Impact of Bacterial Infections on Osteogenesis: Evidence From In Vivo Studies

Michiel Croes, Bart C. H. van der Wal, H. Charles Vogely

Department of Orthopaedics, University Medical Center Utrecht, Heidelberglaan 100, 3508 GA, Utrecht, The Netherlands

Received 12 April 2019; accepted 15 July 2019

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.24422

ABSTRACT: The clinical impact of bacterial infections on bone regeneration has been incompletely quantified and documented. As a result, controversy exists about the optimal treatment strategy to maximize healing of a contaminated defect. Animal models are extremely useful in this respect, as they can elucidate how a bacterial burden influences quantitative healing of various types of defects relative to non-infected controls. Moreover, they may demonstrate how antibacterial treatment and/or bone grafting techniques facilitate the osteogenic response in the harsh environment of a bacterial infection. Finally, it a well-known contradiction that osteomyelitis is characterized by uncontrolled bone remodeling and bone loss, but at the same time, it can be associated with excessive new bone apposition. Animal studies can provide a better understanding of how osteolytic and osteogenic responses are related to each other during infection. This review discusses the in vivo impact of bacterial infection on osteogenesis by addressing the following questions (i) How does osteomyelitis affect the radiographic bone appearance? (ii) What is the influence of bacterial infection on histological bone healing? (iii) How do bacterial infections affect quantitative bone healing? (iv) What is the effect of antibacterial treatment on the healing outcome during infection? (v) What is the efficacy of osteoinductive proteins in infected bones? (vi) What is the balance between the osteoelastic and osteoblastic response during bacterial infections? (vii) What is the mechanism of the observed pro-osteogenic response as observed in osteomyelitis? © 2019 The Authors. Journal of Orthopaedic Research®

Keywords: fracture; union; osteomyelitis; infection; animal models

Osteomyelitis is a frequent complication of trauma or surgery, often in conjunction with prosthetic implantation, but may also occur secondary to vascular insufficiency or hematogenous infections. Microorganisms that are either competent in binding to the surface of the host tissue/implant (i.e., coagulase-negative staphylococci), or specialized in evading the host defense system and destructing host cells/extracellular matrix (i.e., Staphylococcus aureus), are the most common causative pathogens in osteomyelitis. After the formation of a persisting biofilm, osteomyelitis can manifest itself as a complicated clinical scenario, necessitating repeated surgical interventions to clear the infection.

It is well-accepted that the exaggerated inflammatory response in osteomyelitis leads to drastic bone changes, which are the result of a dysregulation of the number of bone-forming osteoblasts and bone-resorbing osteoclasts. The cytokine receptor activator of NF-κB ligand (RANKL) is a critical regulator of bone remodeling and regeneration by controlling osteoclast formation and activity. An enhanced expression of RANKL is a hallmark of osteomyelitis, and occurs in direct response to bacterial antigens and their secreted products, or in an indirect response to exaggerated tissue inflammation. Osteoblasts actively participate in the osteolytic process by internalizing bacteria and aggravating the inflammatory response through secretion of pro-inflammatory or osteoclast-modulating factors. In addition to the enhanced osteoclast-mediated bone resorption, several processes may cause a lack of sufficient bone matrix-depositing cells at the bacterial burden. Osteomyelitis is known to cause an uncontrolled cell death by compression of vascular channels and the local release of nitric oxide. Moreover, osteoblasts undergo programmed cell death and necrotic cell death after uptake of bacteria, or after exposure to high levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interferon γ (IFN-γ). These many bone changes result into life-long diminished resistance of the tissue to bacterial adherence and infection, explaining the relatively high chance of reinfection even years after the first episode.

In quantitative terms, the actual clinical impact of bacterial infections on bone regeneration remains largely elusive. Clinicians recognize that osteomyelitis impairs the regenerative response after injury, but the underlying mechanism, the course of the disease, and the healing outcome cannot be measured in a standardized way in a clinical situation. Moreover, as bone infections are usually treated as soon as clinical manifestations occur, it is difficult to assess the long-term effect of infection on bone healing without any intervention. Animal models can provide valuable insight into the correlation between the bacterial burden, bone loss, and callus formation. Furthermore, infection...
From a basic point of view, the current review summarizes how bacterial infection affects the radiographic and histological appearance of bone tissue in the context of fracture healing, and whether this is related to changes in mechanical stability and callus formation. Whereas bone fracture healing normally is a well-orchestrated event, it is unclear if, and to what extent, the sequential stages of fracture healing (i.e., inflammation, repair, and remodeling) are disturbed following bacterial contamination. Moreover, it is discussed how bone loss and new bone formation coincide as part of the local tissue response to a bacterial infection, and how these seemingly opposite processes are related to each other. Together, this can elucidate which cellular processes are most affected in osteomyelitis. From a more translational point of view, this review addresses how antibacterial treatment and/or bone grafting techniques facilitate the osteogenic response in the harsh environment of a bacterial infection. In this respect, there are several uncertainties that complicate the decision-making in osteomyelitis treatment. For example, although early removal of an implant increases the chance of bacterial eradication, defect healing is compromised by defect instability following implant removal. Using available in vivo data, we aim to answer the following questions (i) How does osteomyelitis affect the radiographic bone appearance? (ii) What is the influence of bacterial infection on histological bone healing? (iii) How do bacterial infections affect quantitative bone healing? (iv) What is the effect of antibacterial treatment on the healing outcome during infection? (v) What is the efficacy of osteoinductive proteins in infected bones? (vi) What is the balance between the osteoclastic and osteoblastic response during bacterial infections? (vii) What is the mechanism of the observed pro-osteogenic response as observed in osteomyelitis?

LITERATURE SEARCH STRATEGY
A literature search was performed in the PubMed database. The following search terms were used: bone OR callus OR osteogenesis AND animal OR in vivo OR pre-clinical OR pre-clinical AND infection OR bacterial OR contamination OR osteomyelitis. Non-English articles or articles published before 2000 were excluded. The resulting ±150 articles were screened for relevance, that is, whether the effect of bacterial infection on bone healing, osteolysis, or new bone formation was reported in a quantitative manner. The main findings from the remaining 40 references were summarized and critically reviewed. The key reports are shown in Supplementary Table S1 and Table S2. Additional clinical or in vitro articles were included to interpret the findings.

