Published in final edited form as:

J Orthop Res. 2020 October ; 38(10): 2091–2103. doi:10.1002/jor.24689.

Therapeutics and delivery vehicles for local treatment of osteomyelitis

Leah H. Cobb¹, Emily M. McCabe^{1,2}, Lauren B. Priddy^{1,*}

¹Department of Agricultural and Biological Engineering, Mississippi State University, Mississippi State, MS, USA

²Department of Mechanical Engineering, Mississippi State University, Mississippi State, MS, USA

Abstract

Osteomyelitis, or the infection of bone, presents a major complication in orthopedics and may lead to prolonged hospital visits, implant failure, and in more extreme cases, amputation of affected limbs. Typical treatment for this disease involves surgical debridement followed by long-term, systemic antibiotic administration, which contributes to the development of antibiotic-resistant bacteria and has limited ability to eradicate challenging biofilm-forming pathogens including *Staphylococcus aureus*—the most common cause of osteomyelitis. Local delivery of high doses of antibiotic via traditional bone cements can reduce systemic side effects of antibiotic. Nonetheless, growing concerns over burst release (then subtherapeutic dose) of antibiotic, along with microbial colonization of the nondegradable cement biomaterial, further exacerbate antibiotic resistance and highlight the need to engineer alternative antimicrobial therapeutics and local delivery vehicles with increased efficacy against, in particular, biofilm-forming, antibiotic-resistant bacteria. Furthermore, limited guidance exists regarding both standardized formulation protocols and validated assays to predict efficacy of a therapeutic against multiple strains of bacteria. Ideally, antimicrobial strategies would be highly specific while exhibiting a broad spectrum of bactericidal activity. With a focus on *Staphylococcus aureus* infection, this review addresses the efficacy of novel therapeutics and local delivery vehicles, as alternatives to the traditional antibiotic regimens. The aim of this review is to discuss these components with regards to long bone osteomyelitis, and to encourage positive directions for future research efforts.

Keywords

Osteomyelitis; Infection; *Staphylococcus aureus*; Biofilm; Therapeutics; Delivery vehicles; Antibiotic resistance

*corresponding author: Contact: lbpriddy@abe.msstate.edu, (662) 325-5988, Department of Agricultural and Biological Engineering, Mississippi State University, 130 Creelman Street, Mississippi State, MS, USA 39762.
Author Contributions

All authors have made substantial contributions to research design, or the acquisition, analysis or interpretation of data; and drafting the paper or revising it critically. All authors have read and approved the final submitted manuscript.

Introduction

Since penicillin was introduced in the 1940s, antibiotics have been the gold standard in the treatment of bacterial infections.¹ Due in part to the widespread, empirical, and often prolonged use of antibiotics, in recent years, a population of antibiotic-resistant pathogens has emerged, rendering many antibiotic regimens futile. An estimated \$20 billion in annual healthcare costs in the United States are due to bacterial antibiotic resistance, with costs estimated as high as \$35 billion when lost productivity in the workforce is accounted for.² Commonly, these infections are caused by the bacterial strain *Staphylococcus aureus* (*S. aureus*), known for its genetic propensity for bacterial resistance. In 2013, in the United States alone there were over 80,000 infections and 11,285 deaths due to antibiotic-resistant strains of *S. aureus*.² Infections commonly caused by *S. aureus* include endocarditis, dermatitis, and osteomyelitis. In osteomyelitis (bone infection), bacteria can access the bone following traumatic injuries, from the blood stream, or contiguously from nearby infections or contaminated hardware.^{3,4} In 2020, it is estimated that the treatment of implant-related osteomyelitis will cost \$1.62 billion dollars.⁵ Osteomyelitis is anticipated to have worsening implications on patient outcomes in the years to come, particularly in the growing diabetic population. In 2014, 108,000 diabetic patients underwent lower limb extremity amputations due to infections not successfully cleared with antibiotics.⁶

Osteomyelitis (OM) infections may be classified as either acute or chronic.⁷ Acute osteomyelitis can generally be cleared with antibiotics if treated in a timely manner.⁴ Chronic osteomyelitis is classified as persistent, long-term infection. Often, biofilms are indicated. Although chronic OM has traditionally been defined as infection persisting longer than six weeks, because infections can vary by causative agent, location, patient health, and other confounding factors, it is now more appropriately classified as a continuum^{8,9} for which long-term administration of high dose antibiotics with or without surgical intervention is considered the minimum effective treatment.^{7,10,11} Typical symptoms include bone necrosis, subsequent involucrum formation, and an increased risk of amputation.⁴

First discovered in the late 1880s, *S. aureus* is a gram positive bacteria (diameter ~1 μm) that appears as grape-like clusters (Figure 1).¹² Several phenotypic states of *S. aureus* are considered hallmarks of bacterial antibiotic resistance: small colony variants (SCVs), biofilms, *Staphylococcus* abscess communities (SAC), and colonization of the osteocytic canalicular networks of cortical bone.^{8,13,14} An SCV refers to an *S. aureus* cell with markedly decreased metabolic activity compared to the wildtype.¹³ The slower metabolic rate may be attributed to auxotrophies, or downregulated citric acid cycle activity as seen in recent *in vitro* testing.¹⁵ Due to altered metabolic activity, SCVs are more resistant to antibiotics and may be more apt to form biofilms.^{8,13,16} A biofilm refers to colonies of bacteria that have adhered to implants, necrotic tissues, or other available niches, via surface adhesins.⁸ These *S. aureus* cells may be surrounded by a self-produced protective exopolysaccharide polymer (Figure 1C–D).^{3,7,15,16} Biofilms present a significant challenge for treatment, as antibiotics may have difficulty penetrating the extracellular matrix that protects the underlying viable cells.³ Additionally, while in this state *S. aureus* often exhibits altered, slower metabolism, rendering some antimicrobials ineffective.¹³

SACs, like biofilm, contribute to infection persistence by hindering access of antimicrobials to the site of viable bacterial cells. The innate immune system, in an effort to contain harmful pathogens, can generate an abscess of *S. aureus*. The physical, fibrin-based barrier of this lesion will confer protection to bacterial cells, typically requiring debridement for sufficient infection clearance.^{8,17} Similarly in osteomyelitis, when *S. aureus* invades the canalicular networks of cortical bone, it achieves protection from the immune system, thus thwarting therapeutic efficacy. The osteocyte lacuno-canalicular network (OLCN) is an ideal architecture for *S. aureus* to attach, form biofilm, and hide from immune attack.^{8,14} Interestingly, *S. aureus* can readily change from its classic cocci shape into sub-micron rods, to facilitate invasion of the OLCN.¹⁴ Severe damage to the bony host, such as demineralization, may also occur due to the acidic byproducts of *S. aureus*.⁸ In any of these four phenotypic hallmarks of osteomyelitis, it is imperative that persister cells not readily killed with therapeutic regimens (whether due to location, or drug resistance mechanisms) are effectively cleared. Otherwise, the patient is at risk of infection recurrence.¹⁸

The increase in antibiotic-resistant pathogens combined with the higher prevalence of comorbidities to osteomyelitis highlight the importance of engineering new therapeutics and related delivery vehicles for *S. aureus*, especially for chronic osteomyelitis cases (Table 1).^{19,20} Ideally, novel treatments would be noninvasive and highly specific, and would not promote antimicrobial resistance. The delivery vehicles for such therapeutics should be biocompatible and enable local administration of a sufficient amount of therapeutic over time, decreasing the risk of systemic toxicity. To develop an appropriate therapeutic agent, these factors, along with the elusive genetic alterations and phenotypic states of *S. aureus*, must be collectively considered (Figure 2).

Therapeutics

Surgical debridement and Antibiotics

Surgical debridement, or the removal of infected tissues, is considered vital to successful treatment of chronic osteomyelitis.^{10,21,22} Its superiority to antibiotic therapy alone has been emphasized in numerous studies.^{22–24} This procedure is followed by post-operative, long-term administration of antibiotics. Bacteria can develop resistance to these drugs over time due to: (i) SCV formation, (ii) biofilm formation, and/or (iii) acquired genetic changes.¹³ Knowledge of whether or not bacterial strains exhibit these traits can help direct antibiotic treatment regimens. For example, antibiotics such as β -lactams, daptomycin, and fosfomycin that act on the bacterial cell wall generally have poor efficacy against SCVs.¹³ In one study, low doses of gentamicin, moxifloxacin, and clindamycin actually induced SCV formation *in vitro*.¹³ In another study, low doses of β -lactam induced not only SCV formation but also biofilm growth.²⁵

Biofilm presence should also influence antibiotic choice; in one recent study, the effects of ten antibiotics commonly used against *S. aureus* were evaluated at various doses (0.06–128 $\mu\text{g/mL}$).²⁶ At sub-minimum inhibitory concentrations (MICs), linezolid appeared most effective in clearing biofilm. Conversely, azithromycin, vancomycin, oxacillin, cotrimoxazole, minocycline, and clindamycin induced biofilm in some of the bacterial strains tested.²⁶

An alternative, more effective approach to antibiotic monotherapy is combination antibiotics.^{27–29} Rifampin has been studied in combinations with linezolid, vancomycin, cloxacillin, and ciproflaxin.^{27–29} Dual therapy of rifampin with either linezolid or vancomycin for *in vivo* tibial infections resulted in reduced bacterial load compared to rifampin monotherapy.²⁷ In another study, rifampin and ciproflaxin together completely cleared a combination *S. aureus* and *Escherichia coli* co-infection.²⁹ Thus, combinations of antibiotics may offer better treatment outcomes.

