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Biomaterials approaches to treating implant-associated osteomyelitis

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Abstract

Orthopaedic devices are the most common surgical devices associated with implant-related infections and *Staphylococcus aureus* (*S. aureus*) is the most common causative pathogen in chronic bone infections (osteomyelitis). Treatment of these chronic bone infections often involves combinations of antibiotics given systemically and locally to the affected site via a biomaterial spacer. The gold standard biomaterial for local antibiotic delivery against osteomyelitis, poly(methyl methacrylate) (PMMA) bone cement, bears many limitations. Such shortcomings include limited antibiotic release, incompatibility with many antimicrobial agents, and the need for follow-up surgeries to remove the non-biodegradable cement before surgical reconstruction of the lost bone. Therefore, extensive research pursuits are targeting alternative, biodegradable materials to replace PMMA in osteomyelitis applications.

Herein, we provide an overview of the primary clinical treatment strategies and emerging biodegradable materials that may be employed for management of implant-related osteomyelitis. We performed a systematic review of experimental biomaterials systems that have been evaluated for treating established *S. aureus* osteomyelitis in an animal model. Many experimental biomaterials were not decisively more efficacious for infection management than PMMA when delivering the same antibiotic. However, alternative biomaterials have reduced the number of follow-up surgeries, enhanced the antimicrobial efficacy by delivering agents that are incompatible with PMMA, and regenerated bone in an infected defect. Understanding the advantages, limitations, and potential for clinical translation of each biomaterial, along with the conditions under which it was evaluated (e.g. animal model), is critical for surgeons and researchers to navigate the plethora of options for local antibiotic delivery.

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Keywords

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1. Introduction to implant-associated osteomyelitis

Osteomyelitis (OM) refers to a microbial pathogen, usually bacteria, infecting the bone, which results in an inflammatory reaction that leads to bone destruction (osteolysis) [74, 118]. The bone infection can develop by direct contamination (e.g. open fractures) or by spreading either via the blood stream (hematogenously) or from a contiguous site or implant. Acute bone infections sometimes progress to chronic infections (clinically referred to as OM). The diagnosis of OM and identification of the infecting pathogen may require multiple bone biopsies. However, the heterogeneity of bone colonization can result in false-negative cultures [74]. Routine systemic antimicrobial therapy is typically sufficient to clear an acute bone infection; but chronic OM can be extremely difficult to treat and requires radical surgical debridement of the necrotic and infected tissues, followed by extensive courses of antibiotics [28, 74, 132, 148].

OM is often associated with an orthopaedic implant, such as a prosthetic joint or fracture fixation device. As of 2004, there were approximately 600,000 artificial joint replacements and 2 million fracture fixation devices implanted each year in the U.S. alone, resulting in >110,000 infections [28]. Further, 65% of military injuries are orthopaedic in nature, with infection rates as high as 50% [89]. The most prevalent causative pathogen in bone infection cases is *Staphylococcus aureus* (*S. aureus*), which is a highly opportunistic species that can be extremely difficult to treat [28, 150]. In fact, *S. aureus* is associated with the highest rates of recurrent OM, even when this organism was not present in the initial positive bacterial cultures [145].

The primary challenge in treating implant-associated *S. aureus* infections is often attributed to the formation of biofilm on the indwelling device and within the bone [28, 148]. A biofilm is a surface-attached bacterial community embedded within dense extracellular matrix. *S. aureus* adheres to the biomaterial implants and host tissues by expressing adhesins that interact with host extracellular matrix proteins, such as fibronectin and collagen [7, 38], or by direct contact with the implant material through hydrophobic or electrostatic interactions that are mediated by molecules such as autolysin and teichoic acid [36]. Maturation of the biofilm proceeds through bacterial proliferation and intercellular adhesion, which is primarily achieved through excretion of polysaccharide intercellular adhesin (PIA) – a major component of the biofilm extracellular matrix [98]. Bacteria within a biofilm can evade the host immunological response, and often transition to a dormant or quiescent state [106]. Considering that many mechanisms of action of antimicrobials interfere with bacterial metabolism or proliferation, the dormancy of bacteria within the biofilm can further impair antibiotic efficacy [63]. Indeed, the minimum inhibitory concentration (MIC) of *S. aureus* in biofilm can be orders of magnitude higher than its planktonic counterparts [85]. Additionally, *S. aureus* has been reported to internalize within host cells, such as osteoblasts, which provides another potential mechanism to evade the host defenses [14].

Due to the dramatic morbidity suffered by patients afflicted with *S. aureus* OM and the extreme challenge imposed on the surgeons for treatment, extensive research efforts aim to improve therapeutic interventions. Much of the focus has been on developing novel antimicrobial biomaterial systems to help eradicate an established bone infection. The Holy Grail is to develop a biomaterial system to not only clear the infection but to also contribute to the subsequent bone regeneration process. Herein, we summarize the current techniques for treatment of implant-associated osteomyelitis and systematically review the progress in biomaterials-based treatments along with the animal models that are employed to evaluate these experimental treatments.

2. Current treatment strategies for established bone infections

2.1. Surgical and medical approaches

A revision surgery that involves aggressive debridement of the pathologic bone and soft tissue, often according to oncologic principles, is a critical component in treating chronic OM. That is, complete excision of the infected tissues provides the greatest chance for reliable eradication of an infection. This radical debridement process often results in a large bone and soft tissue defect, where the dead space must be effectively managed to help reduce the chance of reinfection. Dead space management typically involves the implantation of a temporary biomaterial spacer for local delivery of antibiotics or transplantation of vascularized tissue such as a bone graft or soft tissue flaps [18, 46].

Despite the aggressive tissue debridement, the spatial heterogeneity of bacterial colonization in the bone and the surrounding tissue makes it impossible to ensure complete elimination. Therefore, systemic antimicrobial treatment is still essential. Antibiotics may be administered intravenously for the first 2–4 weeks after the revision surgery, followed by oral therapy that can last an additional 8–10 weeks [132, 133, 148]. During revision, the surgeon may retain the implant, perform a partial exchange (e.g. replace the plastic components of an artificial joint), or completely exchange the device. The choice of implant retention versus exchange depends on multiple factors and the decision algorithms can vary between artificial joints and fracture fixation devices. In general, clinical algorithms recommend retention of the implant if it is well fixed into the bone, the patient is being treated within 2–3 weeks of presenting infection symptoms, and the infection site has undergone aggressive surgical debridement [28, 133]. Complete exchange may be preferred, however, if the bacterial cultures are positive for resistant (e.g. methicillin-resistant *S. aureus*; MRSA) or otherwise challenging organisms (e.g. small-colony variants of *Staphylococci*) [132].

The choice of antibiotics is also a critical factor that largely depends on the infecting organism and its susceptibility. For an in-depth discussion on antibiotics for treating osteomyelitis, the interested reader is referred to the excellent comprehensive review of the topic [122]. In cases where the orthopaedic implant will be retained, rifampin has been regarded as an essential component of the therapy [28, 76, 150], considering that this antibiotic may be effective against *Staphylococci* in biofilm [85, 128]. Rifampin must always be combined with another antibiotic, because bacteria can develop resistance to rifampin very rapidly when it is used as a monotherapy [136, 150].

3. Biomaterials approaches

3.1. Non-biodegradable materials

Poly(methyl methacrylate) (PMMA) bone cement is the gold standard biomaterial for local antibiotic therapy in orthopaedics and has been used for over 35 years for both prophylaxis and therapy. Seminal studies conducted by Buchholz and Engelbrecht in 1970 [17] and Klemm in 1979 [64] described the use of antibiotic-laden PMMA to prevent infection and treat chronic bone infections, respectively. PMMA bone cements containing antibiotics are commercially available and contain either gentamicin or tobramycin at low doses (0.5–1 g per 40 g cement), which are sufficient for prophylaxis, but not for therapeutic applications in established OM [56]. In contrast, Septopal is a commercially available chain of gentamicin-impregnated PMMA beads (7.5 mg gentamicin/bead) that have successfully been used for infection treatment [19, 90, 100, 139]; but Septopal is not currently approved in many countries, including the U.S. Therefore, many surgeons still intraoperatively mix antibiotics into PMMA and mold beads or spacer blocks for implantation into a septic bone defect during a revision procedure [56, 140].

