, of limp of lameness during activity.

intermediate in size, have allowed the study of local antibiotics [53,54], implant devices [56] and the use of pulsatile lavage irrigation systems [58]. With the development of smaller implants and better fixation techniques, it has provided more possibilities of small animal experiments in recent years. Although a Haversian system is absent in the bone structure of rodents, their bone remodelling mechanism using resorptive cavities remains similar to that of human [67]. Furthermore, the use of small animals is less costly and easy to handle. In respect to their immune functions, however, the immune genes of rodents are thought to be more phylogenetically distant to humans than rabbits and some larger animals [68–70]. Mice engrafted with humanized immune systems could be a possible solution if all facets of pathogenesis in clinical infections are warranted [71]. In our reviewed literature, murine models had been utilised to explore a range of osteo-inductive agents [40,45–47,49,51] and antibiotic strategies [32,36,40,44–46,48,52] in combating infection on the course of bone healing. Whether these interventions conducted in small animals can be extrapolated to clinical practice, however will require further investigations for delivery dosage in clinical trials. Clinicians and researchers should consider their individual study needs, surgical skills and resources, and are encouraged to select a lower-order animal initially.

S. aureus is the most common organism in bone infection [72]. As demonstrated in our reviewed studies, the bacteria can form a potent biofilm and can cause osteolysis [55,56], periosteal reaction [55] and fracture non-union [35,43,56] which are frequently encountered in clinical bone infection. Therefore, most infection studies used *S. aureus* as the organism of choice. However, although Staphylococcus infection is often encountered, polymicrobial infections are also common and often cause complications [63]. Retrospective studies in health settings have reported that 21%–31% of cultured positive, osteomyelitis cases were polymicrobial [63,73,74]. Animal models that mimic this situation would also closely resemble the clinical settings of bone infection associated with open fracture [75,76]. These models are lacking and future studies should have development in this area.

The inoculum load ranged from 10^2 to 10^{10} CFU in the current literature. In 2 dose responsive studies, it was found that a bacterial inoculum of less than 10^4 CFU resulted in unsuccessful and inconsistent infection [39,56]. The low infection rates was consistent to pilot work performed by Chen et al. [47]. In 1 model using a 10^2 CFU inoculation, infection rate was not specified in the non-treatment group. Increase in bone volume for the treatment group, compared to the control group, may have been more pronounced if a higher inoculation load was sought [48]. However, an inoculum of 10^3 CFU *S. aureus* in a closed fracture model was found to have achieved a 100% of infection rate based on bacterial quantification assessment [42]. While it is also expected a higher inoculum load was needed for larger sized animal, such pattern was not observed in our review. A medium grade animal infection model with chronic, localized bone infection could be achieved using an inoculum of 10^4 CFU as demonstrated in several studies [43–46,59]. An inoculum of 10^8 CFU were used in one ewe [60] and one rat model [41]. This mimics severe clinical cases where sepsis is presented along with acute osteomyelitis [39,41]. In overview, there is no consensus in the literature to indicate a standardized threshold or inoculum dose for the creation of a bone infection model, which may be variable due to the animal species, bacterial strain of individual studies and a number of factors such as fracture type and location [77]. Clinicians should consider the severity of bacterial infection required for the specific clinical scenario they wish to mimic.

Inoculums primarily using planktonic [34,35,39–44,47,53,54,56,57,59,60] and biofilm [55] bacteria were featured in our reviewed models. The majority of studies favor the use of planktonic inoculum, in which, bacteria were grown by batch culture, prepared as suspension and induced to the fracture site with an injectable device. In Zhang's model, fixation devices were incubated in bacterial solution overnight and washed with phosphate-buffered saline (PBS) prior to fracture fixation [55]. The pretreatment allowed biofilm formation on the implant surface beforehand. Rochford et al. also performed a similar model, but the immersion time was shortened to 20 min followed by 5-min air-drying of the implants [33,37]. Other models employed the use of foreign objects that were soaked or premixed with bacteria solution, and placed in the fracture [32,36,40,45,46,50,52,58]. However, biofilm formation was not intended in these studies. Compared to planktonic bacteria, biofilm bounded bacterial cells are more likely to escape assault from the host immune defense [78] and antibiotics modalities [79,80]. Zhang's model is thus more suitable in chronic infection models with established biofilm formation. The inoculation method was also claimed to be a more clinically relevant model [81] as a majority of the bacteria in the natural ecosystems reside as biofilm phenotype [82]. Theoretically, wound surface is more prone to contamination of biofilm bounded bacteria in orthopaedic trauma. However, given that both planktonic and biofilm had successfully produced consistent grade and highly reproducible infection models in our reviewed studies, either inoculation technique can be used to meet different research needs.