Bacteriophages

For nearly 70 years, successful clinical use of bacteriophage (phage) in the treatment of long-term, antibiotic-resistant infections has been reported.^{30–32} Phage use is appealing, as they have high efficacy in lysing bacteria for which they are specific, and proliferate in the presence of their bacterial hosts, allowing for increased “dosage” of treatment as needed.^{33–35} In the United States, phages were largely abandoned after the widespread commercialization of antibiotics, although they are still used with great success across the world, particularly in the country of Georgia.³¹

Ongoing phage therapeutic research has been promising. In one *in vitro* study, bacteriophage SAP-26 alone was shown to have bactericidal activity on over 100 MRSA and 56 methicillin-sensitive *S. aureus* (MSSA) strains. With the antibiotics rifampin, azithromycin, and vancomycin, compared to bacteriophage, bacterial biofilms were reduced by 40%, 25%, 17%, and 28%, respectively. When testing combinations of the antibiotics with SAP-26, rifampin + SAP-26 reduced bacterial biofilms to the greatest extent (65%).³⁶ The superiority of phage and antibiotic combination treatment to monotherapy of either has been reported, commonly with rifampin, implying a potential synergistic effect.^{35,37–40} *In vivo*, phage has also shown significant clearance of soft tissue infections.^{41,42} In one abscess study, 10⁹ PFU of bacteriophage M^{Sa} eradicated infection in 97% of mice, when treated immediately after inoculation of 10⁸ CFU of *S. aureus* intravenously.⁴¹ The same treatment administered 10 days after intravenous (IV) delivery of 5 × 10⁶ CFU *S. aureus* resulted in clearance in all phage treated mice 10 days after treatment, whereas all non-treated mice remained infected through 20 days.

Before phages become widely used clinically, several hurdles must be overcome. First, phages are typically specific for one strain of bacteria, so rapid treatment of polymicrobial infections, or infections whose causative agents are unidentified, is problematic. To overcome this problem, phage cocktails containing multiple strains of phages have been developed, and may be necessary for clinical efficacy.^{40,43} There is also concern over the use of viruses within human hosts, without knowledge of their potential side effects. However, little if any side effects have been identified in numerous clinical reports of bacteriophage use, with no reports of anaphylaxis.³²

Phage may be inactivated in human sera,⁴⁴ and bacteria can develop resistance to phage.⁴⁵ Additionally, since phages die without a bacterial host, efficient and precise delivery to the site of infection is critical for bactericidal activity.^{34,40,42} In one recent study, alginate hydrogel was used to deliver CRISPR-Cas9 bacteriophage to *S. aureus* infected femora. Although the surrounding soft tissue infection was reduced, bacterial load in the bone was

unchanged, presumably due to insufficient localization of phage at the bony site.⁴⁶ Recent research has focused on engineering hydrogels, powders, nanoparticles, and implant coatings for delivery and prolonged bioavailability of phages.^{34,35,47,48} Long term, another hurdle will be reliable generation and storage of phages needed for widespread commercialization. In some phage lysate preparations, toxins have been found.⁴⁹

An alternative to using phages themselves is to utilize a class of phage enzymes called endolysins, which have hydrolase activity to dismantle bacterial cell walls.⁵⁰ In a recent study utilizing the endolysin CHAP_K, delivered with the antimicrobial peptide lysostaphin, bacterial clearance was reported.⁵⁰ The role of bacteriophage in future treatment of multi-drug-resistant osteomyelitis is unclear; yet its history of successful use, positive attributes, and ability to act on resistant strains of bacteria are compelling motivators for the continued, necessary research on these viral therapeutics.^{51–53}

Antimicrobial peptides

Antimicrobial peptides (AMPs), found naturally within human neutrophils and in epithelial secretions, have gained popularity in recent years as therapeutics for osteomyelitis.⁵⁴ The list of AMPs is extensive,⁵⁵ but some investigated for *S. aureus* clearance include: β -defensins,^{56,57} Magainin 2 (also known by its analog, pexiganan acetate),^{58,59} human lactoferrin 1–11 (hLF1–11),^{60,61} isolates of bee venom,^{62–65} snake venom,⁶⁶ and L-homocarnosine.⁶⁷

In one interesting study, a murine model for the study of human β -defensin-3 (HBD-3) was established. Mice naturally have a structural and functional homolog of HBD-3 called mouse β -defensin-14 (MBD-14), whose expression may be increased following intraperitoneal administration of anisomycin (15.0 mg/kg or 5.0 mg/kg), an activator of the signaling pathway p38 mitogen-activated protein kinase (MAPK).⁵⁶ In a tibial OM model, mice with increased MBD-14 levels had a significantly lowered bacterial load after 7 days of treatment, relative to the untreated controls with unaltered MBD-14 levels.⁵⁶ In a following study, infections in mice treated with HBD-3 were cleared to the same extent as those treated with the antibiotic vancomycin.⁵⁷ In some studies, AMPs have exhibited greater bactericidal activity than antibiotics. In an *ex vivo* study of infected human femoral heads, calcium phosphate or poly(methyl methacrylate) (PMMA) cements were used to deliver analogs of bee venom halcitides (HAL-1 and HAL-2), with significantly greater efficacy than vancomycin and gentamicin, with 3 and 2 log (CFU) differences, respectively.⁶⁸

Alternatively, vancomycin has been used as a synergistic agent with AMPs. L-homocarnosine (a peptide abundant in brain and skeletal muscle) and vancomycin together yielded the greatest antimicrobial effect as compared to either agents delivered alone, with nearly a 5 log difference in bacterial load (versus ~1–2 log difference as monotherapy).⁶⁷

It should be noted that, similarly to bacteriophages, antimicrobial peptides exhibit low metabolic stability, with low oral bioavailability and poor penetration of the intestinal wall.⁶⁹ For this reason, IV administration is not efficacious, as the peptides would readily be degraded or cleared within the blood plasma, liver, or kidneys.⁷⁰ To improve the stability of these peptides for local delivery regimens, chemical modifications of the peptides have been explored.^{69,71,72} One specific method is blockade of the N-terminal and C-terminal ends of AMPs, to hinder host peptidases.^{69,70}

Enzymes

Another interesting area of research involves enzymes as therapeutics. One currently popular enzyme is lysostaphin, a commercially available bacteriocin secreted by *S. simulans biovar staphylolyticus* that can lyse the cell wall of *S. aureus*.^{50,73–75} This enzyme has been shown to act on both biofilms and resistant strains of bacteria (Figure 3).⁷³ The attractiveness of lysostaphin is heightened by its ability to work synergistically with other therapeutics such as BMP-2 (for bony defect healing),⁷⁶ endolysins,^{50,77} and some antibiotics.⁷⁸ Lysostaphin's efficacy may be attributed to its rapid action, which aids the initial attack by antibiotics on bacteria and helps increase efficacy.⁷⁸ However, a key feature of this peptide is also a major limitation: lysostaphin is only efficacious on *S. aureus*, and thus in polymicrobial infections would not kill any other genera.⁷⁵

As mentioned previously, endolysins (hydrolases) produced by bacteriophages may also be a potential osteomyelitis therapeutic, as they effectively disrupt the bacterial cell wall and act proficiently on biofilm.^{50,79} A recent focus has been on chimeric endolysins, which may enhance the bactericidal potential of these enzymes.^{80–82} One recently engineered chimeric endolysin exerted bactericidal efficacy on *S. aureus*, *S. epidermidis*, and *enterococci*, which is promising for polymicrobial osteomyelitis infection treatment.⁸⁰ Furthermore, this chimeric enzyme exerted synergistic effects with vancomycin.⁸⁰ As with other previously mentioned therapeutics, host enzymes are not stable *in vivo* and thus must be applied locally for optimal efficacy.