Unfortunately, the surgeon's options of antibiotics to mix into PMMA can be limited. The most common antibiotics to mix into PMMA are gentamicin, tobramycin, vancomycin, or cephalosporins [56]. Some antibiotics are incompatible with PMMA because they are heat-labile and cannot withstand the exothermic polymerization reaction [140]. Other antibiotics, including rifampin, are incompatible with PMMA due to their ability to scavenge free radicals and impair PMMA polymerization [10].

The elution kinetics of antibiotics from PMMA are highly variable and depend on the type of antibiotic as well as the mixing procedure and additives, which affect the porosity of the PMMA spacer. Combinations of antibiotics in PMMA, such as tobramycin plus vancomycin, can produce higher in vitro elution efficiency than when either is incorporated alone [102]; but less than 10% of the incorporated antibiotic(s) is ever released [87, 102, 135], and this release typically happens in the first 3–7 days. The commercially prepared Septopal beads are an exception, as they are able to release 35% of the loaded gentamicin due to higher porosity [87]. Based on this concept, many fillers and mixing techniques have been studied to increase the PMMA porosity and release efficiency from manually prepared beads and spacers [6, 82, 83, 107], but there are no widely accepted standards for intraoperative preparation of antibiotic-laden PMMA.

In addition to the limitations on antibiotic choice and the elution kinetics and efficiency, the other major shortcoming of PMMA is that it is non-biodegradable. Consequently, it must be removed after infection management as it could impair healing of the debrided bone defect. Furthermore, PMMA has been reported to serve as an additional substratum for bacterial colonization. For example, clinical studies have found bacterial colonization of antibiotic-laden PMMA spacers during 2-stage exchange arthroplasty procedures [79, 113].

For bone defect cases, replacing a PMMA spacer with a bone graft in a second revision surgery according to the Masquelet technique (also known as the induced-membrane technique) has shown efficacy. After initial placement of the PMMA, a fibrous, vascularized

membrane that secretes angiogenic and osteoinductive growth factors forms around the foreign body [101]. Retention of this membrane during the second revision surgery, when the PMMA is exchanged for a bone graft, has been shown to improve healing of the bone defect [80]. The drawback of this approach, however, is that it requires considerable time and additional surgeries, which increase treatment costs and patient burden. Therefore, development of novel biomaterials for local antibiotic delivery has primarily focused on biodegradable solutions that could aid in subsequent bone regeneration following successful infection management.

3.2. Biodegradable and resorbable materials

At the time of this review, calcium sulfate (CS) is the primary resorbable material that has been used clinically for local antibiotic delivery. Multiple companies have made CS-based biomaterials commercially available in the U.S. as bone void fillers only, which nevertheless can be prepared to carry antibiotics for off-label use [13]. Commercial preparations of tobramycin-loaded CS are approved for clinical use in many other countries, such as Canada and throughout Europe. Antibiotic-laden CS has been utilized to treat patients with OM in multiple studies [35, 37, 81]. McKee and colleagues reported that the efficacy of local tobramycin delivered from CS pellets is comparable with handmade PMMA beads for treatment of chronic OM or infected non-union in a prospective, randomized clinical trial of 30 patients [81]. Clinically, infection clearance was achieved in 86% of the patients in each of the treatment groups at 24 months follow-up. However, the total number of subsequent surgical procedures was significantly reduced in the CS group compared to the PMMA group (7 CS vs. 15 PMMA, $p=0.04$). Ferguson et al. reported similar success for treatment of OM when local tobramycin-laden CS pellets were combined with systemic antibiotic treatment, with 90.8% (177/195) of patients not presenting a recurrent infection at a mean follow-up of 3.7 years (range: 1.3–7.1 years) [35].

On average, CS pellets take approximately 2–3 months to radiographically resorb [13]. These calcium-based antibiotic-delivery systems have advantages over PMMA in that they can carry a wider range of antibiotics and do not need a second surgery to remove them. Clinical studies consistently reported that approximately 5% of patients treated with CS pellets, with or without antibiotics, developed a seroma and fluid drainage [13, 35, 81]. This exudate is usually sterile, has not been associated with reinfection, and generally subsides without further treatment.

Other biodegradable materials have been used clinically for the local administration of antibiotics, including bioactive glass [111], calcium phosphates [54, 111, 125], collagen implants [65], demineralized bone matrix [111], and allograft bone [141]. Yet, comparing the efficacy between all of these materials is inconclusive considering that all of these therapies had at least 80% success rates in clinical reports and have not been compared head to head in a large prospective, randomized clinical trial. Studies that compared biodegradable materials with PMMA beads observed comparable rates of infection clearance and less subsequent surgeries required in the biodegradable materials groups [12, 73, 81]. Thus far, the only apparent clinical advantage of these alternative biodegradable materials over PMMA is the potential to reduce the number of follow-up surgeries.

However, this conclusion is only based on the limited clinical evidence that is currently available and large prospective, randomized clinical trials are required to better understand the efficacy of biodegradable materials for infection therapy.

4. Systematic review of experimental biomaterials for treatment of *S. aureus* bone infections

4.1. Search methods and inclusion criteria

The PubMed database was searched using the string ‘((biomaterial OR polymer OR ceramic OR cement OR gel OR allograft) AND (antibiotic) AND (bone infection OR osteomyelitis) AND (in vivo OR animal model))’ to find studies that investigated biomaterials for local antibiotic delivery in an animal model of bone infection. This search, performed on August 16, 2015, returned 232 results. Only studies that evaluated biomaterial-based treatments in an animal model with an established *S. aureus* bone infection were considered. That is, the antimicrobial biomaterial could not be implanted at the same time as inoculation, as this represents a prophylactic study rather than a therapeutic study. The presence of an orthopaedic device or implant in the animal model was not required, since many studies did not include this component and patients are sometimes treated with complete removal of the device in exchange for an antibiotic PMMA spacer (e.g. antibiotic-laden articulating knee spacers [114]). Articles in languages other than English were excluded. Studies on PMMA and calcium sulfates were excluded unless they were incorporated as an experimental composite or used as a control. Studies were also excluded if no controls were used or quantitative results were not presented. Additional studies that fit these criteria, but were not returned in the PubMed search, were identified through reference sections of reviewed articles. A total of 43 articles describing 40 unique studies were fully reviewed for discussion and are summarized in Tables 1–4.

5. Experimental biomaterials for local antibiotic delivery

5.1. Summary of biomaterials and antibiotics used in the reviewed studies

Of the reviewed studies, 40% (16/40) used synthetic polymers such as poly(D,L-lactide-co-glycolide) (PLGA), 28% (11/40) used minerals or ceramics such as hydroxyapatite (HA), 15% (6/40) used natural polymers such as collagen, and 20% (8/40) used a composite. The most common materials were PLGA (6/40) and HA in a variety of forms (7/40). These materials were most often used to locally deliver vancomycin (14/40) or gentamicin (12/40), but other studies administered ciprofloxacin (3/40), tobramycin (2/40), teicoplanin (2/40), rifampin (1/40) or other antimicrobials. Some studies evaluated more than one experimental material and more than one antibiotic. The evaluation time after placing the antibiotic-laden biomaterials ranged from 1 week to 3 months, with a median and mode of 4 weeks.

5.2. Natural Polymers

Natural polymers, such as collagen, fibrin, and chitosan, have been employed in a wide variety of drug delivery applications (Table 1). Collagen is the most extensively utilized natural polymer in orthopaedic applications because of its biocompatibility, importance as an extracellular matrix protein, its implications for tissue regeneration, and the wide

commercial availability of collagen products [1]. In fact, type I collagen is the most abundant structural protein in the human body and is a critical component of bone's extracellular matrix. Antibiotics are most commonly incorporated into collagen products by soaking the material in the antibiotic solution and allowing absorption by the hydrophilic matrix.