Biomechanical factor is a major element in animal fracture models. Mechanically stable fixtures contribute to early callus formation, increased vascularity and tissue differentiation and thus shorten time course of osseous union [83]. In general implant devices such as intramedullary wires, metal plates and screws were shown to provide considerate stability in animal fracture and bone defects experiments. Nevertheless, when an infection component is introduced, loss of fixation is observed due to extensive osteolysis and sequestra formation [1]. Fixation systems should therefore be rigid since instability can greatly influence healing outcomes and cause further soft tissue damage [84]. For animal models, there have been controversies on the fixation stability of K-wires compared to plate and screws [85]. However, more recent studies have shown K-wire fixation are able to promote osseous healing within a timeframe similar to plate devices in bacterial inoculated models [56]. The use of the intramedullary nailing device in open tibia fractures is also commonly used in the clinical setting [86]. Other fixation techniques include polyether ether ketone (PEEK) plate, polyacetate plates and cerclage wire in the animal studies [32,37,45–47,57]. The PEEK material is recently FDA approved and used in orthopaedics and dental implants. Different from metallic implant fixture, its radiolucency had allowed clearer visualisation of osteolysis and reactive bone formation [32,37]. Compared to traditional stainless and titanium, however, PEEK material is less stiff and has a similar bone modulus to human bone which makes it less attractive for long bone fixation [87,88]. For polyacetate plates, there were no comparison studies for this polymer with metal related to strength [45–47]. In animal studies, it is important to note that not all material represent a clinically relevant scenario. If a specific implant material is used, researchers could provide more information related to implant rigidity. Further studies on fixation rigidity in an infection model could also guide evolvingnew implant devices and materials in combating bone infection. In summary, researchers should select a fracture and fixation technique that best replicate the common clinical situations in devising translational therapeutics treatment.

A range of assessments was performed in the literature. According to Centre for Disease Control and Prevention/National Healthcare Safety Network's (CDC/NHSN) diagnosis for bone infection, patients were clinically identified with osteomyelitis based on four major criteria: microbiological testing of blood and bone; anatomic and histological evaluation; localized signs and symptoms; and most importantly, radiographic examination supported by clinician correlation [89]. To closely replicate the clinical diagnosis on osteomyelitis, it is recommended that an animal model should make reference to the CDC/NSDH's, in which radiological evaluation and assessment of at least one of the other three criteria should be performed. All reviewed literature had included these components and provided sound evidence. However, quality difference was observed among the literature evidence. In respect to microbiological evidence, tissue culture such as bone, soft tissue and synovial fluid should be preferred than swab culture. In clinical settings, a lack of concordance was seen in bacterial recovery of tissue biopsies and swab specimens [90–92]. Despite not encountered in our reviewed literature, swab culture is easily contaminated, leading to false positive result [93], and has a lower sensitivity in detecting biofilm-bounded bacteria [94]. Sonication of the implant to dislodge bacterial cells is also a viable approach in bacteria culture [2]. One study utilised bioluminescent microbe which enables longitudinal capturing of bacterial metabolic activity [32]. The technology was innovative; however results should be examined together with other microbiological assessment due to limited penetration depth [95]. When assessing bone healing complicating with infection, radiographical assessment could be achieved semi-quantitatively with scoring systems. Some grading systems included the radiographic union score for tibia (RUST) [50,54], the Lane and Sandhu score [42,49] and one developed by An YH and Friedman RJ [49,60]. Additionally, a histological score system proposed by Büren et al. achieved high sensitivity and specificity for assessing the severity in OAI model in mice [61]. Some employed a self-defined rating scheme that was not validated [34,35,47,57]. A reason for lack of coherence in grading system used in the reviewed literature could be due to missing components of bony lysis, sequestration, periosteal reactions and implant instability. Establishing a rating scale specific in preclinical bone infection studies in the future could provide researchers consistency and accuracy in performing bone assessment. Regarding the preclinical studies of OAI, a recent review study by Moriarty et al. also show the basic principles as well as common errors for outcomes assessment [96].

Complications appear to be a concern in reviewed literature. Due to fracture dislocation, surgical inaccuracy, severe loss of fixation and septic infection, deaths were unavoidable. Before the experiment, researchers should intelligently design the study to answer the research question; have standardized protocols for anaesthesia and pain management; prepare details for materials and methods; ensure protocols meet criteria established for a "justified animal study"; design humane end-points with scientific output; and address confounding variables. A pilot study is also recommended for model validation and powering. During the experiment, skillful surgeons should perform animal surgeries and a complication rate of less than 10% should be targeted [97].