Quorum sensing inhibitors

In *S. aureus*, virulence is maintained through quorum sensing, a method of chemical communication among neighboring *S. aureus* cocci.⁸³ Thus, quorum sensing inhibitors, also called “quorum quenchers,” have been explored as antimicrobials.⁸³ These drugs are not direct antimicrobials; rather, they serve to improve the efficacy of pre-existing therapeutics for infection treatment. Thus, this mode of treatment would require the administration of antibiotics or other bactericidal agents.⁸⁴ Some identified quorum sensing inhibitors include savirin,⁸⁵ Hamamelitannin,^{84,86} farnesol,^{87–89} and the nonsteroidal anti-inflammatory drug (NSAID) diflunisal.⁹⁰ In one study, the antimicrobials cefazolin, cefalonium, cephalixin, cefoxitin, daptomycin, linezolid, tobramycin, and fusidic acid were tested alone or with Hamamelitannin against *in vitro* *S. aureus* biofilms. In all cases except fusidic acid, treatment with the quorum sensing inhibitor was superior to antimicrobial monotherapy.⁸⁴ In another study, synergy between gentamicin and farnesol was noted with reduction of bacterial populations by 2 log units. However, both agents were required at high doses (gentamicin at 2.5 times the MIC, and farnesol at 100 μ M (22 μ g/ml)), which indicates the need for further studies for optimization.⁸⁷ As an implant coating, farnesol also modestly, but significantly, reduced the bacterial load of Ti6Al4V pins (from 4.86 to 4.26 Log₁₀ CFU).⁸⁹

Biofilm-dispersing agents have also gained interest, as penetrating the biofilm is one of the most difficult burdens for clinicians. One example are D-amino acids, protein residues in the D-configuration.^{91–93} In one *in vivo* study, polyurethane (PUR) scaffolds made with 1, 5, or 10 wt% D-Met:D-Pro:D-Trp (in a 1:1:1 ratio) had a 4-log reduction in bacterial load relative to PUR scaffolds.⁹¹ When applied into a segmental defect model of osteomyelitis, in

which all subjects received cefazolin treatment, the 5 and 10% wt PUR scaffolds prevented implant contamination entirely (n=10), whereas 4/10 of the PUR scaffolds recovered had $> 3\text{-log}_{10}\text{CFU/g}$ per bone.⁹¹ A different formulation, poly(ethylene glycol) (PEG) scaffolds incorporated with D-Pro:D-Met:D-Phe (1:1:1 ratio), has also been effective at preventing MRSA and MSSA colonization *in vitro* at concentrations of 13.5 mmol/L or greater. More novel combinations include the use of thermoresponsive, magnetic glycol chitin-based nanocomposites for release of D-amino acids and iron oxide nanoparticles. The combined disruption of biofilms by D-amino acids followed by magnetic hyperthermia successfully eradicated *S. aureus* biofilms *in vitro*.⁹³

Metals

A materials-based outlook on antimicrobials for osteomyelitis has also been considered, largely focusing on metal alloys used for orthopedic hardware. One novel metal formulation, stainless steel with a 4.5% copper alloy (317L-Cu), has been shown to exhibit antimicrobial properties while maintaining biocompatibility *in vitro* and *in vivo*, making it a promising material choice for future implant devices.⁹⁴ A magnesium-copper Mg25Cu alloy (Mg with 0.25 wt% Cu) may also provide desired antimicrobial activity, as supported by a recent *in vitro* and *in vivo* study. *In vitro*, the use of Mg25Cu alloy resulted in a nearly twofold logarithmic reduction in bacterial load compared to pure Ti and Mg alone. When utilized in an *in vivo* tibial osteomyelitis study, rabbits treated with intramedullary nails made of Mg0.25Cu had significantly improved histological and radiological scores. No quantitative bacterial counts from the *in vivo* study were reported.⁹⁵

Gold is an attractive option for a metal-based therapeutic. As a nanoparticle, it has exhibited cytocompatibility, efficacy against planktonic and sessile bacteria, and bactericidal activity against MSSA and MRSA strains.⁹⁶ Similarly, silver (Ag) has been explored for both orthopedic implants and as an antimicrobial therapeutic. In a study evaluating the delivery of Ag nanoparticles from a silk-fibroin hydrogel, Ag had positive cytocompatibility and bactericidal activity at a concentration 0.5%, with adverse effects on osteocytes at higher concentrations.⁹⁶ Variability in its efficacy as an antimicrobial was dependent on the strain of *S. aureus*.⁹⁷ Against some strains, Ag may be more beneficial at higher doses, in conjunction with other materials, or when utilized in a silver ion state.^{97–99}

Delivery Vehicles

Hydrogels

Commonly used by clinicians and researchers, hydrogels are a versatile and compatible delivery vehicle for therapeutics of osteomyelitis. Hydrogels are water-based materials, composed of synthetic or natural hydrophilic polymers in various combinations. Some natural materials that constitute hydrogels include, but are not limited to: alginate,^{46,100,101} chitosan,¹⁰² silk,⁹⁶ gelatin,^{103,104} and keratin.¹⁰⁵ Alternatively, synthetic materials can be used, including: poly(acrylic acid),^{103,106} poly(lactic-co-glycolic acid) (PLGA),¹⁰⁷ and PEG.^{73,108} The list of these materials is extensive, as various combinations and novel formulations are often explored.^{96,109,110}

The release profiles of drugs from hydrogels vary depending on the nature of the interaction of drug with polymer, but there is typically an initial burst of therapeutic, which may be followed by a slow, low dose elution of residual therapeutic.^{103,111} Hydrogels are generally biodegradable and may be cleared by the body in as early as 2–6 weeks.^{103,107,111}

Hydrogels have been used as carriers for antibiotics,^{105,107,110,112,113} bacteriophages,^{34,46,48,108} and proteins,^{46,50,73,76} as well as nanoparticles and microparticles containing therapeutics.^{96,107,112}

One of the advantages of hydrogels is the ability to tailor their properties including porosity, viscosity, elasticity, degree of swelling, and rate of drug elution. To alter drug elution rates, the sensitivity of hydrogels to temperature, pH, or both can be altered to increase or decrease degradation rates.^{21,106,107,110,112} Another way to modify drug elution rate is through changes in the composition of the gels. Decreasing gelatin concentration in poly(acrylic acid) hydrogels was directly related to a decreased antibiotic elution rate.¹⁰³ Modulating keratin concentration through an oxidative extraction process resulted in a desired ciprofloxacin release of 60% elution over 10 days, followed by a slow, sustained release for 3 weeks.¹⁰⁵ This specific formula showed success inhibiting *S. aureus* growth.

A more unique way to control drug release may be through conformational changes. Recently, a layered hyaluronic acid methacrylate (HAMA) system delivered triggered release of phages *in vivo*.³⁴ The success of the system was due to the outer HAMA layer, which, upon contact with hyaluronidase in *S. aureus*, degraded and released bacteriophage to treat *S. aureus* biofilms.³⁴ Another alteration of hydrogels involved the utilization of gellan gum, in which the hydrogel took on a threefold double helical shape and was used to deliver both soluble vancomycin and vancomycin encapsulated in PLGA nanoparticles.¹¹⁴

Cements

Traditionally, bone cements have been used by surgeons for placement of orthopedic hardware. In the past, these cements were largely non-degradable which impeded bone healing and necessitated additional surgeries. Recent modifications of bone cement have improved degradation rates and enabled them to serve as local drug carriers for antibiotics such as teicoplanin,¹⁰⁷ vancomycin,^{115,116} gentamicin,^{117,118} and doxycycline,¹¹⁹ along with antimicrobial peptides,^{61,68} and other multifactor therapies.^{120–123} Nonetheless, empirical use of high-dose, antibiotic-loaded bone cement (ALBC) typically elicits a burst release (then subtherapeutic dose), contributing to antibiotic resistance.¹²⁴ For enhanced antimicrobial properties and extended duration of drug elution, new formulations of bone cements are being engineered including core materials of calcium sulfate¹²², calcium phosphate,^{117,120} PMMA,¹¹⁵ and even bioactive glasses.^{121,123} PMMA has been the most commonly utilized and modified bone cement. One interesting formulation is hydrophilized PMMA containing a pluronic F68 additive (7% wt), which was able to effectively deliver vancomycin over 11 weeks while maintaining adequate mechanical stability at the site of fracture. It was hypothesized that the extended drug release profile of this formulation can be directly attributed to the hydrophilic properties of F68.¹¹⁵ Other research has been dedicated to exploring novel formulations of orthopedic cements. In one such study, an implanted calcium phosphate-calcium sulfate mixture (Cerament™), mirrored the antibacterial efficacy

of traditional PMMA, while also demonstrating partial resorption and a 20% increase in bone volume fraction.¹²⁵ As foreign objects provide a niche for bacterial colonization and mechanical incongruencies, and PMMA is a non-resorbable material, Cerament™ could be a promising alternative.¹²⁵ Despite successes with ALBC, standardized protocols, studies elucidating minimum effective dosing against biofilm, and efficacy in randomized controlled trials are warranted.¹²⁴