Fibrin is a biopolymer involved in blood clotting, which results from the polymerization of fibrinogen following cleavage by the protease thrombin. Considering the natural presence of fibrin in the healing process, fibrin is employed by surgeons as a sealant for general wound hemostasis. Fibrin sealants have also been utilized for infection prophylaxis by adding powdered antibiotic to the polymerizing fibrin gel at the site of wound closure [131].

Chitosan is a polysaccharide biopolymer that is produced through deacetylation of chitin, which is often obtained from the exoskeletons of crustaceans. Chitosan interestingly possesses innate antimicrobial properties against a broad spectrum of pathogens [127]. The antibacterial mechanism of action has not been fully elucidated; but evidence suggests that the antibacterial effects of chitosan are related to its polycationic nature, which enables interactions with negatively charged bacterial surface molecules, such as teichoic acids [105].

Antibiotic release from collagen and fibrin gels is typically characterized by a rapid bolus release, which is driven solely by diffusion. In vitro, at least 90% of the antibiotic will release within the first day, with complete elution occurring by 4 days [137, 144]. In vivo, however, the release time may be longer considering the potentially restricted fluid volume and mass transfer around the implant. Due to the short-term release profile, these biopolymers may be better suited for acute infections and prophylaxis rather than treatment of chronic infections [9]. In contrast, chitosan gels and sponges typically demonstrate a more sustained release of antibiotics that can last weeks in vitro [120]; and, as a coating to ceramics, can help control the initial bolus antibiotic release [11].

5.3. Synthetic Polymers

Synthetic polymers were the most extensively studied materials among the reviewed literature. These types of materials have gained popularity due to the ample control over release kinetics, degradation rates, predictability/quality control, and mechanical properties. Polyesters, including poly(lactic acid) (PLA), poly(caprolactone) (PCL), and poly(lactic-co-glycolic acid) (PLGA), were the most commonly employed synthetic polymers (Table 2). These materials degrade through hydrolysis of the ester linkages in a bulk erosion process. The rate of degradation for PLGA depends on the lactide:glycolide ratio, with 50:50 achieving the fastest degradation rate [78]. Antibiotic release from these materials is regulated by diffusion and hydrolytic bulk degradation of the polymer, which allows for sustained release profiles on the order of weeks to months depending on the formulation [41, 78]. When PLGA degrades, however, it is converted back to lactic and glycolic acids that lower the local pH. Depending on the degradation rate and the local fluid exchange rate, a reduction in the pH can accelerate the hydrolytic erosion process and cause an autocatalytic degradation of the polymer and accelerated release of the drugs. Erosion of the polymer that is too rapid can also result in an acidic environment that will elicit a host inflammatory

response [41] and may reduce the functional efficacy of the delivered antibiotics. Nonetheless, many types of polyesters, such as PLGA, are generally regarded as safe and biocompatible and are approved for clinical use by the U.S. Food and Drug Administration (FDA).

In contrast to the bulk-eroding polyesters, polyanhydrides were the second most common class of synthetic polymers. Polyanhydrides predominantly degrade through surface erosion, which enables zero-order release kinetics (release rate is constant over time) of the antibiotics [58]. Polyanhydrides are regarded as biocompatible [50, 58], although some studies have reported excessive inflammatory reactions with sebacic acid-based polymers [57].

5.4. Ceramics

Ceramics, such as HA or bioactive glass, are commonly utilized as bone graft substitutes due to their excellent biocompatibility and osteoconductive properties. These materials are either biodegradable or resorbable by osteoclasts and can be incorporated into newly forming bone [70]. For antibiotic delivery applications, injectable calcium phosphate cements were the most commonly used (Table 3). These cements are typically formed by combining a soluble calcium phosphate (e.g. tricalcium phosphate) with water, or another aqueous solution, to form a paste that will re-precipitate into HA or a calcium-deficient apatite (CDA). Antibiotic release is diffusion-controlled and does not significantly rely on degradation of the matrix. The duration of antibiotic release from these materials is generally on the order of days to weeks, depending on the formulation [45].

5.5. Composites

Individually, all biomaterials are constrained by limitations that may be related to the mechanical properties, drug elution kinetics, or ability to contribute to tissue regeneration. Development of composite materials that can surpass the shortcomings of the individual constituents is a rapidly growing area of research. The studies reviewed herein utilized a variety of synthetic or natural polymers in combination with a ceramic bone graft substitute to achieve sustained antibiotic release from an osteoconductive carrier (Table 4). Ceramics and cements can provide high compressive strength and structural support, but are very brittle. Integrating polymers can help improve the toughness in addition to controlled antibiotic release [104]. Supplementing the inorganic materials with natural polymers, such as collagen, can also help improve the biological performance by enhancing cellular interaction [52, 88, 103, 126, 130]. Given the expanse of potential formulations and the superior functional aspects, composites may be the most promising solutions for the complex tasks of infection management followed by bone regeneration.

6. Animal models used to study experimental biomaterials

The details of the animal model are critical when evaluating the efficacy of antimicrobials and biomaterials, particularly when comparing results across studies. A recent systematic review provides an extensive summary and discussion on animal models of *S. aureus* osteomyelitis [109]. In the animal model studies that were reviewed by Reizner and

colleagues [109] as well as in the treatment studies reviewed herein, a rabbit model of chronic OM that was established by Norden et al. [94], or a close variant, was most commonly employed. Briefly, the marrow cavity of the proximal tibial metaphysis was inoculated with *S. aureus* through a needle and the cortical hole was plugged with bone wax or a similar material and the infection was allowed to establish for a predefined time period. The proximal tibial metaphysis was inoculated in this way in 73% (29/40) of the reviewed studies, with 18 studies using rabbits, 10 using rats, and 1 using dogs. Of these 29 studies, 11 of the rabbit studies coupled the inoculation with a sclerosing agent (5% sodium morrhuate), as Norden et al. did, which causes local occlusion of the microvasculature and focal necrosis that makes the bone more susceptible to infection establishment [110, 112]. One rat study used arachidonic acid as a sclerosing agent [84]. The use of sclerosing agents is controversial and may artificially confound the results of treatment studies, particularly those that rely on vascular distribution of systemic antibiotics.

As an alternative to sclerosing agents, foreign body implants are a more clinically relevant mechanism to increase the infection susceptibility [149]. Implants were placed during the inoculation procedure in 48% (19/40) of the studies. These implants were most commonly stainless steel K-wire or needles (9/19), but other materials included PMMA (2/19), crushed allograft bone (2/19), suture (2/19), a hemostatic compress (1/19), or plastic fixation plates with metal screws to stabilize a segmental femoral defect (3/19). All of the implants that were placed at the time of inoculation were removed or exchanged during the revision surgery, except for the crushed allograft bone and fixation plates and screws. During the revision surgery, 83% (33/40) of the studies debrided the inoculation site and/or marrow cavity.

When the proximal tibial metaphysis was not used as the infection site, the marrow cavity was inoculated through the intercondylar notch of the distal femur (3/40), between the greater and lesser trochanters of the proximal femur (1/40), or into the mid-shaft of the radius (4/40) in rabbits. Three studies created segmental defects in rat femurs [49, 75] or mouse femurs [51] and stabilized the defect with a plastic plate and metal screws.