Figure 1. Commonly observed radiographic signs of osteomyelitis. (A) A closed fracture was created in the rat femur and stabilized with an intramedullary nail, either with (right image) or without (left image) Staphylococcus aureus contamination. After 3 weeks, the radiographs show complete healing in the absence of infection. In the presence of infection, the defects were unable to heal. Osteolysis (asterisk) is seen in proximity of the fracture gap (arrow), while pronounced periosteal bone formation (arrowheads) can be seen distally and proximally to the defect. Reprinted from Robinson et al. (B) S. aureus infection in a rabbit tibia model of periprosthetic infection leading to periosteal bone formation (arrows) and osteolysis (asterisk) as observed by micro-CT. The amount of periosteal bone formation and cortical resorption is associated with the number of colony-forming units (CFU) after 4 weeks. Reprinted from Croes et al.
HOW DOES OSTEOMYELITIS AFFECT THE RADIOGRAPHIC BONE APPEARANCE?
Bone resorption, cortical thickening, and periosteal new bone apposition are the key signs of osteomyelitis by plain radiography or computed tomography (CT) imaging.\textsuperscript{31–35} The osteomyelitis-related bone changes are consistent among small or large animal models, and are similar to those seen in patients\textsuperscript{36,37} (Fig. 1). This leads to the suggestion that also the underlying pathologic abnormalities (i.e., bacterial spread through the cortex, induction of an inflammatory milieu, abscess formation)\textsuperscript{37} are comparable from mice to men. Periosteal new bone formation generally has an earlier onset than cortical osteolysis.\textsuperscript{31,33,38} As an explanation, reactive bone formation occurs in a direct response to the local inflammatory milieu,\textsuperscript{39} while in comparison, cortical osteolysis is also caused by bacterial spread into the cortical Haversian and Volkmann canals.\textsuperscript{36,37} Several studies have utilized radiographic scoring systems to quantify the severity of osteomyelitis. These grading systems can clearly distinguish between infected animals and non-infected controls.\textsuperscript{31,40–44} Nevertheless, there is no evident relationship between the radiographic score and the actual bacterial count, making radiography by itself an unreliable tool to assess the effectiveness of antibacterial strategies.\textsuperscript{45–48} There are several explanations as to why the radiographic appearance of bone is not directly related to the bacterial burden. First, even if the bacterial burden is completely removed, the bone would require several weeks to remodel to its original architecture.\textsuperscript{49} Second, bacterial effects on bone remodeling are very dependent on the specific strain being utilized, for example, by having a different capacity to secrete toxins modulating osteogenesis or osteoclastogenesis,\textsuperscript{4,40} or by engaging different immune response through extracellular or intracellular pathogen-associated molecular patterns (PAMPs).\textsuperscript{50–53} Third, the presence of a foreign body or delivered compounds alone can already lead to osteomyelitis-like bone changes and exaggerate the radiographic scores, as has been observed with silver nanoparticle-based implant coatings.\textsuperscript{48}

WHAT IS THE INFLUENCE OF BACTERIAL INFECTION ON HISTOLOGICAL BONE HEALING?
In this section, we discuss how the complex immunological and tissue response to bacterial infection impacts the process of histological bone healing. In the normal situation, fracture healing follows a characteristic time-course, with the three overlapping phases of inflammation, repair, and remodeling.\textsuperscript{54} The phases of bone repair are similar among different species, although it should be realized that small animals generally show faster fracture healing as compared to larger animals or humans.\textsuperscript{54,55} Furthermore, the type of bone healing depends on the anatomical location of the fracture. As compared with diaphyseal bone healing, metaphyseal/epiphyseal fracture healing occurs in a direct manner, with limited or no periosteal callus formation.\textsuperscript{56} The acute inflammatory response, essential for the recruitment of progenitor cells from distant sources, also seems less important for metaphyseal bone healing.\textsuperscript{57} Since fracture (infection) models commonly involve defects created in the diaphyseal bone in rodents, the current discussion focuses on bone healing at this anatomical location.

In response to tissue injury and blood clot formation, an inflammatory response is initiated (duration: 0–5 days), which is critical for bone healing.\textsuperscript{55,58,59} Neutrophils enter the injured site already within minutes.\textsuperscript{60} Their main contribution is to phagocytose bacteria, remove cellular debris, and produce cytokines and chemokines to direct the infiltration of monocyte/macrophages.\textsuperscript{60,61} Macrophages play a key role in the inflammatory response, as they produce key regulatory factors needed for fibroblast and mesenchymal stem cell (MSC) recruitment from their local niches, and growth factors inducing osteogenesis and angiogenesis.\textsuperscript{58,62} The initial fracture hematoma is replaced by granulation tissue with proliferating fibroblasts and MSCs in the first-week post-fracture. The subsequent repair stage (duration: 1–4 weeks) involves a combination of both direct (intramembranous) and indirect (endochondral) forms of bone healing, with the relative contribution of these processes being influenced by the mechanical stability of the defect, that is, with relative more intramembranous healing when there is less interfragmentary motion.\textsuperscript{55} Chondrocytes contribute to the formation of a soft callus, that is, cartilage tissue accompanied by fibrotic tissue. This serves as the template for subsequent endochondral bone formation. At the same moment, direct bone formation originates from recruited and periosteal-derived progenitor cells that differentiate into osteoblasts to deposit woven bone. The stability provided by the initial fibrocartilage and woven bone scaffold allows the formation of hard callus, characterized by hypertrophy of chondrocytes, revascularization, and the replacement of the cartilage scaffold by woven bone. In the final stage (duration: 5–8 weeks), the woven bone and cartilage is removed by osteoclasts and replaced by the typical osteon and Haversian bone structure under influence of the mechanical stresses applied to the bone.\textsuperscript{55,58,63} Most of the in vivo studies have characterized the histological appearance of the bone after 4 or more weeks, which corresponds to the reparative (1–4 weeks) or remodeling (5–8 weeks) phases of healing. The studies collectively show that the well orchestrated event of callus formation is disrupted in osteomyelitis. Instead of the expected fibrocartilage and woven bone, an inflammatory medullary reaction is observed composed of fibrous tissue and abscess formation with only occasionally fibrocartilaginous tissue deposition. The cortical response is characterized by osteolysis, necrotic bone sequestra, and periosteal new bone formation. In contrast to non-infected controls, active inflammation is always observed in infection animals, irrespective of the time point of evaluation.\textsuperscript{21,22,25,33,34,44} Whereas neutrophils and
monocytes normally only play a role in the acute phase of healing, they remain predominant cell populations in infected defects even after weeks to months. The total absence of hyaline cartilage in the defects of severely-infected animals compared with low-grade or non-infected animals, leads to the suggestion that bacterial contamination already disrupts the early stages of callus formation. Histology reports suggest that the sustained presence of neutrophils may be a limiting factor for normal callus formation. Neutrophils are normally only short-lived (hours-days), and are quickly replaced by macrophages in the fracture hematoma. In osteomyelitis, they remain present in high numbers both locally and systemically, outnumbering monocytes and lymphocytes. Recent reports have shed light on possible mechanisms by which neutrophils could inhibit callus formation. First, a sustained presence of neutrophils will dysregulate the osteogenic differentiation and matrix production of bone progenitor cells. Second, sustained neutrophil activity can limit the recruitment of monocytes/macrophages to the defect site, thus delaying the angiogenic and osteogenic responses.