Micro- and Nanoparticles

Recently, micro- and nanoparticles have been explored for their favorable characteristics including biocompatibility, use for local or systemic delivery, and easy manipulation.¹²⁶ Micro- and nanoparticles can vary by size and composition, as well as by mode of fabrication and administration. Micro- and nanoparticles have been used to deliver antibiotics^{114,126–130} and bacteriophages,^{48,131,132} among other antimicrobial therapeutics. Materials utilized for favorable micro- and nanoparticle synthesis include: PEG,¹⁰⁷ poly(ester-amide)-PEGs (PEAE), poly(N-isopropylacrylamide) (PNIPAM),¹³¹ gold,^{48,96,132} silver,^{96,133} silk fibroin,^{96,129} chitosan,¹³⁴ chitosan/silver-hydroxyapatite blends,¹³⁵ and PLGA.^{107,114,128,130} Chitosan-glycerophosphate,¹¹² gelatin,¹⁰⁴ and a mixture of chitosan and gelatin cross-linked with genipin have also been used.¹³⁶ As an alternative to the more expensive gold micro- and nanoparticles, recent *in vitro* studies of aragonite nanoparticles, derived from cockle shells, indicated 85% of vancomycin was released within the first 15 hours followed by a slow release period of approximately 120 hours, during which the MIC of the *S. aureus* strain was exceeded.¹³⁷ Another interesting material is raspberry-like gelatin microspheres, which have multiple compartments inside a single microsphere, making them a desirable blend of nanoparticles and microspheres. Various biomolecules can be released from the microspheres by diffusion, erosion, or degradation-controlled release, which shows promise for administering therapeutics such as vancomycin and growth factors.¹⁰⁴

Micro- and nanoparticles may be used for local or systemic delivery of therapeutics. Hydrogels are a common way these particles have been delivered to the site of infection.^{112,114} Oral ingestion has been considered, although stomach acid can hinder the effectiveness of phages and antibiotics. Thus, extra measures must be taken to protect microspheres, which can involve encapsulation by a basic protectant such as calcium carbonate.^{47,138} Alginate has also been shown to increase the survival of many bioactive substances in simulated stomach acid.^{47,138} For greater protection of phage, a combination of alginate and calcium carbonate has been used to buffer the acid.¹³⁸

Coatings/Films

Another effective class of local delivery systems are coatings and films, which can serve as a preventative measure or an active treatment against contaminated implants. In recent studies, antibiotics, phages, and lysostaphin released from films/coatings made of phosphatidylcholine,^{139,140} hydroxypropylmethlycellulose (HPMC),³⁵ poly(D,L-lactide) (PDLLA),¹⁴¹ and a hyaluronic acid/poly-lactic acid hydrogel (DAC®)¹¹³ have been explored, among others.^{99,142–148} Interestingly, silver as a coating material may be used as both an antimicrobial and a delivery vehicle, or combined with other materials such as zeolites (made of aluminum and silicon), which are beneficial in this pairing because of their

crystal-like porous structure.⁹⁹ In one study, zeolite alone decreased bacterial counts by approximately 3000 units. Through ion exchange, the silver-zeolite coating decreased bacteria by an additional 1500 units.⁹⁹ Another metal silver has been alloyed with for a coating is titanium, to limit any potential systemic effects of silver ion.⁹⁸ In a recent study, silver nanoparticle coating of a stainless steel orthopedic implant (316L) completely eradicated infection in an *in vivo* study spanning 42 days.¹³³ Thus, it may be possible to alter both the orthopedic hardware materials and nanoparticle coatings for optimal anti-infection properties. Poloxamer 407 (Pluronic® F-127) is a hydrogel that may also serve as a coating to prevent adhesion of bacteria to orthopedic hardware and bone cements such as PMMA.¹⁴⁶

Scaffolding

Bone scaffolds that serve as delivery vehicles for osteomyelitis treatment are desirable, as they could offer support for a fracture and direct bone healing. Some scaffolds may even be engineered to degrade, ideally at the rate of bone regeneration.¹⁴⁹ Scaffolds composed of combinations of PLGA,¹⁵⁰ polyurethane,^{91,151} PDLLA,¹⁵² β -tricalcium phosphate (β -TCP),¹⁵³ and other materials have been used to deliver a multitude of therapeutics including antibiotics and proteins.

A recently engineered bone graft highlights the wide range of alterations that can be made to these scaffolds. In this study, calcium sulfate, hyaluronan, simvastatin, and PLGA microspheres loaded with vancomycin were combined to create a bone graft substitute with a hard “core,” surrounded by a moldable protective shell. The goal of this design was to allow release of antibiotic upon implantation, followed by release of an osteogenic agent (simvastatin) to promote bone union.¹⁵⁰ Another novel approach includes the use of 3D printing for scaffold production. In one study, a PDLLA cylinder was crafted of a series of four concentric layers for specific, alternating delivery of either levofloxacin or tobramycin.¹⁵²

Sponges

Sponges for local and rapid treatment of osteomyelitis appears to be a growing area of interest, particularly in military applications, as they are readily available to pack into traumatic wounds and rapidly deliver therapeutics directly to the site of infection.²⁹ Reported materials of sponges include chitosan, chitosan-polycaprolactone blends, and collagen, which have been used to deliver gentamicin,^{118,154} ciprofloxacin,^{29,155} amikacin,^{156,157} vancomycin,^{156–158} rifampin²⁹, and others.¹⁵⁹ In two comparative studies in humans, gentamicin loaded collagen sponges matched the efficacy of traditionally used PMMA beads in osteomyelitis treatment, while reducing the need for secondary surgical procedures.^{118,160} In one polymicrobial (*S. aureus* and *E. coli*) murine model of implant-related osteomyelitis, dual ciprofloxacin and rifampin drugs delivered locally by chitosan sponges completely cleared infection. Furthermore, efficacy against biofilm was also noted.²⁹ Thus, sponges as delivery vehicles for antimicrobials may be a promising area for future research.

Conclusion

A multitude of therapeutics and delivery vehicles for osteomyelitis treatment have been engineered, with promising results, although largely observed only in animal models. In these research studies, we must continue to address the growing concerns of clinicians regarding osteomyelitis infections, including but not limited to: biofilm, SCVs, immunocompromised (e.g., diabetic) patients, antimicrobial-resistant bacterial strains, and simultaneous treatment of infection and fracture. Therapeutics/regimens with reported bactericidal success discussed herein include: surgical debridement, antibiotics, bacteriophages, antimicrobial peptides, enzymes, quorum-sensing inhibitors, and metals. To optimize their success, delivery vehicles including hydrogels, cements, micro/nanoparticles, coatings/films, scaffolds, and sponges must continue to be studied and modified. History has shown that bacteria will continue to develop resistance to commonly used antibiotics, so it is imperative that we continue to explore alternative means of infection mitigation.

Acknowledgements

The authors would like to acknowledge support from the Mississippi State University Office of Research and Economic Development. L. Priddy is supported in part by NIH grant P20GM103646-07.

References

1. Deurenberg RH & Stobberingh EE The evolution of *Staphylococcus aureus*. *Infection, Genetics and Evolution* (2008). doi:10.1016/j.meegid.2008.07.007
2. Frieden Tom. Antibiotic Resistance Threats. Cdc (2013). doi:CS239559-B
3. Gil C et al. Biofilm matrix exoproteins induce a protective immune response against *Staphylococcus aureus* biofilm infection. *Infect. Immun.* (2014). doi:10.1128/IAI.01419-13
4. Bhowmik D, Bhanot R, Gautam D, Rai P & Kumar KPS Osteomyelitis-Symptoms, Causes and Treatment. *Res. J. Sci. Technol.* (2018). doi:10.5958/2349-2988.2018.00024.4
5. Kurtz SM, Lau E, Watson H, Schmier JK & Parvizi J Economic burden of periprosthetic joint infection in the united states. *J. Arthroplasty* (2012). doi:10.1016/j.arth.2012.02.022
6. CDC. New CDC report: More than 100 million Americans have diabetes or prediabetes | CDC Online Newsroom | CDC. Cdc (2017).
7. Sanders J & Mauffrey C Long Bone Osteomyelitis in Adults: Fundamental Concepts and Current Techniques. *Orthopedics* (2013). doi:10.3928/01477447-20130426-07
8. Masters EA et al. Evolving concepts in bone infection: redefining “biofilm”, “acute vs. chronic osteomyelitis”, “the immune proteome” and “local antibiotic therapy”. *Bone Research* (2019). doi:10.1038/s41413-019-0061-z
9. Schwarz EM et al. 2018 International Consensus Meeting on Musculoskeletal Infection: Research Priorities from the General Assembly Questions. *Journal of Orthopaedic Research* (2019). doi:10.1002/jor.24293
10. Kaarsemaker S, Walenkamp GHIM & Anthony AEJ New model for chronic osteomyelitis with *Staphylococcus aureus* in sheep. *Clin. Orthop. Relat. Res.* (1997). doi:10.1097/00003086-199706000-00033
11. Hill PF & Watkins PE The prevention of experimental osteomyelitis in a model of gunshot fracture in the pig. *Eur. J. Orthop. Surg. Traumatol.* (2001). doi:10.1007/BF01686897
12. Monteiro JM et al. Cell shape dynamics during the staphylococcal cell cycle. *Nat. Commun.* (2015). doi:10.1038/ncomms9055
13. Tuscherr L et al. *Staphylococcus aureus* develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis. *J. Antimicrob. Chemother.* (2016). doi:10.1093/jac/dkv371