Infected segmental defects are difficult to treat clinically and to model experimentally due to the complexities of dead space management, maintenance of anatomical reduction and mechanical fixation of the bone, and the presence of a large implant that is susceptible to biofilm. Chen and colleagues developed a rat model of an infected segmental defect in the rat femur [24], which was employed by Li et al. [75] and Guelcher et al. [49] to study antibiotic and Bone Morphogenetic Protein-2 (BMP-2) delivery from polyurethane spacers. Inzana et al. developed a similar model using the mouse femur and characterized the response to vancomycin therapy delivered systemically as well as locally via PMMA spacers [53]. In a subsequent study, Inzana et al. utilized this model to evaluate the local combinational delivery of vancomycin and rifampin from calcium phosphate scaffolds, with or without a polymeric coating, compared to vancomycin delivered from PMMA [51]. In the models developed by Chen et al. [24] and Inzana et al. [53], the inoculation dose and time for infection establishment were carefully selected such that mechanical fixation would not be lost due to rapid and extensive osteolysis around the screws. A potentially important difference between the designs of the two models was the initial defect size and debridement

strategy. Chen et al. created a 6 mm segmental defect in the rat femur at the time of inoculation, but did not remove any additional bone during debridement, which would likely be required in a clinical case of chronic osteomyelitis [24]. In contrast, Inzana et al. created an initial defect of 0.7 mm in the mouse femur at the time of inoculation to simulate an infected fracture and later widened the osteotomy to 3 mm during the revision surgery to debride the necrotic and infected bone around the fracture site [53].

It is important to account for the time of infection establishment as well as the size and type of animal when comparing treatments across studies. A longer time for infection establishment may increase the probability of septic implant loosening, necrosis, impairment of vascular perfusion, biofilm formation, and sequestration, which could impact the relative efficacy of each therapeutic strategy. Features of chronic osteomyelitis such as necrosis and severe osteolysis may develop in smaller animals within a shorter time frame compared with larger animals due to the differences in tissue size (e.g. cortical thickness). The length of time allotted for infection establishment across all of the studies ranged from 6 hours to 3 months, with a median and mode of 3 weeks. Infection establishment for 6 hours was chosen by three studies to represent the time between a trauma and surgical treatment [49, 75, 123]. While 6 hours will not represent chronic OM, it may be sufficient for infection establishment and initial biofilm formation [16, 47, 115].

The inoculated *S. aureus* strain was methicillin-sensitive (MSSA) in 48% (19/40) of the studies, methicillin-resistant (MRSA) in 38% (15/40) of the studies, and was unspecified in 20% (8/40) of the studies (2 studies examined more than 1 strain). One study also examined a small-colony variant (SCV) of *S. aureus* [61], which can have altered antibiotic susceptibility profiles among other phenotypic variations relative to the parental strain [40]. Different *S. aureus* strains can express different virulence factors, which Smeltzer and colleagues demonstrated to be an important component in the pathogenesis of musculoskeletal infections [119]. Similarly, the variation in virulence profiles across different strains may have important implications for therapeutic studies as well.

All of the above factors are important to consider when comparing the efficacy of antimicrobial therapeutics across studies. It is imperative to carefully consider the clinical relevance and implications when designing an animal model and the subsequent therapeutic experiments. Authors should provide detailed descriptions of the animal model as well as a thorough evaluation of the microbiology, histopathology, and radiopathology alongside appropriate controls (e.g. untreated, systemic treatment, and standard clinical treatment) to determine the efficacy of the experimental therapeutic relative to current standards of care. Utilizing more than one *S. aureus* strain within each study could further substantiate the efficacy of a novel therapeutic or provide additional insights for failed treatments.

7. Methods for evaluating the treatment efficacy

7.1. Microbiological analysis

Experimental animal models enable more rigorous analysis of the bacterial infection compared with human studies. With animal models, endpoint histological or microbiological evaluations are possible for all involved tissues, whereas human studies are limited to tissue

7.3. Radiographic analysis and other imaging modalities

Radiologic scoring was generally conducted according to the system described by Norden et al. [93] or Smeltzer et al. [119]. The Norden criteria included new periosteal bone formation, sequestra, destruction of bone, and the extent of involvement along the tibia, where higher scores are indicative of a worse outcome. The Smeltzer system grades the periosteal elevation, architectural deformation, widening of the bone shaft, and new bone formation from 0–4, with higher scores indicating greater disease severity. The time course over which radiographic analyses of therapeutic efficacy are performed can affect the interpretation of results. While radiographic worsening with continued osteolysis may be apparent in failed treatments, radiographic improvements may lag by 4–6 weeks after successful infection management. Thus, other analysis techniques are likely required to help delineate the relative success of different treatments.

Despite the popularization of micro-computed tomography (micro-CT) over the last decade, quantitative 3D radiography was only employed in 2 studies to evaluate the volumetric bone changes [49, 51]. Guelcher et al. employed endpoint micro-CT to evaluate the extent of bone regeneration in the infected segmental femoral defect to evaluate the combinational delivery of vancomycin and BMP-2 [49]. They observed that low-dose BMP-2 in combination with vancomycin or high-dose BMP-2, with or without vancomycin, significantly increased bone regeneration in the segmental defect. Inzana et al. utilized longitudinal micro-CT analysis to evaluate the extent of infection-induced osteolysis that occurred over the 3 weeks of treatment, between revision surgery and the study endpoint [51]. In these studies, treatments that locally delivered rifampin in combination with vancomycin via 3D printed calcium phosphate scaffolds significantly reduced the osteolytic bone loss compared to vancomycin delivered from PMMA, without compromising the volume of new bone formation.

Positron emission tomography (PET) is another advanced imaging technique that was utilized to evaluate infection management [67]. Accumulation of the [¹⁸F]fluorodeoxyglucose (FDG) tracer has been associated with the high uptake by granulocytes and inflammatory cells [68, 124], which can be employed to distinguish osteomyelitis from the increased cellular activity associated with aseptic bone healing [66]. A recent review of the literature concluded that FDG-PET reported reliable success in detecting orthopaedic infections associated with implanted prostheses and OM, which demonstrates the great promise for this diagnostic technology in complex infections [8].

8. Efficacy of experimental biomaterials compared with PMMA

Considering that the shortcomings of PMMA are the primary motivation behind many of these novel biomaterial studies, it is important to compare the efficacy of the experimental solution with the current clinical standard of care. Only 30% (12/40) of the studies reviewed here analyzed the experimental material in parallel with a material that is currently used clinically, with 10 of the 12 studies comparing to PMMA and 2 comparing with a CS-based products. Ding et al. reported that local delivery of vancomycin via composite chitosan gel with bioactive glass particles was comparable to AlloMatrix® based on bacterial cultures as well as histological and radiographic scoring [29]. Similarly, Beenken et al. reported that local delivery of daptomycin via chitosan-coated CS pellets was comparable to uncoated CS

pellets based on bacterial culture swabs as well as histological and radiographic scoring [11]. Across the 10 studies that compared with PMMA, 2 studies found degradable synthetic polymers (PLGA [5] and polyanhydrides [71]) or composites and ceramics [51] to outperform PMMA, 8 studies found the experimental material (including natural and synthetic polymers as well as ceramics) to be comparable to PMMA, and 1 study observed that PMMA was more effective at infection management than an injectable HA paste [146]. In the study by Inzana et al., the vancomycin-laden calcium phosphate scaffolds that were supplemented with rifampin significantly outperformed the PMMA, which is not a compatible local delivery material for rifampin [51]. Studies that examined gentamicin delivery from collagen [84] or injectable HA cement [121] observed greater CFU reductions by the experimental material compared to PMMA at earlier treatment time points (2 and 3 weeks, respectively), followed by comparable CFU reductions by the end of the studies (4 and 7 weeks, respectively). Inzana et al. observed the same trend towards improved early infection management, but comparable final outcomes, when delivering vancomycin from 3D printed calcium phosphate scaffolds compared to PMMA [51]. Although the studies using collagen [84] or injectable HA cement [121] did not describe the in vitro release kinetics, these types of materials are typically characterized by a greater initial bolus release than PMMA [9, 33]. Similarly, a much greater cumulative release and release rate of vancomycin was observed from 3D printed calcium phosphates compared with PMMA [51]. This early bolus release from the experimental materials may explain the early reductions in bacterial load, but comparable endpoint outcomes, compared with PMMA.