The histological appearance of the fractured bone has a large impact on its mechanical stability. Beyond the four-week time point, fibrous tissue formation and limited mineralized tissue formation is associated with biomechanically weaker bone based on torsional testing. Studies even report that biomechanical analyses could not be performed on the healing calluses due to the unexpected lack of bone union in the presence of infection. A reduced bone-implant fixation may result into further mechanical instability of the bones. Osseointegration studies have shown that bacterial contamination promotes high osteoclast activity and fibrous capsule development directly around the implant, reducing the strength of implant fixation already in the first-week.

HOW DO BACTERIAL INFECTIONS AFFECT QUANTITATIVE BONE HEALING?
Plain radiography or micro-CT has been the main method to quantify healing outcomes in osteomyelitis models. As presented in Supplementary Table S1, the results of our literature search demonstrate that the impact of bacterial infections on osteogenesis has been mainly examined using closed fracture or non-critical size osteotomies in the long bones of rodents, in combination with a stabilizing metal implant or plate. The radiographic or micro-CT outcomes of the studies show that the defects generally bridge uneventfully under asetic conditions. Strikingly, in spite of the relatively small defects that are often created, many studies report non-union at follow-up once a chronic infection is established. This negative influence of bacterial infection on healing depends on the severity of infection and correlates with the initial bacterial inoculation dose. In comparison, normal defect healing is often reported in cases where antibacterial treatment successfully eliminated all bacteria from the defect site.

WHAT IS THE EFFECT OF ANTIBACTERIAL TREATMENT ON THE HEALING OUTCOME DURING INFECTION?
Next, it was asked how antibacterial treatment restores bone healing in osteomyelitis models. The data from animal models show that complete eradication of implant-associated infections is challenging using systemic antibiotics alone, which is also not uncommon in the clinical situation. Consequently, a partial reduction in bacterial burden only leads to a moderate improvement in callus formation in rats. Although some studies have added a debridement and lavage step in their model, these interventions had little additive value in terms of the osteogenic response. In comparison to systemic treatment, the coating of osteosynthesis plates with antibacterial agents has resulted in more successful bacterial killing and prevention of implant infections. In these specific models, similar healing can be seen as in non-infected controls.

While aforementioned studies indicate that complete killing of bacteria is needed for optimal bone regeneration, there are also reports of contrasting effects of antibiotics in terms of infection or bone healing results. For example, Shiels et al. showed that local application of vancomycin could not successfully reduce the bacterial count, but nevertheless, the clinical signs of infection and radiographic bone healing were improved. In agreement, antibiotic treatment increases the effectiveness of bone-promoting growth factors without actual reduction in infection. Finally, Lovati et al. observed that a low-grade Staphylococcus aureus infection did not reveal clinical signs of infection in rats, yet, the animals displayed impaired bone healing. These studies show that there can be incongruency in the effect of antibiotics on microbiological, clinical, and bone healing results during or latent or "silent" infection, which can occur due to incomplete bacterial eradication or a persisting low-grade infection.

WHAT IS THE EFFICACY OF OSTEOINDUCTIVE PROTEINS IN INFECTED BONES?
The dual role of fracture fixation devices complicates the management of infected bone defects. On the one hand, the formation of an implant-associated biofilm contributes to the chronicity of osteomyelitis, and consequently, the removal of the fixation device facilitates bacterial clearance and gaining bone union. On the other hand, the stability provided by the fracture fixation device aids in callus formation and healing outcome. Hence, there is need of techniques that are capable of rescuing bone union under infectious conditions, but permit removal of the fixation device. Different bone grafts can be applied to promote bone healing by directing osteoconduction and/or...
osteoid; however, it is currently unclear what the effectiveness is of different bone grafts in the harsh environment of a bacterial infection. Even though autologous bone remains the gold standard bone graft, the current literature search did not yield any studies that evaluated the effectiveness of autologous bone in an osteomyelitis environment. The current section will, therefore, focus on the use of bone morphogenetic proteins (BMPs), and of which the BMP-2 (Infuse) and BMP-7 (OP-1) forms are clinically applied as bone graft extender/substitute.83

BMPs are contraindicated in the case of an active infection due to insufficient clinical comparison with autografting or allografting.84 It, therefore, remains unanswered if BMPs are suitable candidates to promote healing in case of an infection. Clinical data indicate that BMPs may be particularly effective in promoting osteogenesis when the local environment is not favorable for healing85 or when there is an increased risk of non-union.86 Several clinical trials have even indicated that the treatment of open tibial fractures with BMP-2 lowers the incidence of implant-related infections.86,87 In addition to the aforementioned clinical studies, animal studies have investigated how BMPs stimulate bone formation in the presence of a clinically relevant infection.

BMP-2/Infuse is FDA-approved for lumbar fusions, however, it is contraindicated in the case of an active infection.84 Miller et al.88 evaluated the efficacy of these proteins in the setting of an infected fusion model in rabbits. Remarkably, following contamination with only 500 colony-forming units (CFU) S. aureus, BMP-2 failed to induce spinal fusion in all 12 rabbits, while fusion occurred in all 13 non-infected control rabbits. Although clinical investigations reported successful fusion rates for BMP-2 in vertebral osteomyelitis, this discrepancy in outcome can be related to the additional debridement and antibiotic treatments performed in these patients.89,90

More numerous in vivo studies have investigated the impact of S. aureus infection on BMP-induced osteogenesis in the long bones, as overviewed in Supplementary Table S2. Critical size defects, by definition, will not heal without intervention and therefore represent the clinical scenario of a non-union where osteoinductive factors may be introduced.91 Chen et al.21,24 investigated the efficacy of BMP-7 or BMP-2 in critical-size femoral defects in rats. By performing a debridement, either with or without subsequent delivery of systemic antibiotics, the authors could compare the effectiveness of the BMPs in high-grade (i.e., without antibiotics) or low-grade (i.e., with antibiotics) infection. In their model, successful defect bridging was only realized for the highest doses (200 µg) of BMP-2 or BMP-7, and only in combination with systemic antibiotics. The BMPs induced minimal callus formation within the infected defects in the absence of antibiotics. In a comparable model, Helbig et al.32 found that neither BMP-2 or BMP-7 was effective in restoring bone healing without additional antibiotics treatment. In other critical size defect models, it was confirmed that BMP-2 was most effective in combination with local antibiotics81,92 or antibacterial nanosilver.93 A comprised of osteoinduction by BMP-2 has even been reported for relatively small defects or closed fracture models.32,93 Together, these studies suggest that a bacterial infection is detrimental for the outcome of BMP-induced bone formation in clinically relevant bone defects, but that a beneficial response to BMPs can be realized in conjunction with appropriate antibacterial treatment.