14. de Mesy Bentley KL et al. Evidence of Staphylococcus Aureus Deformation, Proliferation, and Migration in Canaliculi of Live Cortical Bone in Murine Models of Osteomyelitis. *J. Bone Miner. Res.* 32, 985–990 (2017). [PubMed: 27933662]
15. Kriegeskorte A et al. Staphylococcus aureus small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. *Front. Cell. Infect. Microbiol.* (2014). doi:10.3389/fcimb.2014.00141
16. Painter KL et al. Staphylococcus aureus adapts to oxidative stress by producing H₂O₂-resistant small-colony variants via the SOS response. *Infect. Immun.* (2015). doi:10.1128/IAI.03016-14
17. Cheng AG, DeDent AC, Schneewind O & Missiakas D A play in four acts: Staphylococcus aureus abscess formation. *Trends in Microbiology* (2011). doi:10.1016/j.tim.2011.01.007
18. Vulin C, Leimer N, Huemer M, Ackermann M & Zinkernagel AS Prolonged bacterial lag time results in small colony variants that represent a sub-population of persisters. *Nat. Commun.* (2018). doi:10.1038/s41467-018-06527-0
19. Dantes R et al. National burden of invasive methicillin-resistant Staphylococcus aureus infections, United States, 2011. *JAMA Intern. Med.* (2013). doi:10.1001/jamainternmed.2013.10423
20. Klein EY, Sun L, Smith DL & Laxminarayan R The changing epidemiology of methicillin-resistant Staphylococcus aureus in the United States: A national observational study. *Am. J. Epidemiol.* (2013). doi:10.1093/aje/kws273
21. Aparna RSL, Prasad RGSV & Nirmal NP An Injectable In-Situ Conducting Thermosensitive Gel for Controlled Delivery of Vancomycin in Osteomyelitis Treatment and Bone Regeneration. *SCIENCE OF ADVANCED MATERIALS* 8, 1470–1477
22. Garvin KL et al. Polylactide/polyglycolide antibiotic implants in the treatment of osteomyelitis: A canine model. *J. Bone Jt. Surg. Am. Vol.* 76, 1500–1506 (1994).
23. Wagner JM et al. Surgical debridement is superior to sole antibiotic therapy in a novel murine posttraumatic osteomyelitis model. *PLoS One* (2016). doi:10.1371/journal.pone.0149389
24. Penn-Barwell JG, Murray CK & Wenke JC Early antibiotics and debridement independently reduce infection in an open fracture model. *Bone Joint J.* (2012). doi:10.1302/0301-620X.94B1.27026
25. Ng M et al. Induction of MRSA biofilm by low-dose β -lactam antibiotics: Specificity, prevalence and dose-response effects. *Dose-Response* (2014). doi:10.2203/dose-response.13-021.Kaplan
26. Majidpour A et al. Dose-Dependent Effects of Common Antibiotics Used to Treat Staphylococcus aureus on Biofilm Formation. *Iran. J. Pathol.* 12, 362–370 (2017). [PubMed: 29563932]
27. Vergidis P et al. Treatment with linezolid or vancomycin in combination with rifampin is effective in an animal model of methicillin-resistant Staphylococcus aureus foreign body osteomyelitis. *Antimicrob. Agents Chemother.* (2011). doi:10.1128/AAC.00740-10
28. Zak O et al. Experimental staphylococcal osteomyelitis in rats: therapy with rifampin and cloxacillin alone or in combination. *Curr. Chemother. Immunother. Am. Soc. Microbiol.* Washington, DC 973–974 (1982).
29. Wells CM et al. Ciprofloxacin and Rifampin Dual Antibiotic-Loaded Biopolymer Chitosan Sponge for Bacterial Inhibition. in *Military Medicine* (2018). doi:10.1093/milmed/usx150
30. Slopek S et al. Results of bacteriophage treatment of suppurative bacterial infections. II. Detailed evaluation of the results. *Arch. Immunol. Ther. Exp. (Warsz.)* (1983).
31. Kutateladze M & Adamia R Phage therapy experience at the Eliava Institute. *Med. Mal. Infect.* (2008). doi:10.1016/j.medmal.2008.06.023
32. Speck P & Smithyman A Safety and efficacy of phage therapy via the intravenous route. *FEMS Microbiol. Lett.* 363, (2015).
33. Balcão VM et al. Structural and functional stabilization of phage particles in carbohydrate matrices for bacterial biosensing. *Enzyme Microb. Technol.* (2013). doi:10.1016/j.enzmictec.2013.03.001
34. Bean JE et al. Triggered Release of Bacteriophage K from Agarose/Hyaluronan Hydrogel Matrixes by Staphylococcus aureus Virulence Factors. *CHEMISTRY OF MATERIALS* 26, 7201–7208
35. Kaur S, Harjai K & Chhibber S Bacteriophage mediated killing of Staphylococcus aureus in vitro on orthopaedic K wires in presence of linezolid prevents implant colonization. *PLoS One* (2014). doi:10.1371/journal.pone.0090411

36. Rahman M, Kim S, Kim SM, Seol SY & Kim J Characterization of induced *Staphylococcus aureus* bacteriophage SAP-26 and its anti-biofilm activity with rifampicin. *Biofouling* (2011). doi:10.1080/08927014.2011.631169
37. Jo A, Ding T & Ahn J Synergistic antimicrobial activity of bacteriophages and antibiotics against *Staphylococcus aureus*. *Food Sci. Biotechnol.* 25, 935–940 (2016). [PubMed: 30263357]
38. Dickey J & Perrot V Adjunct phage treatment enhances the effectiveness of low antibiotic concentration against *Staphylococcus aureus* biofilms in vitro. *PLoS One* (2019). doi:10.1371/journal.pone.0209390
39. Nir-Paz R et al. Successful Treatment of Antibiotic-resistant, Poly-microbial Bone Infection With Bacteriophages and Antibiotics Combination. *Clin. Infect. Dis.* (2019). doi:10.1093/cid/ciz222
40. Yilmaz C et al. Bacteriophage therapy in implant-related infections: An experimental study. *J. Bone Jt. Surg. - Ser. A* (2013). doi:10.2106/JBJS.K.01135
41. Capparelli R, Parlato M, Borriello G, Salvatore P & Iannelli D Experimental phage therapy against *Staphylococcus aureus* in mice. *Antimicrob. Agents Chemother.* (2007). doi:10.1128/AAC.01513-06
42. Cobb LH et al. CRISPR-Cas9 modified bacteriophage for treatment of *Staphylococcus aureus* induced osteomyelitis and soft tissue infection. *PLoS One* 14, e0220421 (2019). [PubMed: 31756187]
43. Kishor C et al. Phage therapy of staphylococcal chronic osteomyelitis in experimental animal model. *Indian J. Med. Res.* (2016). doi:10.4103/0971-5916.178615
44. Łusiak-Szelachowska M et al. Phage neutralization by sera of patients receiving phage therapy. *Viral Immunol.* (2014). doi:10.1089/vim.2013.0128
45. Oechslin F Resistance development to bacteriophages occurring during bacteriophage therapy. *Viruses* 10, (2018).
46. Cobb LH et al. CRISPR-Cas9 modified bacteriophage for treatment of *Staphylococcus aureus* induced osteomyelitis and soft tissue infection. *PLoS One* 14, e0220421 (2019). [PubMed: 31756187]
47. Tang Z, Huang X, Sabour PM, Chambers JR & Wang Q Preparation and characterization of dry powder bacteriophage K for intestinal delivery through oral administration. *LWT - Food Sci. Technol.* (2015). doi:10.1016/j.lwt.2014.08.012
48. Souza GR et al. Bottom-up assembly of hydrogels from bacteriophage and Au nanoparticles: The effect of Cis- and trans-acting factors. *PLoS One* (2008). doi:10.1371/journal.pone.0002242
49. Merrill CR et al. Long-circulating bacteriophage as antibacterial agents. *Proc. Natl. Acad. Sci. U. S. A.* (1996). doi:10.1073/pnas.93.8.3188
50. Hathaway H et al. Thermally triggered release of the bacteriophage endolysin CHAPK and the bacteriocin lysostaphin for the control of methicillin resistant *Staphylococcus aureus* (MRSA). *J. Control. Release* (2017). doi:10.1016/j.jconrel.2016.11.030
51. Agarwal R et al. Inhaled bacteriophage-loaded polymeric microparticles ameliorate acute lung infections. *Nat. Biomed. Eng.* 2, 841–849 (2018). [PubMed: 30854250]
52. Furfaro LL, Payne MS & Chang BJ Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles. *Front. Cell. Infect. Microbiol.* (2018). doi:10.3389/fcimb.2018.00376
53. Ul Haq I, Chaudhry WN, Akhtar MN, Andleeb S & Qadri I Bacteriophages and their implications on future biotechnology: A review. *Virology Journal* (2012). doi:10.1186/1743-422X-9-9
54. Lai Y & Gallo RL AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends in Immunology* (2009). doi:10.1016/j.it.2008.12.003
55. Wang G, Li X & Wang Z APD3: The antimicrobial peptide database as a tool for research and education. *Nucleic Acids Res.* (2016). doi:10.1093/nar/gkv1278
56. Zhu C et al. The potential role of increasing the release of mouse β -defensin-14 in the treatment of osteomyelitis in mice: A primary study. *PLoS One* (2014). doi:10.1371/journal.pone.0086874
57. Zhu C, Bao NR, Chen S & Zhao JN The mechanism of human β -defensin 3 in MRSA-induced infection of implant drug-resistant bacteria biofilm in the mouse tibial bone marrow. *Exp. Ther. Med.* (2017). doi:10.3892/etm.2017.4112