Taken together, these studies and the clinical studies on CS pellets do not make a strong case for improved infection management by biodegradable materials relative to PMMA thus far. Some significant advantages that these experimental biodegradable materials may have over PMMA were minimally examined in the reviewed studies. First, many of these experimental materials may be capable of delivering a wider range of antimicrobial agents than PMMA. Considering the potential importance of local delivery of combinations of antibiotics, more research on biomaterial-based delivery approaches is needed. For example, given the reported effectiveness of rifampin in implant-associated staphylococcal infections and the incompatibility of rifampin with PMMA, Inzana et al. demonstrated the enhanced efficacy of calcium phosphate scaffolds delivering both vancomycin and rifampin compared to vancomycin-laden PMMA [51]. Second, many of these materials could potentially be useful in facilitating subsequent bone healing following infection management. Guelcher et al. delivered vancomycin and low- or high-dose BMP-2 via polyurethane scaffolds to a septic segmental femoral defect and observed enhanced bone healing with osseous bridging of the defect when vancomycin was combined with low- or high-dose BMP-2, or with high-dose BMP-2 alone [49]. Cornell and colleagues observed complete healing of a cortical window in the proximal tibial metaphysis in 6 of 9 rabbits after 120 days of treatment with local gentamicin delivered by HA beads [26]. Although these two studies did not compare directly with PMMA, such results could not be achieved with a non-biodegradable material.

9. Efficacy of local vs. parenteral antibiotic administration

Local antibiotic therapy is well regarded for its advantages over systemic therapy based on the administration of higher doses directly at the infection site, with lower risks of systemic

toxicity [46]. Of the reviewed studies, 40% (16/40) evaluated the efficacy of the local delivery in comparison with parenteral administration of the same antibiotic. Local delivery was found to be more effective than systemic in 81% (13/16) of those studies. Impaired blood flow to sites of necrotic bone is characteristic of chronic osteomyelitis, which can inhibit the bioavailability of systemically administered antibiotics [25]. Of the 13 studies that found local antibiotic administration to be more effective than systemic, 5 studies used a sclerosing agent during the initial inoculation and 4 different studies allowed the infection to establish for at least 6 weeks. Using sclerosing agents in studies that will systemically administer antibiotics may confound the results in a non-physiological manner, considering that the vascular insufficiency is induced artificially. In contrast, reduced blood supply that develops naturally as part of the infection pathogenesis over an extended time period (e.g. 6 weeks instead of 1 week) is an appropriate model. The three studies that found local and systemic therapy to be comparable did not use a sclerosing agent and allowed the infection to establish for 4 days, 2 weeks, or 4 weeks [5, 72, 77].

Considering that there were only 3 studies where local antibiotic administration was not more effective than parenteral [5, 72, 77], it is not possible to discern whether any specific antibiotic or local delivery material is associated with the success of local therapy over systemic in the reviewed studies. Of note, however, is that tobramycin was used in 2 of those 3 studies [5, 77] and was not used in any of the 13 studies that found systemic therapy to be less effective than local.

10. Discussion and conclusions

Treatment of osteomyelitis remains a significant clinical challenge despite the extensive surgical and antibiotic treatments that are currently used. The general advantages of local antibiotic delivery over systemic administration are well established for osteomyelitis [46] and are further supported by the currently reviewed literature in experimental animal models. PMMA has been well established for local antibiotic delivery to manage orthopaedic infections, but is still fraught with limitations that could be overcome by novel biomaterials strategies. The high rates of reinfection suggest that the bactericidal efficiency needs to be improved, the limitations on antibiotic choice could be alleviated by alternate biomaterials, and the need for subsequent surgeries to remove PMMA and promote bone healing may be eliminated by a biodegradable, osteoconductive or osteoinductive material.

Regarding the limitations on antibiotic choice with PMMA, few studies have examined the potential efficacy of local rifampin delivery for the treatment of established osteomyelitis, despite many studies and reviews suggesting rifampin's importance in implant-associated staphylococcal infections [28, 76, 150]. A handful of studies have characterized rifampin-laden biomaterials in vitro [2, 48, 86, 95, 138] and one study demonstrated the efficacy of a rifampin and minocycline combination in a prophylactic implant coating in rabbits [27]. One possible reason that local rifampin delivery may not have been pursued extensively is that previous in vitro studies indicated that rifampin is significantly more cytotoxic to osteogenic cells than many other antibiotics [31, 108]. However, these results have not been investigated in vivo and the MIC of the antibiotic must be considered in combination with its toxicity limit to determine the therapeutic window. The in vitro MIC of rifampin for *S.*

aureus is 0.04 µg/mL [85] and the in vitro toxicity limit is 10 µg/mL [108], while the MIC of vancomycin is 1 µg/mL [85] and the in vitro toxicity limit is 2000 µg/mL [108]. Thus, both drugs possess approximately a 3-log₁₀ therapeutic window. Considering the previous lack of investigations of local rifampin delivery for implant-associated osteomyelitis, the authors of this review recently studied the efficacy of combined vancomycin and rifampin-laden calcium phosphates compared to vancomycin monotherapy, delivered from calcium phosphate scaffolds as well as PMMA spacers [51]. In these studies, supplementing the local vancomycin therapy with localized rifampin delivery significantly reduced the bacterial load and produced higher rates of culture negative tissues compared to vancomycin monotherapy. However, the retained fixation plate and screws were culture positive in 100% of the animals, regardless of treatment. While the vancomycin-rifampin combination might be effective in reducing the pathogenic burden in the biological tissues of the host, the treatment was not sufficiently effective against the bacteria in biofilm colonizing the orthopaedic device. While this can be attributed to sub-optimal choice or concentrations of the locally delivered drugs, the results caution against the retention of orthopaedic implants in complex infection scenarios.

Further research is also required for the dual-purpose bone graft substitute, which is designed to manage the bacterial infection and subsequently orchestrate bone regeneration. To this end, Guelcher and colleagues have conducted promising preliminary studies with combinational vancomycin and BMP-2 delivery from polyurethane scaffolds; but all of the bones remained culture positive for *S. aureus*, despite the enhanced bone healing and osseous bridging of the critical segmental defect [49]. This finding underscores the concern for recurrent infection after conclusion of the antibiotic therapy, particularly if the fixation device is contaminated with biofilm, but must remain in place until the bone is able to independently bear the loads. Combating infection in the presence of an orthopaedic implant, which may be biofilm-contaminated, is a critical clinical issue that was only addressed in 8% (3/40) of the reviewed studies [49, 51, 75].

Finally, antimicrobial implant coatings are a very important class of biomaterials that have only been evaluated for their prophylactic efficacy and thus were not discussed in this systematic review. Nonetheless, these implant coatings could be critical for treatment of established infections to help prevent colonization and biofilm formation on an exchanged implant or treat the biofilm on a retained implant. These technologies typically include hydrogel-based systems that can be coated onto an implant intraoperatively [30] or precoated devices, such as the Expert Tibial Nail PROtect from DePuy Synthes, as discussed in detail by a recent review on antimicrobial delivery systems for prophylaxis of orthopaedic infections [129].

Future experimental designs should pay special attention to the clinical implications and relevance of the animal models being employed to evaluate biomaterial delivery systems for antibiotics. Additionally, the details of the evaluation methods for the chosen animal model are critical to efficacy interpretation and should be comprehensively reported. While biopsies and swabs allow for histopathological analysis, these culture techniques increase the probability of false negative results, particularly considering the spatial heterogeneity of bacterial colonization within the bone. Therefore, homogenized whole bone cultures should

be considered for the smaller animal models (e.g. rabbit, rat, mouse), if appropriate within the ethical constraints of the study design. In studies employing histopathological analysis, crystal violet stains and high magnification microscopy are essential to detect the gram-positive bacteria within the tissue – a simple method that surprisingly was neglected in most of the reviewed histopathological studies.