Clinically, OAI with biofilm formation remains an unresolved dilemma. In the human body, bacteria residing in the biofilm usually has high number of cells. In fact, it has been shown that a biofilm consisting of as few as 10^2 to 10^4 bacteria cells could be sufficient for causing valid wound contamination [81]. The components of biofilm including polysaccharides and proteins would secure its structural integrity and cause high resistance to antibiotics and host defense. Hence, it is difficult to eradicate the infection and achieve solid bone union even with long-term systemic antibiotic treatment. This was also confirmed by a recent review study of Croes et al., which suggested that there could be an inconsistency in the effect of systemic antibiotics on microbiological, clinical, and bone healing results in the context of latent infection [98].

To prevent or eradicate infection with biofilm formation, various strategies including Cooper bearing stainless steel, anti-adhesive surface, antibiotic-coated implant, and hydrogel delivery of antibiotics and antimicrobial peptide have been proposed [99,100]. Based on current results, the use of systemic antibiotics in general improved bridging of the infected fracture. However, the most useful interventions were antibiotic-coated implants or the use of antibiotic loaded hydrogels, which allows effective fracture healing. The authors would therefore recommend the use of these interventions for potential translation into clinical use after further clinical trials.

There is an emerging need to develop new treatment strategies for osteosynthesis associated infection. In conclusion, most studies concentrate on *S. aureus* species, which is the most common bacteria in osteosynthesis-associated infections. Based on the fracture model, different interventions have been investigated on their efficacy in eradicating the bacteria. However, the emergence of antibiotic resistance organisms is a rising issue and more effort is needed to simulate other clinical scenarios of osteosynthesis associated infection. Furthermore, recently there has also been an increasing concern of an aging population. As immunity is strongly linked to estrogen deficiency [101,102], postmenopausal women are also at high-risk group for bone infection. An animal model that looks at fracture healing complicated with both bone infection and osteoporosis is thus worthwhile for future studies. Further studies on animal models should also investigate on poly-microbes and antibiotic resistant strains, as these will no doubt play a major role in bone infections.

Funding/support

This review was supported by grants from Osteosynthesis and Trauma Care (OTC) Foundation, Switzerland (Ref: 2018-RWMI), the Health and Medical Research Fund (HMRF), the Food and Health Bureau, The Government of the Hong Kong Special Administrative Region (Ref: 19180062), and Direct Grant for Research, CUHK (Ref: 2018.062).

Conflict of Interest

The authors have no conflicts of interest to disclose in relation to this article.