WHAT IS THE BALANCE BETWEEN THE OSTEOCLASTIC AND OSTEOBLASTIC RESPONSE DURING BACTERIAL INFECTIONS?

Paradoxically, new bone formation often occurs in parallel to the bone loss in osteomyelitis,31–35 suggesting that the local response to bacteria also activates pro-osteogenic pathways. However, it is unknown which of the two processes (i.e., osteolysis vs. osteogenesis) predominates during infection and if there are common pathways involved in both processes. While fracture models have demonstrated a clear negative correlation between bacterial burden and callus formation within the fracture, in the same studies, reactive new bone formation is often also apparent. Numerous reports even indicate a positive correlation between the measured bacterial CFU and the amount of reactive new bone formation.23,39,43,44,66,77,78 Several lines of evidence indicate that new bone formation in osteomyelitis is usually not observed in vicinity of the bacterial burden, but that it often predominates at more distant sites. In the case of plate osteosynthesis, woven bone deposition is more evident in bone regions distant to the implant-related infection, that is, opposite or further away from the plate.23,65,77,94 In the case of intramedullary fixation, bone apposition is observed in the periosteal region along the length of the implant.34,44,93 Studies that have incorporated micro-CT algorithms to quantify bone formation and bone destruction separately from each other, have confirmed that significantly enhanced bone volume is measured more peripheral to the infected site.35,40

WHAT IS THE MECHANISM OF THE OBSERVED PRO-OSTEOGENIC RESPONSE AS OBSERVED IN OSTEOMYELITIS?

Considering that the reactive bone formation starts almost immediately after the onset of infection,39 the bone deposition is unlikely the result of biomechanical adaptation to compensate for osteolysis. Alternatively, it is known that pro-inflammatory signals can directly target bone-lining osteoprogenitor and immune cells to propagate osteogenesis,39,95,96 which can occur uncoupled from an increased osteoclast activity.39,97,98 The finding that infection-induced bone formation is enhanced in mice with metabolic syndrome strengthens
the hypothesis that the inflammatory milieu is a key mediator of the osteogenic response.\textsuperscript{41}

The same cytokines that are needed for efficient antibacterial immune responses may also drastically affect the activity of bone cells.\textsuperscript{99} It can be reasoned that the inflammatory response in bacterial infection is a double-edge sword in terms of its opposite effects on osteogenesis. On the one hand, a mild inflammatory milieu distant from the infection site may stimulate osteogenesis,\textsuperscript{50,100,101} resembling the normal bone healing response after injury.\textsuperscript{95} To illustrate, the pro-inflammatory cytokines TNF-α and interleukin (IL)-17 are upregulated during bacterial infection,\textsuperscript{22} and their transient expression is known to have pro-osteogenic effects on osteoprogenitor cells.\textsuperscript{50,100,102,103} On the other hand, increased cell death, comprised vascularization, and uncontrolled osteoclast activity will be most profound in the vicinity of the bacterial burden.\textsuperscript{17,14,15,17}

Moreover, the increased influx of immune cells can lead to the production of soluble factors hampering osteogenesis.\textsuperscript{104} Histology performed on infected bone tissue generally shows a high number of neutrophils, and also monocytes/macrophages to a lesser extent.\textsuperscript{35,41,68}

Under acute or mild inflammatory conditions, neutrophils contribute to bone fracture healing via yet unknown mechanisms.\textsuperscript{60,105} The finding that neutrophils inhibit the mineralized extracellular matrix production by MSC might explain how their prolonged activity at the infected tissue impairs the early reparative phase of fracture healing.\textsuperscript{74} In the same line of reasoning, macrophages are considered the most important immune regulators in bone regeneration,\textsuperscript{62,72} but can inhibit osteogenesis via IL-1β secretion during exaggerated inflammation. In this context, the activation of various pattern recognition receptors (PRRs) in osteomyelitis may be an important shared feature of the different, sometimes paradoxical, cellular responses seen in osteomyelitis. In recent years, it has been shown that PRRs not only play a key role in the antibacterial immune response,\textsuperscript{106} but that they also modulate the osteoblastic and osteoclastic responses.\textsuperscript{49,99}

**SUMMARY**

Animal studies collectively show a strong negative association between bacterial infection and bone regeneration. In the long bones, callus formation is impaired and the defect is instead replaced by fibrous tissue formation characteristic of an atrophic non-union, even in the case of non-critical size defects. The lack of fibrocartilage and woven bone formation leads to significantly reduced mechanical stability. Neutrophils are the major immune cell type in the infected tissue, and their prolonged presence is associated with an abnormal bone healing response. The histopathology results agree with radiography or micro-CT imaging, showing that osteomyelitis-related bone changes are consistent among different animal models and humans. It should be noted that the outcomes of radiographic or histological scoring systems are not related to the actual bacterial counts, limiting them as a tool to assess the effectiveness of antibacterial strategies.

It was found that even relatively small defects do not consolidate when infected, despite the use of various fixation or stabilization techniques. Instead, the following prerequisites were found to exist to achieve successful bone regeneration of contaminated defects: for small defects—that is, closed fractures and small osteotomies—full bridging requires complete elimination of infection by adequate antibacterial treatment. For large segmental defects, an optimal environment for osteogenesis requires a combinatorial approach with antibiotics and an osteoinductive graft.

Osteoinductive BMPs support promote bone healing in rodent infection models, but relatively high amounts of BMPs are needed to overcome the detrimental effects of bacteria on bone healing.\textsuperscript{92,107–109} This inevitably leads to increasing concerns about unwanted effects at supraphysiologic doses, such as the possibility for complications and ectopic bone formation.\textsuperscript{83} Considering the species-specific requirements in the minimal BMP-2 dose,\textsuperscript{110} more elaborate large animal studies are needed to answer whether BMPs are appropriate bone grafts in case of a clinically relevant infection. Even though it is the gold standard bone graft, it is impossible to draw any conclusions regarding the use of autologous bone transplantation in the context of osteomyelitis due to a gap in the current literature.