Many of the biomaterials discussed herein are promising for improving patient outcomes in the treatment of osteomyelitis, but this review highlights the fact that extensive research is still required to definitively determine the benefits and limitations of these potential solutions. Important imminent areas of research include local delivery of alternative antimicrobial agents that PMMA cannot carry or elute efficiently, more extensive analysis of treatments in the presence of a clinically relevant orthopaedic device, combinational delivery of antibiotics and biofilm dispersal agents, as well as further investigations into post-infection bone regeneration with osteoconductive delivery materials and osteoinductive adjuvants.

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Table 1a

Natural Polymers for Local Antibiotic Delivery

Reference	Material	Antibiotic	Tx Time	Controls	Sample Size	Cultures	Primary Findings
(Itokazu <i>et al.</i> , 1997)	Fibrin	Arbekacin sulfate (ABK)	13 wk	PBO, Untreated	6	Ground bone	Fibrin + ABK significantly reduced CFU vs. controls
(Mader <i>et al.</i> , 2002)	Fibrin	Tobramycin	4 wk	Systemic tobramycin, PMMA, PBO, Untreated	15	Vortex bone chips & marrow	Fibrin, PMMA and systemic tobramycin had similar CFU counts; PMMA had the highest (-) culture rate
(Mendel <i>et al.</i> , 2005)	Collagen	Gentamicin	4 wk	Systemic cefazolin (cef), PMMA +/- sys. cef., Collagen +/- sys. cef., Untreated	10-12	Ground bone	All Tx reduced CFU vs. untreated. Systemic cef. tended to additionally augment local Tx. Collagen had 82% (-) cultures vs. 0% with PMMA
(Cevher <i>et al.</i> , 2006; Orhan <i>et al.</i> , 2006)	Chitosan or Pectin microspheres	Ciprofloxacin	3 wk	Systemic ciprofloxacin (cipro), PBO	8	Ground bone	Significant reduction in CFU by chitosan, but not pectin, vs. sys cipro; sys cipro was not different from PBO
(Stinner <i>et al.</i> , 2010)	Chitosan sponge	Vancomycin	42 hr	Untreated	5	None	Vancomycin-chitosan sponge reduced bioluminescence of bacteria after 42 hrs of Tx
(Xing <i>et al.</i> , 2013)	Alginate beads in fibrin gel	Vancomycin	3 mo	PBO	3	Biopsies in culture broth	100% (-) cultures for Vanco-beads vs. 0% for PBO; Tx improved radiographic scores.

PBO – Placebo (material without antibiotics); (-) – negative; Tx – treatment; sys – systemic

Table 1b

Summary of Animal Models Used for Natural Polymer Studies

Reference	Animal	Site	Implant	Strain	CFU	Sclerosant	Infection Time	Debridement
(Itokazu <i>et al.</i> , 1997)	Rat - Wistar	Proximal Tibia	Vicryl Suture	Im2-42	Not specified	None	4 wk	Yes
(Mader <i>et al.</i> , 2002)	Rabbit -NZW	Proximal Tibia	Allograft bone	MSSA clinical isolate	10 ⁶	None	2 wk	Yes
(Mendel <i>et al.</i> , 2005)	Rat - Wistar	Proximal Tibia	None	MSSA (ATCC 29213)	2×10 ⁵	Arachidonic Acid	3 wk	Yes
(Cevher <i>et al.</i> , 2006; Orhan <i>et al.</i> , 2006)	Rat - Wistar	Proximal Tibia	K-wire	MRSA	2×10 ⁶	None	6 wk	Yes & K-wire removed
(Stinner <i>et al.</i> , 2010)	Goat	Proximal Tibia	None	MSSA (Xen29)	>10 ⁸	None	6 hr	Yes
(Xing <i>et al.</i> , 2013)	Rabbit -NZW	Proximal Tibia	None	MSSA (ATCC 25923)	5×10 ⁷	Sodium morphuate	4 wk	Yes

NZW – New Zealand White

Table 2a

Synthetic Polymers for Local Antibiotic Delivery

Reference	Material	Antibiotic	Tx Time	Controls	Sample Size	Cultures	Primary Findings
(Garvin <i>et al.</i> , 1994)	PLGA (50:50)	Gentamicin	4 wk	Systemic gentamicin, PMMA	8-9	Biopsy	Culture (-) in 63% with systemic gent, 89% with PMMA, and 100% with PLGA; PLGA and PMMA not significantly different
(Nelson <i>et al.</i> , 1997)	PLGA (50:50)	Gentamicin	4 wk	Systemic gentamicin, PBO, Untreated	14-16	Biopsy	93% or 73% culture (-) with PLGA + 20% or 10% gent, 31% with systemic gent, 36% with PBO, 25% with untreated
(Calhoun and Mader, 1997)	PLGA (70:30) microspheres	Vancomycin	4 wk	Systemic vancomycin, PLGA +/- systemic vancomycin, PBO, Untreated	12	Ground bone	PLGA Tx significantly reduced CFU. Systemic vanco did not; No radiographic changes with any Tx, but PBO and Untreated became worse
(Ambrose <i>et al.</i> , 2004)	PLGA (50:50) microspheres	Tobramycin	4 wk	Systemic tobramycin, PMMA, PLGA +/- systemic tobramycin, PBO	8	Swabs	75% culture and histo (-) with PLGA + systemic tobra vs. 25% with PBO. PMMA + systemic was 33% culture and histo (-).
(Cevher <i>et al.</i> , 2007)	PLGA (50:50) microspheres	Sodium Fusidate	3 wk	PBO, Untreated	6	Ground bone	Significant reductions in CFU with PLGA Tx vs. controls; 0% culture (-) bones
(Orhan <i>et al.</i> , 2010)	PLGA (50:50) microspheres	Teicoplanin	20 d	Systemic teicoplanin, PBO, Untreated	7	Crushed bone	Significant reductions in CFU by PLGA vs. systemic teicoplanin; Necrosis and inflammation levels were not different
(Tsiolis <i>et al.</i> , 2011)	PLA	Linezolid	10 wk	Untreated	4	Biopsy	PLA had significantly lower CFU at 6 weeks, but not at 2, 4, 8, or 10 weeks
(Le Ray <i>et al.</i> , 2005)	PCL microparticles	Vancomycin	11 d	Systemic vancomycin	5	Bone marrow	5 log reduction by systemic vanco vs. 4.1 log reduction with local Tx; not significant
(El-Kamel and Baddour, 2007)	PCL	Gatifloxacin	4 wk	Untreated	6	Ground bone	Significant reduction (5 log) in CFU vs. Untreated;

Reference	Material	Antibiotic	Tx Time	Controls	Sample Size	Cultures	Primary Findings
(Yagmurhu <i>et al.</i> , 1999)	PHBV	Cefoperazone + Sulbactam	4 wk	PBO in contralateral limb	8	Swabs	88% culture (-) at 2 wks, 100% by 4 wks with Tx, 0% with PBO.
(Laurencin <i>et al.</i> , 1993)	Polyanhydride co-polymer	Gentamicin	3 wk	PMMA, PBO, Untreated	5	Biopsy	Significantly reduced CFU by polyanhydride, but not by PMMA or PBO, vs. untreated
(Chen <i>et al.</i> , 2007)	Poly(sebacic anhydride)/PLA (10:90)	Ofloxacin	6 wk	PBO	7-10	Biopsy	100% culture (-) with Tx, 0% with PBO; No gram (+) stain with Tx, but necrosis in medullary cavity and fibrous capsule around implant
(Brin <i>et al.</i> , 2008)	Polyanhydride co-polymer	Gentamicin	3 wk	PBO in contralateral limb	13	None	Abscesses in PBO that were not present with Tx. No gram (+) staining in Tx tibiae; No radiographic differences;
(Li <i>et al.</i> , 2010)	Polyurethane (PUR)	Vancomycin (HCl and Free Base (FB))	4 wk	PMMA beads, Untreated	10	Ground bone	PMMA + Vanco-HCl and PUR + Vanco-FB significantly reduced CFU vs. Untreated. PUR + Vanco-HCl did not.
(Guelcher <i>et al.</i> , 2011)	Polyurethane	Vancomycin	8 wk	Polyurethane + BMP-2 +/- vancomycin, Collagen + BMP-2	15	Ground bone	Vanco significantly reduced CFU; Vanco + BMP-2 or high-dose BMP-2 without vanco significantly improved healing of segmental bone defect.
(Overstreet <i>et al.</i> , 2015)	PNDJ	Gentamicin	4 wk	Untreated (Debrided)	8	Biopsy + Swabs	100% culture (-) with PNDJ, 0% culture (-) control. No histo signs of infection with PNDJ, normal healing.