References

[1] Metsemakers WJ, Kuehl R, Moriarty TF, Richards RG, Verhofstad MHJ, Borens O, Kates S, Morgenstern M. Infection after fracture fixation: current surgical and microbiological concepts. Injury 2018;49:511–22.
[2] Yano MH, Klautau GB, da Silva CB, Nigro S, Avanzi O, Mercadante MT, Salles MJ. Improved diagnosis of infection associated with osteosynthesis by use of sonication of fracture fixation implants. J Clin Microbiol 2014;52:4176–82.
[3] Zimmerli W, Sendi P. Pathogenesis of implant-associated infection: the role of the host. Semin Immunopathol 2011;33:295–306.
[4] Thakore RV, Greenberg SE, Shi H, Foxx AM, Francois EL, Prablek MA, Nwosu SK, Archer KR, Ehrenfeld JM, Obremskey WT, Sethi MK. Surgical site infection in orthopedic trauma: a case-control study evaluating risk factors and cost. J Clin Orthop Trauma 2015;6:220–6.
[5] Penn-Barwell JG, Rand BC, Brown KV, Wenke JC. A versatile model of open-fracture infection : a contaminated segmental rat femur defect. Bone Joint Res 2014;3:187–92.
[6] Liu X, Yang L, Li J, Zhang Y, Xu W, Ren Y, Liu B, Yang B, Li B. GS/DBM/PLA porous composite biomaterial for the treatment of infective femoral condyle defect in rats. Exp Therap Medicine 2016;11:2107–16.
[7] Buxton TB, Travis MT, O'Shea KJ, McPherson 3rd JC, Harvey SB, Plowman KM, Walsh DS. Low-dose infectivity of Staphylococcus aureus (SMH strain) in traumatized rat tibiae provides a model for studying early events in contaminated bone injuries. Comp Med 2005;55:123–8.
[8] Petri 3rd WH, Schaberg SJ. The effects of antibiotic-supplemented bone allografts on contaminated, partially avulsive fractures of the canine ulna. J Oral Maxillofac Surg : Off J Am Assoc Oral Maxillofac Surg 1984;42:699–704.
[9] Huang JG, Pang L, Chen ZR, Tan XP. Dual-delivery of vancomycin and icariin from an injectable calcium phosphate cement-release system for controlling infection and improving bone healing. Mol Med Rep 2013;8:1221–7.
[10] Xu H, Zhang G, Xu K, Wang L, Yu L, Xing MMQ, Qiu X. Mussel-inspired dual-functional PEG hydrogel inducing mineralization and inhibiting infection in maxillary bone reconstruction. Mater Sci Eng C, Mater Biol Appl 2018;90:379–86.
[11] Lankinen P, Noponen T, Autio A, Luoto P, Frantzen J, Loyttyniemi E, Hakanen AJ, Aro HT, Roivainen A. A comparative (68)Ga-citrate and (68)Ga-chloride PET/CT imaging of Staphylococcus aureus osteomyelitis in the rat tibia. Contrast Media Mol Imag, 2018 2018:9892604.
[12] Xing J, Hou T, Luobu B, Luo F, Chen Q, Li Z, Jin H, Xu J. Anti-infection tissue engineering construct treating osteomyelitis in rabbit tibia, Tissue engineering. Part A 2013;19:255–63.
[13] Gahukamble AD, McDowell A, Post V, Salavarrieta Varela J, Rochford ET, Richards RG, Patrick S, Moriarty TF. Propionibacterium acnes and Staphylococcus lugdunensis cause pyogenic osteomyelitis in an intramedullary nail model in rabbits. J Clin Microbiol 2014;52:1595–606.
[14] McLaren JS, White LJ, Cox HC, Ashraf W, Rahman CV, Blunn GW, Goodship AE, Quirk RA, Shakesheff KM, Bayston R, Scammell BE. A biodegradable antibiotic-impregnated scaffold to prevent osteomyelitis in a contaminated in vivo bone defect model. Eur Cells Mater J Trans Name Eur Cells Mater 2014;27:332–49.
[15] Tennent DJ, Shiels SM, Jennings JA, Haggard WO, Wenke JC. Local control of polymicrobial infections via a dual antibiotic delivery system. J Orthop Surg Res 2018;13:53.
[16] Yokogawa N, Ishikawa M, Nishitani K, Beck CA, Tsuchiya H, Mesfin A, Kates SL, Daiss JL, Xie C, Schwarz EM. Immunotherapy synergizes with debridement and antibiotic therapy in a murine 1-stage exchange model of MRSA implant-associated osteomyelitis. J Orthop Res : Off Publ Orthop Res Soc 2018;36:1590–8.
[17] Raehtz S, Hargis BM, Kuttappan VA, Pamukcu R, Bielke LR, McCabe LR. High molecular weight polymer promotes bone health and prevents bone loss under Salmonella challenge in broiler chickens. Front Physiol 2018;9:384.
[18] Brunotte M, Rupp M, Stotzel S, Sommer U, Mohammed W, Thormann U, Heiss C, Lips KS, Domann E, Alt V. A new small animal model for simulating a two-stage-revision procedure in implant-related methicillin-resistant Staphylococcus aureus bone infection. Injury 2019;50:1921–8.
[19] Jiang Y, Wang SN, Wu HT, Qin HJ, Ren ML, Lin JC, Yu B. Aspirin alleviates orthopedic implantassociated infection. Int J Mol Med 2019;44:1281–8.
[20] Boyle KK, Sosa B, Osagie L, Turajane K, Bostrom MPG, Yang X. Vancomycin-laden calcium phosphate-calcium sulfate composite allows bone formation in a rat infection model. PloS One 2019;14(9) (no pagination).
[21] Merritt K, Dowd JD. Role of internal fixation in infection of open fractures: studies with Staphylococcus aureus and Proteus mirabilis. J Orthop Res : Off Publ Orthop Res Soc 1987;5:23–8.
[22] Kose N, Çaylak R, Pekşen C, Kiremitçi A, Burukoglu D, Koparal S, Doğan A. Silver ion doped ceramic nano-powder coated nails prevent infection in open fractures: in vivo study. Injury 2016;47:320–4.
[23] 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;36:3136–41.
[24] Surdu-Bob CC, Vlase E, Barbuceanu F, Turcu D, Coman M, Badulescu M, Oporanu M, Coman C. Copper bead therapy in severe bone infection: a rabbit tibial model. Vet Comp Orthop Traumatol 2019;32:41–50.
[25] Darouiche RO, Farmer J, Chaput C, Mansouri M, Saleh G, Landon GC. Anti-infective efficacy of antiseptic-coated intramedullary nails, the Journal of bone and joint surgery. Am Vol 1998;80:1336–40.
[26] Friedrich B, Klaue P. Mechanical stability and post-traumatic osteitis: an experimental evaluation of the relation between infection of bone and internal fixation. Injury 1977;9:23–9.
[27] Stavrakis AI, Zhu S, Loftin AH, Weixian X, Niska J, Hegde V, Segura T, Bernthal NM. Controlled release of vancomycin and tigecycline from an orthopaedic implant coating prevents Staphylococcus aureus infection in an open fracture animal model. BioMed Res Int 2019:1638508.
[28] Vallejo A, Deimling C, Morgenstern M, D'Este M, Puetzler J, Zeiter S, Arens D, Metsemakers WJ, Richards RG, Eglin D, Moriarty TF. Local application of a gentamicin-loaded hydrogel early after injury is superior to peri-operative systemic prophylaxis in a rabbit open fracture model. J Orthop Trauma 2019:6.
[29] Xu Z, Li J, Zhou X, Dai J, Zhang J, Huang Y, Xu N. 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 Mon Int Med J Exp Clin Res 2019;25:312–7.
[30] Puetzler J, Milstrey A, Everding J, Raschke M, Arens D, Zeiter S, Geoff Richards R, Fintan Moriarty T. Focused high-energy extracorporeal shockwaves as supplemental treatment in a rabbit model of fracture-related infection. J Orthop Res 2019 [Epub ahead of print].
[31] 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;44:391–8.
[32] 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.
[33] Rochford ETJ, Sabate Bresco M, Zeiter S, Kluge K, Poulsson A, Ziegler M, Richards RG, O'Mahony L, Moriarty TF. Monitoring immune responses in a mouse model of fracture fixation with and without Staphylococcus aureus osteomyelitis. Bone 2016;83:82–92.
[34] Windolf CD, Logters T, Scholz M, Windolf J, Flohe S. Lysostaphin-coated titan-implants preventing localized osteitis by Staphylococcus aureus in a mouse model. PloS One 2014;9. e115940.
[35] Windolf CD, Meng W, Logters TT, MacKenzie CR, Windolf J, Flohe S. Implant-associated localized osteitis in murine femur fracture by biofilm forming Staphylococcus aureus: a novel experimental model. J Orthop Res : Off Publ Orthop Res Soc 2013;31:2013–20.
[36] Johnson CT, Wroe JA, Agarwal R, Martin KE, Guldberg RE, Donlan RM, Westblade LF, Garcia AJ. Hydrogel delivery of lysostaphin eliminates orthopedic implant infection by Staphylococcus aureus and supports fracture healing. Proc Natl Acad Sci USA 2018;115:E4960–e4969.
[37] Rochford ETJ, Sabate Bresco M, Poulsson AHC, Kluge K, Zeiter S, Ziegler M, O'Mahony L, Richards RG, Moriarty TF. Infection burden and immunological responses are equivalent for polymeric and metallic implant materials in vitro and in a murine model of fracture-related infection. J Biomed Mater Res B Appl Biomater 2018. https://doi.org/10.1002/jbm.b.34202.
[38] Buren C, Logters T, Oezel L, Rommelfanger G, Scholz AO, Windolf J, Windolf CD. Effect of hyperbaric oxygen therapy (HBO) on implant-associated osteitis in a femur fracture model in mice. PloS One 2018;13. e0191594.
[39] Lovati AB, Romano CL, Bottagisio M, Monti L, De Vecchi E, Previdi S, Accetta R, Drago L. Modeling Staphylococcus epidermidis-induced non-unions: subclinical and clinical evidence in rats. PloS One 2016;11. e0147447.