Paradoxically, animal studies show that antibiotics generally improve bone healing compared with untreated controls even if a "silent" S. aureus infection persists. Antibiotics may, therefore, inhibit some detrimental effects of infection without necessarily reducing the bacterial burden. Indeed, subinhibitory concentrations of antibiotics modulate the expression of global virulence loci in S. aureus (i.e., SarA, Sae, and Agr).\textsuperscript{111} The resulting changes in the profile of secreted virulence factors may lead to a reduced impact on tissue degradation or cell cytotoxicity.\textsuperscript{40,112,113} Alternatively, it is possible that antibiotics initially reduce the bacterial burden, allowing bone healing to be initiated before the infection reactivates.

While bacterial contamination impairs callus formation within the bone defect, excessive new bone formation is often seen more distant to the infection.\textsuperscript{21,24,114} The dual effect of infection—that is, on the one hand, impaired bone healing and on the other hand new bone formation—is likely related to a different inflammatory response in the vicinity or more distant to the bacterial burden. The high density of osteoprogenitor cells found in the periosteum, and their responsiveness to inflammatory cues, can explain why reactive bone formation occurs predominantly in this tissue.

Only under mild inflammatory conditions may pro-inflammatory cytokines or bacteria-derived antigens promote osteoblast differentiation in resident bone-lining or recruited osteoprogenitor cells. In line with this hypothesis, it has been shown that a transient
inflammatory reaction to a low-dose of bacterial stimuli promotes periosteal or ectopic bone formation, but that the sustained inflammation caused by a high-dose of bacterial stimuli leads to predominantly osteolysis or impaired ectopic bone formation.39,49,50,115

Of final note, the current review shows that most of the in vivo bone infection studies have used rodent models. On the one hand, different forms of osteosynthesis can be applied in rodents,91 with many research tools available for rodents to study the cellular and molecular aspects of bone healing. Whereas the stages of callus formation in rodents are comparable with humans, the speed of healing is species-dependent.116 To illustrate, we found reports of normal regeneration of infected defects in selective rabbit models,45,46 which could be due to the relatively high bone turnover in these particular species.116,117

The conclusions of the current review are to be interpreted in the context of the simplified models that have been used. Very few of the animal models have included all the multiple elements of traumatic bone infections in patients, that is, soft tissue damage, open fracture, or a delay in treatment. Moreover, since the current applied animal models leave the infected implant in place, they cannot support or challenge the general clinical consensus that bone healing is optimal when an infected fracture fixation system is retained,28–30 even though the basic rule in prosthetic joint infections is to remove the implant to facilitate bacterial eradication.1 As another limitation of the current bone infection models, they almost exclusively use S. aureus as the causative microorganism of infection, whereas in clinical practice, S. aureus is responsible for 30% of the fracture-related infections.118 As bacterial species express unique molecules associated with osteoclastogenesis or osteoblastogenesis, the effect of bacterial infection on bone remodeling, regeneration, and new bone formation is likely very species-dependent.51–53,119 Finally, inter-individual variations in the immune system are also not well-reflected in animal studies due to genetic similarities and housing conditions, even though immune status determines both resistance to bacterial challenge and bone healing capacity.120,121

AUTHORS’ CONTRIBUTION
H.C.V. conceived the original idea. H.C.V. and M.C. made the research design. M.C. performed the literature research and wrote the manuscript in consultation with B.C.H. and H.C.V. All authors have read and approved the final submitted manuscript.

ACKNOWLEDGMENTS
The funding source had no role in the design, preparation, or submission of the manuscript. None of the authors received payments or services from a third party for the submitted work.

REFERENCES
1. Lew DP, Waldvogel FA. 2004. Osteomyelitis. Lancet 364: 369–379.
2. Roojakkers SH, van Kessel KP, van Strijp JA. 2005. Staphylococcal innate immune evasion. Trends Microbiol 13:596–601.
3. Darouiche RO. 2004. Treatment of infections associated with surgical implants. N Engl J Med 350:1422–1429.
4. Nair SP, Meghi S, Wilson M, et al. 1996. Bacterially induced bone destruction: mechanisms and misconceptions. Infect Immun 64:2371–2380.
5. Takayanagi H, Ogasawara K, Hida S, et al. 2000. T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. Nature 408:600–605.
6. Claro T, Widaa A, O’Searghda M, et al. 2011. Staphylococcus aureus protein A binds to osteoblasts and triggers signals that weaken bone in osteomyelitis. PLoS ONE 6:e18748.
7. Montonen M, Li TF, Lukinmaa PL, et al. 2006. RANKL and cathepsin K in diffuse sclerosing osteomyelitis of the mandible. J Oral Pathol Med 35:620–625.
8. Ellington JK, Reilly SS, Ramp WK, et al. 1999. Mechanisms of Staphylococcus aureus invasion of cultured osteoblasts. Microb Pathog 26:317–332.
9. Hudson MC, Ramp WK, Nicholson NC, et al. 1995. Internalization of Staphylococcus aureus by cultured osteoblasts. Microb Pathog 19:409–419.
10. Gasper NA, Petty CC, Schrum LW, et al. 2002. Bacterium-induced CXCL10 secretion by osteoblasts can be mediated in part through toll-like receptor 4. Infect Immun 70:4075–4082.
11. Marriott I, Gray DL, Tranguch SL, et al. 2004. Osteoblasts express the inflammatory cytokine interleukin-6 in a murine model of Staphylococcus aureus osteomyelitis and infected human bone tissue. Am J Pathol 164:1399–1406.
12. Marriott I, Rati DM, McCall SH, et al. 2005. Induction of Nod1 and Nod2 intracellular pattern recognition receptors in murine osteoblasts following bacterial challenge. Infect Immun 73:2967–2973.
13. Somayaji SN, Ritchie S, Sahraei M, et al. 2008. Staphylococcus aureus induces expression of receptor activator of NF-κB ligand and prostaglandin E2 in infected murine osteoblasts. Infect Immun 76:6810–6815.
14. Marriott I. 2013. Apoptosis-associated uncoupling of bone formation and resorption in osteomyelitis. Front Cell Infect Microbiol 3:101.
15. Wiggers EC, Johnson W, Tucci M, et al. 2011. Biochemical and morphological changes associated with macrophages and osteoclasts when challenged with infection—biomed 2011. Biomed Sci Instrum 47:183–188.
16. Alexander EH, Rivera F, Marriott I, et al. 2003. Staphylococcus aureus-induced tumor necrosis factor-related apoptosis-inducing ligand expression mediates apoptosis and caspase-8 activation in infected osteoblasts. BMC Microbiol 3:5.
17. Tucker KA, Reilly SS, Leslie CS, et al. 2000. Intracellular Staphylococcus aureus induces apoptosis in mouse osteoblasts. FEBS Microbiol Lett 186:151–156.
18. Young AB, Cooley ID, Chauhan VS, et al. 2011. Causative agents of osteomyelitis induce death domain-containing TNF-related apoptosis-inducing ligand receptor expression on osteoblasts. Bone 48:857–863.
19. Liu Y, Wang L, Kikuiru T, et al. 2011. Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-γ and TNF-α. Nature Med 17:1594–1601.
20. Uckay I, Assal M, Legout L, et al. 2006. Recurrent osteomyelitis caused by infection with different bacterial strains without obvious source of reinfection. J Clin Microbiol 44: 1194–1196.
21. Chen X, Schmidt AH, Tsukayama DT, et al. 2006. Recombinant human osteogenic protein-1 induces bone formation in a.