PBO – Placebo (material without antibiotics); (-) – negative; (+) – positive; Tx – treatment; PCL – poly(ϵ -caprolactone); PLA – poly(D,L-lactide); PHBV – poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PLGA – poly(D,L-lactide-co-glycolide); PNDJ – poly(N-isopropylacrylamide-co-dimethyl-L-c-butylolactone acrylate-co-*l*-leffamine I M-1000 acrylamide)

Table 2b

Summary of Animal Models Used for Synthetic Polymer Studies

Reference	Animal	Site	Implant	Strain	CFU	Sclerosant	Infection Time	Debridement
(Garvin <i>et al.</i> , 1994)	Dog	Proximal Tibia	PMMA	MSSA (ATCC 25923)	10 ⁸	None	4 wk	Yes & PMMA removed
(Nelson <i>et al.</i> , 1997)	Rabbit -NZW	Radius	None	MRSA (ATCC 49230)	5×10 ⁶	None	4 wk	Yes
(Calhoun and Mader, 1997)	Rabbit -NZW	Proximal Tibia	Crushed allograft bone	Phage type 52/52A/80	10 ⁴	None	4 wk	Yes, in select groups
(Ambrose <i>et al.</i> , 2004)	Rabbit -NZW	Radius	None	MSSA (ATCC 49230)	2×10 ⁶	None	4 wk	Yes
(Cevher <i>et al.</i> , 2007)	Rat -Wistar	Proximal Tibia	K-wire	MRSA clinical isolate	2×10 ⁶	None	3 mo	Yes & K-wire removed
(Orhan <i>et al.</i> , 2010)	Rat -Wistar	Proximal Tibia	K-wire	MRSA clinical isolate	2×10 ⁶	None	3 mo	Yes & K-wire removed
(Tsiolis <i>et al.</i> , 2011)	Rabbit -NZW	Proximal Femur	Needle	MRSA clinical isolate	2×10 ⁷	None	3 wk	No; Need removed
(Le Ray <i>et al.</i> , 2005)	Rabbit	Proximal Tibia	Hemostatic compress	MSSA (ATCC 25922) or MRSA (P9)	10 ⁷	None	4 d	No; Compress removed
(El-Kamel and Baddour, 2007)	Rabbit -NZW	Proximal Tibia	Needle	MRSA (ATCC 33591)	10 ⁶	Sodium morrhuate	2 wk	No
(Yagmurcu <i>et al.</i> , 1999)	Rabbit	Proximal Tibia	K-wire	Clinical isolate (phage type 52/52b)	3×10 ⁵	None	3 wk	No & wire not removed
(Laurençin <i>et al.</i> , 1993)	Rat -SD	Proximal Tibia	PMMA + K-wire	MSSA clinical isolate	10 ⁴	None	3 wk	No; Implant removed
(Chen <i>et al.</i> , 2007)	Rabbit	Proximal Tibia	Silk thread	MSSA (ATCC 25923)	6×10 ⁶	None	4 wk	Yes & thread removed
(Brin <i>et al.</i> , 2008)	Rat -Fischer	Proximal Tibia	None	MSSA (ATCC 29213)	10 ⁴	None	3 wk	Yes
(Li <i>et al.</i> , 2010)	Rat -SD	Femur Mid-shaft	Polyacetal plate + K-wire	MSSA (Xen36)	10 ⁶	None	6 hr	Yes; Implant not removed
(Guelcher <i>et al.</i> , 2011)	Rat -SD	Femur Mid-shaft	Polyacetal plate + K-wire	MSSA (Xen36)	10 ⁵	None	6 hr	Yes; Implant not removed
(Overstreet <i>et al.</i> , 2015)	Rabbit	Radius	K-wire	MSSA (ATCC 49230)	5×10 ⁶	None	3 wk	Yes & new K-wire placed

NZW – New Zealand White; SD – Sprague Dawley

Table 3a

Ceramics for Local Antibiotic Delivery

Reference	Material	Antibiotic	Tx Time	Controls	Sample Size	Cultures	Primary Findings
(Inzana <i>et al.</i> , 2015b)	3D printed calcium phosphate scaffold	Vancomycin or Vancomycin + Rifampin	3 wk	+/- Systemic vanco, PMMA, PBO	7-15	Ground bone, soft tissue, implants	Rifampin + vancomycin from scaffolds significantly reduced CFU counts and volumetric osteolysis compared to vancomycin from PMMA. Only groups with rifampin achieved culture (-) bones
(Kundu <i>et al.</i> , 2011)	Bioactive glass	Ceftriaxone + Sulbactam	6 wk	Systemic ceftriaxone + sulbactam, Untreated	3	None	Histopathologic scores significantly improved with bioactive glass vs. systemic at 12 and 21 days but not at 42 days; radiographic scores were better through 42 days with bioactive glass
(Amador <i>et al.</i> , 2010)	Calcium-deficient apatite (CDA)	Vancomycin	7 d	Systemic vanco, CDA +/- sys. vanco, PBO	5-11	Joint fluid, marrow, & bone	Systemic vanco tended to reduce CFU and mortality rates; CDA significantly reduced CFU and mortality vs. PBO; Systemic vanco tended to additively augment effect of local vanco
(Faber <i>et al.</i> , 2005)	Calcium phosphate paste	Gentamicin or Human Lactoferrin 1-11	3 wk	PBO, Untreated	5-8	Swabs of biopsies	CFU counts were significantly reduced and histological scores significantly improved by gent and hLFI-11 vs. PBO
(Solberg <i>et al.</i> , 1999)	HA paste	Gentamicin	7 wk	Systemic gentamicin, PMMA, Untreated	4	Ground bone	HA and PMMA steadily decreased CFU counts over time course to 100% culture (-) by 7 wks; HA was ~1 log CFU lower than PMMA at 3 wks. Systemic gent was not different from untreated
(Shiriff <i>et al.</i> , 2002)	HA paste	Vancomycin	4 wk	Systemic vanco, PMMA, PBO +/- systemic vanco, Untreated	8-12	Ground bone	Rates of culture (-) bones were significantly reduced by PMMA and HA with vanco. PMMA and HA were comparable. Systemic vanco did not have a significant effect.
(Joosten <i>et al.</i> , 2004)	HA paste	Gentamicin	3 wk	Systemic gentamicin, PBO, Untreated	6	Biopsy and aspirate	100% culture (-) with HA vs. 0% with systemic gent. Histological scores significantly reduced vs. Untreated with HA, but not systemic gent
(Joosten <i>et al.</i> , 2005)	HA paste	Vancomycin	3 wk	PBO	7-8	Biopsy	100% culture (-) with Tx vs. 0% with PBO; WBC counts and histo scores significantly reduced by Tx vs. PBO;

Reference	Material	Antibiotic	Tx Time	Controls	Sample Size	Cultures	Primary Findings
(Zelken <i>et al.</i> , 2007)	HA paste	Vancomycin	3 wk	PMMA, Untreated	16	Ground bone	CFU counts significantly reduced with PMMA vs. HA paste and untreated
(Cornell <i>et al.</i> , 1993)	HA beads	Gentamicin	40 d, 120 d	PBO, untreated	8-13	Biopsy and swabs	At 40 days, 73% culture (-) with HA vs. 0% of PBO and untreated; In the 120 d subset, 66% with HA Tx completely healed the osseous defect, controls were not considered for 120 d.
(Jiang <i>et al.</i> , 2012)	Nano-HA pellets	Vancomycin	12 wk	PBO, Untreated	4	Biopsy	100% culture (-) with HA vs. 0% in PBO and untreated. Histo scores significantly improved with HA vs. PBO or untreated.