[40] Schindeler A, Yu NY, Cheng TL, Sullivan K, Mikulec K, Peacock L, Matthews R, Little DG. Local delivery of the cationic steroid antibiotic CSA-90 enables osseous union in a rat open fracture model of Staphylococcus aureus infection, the Journal of bone and joint surgery. Am Vol 2015;97:302–9.

[41] Bilgili F, Balci HI, Karaytug K, Sariyilmaz K, Atalar AC, Bozdag E, Tuna M, Bilgic B, Gurler N. 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;473:3190–6.

[42] Helbig L, Guehring T, Rosenberger S, Ivanova A, Kaeppler K, Fischer CA, Moghaddam A, Schmidmaier G. A new animal model for delayed osseous union secondary to osteitis. BMC Muscoskel Disord 2015;16:362.

[43] Alt V, Lips KS, Henkenbehrens C, Muhrer D, Oliveira Cavalcanti MC, Sommer U, Thormann U, Szalay G, Heiss C, Pavlidis T, Domann E, Schnettler R. 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;48:1146–53.

[44] Robinson DA, Bechtold JE, Carlson CS, Evans RB, Conzemius MG. Development of a fracture osteomyelitis model in the rat femur. J Orthop Res 2011;29:131–7.

[45] Chen X, Schmidt AH, Mahjouri S, Polly Jr 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: 693–700.