JOURNAL OF ORTHOPAEDIC RESEARCH© OCTOBER 2019
chronically infected, internally stabilized segmental defect in the rat femur. J Bone Joint Surg Am 88:1510–1523.

22. Rochford ET, Sabaté brescó M, Zeiter S, et al. 2016. Monitoring immune responses in a mouse model of fracture fixation with and without Staphylococcus aureus osteomyelitis. Bone 83:82–92.

23. Zheng Z, Yin W, Zara JN, et al. 2010. The use of BMP-2 coupled—nansilver-PGLA composite grafts to induce bone repair in grossly infected segmental defects. Biomaterials 31:9293–9300.

24. Chen X, Schmidt AH, Mahjouri S, et al. 2007. 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 21:693–700.

25. Bilgili F, Balci HI, Karayigit K, et al. 2015. Can normal fracture healing be achieved when the implant is retained on the basis of infection? An experimental animal model. Clin Orthop Relat Res 473:3190–3196.

26. Bonsignore LA, Anderson JR, Lee Z, et al. 2013. Adherent lipo-polysaccharide inhibits the osseointegration of orthopedic implants by impairing osteoblast differentiation. Bone 52:93–101.

27. Harris LG, Richards RG. 2006. Staphylococci and implant surfaces: a review. Injury 37(Suppl 2):S3–S14.

28. Claes LE, Heigle CA, Neidlinger-Wilke C, et al. 1998. Effects of mechanical factors on the fracture healing process. Clin Orthop Relat Res 355:S132–S147.

29. Klein P, Schell H, Streitparth F, et al. 2003. The initial phase of fracture healing is specifically sensitive to mechanical conditions. J Orthop Res 21:662–669.

30. Mark H, Nilsson A, Nannmark U, et al. 2004. Effects of fracture fixation stability on ossification in healing fractures. Clin Orthop Relat Res 419:245–259.

31. Odekerken JC, Arts JJ, Surtel DA, et al. 2013. A rabbit osteomyelitis model for the longitudinal assessment of early post-operative implant infections. J Orthop Surg 8:38.

32. Li D, Gromov K, Seballe K, et al. 2008. Quantitative mouse model of implant-associated osteomyelitis and the kinetics of microbial growth, osteolysis, and humoral immunity. J Orthop Res 26:96–105.

33. Shiels SM, Bedigrew KM, Wenke JC. 2015. Development of a hematogenous implant-related infection in a rat model. BMC Musculoskelet Disord 16:255.

34. Alt V, Lips KS, Henkenbehrens C, et al. 2011. A new animal model for implant-related infected non-unions after intra-medullary fixation of the tibia in rats with fluorescent in situ hybridization of bacteria in bone infection. Bone 48:1146–1153.

35. Stadelmann VA, Potapova I, Camenisch K, et al. 2015. In vivo MicroCT monitoring of osteomyelitis in a rat model. BioMed Res Int 2015:1–12.

36. Kothari NA, Pelchovitz DJ, Meyer JS. 2001. Imaging of musculoskeletal infections. Radiol Clin North Am 39:653–671.

37. Pineda C, Espinosa R, Pena A. 2009. Radiographic imaging in osteomyelitis: the role of plain radiography, computed tomography, ultrasonography, magnetic resonance imaging, and scintigraphy. Semin Plast Surg 23:80–89.

38. Lovati AB, Drago L, Monti L, et al. 2013. Diabetic mouse model of orthopaedic implant-related Staphylococcus aureus infection. PLoS ONE 8:e67628.

39. Croes M, Boot W, Kruyt MC, et al. 2017. Inflammation-induced osteogenesis in a rabbit tibia model. Tissue Eng Part C Methods 23:673–685.

40. Cassat JE, Hammer ND, Campbell JP, et al. 2013. A secreted bacterial protease tailors the Staphylococcus aureus virulence repertoire to modulate bone remodeling during osteomyelitis. Cell Host Microbe 13:759–772.

41. Farnsworth CW, Schott EM, Bennie AM, et al. 2018. Obesity/type-2 diabetes increases inflammation, periostealreactive bone formation, and osteolysis during Staphylococcus aureus implant-associated bone infection. J Orthop Res 36:1614–1623.

42. Helbig L, Omlor GW, Ivanova A, et al. 2018. Bone morphogenetic proteins-7 and -2 in the treatment of delayed osseous union secondary to bacterial osteitis in a rat model. BMC Musculoskelet Disord 19:261.

43. Lovati AB, Romanó CL, Bottagisio M, et al. 2016. Modeling Staphylococcus epidemium-induced non-unions: subclinical and clinical evidence in rats. PLoS ONE 11:e0147447.

44. Robinson DA, Bechtold JE, Carlson CS, et al. 2011. Development of a fracture osteomyelitis model in the rat femur. J Orthop Res 29:131–137.

45. Ambrose CG, Clyburn TA, Louden K, et al. 2004. Effective treatment of osteomyelitis with biodegradable microspheres in a rabbit model. Clin Orthop Relat Res 421:293–299.

46. Andriele VT, Nagel DA, Southwick WO. 1974. Chronic staphylococcal osteomyelitis: an experimental model. Yale J Biol Med 47:33–39.

47. Inzana JA, Schwarz EM, Kates SL, et al. 2015. 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 72:128–136.

48. Croes M, Bakhshandeh S, van Hengel IAJ, et al. 2018. Antibacterial and immunogenic behavior of silver coatings on additively manufactured porous titanium. Acta Biomater 81:315–327.

49. Croes M, Kruyt MC, Boot W, et al. 2019. The role of bacterial stimuli in inflammation-driven bone formation. Eur Cell Mater 37:402–419.

50. Croes M, Kruyt MC, Loonen L, et al. 2017. Local induction of inflammation affects bone formation. Eur Cell Mater 33:211–226.