58. Rosenbaum Chou TG, Petti CA, Szakacs J & Bloebaum RD Evaluating antimicrobials and implant materials for infection prevention around transcutaneous osseointegrated implants in a rabbit model. *J. Biomed. Mater. Res. - Part A* (2010). doi:10.1002/jbm.a.32413
59. Lipsky BA, Holroyd KJ & Zasloff M Topical versus Systemic Antimicrobial Therapy for Treating Mildly Infected Diabetic Foot Ulcers: A Randomized, Controlled, Double-Blinded, Multicenter Trial of Pexiganan Cream. *Clin. Infect. Dis.* (2008). doi:10.1086/593185
60. Stallmann HP, Faber C, Bronckers ALJJ, Nieuw Amerongen AV & Wuismann PIJM Osteomyelitis prevention in rabbits using antimicrobial peptide hLF1–11- or gentamicin-containing calcium phosphate cement. *J. Antimicrob. Chemother.* (2004). doi:10.1093/jac/dkh346
61. Faber C et al. Comparable efficacies of the antimicrobial peptide human lactoferrin 1–11 and gentamicin in a chronic methicillin-resistant *Staphylococcus aureus* osteomyelitis model. *Antimicrob. Agents Chemother.* (2005). doi:10.1038/sj.mp.4002127
62. Monincová L et al. Novel antimicrobial peptides from the venom of the eusocial bee *Halictus sexcinctus* (Hymenoptera: Halictidae) and their analogs. *Amino Acids* (2010). doi:10.1007/s00726-010-0519-1
63. e ovský V et al. Lasioglossins: Three novel antimicrobial peptides from the venom of the eusocial bee *Lasioglossum laticeps* (hymenoptera: halictidae). *ChemBioChem* (2009). doi:10.1002/cbic.200900133
64. Melicher ík P et al. Testing the efficacy of antimicrobial peptides in the topical treatment of induced osteomyelitis in rats. *Folia Microbiol. (Praha)*. (2018). doi:10.1007/s12223-017-0540-9
65. Melicher ík P, Neřuta O & e ovský V Antimicrobial peptides for topical treatment of osteomyelitis and implant-related infections: Study in the spongy bone. *Pharmaceuticals* (2018). doi:10.3390/ph11010020
66. Mehrdar MT, Madani R, Hajihosseini R & Bidhendi SM Antibacterial activity of isolated immunodominant proteins of *Naja Naja* (Oxiana) Venom. *Iran. J. Pharm. Res.* (2017).
67. Zhou P et al. Synergistic effect of vancomycin and l-homocarnosine alleviates *Staphylococcus aureus*-induced osteomyelitis in rats. *Biomed. Pharmacother.* 111, 31–35 (2019). [PubMed: 30572244]
68. Melicher ík P, Neřuta O & e ovský V Antimicrobial peptides for topical treatment of osteomyelitis and implant-related infections: Study in the spongy bone. *Pharmaceuticals* (2018). doi:10.3390/ph11010020
69. Rink R et al. To protect peptide pharmaceuticals against peptidases. *J. Pharmacol. Toxicol. Methods* (2010). doi:10.1016/j.vascn.2010.02.010
70. Malmsten M, Kasetty G, Pasupuleti M, Alenfall J & Schmidtchen A Highly selective end-tagged antimicrobial peptides derived from prelp. *PLoS One* (2011). doi:10.1371/journal.pone.0016400
71. Fjell CD, Hiss JA, Hancock REW & Schneider G Designing antimicrobial peptides: Form follows function. *Nature Reviews Drug Discovery* (2012). doi:10.1038/nrd3591
72. Waghv FH et al. Designing antibacterial peptides with enhanced killing kinetics. *Front. Microbiol.* (2018). doi:10.3389/fmicb.2018.00325
73. Johnson CT et al. Hydrogel delivery of lysostaphin eliminates orthopedic implant infection by *Staphylococcus aureus* and supports fracture healing. *Proc. Natl. Acad. Sci.* (2018). doi:10.1073/pnas.1801013115
74. Wu JA, Kusuma C, Mond JJ & Kokai-Kun JF Lysostaphin Disrupts *Staphylococcus aureus* and *Staphylococcus epidermidis* Biofilms on Artificial Surfaces. *Antimicrob. Agents Chemother.* (2003). doi:10.1128/AAC.47.11.3407-3414.2003
75. Bastos M, do C. de F., Coutinho BG & Coelho MLV. Lysostaphin: A staphylococcal bacteriolysin with potential clinical applications. *Pharmaceuticals* (2010). doi:10.3390/ph3041139
76. Johnson CT et al. Lysostaphin and BMP-2 co-delivery reduces *S. Aureus* infection and regenerates critical-sized segmental bone defects. *Sci. Adv.* (2019). doi:10.1126/sciadv.aaw1228
77. Becker SC, Foster-Frey J & Donovan DM The phage K lytic enzyme LysK and lysostaphin act synergistically to kill MRSA. *FEMS Microbiol. Lett.* (2008). doi:10.1111/j.1574-6968.2008.01308.x

78. Narasimhaswamy N, Bairy I, Shenoy G & Bairy L In vitro activity of recombinant lysostaphin in combination with linezolid, vancomycin and oxacillin against methicillin-resistant *Staphylococcus aureus*. *Iran. J. Microbiol.* 9, 208–212 (2017). [PubMed: 29238455]
79. Sass P & Bierbaum G Lytic activity of recombinant bacteriophage ϕ 11 and ϕ 12 endolysins on whole cells and biofilms of *Staphylococcus aureus*. *Appl. Environ. Microbiol.* (2007). doi:10.1128/AEM.01616-06
80. Kashani HH, Fahimi H, Goli YD & Moniri R A novel chimeric endolysin with antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *Front. Cell. Infect. Microbiol.* (2017). doi:10.3389/fcimb.2017.00290
81. Mao J, Schmelcher M, Harty WJ, Foster-Frey J & Donovan DM Chimeric Ply187 endolysin kills *Staphylococcus aureus* more effectively than the parental enzyme. *FEMS Microbiol. Lett.* (2013). doi:10.1111/1574-6968.12104
82. Fernandes S et al. Novel chimerical endolysins with broad antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. *Microb. Drug Resist.* (2012). doi:10.1089/mdr.2012.0025
83. Salam AM & Quave CL Targeting Virulence in *Staphylococcus aureus* by Chemical Inhibition of the Accessory Gene Regulator System In Vivo. *mSphere* 3, e00500–17 (2018). [PubMed: 29359191]
84. Brackman G et al. The Quorum Sensing Inhibitor Hamamelitannin Increases Antibiotic Susceptibility of *Staphylococcus aureus* Biofilms by Affecting Peptidoglycan Biosynthesis and eDNA Release. *Sci. Rep.* (2016). doi:10.1038/srep20321
85. Sully EK et al. Selective Chemical Inhibition of agr Quorum Sensing in *Staphylococcus aureus* Promotes Host Defense with Minimal Impact on Resistance. *PLoS Pathog.* (2014). doi:10.1371/journal.ppat.1004174
86. Brackman G, Cos P, Maes L, Nelis HJ & Coenye T Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. *Antimicrob. Agents Chemother.* (2011). doi:10.1128/AAC.00045-11
87. Jabra-Rizk MA, Meiller TF, James CE & Shirtliff ME Effect of farnesol on *Staphylococcus aureus* biofilm formation and antimicrobial susceptibility. *Antimicrob. Agents Chemother.* (2006). doi:10.1128/AAC.50.4.1463-1469.2006
88. Unnanuntana A, Bonsignore L, Shirtliff ME & Greenfield EM The effects of farnesol on *Staphylococcus aureus* biofilms and osteoblasts: An in vitro study. *J. Bone Jt. Surg. - Ser. A* (2009). doi:10.2106/JBJS.H.01699
89. Constantino JA et al. In vivo bactericidal efficacy of farnesol on Ti6Al4V implants TT - Eficacia bactericida in vivo del farnesol sobre implantes de Ti6Al4V. *Rev. Esp. Cir. Ortop. Traumatol.* (2016). doi:10.1016/j.recot.2016.04.004
90. Hendrix AS et al. Repurposing the nonsteroidal anti-inflammatory drug diflunisal as an osteoprotective, antivirulence therapy for *Staphylococcus aureus* osteomyelitis. *Antimicrob. Agents Chemother.* (2016). doi:10.1128/AAC.00834-16
91. Sanchez CJ et al. Effects of local delivery of d-amino acids from biofilm-dispersive scaffolds on infection in contaminated rat segmental defects. *Biomaterials* (2013). doi:10.1016/j.biomaterials.2013.06.026
92. Sanchez CJ et al. D-amino acids enhance the activity of antimicrobials against biofilms of clinical wound isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* (2014). doi:10.1128/AAC.02468-14
93. Abenojar EC et al. Magnetic Glycol Chitin-Based Hydrogel Nanocomposite for Combined Thermal and d -Amino-Acid-Assisted Biofilm Disruption. *ACS Infect. Dis.* (2018). doi:10.1021/acsinfecdis.8b00076
94. Chai H et al. Antibacterial effect of 317L stainless steel contained copper in prevention of implant-related infection in vitro and in vivo. *J. Mater. Sci. Mater. Med.* (2011). doi:10.1007/s10856-011-4427-z
95. Li Y et al. Biodegradable Mg-Cu alloy implants with antibacterial activity for the treatment of osteomyelitis: In vitro and in vivo evaluations. *Biomaterials* (2016). doi:10.1016/j.biomaterials.2016.08.031