[46] 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, the Journal of bone and joint surgery. Am Vol 2006;88:1510–23.

[47] Chen X, Kidder LS, Lew WD. Osteogenic protein-1 induced bone formation in an infected segmental defect in the rat femur. J Orthop Res : Off Publ Orthop Res Soc 2002;20:142–50.

[48] 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.

[49] Helbig L, Omlor GW, Ivanova A, Guehring T, Sonntag R, Kretzer JP, Minkwitz S, Wildemann B, Schmidmaier G. Bone morphogenetic proteins - 7 and - 2 in the treatment of delayed osseous union secondary to bacterial osteitis in a rat model. BMC Muscoskel Disord 2018;19:261.

[50] Mills R, Cheng TL, Mikulec K, Peacock L, Isaacs D, Genberg C, Savage PB, Little DG, Schindeler A. CSA-90 promotes bone formation and mitigates methicillin-resistant Staphylococcus aureus infection in a rat open fracture model. Clin Orthop Relat Res 2018;476:1311–23.

[51] Cui T, Li J, Zhen P, Gao Q, Fan X, Li C. Masquelet induced membrane technique for treatment of rat chronic osteomyelitis. Exp Therap Medicine 2018;16:3060–4.

[52] Shiels SM, Tennent DJ, Lofgren AL, Wenke JC. Topical rifampin powder for orthopaedic trauma part II: topical rifampin allows for spontaneous bone healing in sterile and contaminated wounds. J Orthop Res : Off Publ Orthop Res Soc 2018; 36:3142–50.

[53] Ter Boo GJ, Schmid T, Zderic I, Nehrbass D, Camenisch K, Richards RG, Grijpma DW, Moriarty TF, Eglin D. Local application of a gentamicin-loaded thermo-responsive hydrogel allows for fracture healing upon clearance of a high Staphylococcus aureus load in a rabbit model. Eur Cell Mater 2018;35:151–64.

[54] Zhou L, Liu Q, Zhou Z, Lu W, Tao J. Efficacy of tobramycin-loaded coating K-wire in an open-fracture rabbit model contaminated by staphylococcus aureus. Int J Clin Exp Med J Trans Name Int J Clin Exp Med 2017;10:6004–16.

[55] Zhang X, Ma YF, Wang L, Jiang N, Qin CH, Hu YJ, Yu B. A rabbit model of implant-related osteomyelitis inoculated with biofilm after open femoral fracture. Exp Therap Medicine 2017;14:4995–5001.

[56] Arens D, Wilke M, Calabro L, Hackl S, Zeiter S, Zderic I, Richards RG, Moriarty TF. A rabbit humerus model of plating and nailing osteosynthesis with and without Staphylococcus aureus osteomyelitis. Eur Cell Mater 2015;30:148–61. discussion 161-142.

[57] 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 : Off Publ Orthop Res Soc 2004;22:66–72.

[58] Caprise Jr PA, Miclau T, Dahners LE, Dirschl DR. High-pressure pulsatile lavage irrigation of contaminated fractures: effects on fracture healing. J Orthop Res : Off Publ Orthop Res Soc 2002;20:1205–9.

[59] Tran N, Tran PA, Jarrell JD, Engiles JB, Thomas NP, Young MD, Hayda RA, Born CT. In vivo caprine model for osteomyelitis and evaluation of biofilm-resistant intramedullary nails. BioMed Res Int 2013;2013:674378.

[60] Schaer TP, Stewart S, Hsu BB, Klibanov AM. Hydrophobic polycationic coatings that inhibit biofilms and support bone healing during infection 2012;33:1245–54.

[61] Buren C, Hambuchen M, Windolf J, Logters T, Windolf CD. Histological score for degrees of severity in an implant-associated infection model in mice. Arch Orthop Trauma Surg 2019;139:1235–44.

[62] Oezel L, Buren 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. e0213590.

[63] Kremers HM, Nwojo ME, Ransom JE, Wood-Wentz CM, Melton LJ, 3rd, Huddleston 3rd PM. Trends in the epidemiology of osteomyelitis: a population-based study, 1969 to 2009, the Journal of bone and joint surgery. Am Vol 2015; 97:837–45.

[64] Vanvelk N, Morgenstern M, Moriarty TF, Richards RG, Nijs S, Metsemakers WJ. Preclinical in vivo models of fracture-related infection: a systematic review and critical appraisal. Eur Cell Mater 2018;36:184–99.

[65] Schaer TP, Stewart S, Hsu BB, Klibanov AM. Hydrophobic polycationic coatings that inhibit biofilms and support bone healing during infection. Biomaterials 2012;33:1245–54.