51. Krischer T, Bar-Shavit Z. 2014. Regulation of osteoclastogenesis by integrated signals from toll-like receptors. J Cell Biochem 115:2146–2154.

52. Reikerås O, Shegarfi H, Wang JE, et al. 2005. Lipo-polysaccharide imparts fracture healing: an experimental study in rats. Acta Orthop 76:749–753.

53. Reikerås O, Wang JE, Foster SJ, et al. 2007. Staphylococcus aureus peptidoglycan impairs fracture healing: an experimental study in rats. J Orthop Res 25:262–266.

54. Einhorn TA. 1998. The cell and molecular biology of fracture healing. Clin Orthop Relat Res 355:S7–S21.

55. Claes L, Recknagel S, Ignatius A. 2012. Fracture healing as a model system for tissue regeneration. Expert Opin Biol Ther 14:247–259.

56. Claes L, Reuss M, Göckelmann M, et al. 2011. Metaphyseal fracture healing follows similar biomechanical rules as diaphyseal healing. J Orthop Res 29:425–432.

57. Sandberg O, Aspengren P. 2015. Different effects of indomethacin on healing of shaft and metaphyseal fractures. Acta Orthop 86:243–247.

58. Loi F, Córdova LA, Pajarinj J, et al. 2016. Inflammation, fracture, and bone repair. Bone 86:119–130.

59. Schmidt-Bleek K, Petersen A, Dienelt A, et al. 2014. Initiation and early control of tissue regeneration - bone healing as a model system for tissue regeneration. Expert Opin Biol Ther 14:247–259.

60. Kottgen A, Bergdolt S, Wiegrner R, et al. 2016. The crucial role of neutrophil granulocytes in bone fracture healing. Eur Cell Mater 32:152–162.

61. Bastian O, Pillay J, Allblas J, et al. 2011. Systemic inflammation and fracture healing. J Leukoc Biol 89:669–673.
62. Sinder BP, Pettit AR, McCauley DK. 2015. Macrophages: their emerging roles in bone. J Bone Miner Res 39: 2140–2149.
63. Marsell R, Einhorn TA. 2011. The biology of fracture healing. Injury 42:551–555.
64. Arens D, Wilke M, Calabro L, et al. 2015. A rabbit humerus model of plating and nailing osteosynthesis with and without *Staphylococcus aureus* osteomyelitis. Eur Cell Mater 30:148–161.
65. Stewart S, Barr S, Engiles J, et al. 2012. 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 94:1406–1415.
66. Li GY, Yin JM, Ding H, et al. 2013. Efficacy of leukocyte-porate and platelet-rich plasma gel (L-PRP gel) in treating osteomyelitis in a rabbit model. J Orthop Res 31:949–956.
67. Lucke M, Schmidmaier G, Sadoni S, et al. 2003. A new model of implant-related osteomyelitis in rats. J Biomed Mater Res B Appl Biomater 67:593–602.
68. Windolf CD, Meng W, Lögters TT, et al. 2013. Implant-associated localized osteitis in murine femur fracture by biofilm forming *Staphylococcus aureus*: a novel experimental model. J Orthop Res 31:2013–2020.
69. Antunes MB, Feldman MD, Cohen NA, et al. 2007. Dose-dependent effects of topical tobramycin in an animal model of *Pseudomonas sinuatis*. Am J Rhinol 21:423–427.
70. Chung R, Cool JC, Scherer MA, et al. 2012. Roles of neutrophil-mediated inflammatory response in the bony repair of injured plate graft cartilage in young rats. J Leukoc Biol 80:1272–1280.
71. Andrew JG, Andrew S, Freemont A, et al. 1994. Inflammatory cells in normal human fracture healing. Acta Orthop Scand 65:462–466.
72. Schmidt-Bleek K, Kwee BJ, Mooney DJ, et al. 2015. Bone and bone of inflammation in bone tissue regeneration and its link with angiogenesis. Tissue Eng Part B Rev 21:354–364.
73. Bastian OW, Croes M, Alblas J, et al. 2018. Neutrophils inhibit synthesis of mineralized extracellular matrix by human bone marrow-derived stromal cells in vitro. Front Immunol 9:945.
74. Helbig L, Guehring T, Rosenberger S, et al. 2015. A new animal model for delayed osseous union secondary to osteitis. BMC Musculoskelet Disord 16:362.
75. Büren C, Lögters T, Oezel L, et al. 2018. Effect of hyperbaric oxygen therapy (HBO) on implant-associated osteitis in a femur fracture model in mice. PLoS ONE 13:e0191594.
76. Johnson CT, Wroe JA, Agarwal R, et al. 2018. Hydrogel delivery of lysostaphin eliminates orthopedic implant infection by *Staphylococcus aureus* and supports fracture healing. Proc Natl Acad Sci USA 115:E4960–E4969.
77. Windolf CD, Lögters T, Scholz M, et al. 2014. Lysostaphin-coated titan-implants preventing localized osteitis by *Staphylococcus aureus* in a mouse model. PLoS ONE 9:e115940.
78. Hamza T, Dietz M, Pham D, et al. 2013. Intra-cellular *Staphylococcus aureus* alone causes infection in vivo. Eur Cells Mater 25:341–350.
79. Byren I, Bejon P, Atkins BL, et al. 2009. One hundred and twelve infected arthroplasties treated with ‘DAIR’ (debridement, antibiotics and implant retention): antibiotic duration and outcome. J Antimicrob Chemother 63:1264–1271.
80. Shiels SM, Tennent DJ, Wenke JC. 2018. Topical rifampin powder for orthopaedic trauma part I: Rifampin powder reduces recalcitrant infection in a delayed treatment musculoskeletal trauma model. J Orthop Res 36:3136–3141.
81. Stewart RL, Cox JT, Volgas D, et al. 2010. The use of a biodegradable, load-bearing scaffold as a carrier for antibiotics in an infected open fracture model. J Orthop Trauma 24:587–591.
82. Moojen DJF, van Hellemont G, Vogely HC, et al. 2010. Incidence of low-grade infection in aseptic loosening of total hip arthroplasty. Acta Orthop 81:667–673.
83. Hustedt JW, Blizzard DJ. 2014. The controversy surrounding bone morphogenetic proteins in the spine: a review of current research. Yale J Biol Med 87:549–561.
84. Medtronic. 2019. Infuse Package Insert.
85. Giannoudis PV, Dinopoulos HT. 2010. Autologous bone graft: when shall we add growth factors? Foot Ankle Clin 15:597–609.
86. Govender S, Casima C, Genant HK, et al. 2002. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J Bone Joint Surg Am 84:2123–2134.
87. Swintokowski MF, Aro HT, Donell S, et al. 2006. Recombinant human bone morphogenetic protein-2 in open tibial fractures: a subgroup analysis of data combined from two prospective randomized studies. J Bone Joint Surg Am 88:1258–1265.
88. Miller CP, Simpson AK, Whang PG, et al. 2009. Effects of recombinant human bone morphogenetic protein 2 on surgical infections in a rabbit posterolateral lumbar fusion model. Am J Orthop 38:578–584.
89. Allen RT, Lee YP, Stimson E, et al. 2007. Bone morphogenetic protein-2 (BMP-2) in the treatment of pyogenic vertebral osteomyelitis. Spine 32:2996–3006.
90. O’Shaughnessy BA, Kuklo TR, Ondra SL. 2008. Surgical treatment of vertebral osteomyelitis with recombinant human bone morphogenetic protein-2. Spine 33:E132–E139.
91. García P, Histing T, Holstein JH, et al. 2013. Rodent animal models of delayed bone healing and non-union formation: a comprehensive review. Eur Cell Mater 26:1–12.
92. Guelcher SA, Brown KV, Li B, et al. 2011. Dual-purpose bone grafts improve healing and reduce infection. J Orthop Trauma 25:477–482.
93. Schindeler A, Yu NYC, Cheng TL, et al. 2015. Local delivery of the cationic steroid antibiotic CSA-90 enables osseous union in a rat open fracture model of *Staphylococcus aureus* infection. J Bone Joint Surg Am 97:302–309.
94. Rochford ETJ, Richards RG, Moriarty TF. 2012. Influence of material on the development of device-associated infections. Clin Microbiol Infect 18:1162–1167.
95. Lin Z, Fateh A, Salem DM, Intini G. 2014. Periosteum: biology and applications in craniofacial bone regeneration. J Dent Res 93:109–116.
96. Chang MK, Raggatt LJ, Alexander KA, et al. 2008. Osteal inflammation and up-regulation of the cationic steroid antibiotic CSA-90 enables osseous union in a rat open fracture model of *Staphylococcus aureus* infection. J Bone Joint Surg Am 97:302–309.
97. Roche E, Richards RG, Moriarty TF. 2012. Influence of material on the development of device-associated infections. Clin Microbiol Infect 18:1162–1167.
98. Min N, Fateh A, Salem DM, Intini G. 2014. Periosteum: biology and applications in craniofacial bone regeneration. J Dent Res 93:109–116.
99. Chang MK, Raggatt LJ, Alexander KA, et al. 2008. Osteal inflammation and up-regulation of the cationic steroid antibiotic CSA-90 enables osseous union in a rat open fracture model of *Staphylococcus aureus* infection. J Bone Joint Surg Am 97:302–309.
100. Roche E, Richards RG, Moriarty TF. 2012. Influence of material on the development of device-associated infections. Clin Microbiol Infect 18:1162–1167.
101. Min N, Fateh A, Salem DM, Intini G. 2014. Periosteum: biology and applications in craniofacial bone regeneration. J Dent Res 93:109–116.
100. Mountziaris PM, Spicer PP, Kasper FK, et al. 2011. Harnessing and modulating inflammation in strategies for bone regeneration. Tissue Eng Part B Rev 17:393–402.