96. Ribeiro M et al. Original Article: Antibacterial silk fibroin/nanohydroxyapatite hydrogels with silver and gold nanoparticles for bone regeneration. *Nanomedicine Nanotechnology, Biol. Med.* 13, 231–239 (2017).
97. Harrasser N et al. A new model of implant-related osteomyelitis in the metaphysis of rat tibiae. *BMC Musculoskelet. Disord.* (2016). doi:10.1186/s12891-016-1005-z
98. Lu M et al. An effective treatment of experimental osteomyelitis using the antimicrobial titanium/silver-containing nHP66 (nano-hydroxyapatite/polyamide-66) nanoscaffold biomaterials. *SCIENTIFIC REPORTS* 6,
99. Wang J et al. Antibacterial and anti-adhesive zeolite coatings on titanium alloy surface. *Microporous Mesoporous Mater.* (2011). doi:10.1016/j.micromeso.2011.04.005
100. Krishnan L et al. Delivery vehicle effects on bone regeneration and heterotopic ossification induced by high dose BMP-2. *Acta Biomater.* (2017). doi:10.1016/j.actbio.2016.12.012
101. Priddy LB et al. Oxidized alginate hydrogels for bone morphogenetic protein-2 delivery in long bone defects. *Acta Biomater.* 10, 4390–4399 (2014). [PubMed: 24954001]
102. Hasan S, Thomas N, Thierry B & Prestidge CA Controlled and Localized Nitric Oxide Precursor Delivery From Chitosan Gels to *Staphylococcus aureus* Biofilms. *J. Pharm. Sci.* (2017). doi:10.1016/j.xphs.2017.08.006
103. Changez M, Burugapalli K, Koul V & Choudhary V The effect of composition of poly(acrylic acid)-gelatin hydrogel on gentamicin sulphate release: In vitro. *Biomaterials* (2003). doi:10.1016/S0142-9612(02)00364-2
104. Diba M et al. Nanostructured raspberry-like gelatin microspheres for local delivery of multiple biomolecules. *Acta Biomater.* (2017). doi:10.1016/j.actbio.2017.05.059
105. Saul JM, Ellenburg MD, De Guzman RC & Dyke M Van. Keratin hydrogels support the sustained release of bioactive ciprofloxacin. *J. Biomed. Mater. Res. - Part A* (2011). doi:10.1002/jbm.a.33147
106. Dong L, chang & Hoffman AS A novel approach for preparation of pH-sensitive hydrogels for enteric drug delivery. *J. Control. Release* (1991). doi:10.1016/0168-3659(91)90072-L
107. Peng KT et al. Treatment of osteomyelitis with teicoplanin-encapsulated biodegradable thermosensitive hydrogel nanoparticles. *Biomaterials* (2010). doi:10.1016/j.biomaterials.2010.03.027
108. Johnson C, Dinjaski N, Prieto M & García A Bacteriophage Encapsulation in Poly(Ethylene Glycol) Hydrogels Significantly Reduces Bacteria Numbers in an Implant-Associated Infection Model of Bone Repair. *Igarss 2014* (2014). doi:10.1007/s13398-014-0173-7.2
109. Ci L et al. Amino-functionalized poloxamer 407 with both mucoadhesive and thermosensitive properties: preparation, characterization and application in a vaginal drug delivery system. *Acta Pharm. Sin. B* (2017). doi:10.1016/j.apsb.2017.03.002
110. Ter Boo GJA et al. Local application of a gentamicin-loaded thermo-responsive hydrogel allows for fracture healing upon clearance of a high *Staphylococcus aureus* load in a rabbit model. *Eur. Cells Mater.* (2018). doi:10.22203/eCM.v035a11
111. Overstreet D, McLaren A, Calara F, Vernon B & McLemore R Local Gentamicin Delivery From Resorbable Viscous Hydrogels Is Therapeutically Effective. *Clin. Orthop. Relat. Res.* (2015). doi:10.1007/s11999-014-3935-9
112. Mahmoudian M & Ganji F Vancomycin-loaded HPMC microparticles embedded within injectable thermosensitive chitosan hydrogels. *Prog. Biomater.* 6, 49–56 (2017). [PubMed: 28447299]
113. Giavaresi G et al. Efficacy of antibacterial-loaded coating in an in vivo model of acutely highly contaminated implant. *Int. Orthop.* (2014). doi:10.1007/s00264-013-2237-2
114. Posadowska U, Brzychczy-Wloch M & Pamula E Injectable gellan gum-based nanoparticles-loaded system for the local delivery of vancomycin in osteomyelitis treatment. *J. Mater. Sci. Mater. Med.* (2016). doi:10.1007/s10856-015-5604-2
115. Oh EJ, Oh SH, Lee IS, Kwon OS & Lee JH Antibiotic-eluting hydrophilized PMMA bone cement with prolonged bactericidal effect for the treatment of osteomyelitis. *J. Biomater. Appl.* (2015). doi:10.1177/0885328216629823

116. Li B, Brown KV, Wenke JC & Guelcher SA Sustained release of vancomycin from polyurethane scaffolds inhibits infection of bone wounds in a rat femoral segmental defect model. *J. Control. Release* (2010). doi:10.1016/j.jconrel.2010.04.002
117. Trombetta PR et al. Calcium Phosphate Spacers for the Local Delivery of Sitafloracin and Rifampin to Treat Orthopedic Infections: Efficacy and Proof of Concept in a Mouse Model of Single-Stage Revision of Device-Associated Osteomyelitis. *Pharmaceutics* 11, (2019).
118. Atan AA, Bajuri MY, Ali AM, Rehir R & Rashid AFA THE EFFECTS OF GENTAMICIN-IMPREGNATED COLLAGEN SPONGE VERSUS GENTAMICIN-IMPREGNATED POLYMETHYLMETHACRYLATE BEADS IN PATIENTS WITH OSTEOMYELITIS. *Asian J. Pharm. Clin. Res.* 11, (2018).
119. Mestres G et al. In vivo efficiency of antimicrobial inorganic bone grafts in osteomyelitis treatments. *Mater. Sci. Eng. C* 97, 84–95 (2019).
120. Joosten U et al. Effectiveness of hydroxyapatite-vancomycin bone cement in the treatment of *Staphylococcus aureus* induced chronic osteomyelitis. *Biomaterials* (2005). doi:10.1016/j.biomaterials.2005.01.001
121. Ding H et al. A novel injectable borate bioactive glass cement as an antibiotic delivery vehicle for treating osteomyelitis. *PLoS One* (2014). doi:10.1371/journal.pone.0085472
122. Mistry S et al. A novel, multi-barrier, drug eluting calcium sulfate/biphasic calcium phosphate biodegradable composite bone cement for treatment of experimental MRSA osteomyelitis in rabbit model. *J. Control. Release* 239, 169–181 (2016). [PubMed: 27582374]
123. Zhang X et al. Teicoplanin-loaded borate bioactive glass implants for treating chronic bone infection in a rabbit tibia osteomyelitis model. *Biomaterials* (2010). doi:10.1016/j.biomaterials.2010.04.005
124. Schwarz EM et al. Adjuvant antibiotic-loaded bone cement: Concerns with current use and research to make it work. *J. Orthop. Res.* (2020). doi:10.1002/jor.24616
125. Boyle KK et al. Vancomycin-laden calcium phosphate-calcium sulfate composite allows bone formation in a rat infection model. *PLoS One* (2019). doi:10.1371/journal.pone.0222034
126. Szczablinska J, Fijalkowski K, Kohn J & El Fray M Antibiotic loaded microspheres as antimicrobial delivery systems for medical applications. *Mater. Sci. Eng. C* (2017). doi:10.1016/j.msec.2017.03.215
127. Akram FE, El-Tayeb T, Abou-Aisha K & El-Azizi M A combination of silver nanoparticles and visible blue light enhances the antibacterial efficacy of ineffective antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA). *Ann. Clin. Microbiol. Antimicrob.* (2016). doi:10.1186/s12941-016-0164-y
128. Posadowska U, Brzychczy-Włoch M & Pamuła E Gentamicin loaded PLGA nanoparticles as local drug delivery system for the osteomyelitis treatment. *Acta Bioeng. Biomech.* (2015). doi:10.5277/ABB-00188-2014-02
129. Hassani Besheli N et al. Sustainable Release of Vancomycin from Silk Fibroin Nanoparticles for Treating Severe Bone Infection in Rat Tibia Osteomyelitis Model. *ACS Appl. Mater. Interfaces* (2017). doi:10.1021/acsami.6b14912
130. Dorati R et al. An experimental design approach to the preparation of pegylated polylactide-co-glicolide gentamicin loaded microparticles for local antibiotic delivery. *Mater. Sci. Eng. C* (2016). doi:10.1016/j.msec.2015.09.053
131. Hathaway H et al. Poly(N-isopropylacrylamide-co-allylamine) (PNIPAM-co-ALA) nanospheres for the thermally triggered release of Bacteriophage K. *Eur. J. Pharm. Biopharm.* (2015). doi:10.1016/j.ejpb.2015.09.013
132. Rastogi L, Kora AJ & Arunachalam J Highly stable, protein capped gold nanoparticles as effective drug delivery vehicles for amino-glycosidic antibiotics. *Mater. Sci. Eng. C* (2012). doi:10.1016/j.msec.2012.04.044
133. Nandi SK, Shivaram A, Bose S & Bandyopadhyay A Silver nanoparticle deposited implants to treat osteomyelitis. *J. Biomed. Mater. Res. - Part B Appl. Biomater.* (2018). doi:10.1002/jbm.b.33910