[66] Cohen C. The case for the use of animals in biomedical research. N Engl J Med 1986;315:865–70. https://www.ncbi.nlm.nih.gov/pubmed/3748104.

[67] Histing T, Garcia P, Holstein JH, Klein M, Matthys R, Nuetzi R, Steck R, Laschke MW, Wehner T, Bindl R, Recknagel S, Stuermer EK, Vollmar B, Wildemann B, Lienau J, Willie B, Peters A, Ignatius A, Pohlemann T, Claes L, Menger MD. Small animal bone healing models: standards, tips, and pitfalls results of a consensus meeting. Bone 2011;49:591–9.

[68] Graur D, Duret L, Gouy M. Phylogenetic position of the order Lagomorpha (rabbits, hares and allies). Nature 1996;379:333–5.

[69] Neves F, Abrantes J, Almeida T, de Matos AL, Costa PP, Esteves PJ. Genetic characterization of interleukins (IL-1alpha, IL-1beta, IL-2, IL-4, IL-8, IL-10, IL-12A, IL-12B, IL-15 and IL-18) with relevant biological roles in lagomorphs. Innate Immun 2015;21:787–801.

[70] Perkins HD, van Leeuwen BH, Hardy CM, Kerr PJ. The complete cDNA sequences of IL-2, IL-4, IL-6 AND IL-10 from the European rabbit (Oryctolagus cuniculus). Cytokine 2000;12:555–65.

[71] Legrand N, Ploss A, Balling R, Becker PD, Borsotti C, Brezillon N, Debarry J, de Jong Y, Deng H, Di Santo JP, Eisenbarth S, Eynon E, Flavell RA, Guzman CA, Huntington ND, Kremsdorf D, Manns MP, Manz MG, Mention JJ, Ott M, Rathinam C, Rice CM, Rongvaux A, Stevens S, Spits H, Strick-Marchand H, Takizawa H, van Lent AU, Wang C, Weijer K, Willinger T, Ziegler P. Humanized mice for modeling human infectious disease: challenges, progress, and outlook. Cell Host Microbe 2009;6:5–9.

[72] Trampuz A, Zimmerli W. Diagnosis and treatment of infections associated with fracture-fixation devices. Injury 2006;37(Suppl 2):S59–66.

[73] Peng J, Ren Y, He W, Li Z, Yang J, Liu Y, Zheng Z, Kates SL, Schwarz EM, Xie C, Xu Y. Epidemiological, clinical and microbiological characteristics of patients with post-traumatic osteomyelitis of limb fractures in southwest China: a hospital-based study. J Bone Joint infect 2017;2:149–53.

[74] Venkata Suresh Babu Tummala D, Suresh Babu Surapaneni D, Siddarth Pigilam D. Bacteriological study of orthopaedic infections. Int J Orthopaed Sci 2017;3(2): 90–2. http://www.orthopaper.com/archives/?year=2017&vol=3&issue=2&part=B&articleid=305.

[75] Jorge LS, Fucuta PS, Oliveira MGL, Nakazone MA, de Matos JA, Chueire AG, Salles MJC. Outcomes and risk factors for polymicrobial posttraumatic osteomyelitis. J Bone Joint infect 2018;3:20–6.

[76] Brady RA, Leid JG, Calhoun JH, Costerton JW, Shirtliff ME. Osteomyelitis and the role of biofilms in chronic infection. FEMS Immunol Med Microbiol 2008;52: 13–22.

[77] Patzakis MJ, Wilkins J. Factors influencing infection rate in open fracture wounds. Clinical orthopaedics and related research; 1989. p. 36–40.

[78] Watters C, Fleming D, Bishop D, Rumbaugh KP. Chapter seven - host responses to biofilm. In: San Francisco M, San Francisco B, editors. Progress in molecular biology and translational science. Academic Press; 2016. p. 193–239.

[79] del Pozo JL, Patel R. The challenge of treating biofilm-associated bacterial infections. Clin Pharmacol Therapeut 2007;82:204–9.

[80] Melchior MB, Fink-Gremmels J, Gaastra W. Comparative assessment of the antimicrobial susceptibility of Staphylococcus aureus isolates from bovine mastitis in biofilm versus planktonic culture. J Vet Med B, Infect Dis Vet Publ Health 2006; 53:326–32.

[81] Williams DL, Costerton JW. Using biofilms as initial inocula in animal models of biofilm-related infections. J Biomed Mater Res B Appl Biomater 2012;100:1163–9.

[82] Wimpenny J, Manz W, Szewzyk U. Heterogeneity in biofilms. FEMS (Fed Eur Microbiol Soc) Microbiol Rev 2006;24:661–71.