101. Thomas MV, Puleo DA. 2011. Infection, inflammation, and bone regeneration: a paradoxical relationship. J Dent Res 90:1052–1061.

102. Ono T, Okamoto K, Nakashima T, et al. 2016. IL-17-producing γδ T cells enhance bone regeneration. Nat Commun 7:10928.

103. Croes M, Kruyt MC, Groen WM, et al. 2018. Interleukin 17 enhances bone morphogenetic protein-2-induced ectopic bone formation. Sci Rep 8:7269.

104. Martino MM, Maruyama K, Kuhn GA, et al. 2016. Inhibition of IL-1R1/MyD88 signalling promotes mesenchymal stem cell-driven tissue regeneration. Nat Commun 7:11051.

105. Bastian OW, Koenderman L, Abelas J, et al. 2016. Neutrophils contribute to fracture healing by synthesizing fibronectin+ extracellular matrix rapidly after injury. Clin Immunol (Orlando, Fla) 164:78–84.

106. Pasare C, Medzhitov R. 2005. Advances in experimental medicine and biology. Adv Exp Med Biol 560:11–18.

107. Angle SR, Sena K, Sumner DR, et al. 2012. Healing of rat femoral segmental defect with bone morphogenetic protein-2: a dose response study. J Musculoskelet Neuronal Interact 12:28–37.

108. van der Stok J, Koolen MK, de Maat MP, et al. 2015. Full regeneration of segmental bone defects using porous titanium implants loaded with BMP-2 containing fibrin gels. Eur Cell Mater 29:141–153.

109. Williams JC, Maitra S, Anderson MJ, et al. 2015. BMP-7 and bone regeneration: evaluation of dose-response in a rodent segmental defect model. J Orthop Trauma 29:e336–e341.

110. Zara JN, Siu RK, Zhang X, et al. 2011. High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo. Tissue Eng Part A 17:1389–1399.

111. Hodille E, Rose W, Diep BA, et al. 2017. The role of antibiotics in modulating virulence in Staphylococcus aureus sarA mutants. Mol Microbiol 92:1299–1312.

112. Loughran AJ, Gaddy D, Beenken KE, et al. 2016. Impact of sarA and phenol-soluble modulins on the pathogenesis of osteomyelitis in diverse clinical isolates of Staphylococcus aureus. Infect Immun 84:2586–2594.

113. Chen X, Kidder LS, Lew WD. 2002. Osteogenic protein-1 induced bone formation in an infected segmental defect in the rat femur. J Orthop Res 20:142–150.

114. Croes M, Oner FC, Kruyt MC, et al. 2015. Proinflammatory mediators enhance the osteogenesis of human mesenchymal stem cells after lineage commitment. PLoS ONE 10:e0132781.

115. Gomes PS, Fernandes MH. 2011. Rodent models in bone-related research: the relevance of calvarial defects in the assessment of bone regeneration strategies. Lab Anim 45:14–24.

116. Loughran AJ, Qureshi AT, Hope DN, et al. 2015. Bioburden increases heterotopic ossification formation in an established rat model. Clin Orthop Relat Res 473:2840–2847.

117. Netea MG, Joosten LAB, Latz E, et al. 2016. Trained immunity: a program of innate immune memory in health and disease. Science (New York, NY) 352:aaf1098.

118. Reinke S, Geissler S, Taylor WR, et al. 2013. Terminally differentiated CD8(+) T cells negatively affect bone regeneration in humans. Sci Trans Med 5:177.

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of this article.