PBO – Placebo (material without antibiotics); (-) – negative; Tx – treatment; HA – hydroxyapatite; WBC – white blood cell

Table 3b

Summary of Animal Models Used for Ceramics Studies

Reference	Animal	Site	Implant	Strain	CFU	Sclerosant	Infection Time	Debridement
(Inzana <i>et al.</i> , 2015b)	Mouse -BALB/cJ	Femur Mid-shaft	PEEK plate + Ti screws	MSSA (Xen36)	8×10 ⁴	None	1 wk	Yes, osteotomy widened & center screws replaced
(Kundu <i>et al.</i> , 2011)	Rabbit -NZW	Proximal Tibia	None	Veterinary isolate	3×10 ⁶	None	6 wk	No
(Amador <i>et al.</i> , 2010)	Rabbit -NZW	Distal Femur	None	MRSA clinical isolate	10 ⁹	None	3 d	Yes
(Faber <i>et al.</i> , 2005)	Rabbit -NZW	Proximal Tibia	None	MRSA (W234)	3×10 ⁶	Sodium morrhuate	3 wk	Yes
(Solberg <i>et al.</i> , 1999)	Rat -SD	Proximal Tibia	K-wire	MSSA (ATCC 49230)	4×10 ⁶	None	7 wk	Yes
(Shirliff <i>et al.</i> , 2002)	Rabbit -NZW	Proximal Tibia	None	MRSA clinical isolate	10 ⁶	Sodium morrhuate	2 wk	Yes
(Joosten <i>et al.</i> , 2004)	Rabbit -NZW	Proximal Tibia	None	Clinical isolate	3×10 ⁶	Sodium morrhuate	3 wk	Yes
(Joosten <i>et al.</i> , 2005)	Rabbit -NZW	Proximal Tibia	None	SCV (A22616/3) or MRSA (W234)	3×10 ⁶	Sodium morrhuate	3 wk	Yes
(Zelken <i>et al.</i> , 2007)	Rat -SD	Proximal Tibia	None	Unspecified MSSA	10 ⁶	None	3 wk	Yes
(Cornell <i>et al.</i> , 1993)	Rabbit -NZW	Proximal Tibia	None	Unspecified	10 ⁶	Sodium morrhuate	3 wk	Yes
(Jiang <i>et al.</i> , 2012)	Rabbit -NZW	Proximal Tibia	None	MRSA clinical isolate	10 ⁸	Sodium morrhuate	3 wk	Yes

NZW – New Zealand White; SD – Sprague Dawley

Table 4a

Composites for Local Antibiotic Delivery

Reference	Material	Antibiotic	Tx Time	Controls	Sample Size	Cultures	Primary Findings
(Inzana <i>et al.</i> , 2015b)	3D printed calcium phosphate scaffold + PLGA coating	Vancomycin + Rifampin	3 wk	PMMA with vanco, Scaffold w/o PLGA	7-15	Ground bone, soft tissue, implants	Group with PLGA coating had the highest culture (-) rate in the bone and was still able to elute functional rifampin after 3 weeks in vivo
(Koort <i>et al.</i> ,	PLA + 2005)bioactive glass particles	Ciprofloxacin	6 wk	PBO, Untreated	5-9	Swabs	100% culture (-) bone and 66% soft tissue with Tx vs. 0% with PBO and untreated; PET values significantly decreased with Tx
(Jia <i>et al.</i> , 2010; Zhang <i>et al.</i> , 2010)	Borate bioactive glass/chitosan gel	Teicoplanin	12 wk	Systemic teicoplanin, PBO, Untreated	10-14	Biopsy	86% culture (-) with bioactive glass, 43% with systemic Tx, 21% with PBO, 0% with Untreated; Histo and radiographic scores significantly improved with bioactive glass vs. systemic Tx;
(Ding <i>et al.</i> , 2014)	Borate bioactive glass/chitosan gel	Vancomycin	2 mo	Systemic vancomycin, AlloMatrix®, Untreated	10-16	Swabs	88% culture (-) with bioactive glass, 81% with AlloMatrix®, 43% systemic vanco, 0% untreated; Histo and radiographic scores improved significantly with local vs. systemic vanco
(Alvarez <i>et al.</i> , 2008)	18% PLA + 72% HA/TCP (25:75)	Ciprofloxacin	6 wk	Untreated	3	Sonicated bone	33% culture (-) by 2 weeks, 66% at 3 weeks, 100% by 4 weeks with Tx vs. 0% with untreated; Clinical scores tended to be better in treated animals and CRP levels were lower
(Shi <i>et al.</i> , 2010)	Ethyl cellulose microspheres/nano-HA/chitosan gel	Gentamicin	8 wk	PBO, Untreated	11	Biopsy	Reduction of CFU to 10 ² with local Tx vs. 10 ⁸ with PBO or untreated. Stats not described, % culture (-) not described
(Giavaresi <i>et al.</i> , 2012a; Giavaresi <i>et al.</i> , 2012b)	PMMA BaSO ₄ + β-TCP	Gentamicin + Vancomycin	3 wk	PMMA BaSO ₄ without β-TCP, PBO	5	Swabs and vortexed bone	100% culture (-) with local vanco vs. 0% with PBO; Necrosis and inflammation reduced to 50% and 75%, respectively, with local vanco vs. PBO; β-TCP in PMMA does not affect efficacy
(Beenken <i>et al.</i> , 2014)	Chitosan-coated CaSO ₄ pellets	Daptomycin	3 wk	PBO (w/& w/o coating), Uncoated	6	Swabs	Chitosan-coated pellets significantly reduced bacteriological score vs. PBO, but no difference vs. Uncoated pellets

PBO – Placebo (material without antibiotics); (-) – negative; Tx – treatment; PLA – poly(D,L-lactide); HA – hydroxyapatite; TCP – tricalcium phosphate; PET – positron emission tomography

Table 4b

Summary of Animal Models Used for Composites Studies

Reference	Animal	Site	Implant	Strain	CFU	Sclerosant	Infection Time	Debridement
(Inzana <i>et al.</i> , 2015b)	Mouse -BALB/cJ	Femur Mid-shaft	PEEK plate + Ti screws	MSSA (Xen36)	8×10 ⁴	None	1 wk	Yes, osteotomy widened & center screws replaced
(Koort <i>et al.</i> , 2005)	Rabbit -NZW	Proximal Tibia	PMMA	MSSA	10 ⁴	No	2 wk	Yes & PMMA removed
(Jia <i>et al.</i> , 2010; Zhang <i>et al.</i> , 2010)	Rabbit -NZW	Proximal Tibia	None	MRSA	10 ⁷	Sodium morrhuate	4 wk	Yes
(Ding <i>et al.</i> , 2014)	Rabbit -NZW	Proximal Tibia	None	MRSA (ATCC43300)	10 ⁷	Sodium morrhuate	4 wk	Yes
(Alvarez <i>et al.</i> , 2008)	Rabbit -NZW	Distal Femur	None	MSSA (ATCC 6538)	3×10 ⁷	No	2 wk	Yes
(Shi <i>et al.</i> , 2010)	Rabbit -NZW	Proximal Tibia	None	MSSA (ATCC 25923)	9×10 ⁸	Sodium morrhuate	4 wk	No
(Giavaresi <i>et al.</i> , 2012a; Giavaresi <i>et al.</i> , 2012b)	Rabbit -NZW	Distal Femur	None	MRSA clinical isolate	10 ⁶	None	4 wk	Yes
(Beenken <i>et al.</i> , 2014)	Rabbit -NZW	Radius	None	MSSA (UAMS-1)	2×10 ⁶	None	3 wk	Yes

NZW – New Zealand White