[83] Lienau J, Schell H, Duda GN, Seebeck P, Muchow S, Bail HJ. Initial vascularization and tissue differentiation are influenced by fixation stability. J Orthop Res : Off Publ Orthop Res Soc 2005;23:639–45.

[84] Goodship AE, Watkins PE, Rigby HS, Kenwright J. The role of fixator frame stiffness in the control of fracture healing. An experimental study. J Biomech 1993;26:1027–35.

[85] Worlock P, Slack R, Harvey L, Mawhinney R. The prevention of infection in open fractures: an experimental study of the effect of fracture stability. Injury 1994;25: 31–8.

[86] Agrawal A, Chauhan VD, Maheshwari RK, Juyal AK. Primary nailing in the open fractures of the tibia-is it worth? J Clin Diagn Res : J Clin Diagn Res 2013;7: 1125–30.

[87] Sanderson J, Nakanishi Y, Murayama N, Nishimura T, Ishi T, Mizuta H, Higaki H. Polyetheretherketone in orthopedic plate design to facilitate fracture recovery. J Biomech Sci Eng 2011;6:125–33.

[88] Mugnai R, Tarallo L, Capra F, Catani F. Biomechanical comparison between stainless steel, titanium and carbon-fiber reinforced polyetheretherketone volar locking plates for distal radius fractures. Orthop Traumatol Surg Res : OTSR 2018; 104:877–82.

[89] Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Contr 2008;36:309–32.

[90] Senneville E, Melliez H, Beltrand E, Legout L, Valette M, Cazaubiel M, Cordonnier M, Caillaux M, Yazdanpanah Y, Mouton Y. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. Clin Infect Dis : An Off Publ Infect Dis Soc Am 2006;42: 57–62.

[91] Aggarwal VK, Higuera C, Deirmengian G, Parvizi J, Austin MS. Swab cultures are not as effective as tissue cultures for diagnosis of periprosthetic joint infection. Clin Orthop Relat Res 2013;471:3196–203.
[92] Omar M, Suero EM, Liodakis E, Reichling M, Guenther D, Decker S, Stiesch M, Krettek C, Eberhard J. Diagnostic performance of swab PCR as an alternative to tissue culture methods for diagnosing infections associated with fracture fixation devices. Injury 2016;47:1421–6.
[93] Wilson ML, Winn W. Laboratory diagnosis of bone, joint, soft-tissue, and skin infections, Clinical infectious diseases. An Off Publ Infect Dis Soc Am 2008;46:453–7.
[94] Ehrlich G, DeMeo P, Palmer M, Sauber TJ, Altman D, Altman G, Sotereanos N, Conti S, Baratz M, Maale G, Hu FZ, Christopher Post J, Nistico L, Melton-Kreft R, Hall-Stoodley L, Costerton JW, Stoodley P. Culture-negative infections in orthopedic surgery. 2012. p. 17–27.
[95] Kim JE, Kalimuthu S, Ahn BC. In vivo cell tracking with bioluminescence imaging. Nucl. Med. Mol. Imag. 2015;49:3–10.
[96] Moriarty TF, Harris LG, Mooney RA, Wenke JC, Riool M, Zaat SAJ, Moter A, Schaer TP, Khanna N, Kuehl R, Alt V, Montali A, Liu J, Zeiter S, Busscher HJ, Grainger DW, Richards RG. Recommendations for design and conduct of preclinical in vivo studies of orthopedic device-related infection. J Orthop Res 2019;37:271–87.
[97] Auer JA, Goodship A, Arnoczky S, Pearce S, Price J, Claes L, von Rechenberg B, Hofmann-Amtenbrinck M, Schneider E, Müller-Terpitz R, Thiele F, Rippe K-P, Grainger DW. Refining animal models in fracture research: seeking consensus in optimising both animal welfare and scientific validity for appropriate biomedical use. BMC Muscoskel Disord 2007;8:72.
[98] Croes M, van der Wal BCH, Vogely HC. Impact of bacterial infections on osteogenesis: evidence from in vivo studies. J Orthop Res 2019. https://doi.org/10.1002/jor.24422.
[99] Yang K, Han Q, Chen B, Zheng Y, Zhang K, Li Q, Wang J. Antimicrobial hydrogels: promising materials for medical application. Int J Nanomed 2018;13:2217–63.
[100] Zhuang Y, Zhang S, Yang K, Ren L, Dai K. Antibacterial activity of copper-bearing 316L stainless steel for the prevention of implant-related infection. J Biomed Mater Res B Appl Biomater 2020;108:484–95.
[101] Sakiani S, Olsen NJ, Kovacs WJ. Gonadal steroids and humoral immunity. Nat Rev Endocrinol 2013;9:56–62.
[102] Whitacre CC. Sex differences in autoimmune disease. Nat Immunol 2001;2:777–80.