134. Cevher E et al. Characterization of biodegradable chitosan microspheres containing vancomycin and treatment of experimental osteomyelitis caused by methicillin-resistant *Staphylococcus aureus* with prepared microspheres. *Int. J. Pharm.* (2006). doi:10.1016/j.ijpharm.2006.03.014
135. Shen J, Jin B, Qi Y, Jiang Q & Gao X Carboxylated chitosan/silver-hydroxyapatite hybrid microspheres with improved antibacterial activity and cytocompatibility. *Mater. Sci. Eng. C* 78, 589–597 (2017).
136. Del Gaudio C et al. Natural polymeric microspheres for modulated drug delivery. *Mater. Sci. Eng. C* (2017). doi:10.1016/j.msec.2017.02.051
137. Saidykhan L, Bakar MZBA, Rukayadi Y, Kura AU & Latifah SY Development of nanoantibiotic delivery system using cockle shell-derived aragonite nanoparticles for treatment of osteomyelitis. *Int. J. Nanomedicine* (2016). doi:10.2147/IJN.S95885
138. Ma Y et al. Enhanced alginate microspheres as means of oral delivery of bacteriophage for reducing *staphylococcus aureus* intestinal carriage. *Food Hydrocoll.* (2012). doi:10.1016/j.foodhyd.2010.11.017
139. Jennings JA et al. Antibiotic-loaded phosphatidylcholine inhibits staphylococcal bone infection. *World J. Orthop.* (2016). doi:10.5312/wjo.v7.i8.467
140. Harris MA, Beenken KE, Smeltzer MS, Haggard WO & Jennings JA Phosphatidylcholine Coatings Deliver Local Antimicrobials and Reduce Infection in a Murine Model: A Preliminary Study. *Clin. Orthop. Relat. Res.* (2017). doi:10.1007/s11999-016-5211-7
141. Lucke M et al. Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats. *Bone* (2003). doi:10.1016/S8756-3282(03)00050-4
142. Moskowitz JS et al. The effectiveness of the controlled release of gentamicin from polyelectrolyte multilayers in the treatment of *Staphylococcus aureus* infection in a rabbit bone model. *Biomaterials* (2010). doi:10.1016/j.biomaterials.2010.04.011
143. Windolf CD et al. Lysostaphin-coated titan-implants preventing localized osteitis by *staphylococcus aureus* in a mouse model. *PLoS One* (2014). doi:10.1371/journal.pone.0115940
144. Paiva Costa L et al. Effectiveness of Chitosan Films Impregnated With Ciprofloxacin for the Prophylaxis of Osteomyelitis in Open Fractures: An Experimental Study in Rats. *Arch. Trauma Res.* (2016). doi:10.5812/atr.36952
145. Beenken KE et al. Chitosan coating to enhance the therapeutic efficacy of calcium sulfate-based antibiotic therapy in the treatment of chronic osteomyelitis. *Journal of Biomaterials Applications* 29, 514–523 (2014). [PubMed: 24854984]
146. Veyries ML, Faurisson F, Joly-Guillou ML & Rouveix B Control of staphylococcal adhesion to polymethylmethacrylate and enhancement of susceptibility to antibiotics by poloxamer 407. *Antimicrob. Agents Chemother.* (2000). doi:10.1128/AAC.44.4.1093-1096.2000
147. Schaer TP, Stewart S, Hsu BB & Klivanov AM Hydrophobic polycationic coatings that inhibit biofilms and support bone healing during infection. *Biomaterials* (2012). doi:10.1016/j.biomaterials.2011.10.038
148. Li B et al. Biological and antibacterial properties of the micro-nanostructured hydroxyapatite/chitosan coating on titanium. *Sci. Rep.* 9, 14052 (2019). [PubMed: 31575877]
149. McLaren JS et al. A biodegradable antibiotic-impregnated scaffold to prevent osteomyelitis in a contaminated in vivo bone defect model. *Eur. Cells Mater.* (2014). doi:10.22203/eCM.v027a24
150. Brown ME et al. Testing of a bioactive, moldable bone graft substitute in an infected, critically sized segmental defect model. *J. Biomed. Mater. Res. - Part B Appl. Biomater.* (2018). doi:10.1002/jbm.b.34001
151. Zhang D et al. Efficacy of novel nano-hydroxyapatite/polyurethane composite scaffolds with silver phosphate particles in chronic osteomyelitis. *J. Mater. Sci. Mater. Med.* 30, 59 (2019). [PubMed: 31127361]
152. Wu W, Ye C, Zheng Q, Wu G & Cheng Z A therapeutic delivery system for chronic osteomyelitis via a multi-drug implant based on three-dimensional printing technology. *J. Biomater. Appl.* (2016). doi:10.1177/0885328216640660
153. Zhou J, Zhou XG, Wang JW, Zhou H & Dong J Treatment of osteomyelitis defects by a vancomycin-loaded gelatin/ β -tricalcium phosphate composite scaffold. *Bone Joint Res.* (2018). doi:10.1302/2046-3758.71.BJR-2017-0129.R2

154. Maczynska B et al. In vitro efficacy of gentamicin released from collagen sponge in eradication of bacterial biofilm preformed on hydroxyapatite surface. PLoS One 14, e0217769 (2019). [PubMed: 31163049]
155. Pawar V & Srivastava R Chitosan-polycaprolactone blend sponges for management of chronic osteomyelitis: A preliminary characterization and in vitro evaluation. Int. J. Pharm. (2019). doi:10.1016/j.ijpharm.2019.118553
156. Boles LR et al. Local Delivery of Amikacin and Vancomycin from Chitosan Sponges Prevent Polymicrobial Implant-Associated Biofilm. in Military Medicine (2018). doi:10.1093/milmed/usx161
157. Jennings JA et al. Polymicrobial Biofilm Inhibition Effects of Acetate-Buffered Chitosan Sponge Delivery Device. Macromol. Biosci. (2016). doi:10.1002/mabi.201500347
158. Parker AC et al. Characterization of local delivery with amphotericin B and vancomycin from modified chitosan sponges and functional biofilm prevention evaluation. J. Orthop. Res. (2015). doi:10.1002/jor.22760
159. Schindeler A et al. Local delivery of the cationic steroid antibiotic CSA-90 enables osseous union in a rat open fracture model of Staphylococcus aureus infection. J. Bone Jt. Surg. - Am. Vol. (2015). doi:10.2106/JBJS.N.00840
160. Ipsen T, Jørgensen PS, Damholt V & Tørholm C Gentamicin-collagen sponge for local applications: 10 cases of chronic osteomyelitis followed for 1 year. Acta Orthop. (1991). doi:10.3109/17453679108994503

Figure 1. Scanning electron microscopy of *Staphylococcus aureus*. A-B, *S. aureus* was found abundantly in ex vivo femoral bone fragments (20 days post infection) of rats with chronic osteomyelitis. Some evidence of residual glycocalyx (biofilm) matrix is noted around the cocci. C-D, *S. aureus* localized within the ridges of an ex vivo orthopedic screw 20 days post infection

Figure 2.

Strategies and criteria for selecting delivery vehicles and therapeutics for osteomyelitis. An array of factors must be considered in the design of delivery vehicles and therapeutics, to offer improvements in both efficacy and ultimately patient outcomes

Figure 3.

Lysostaphin-delivering hydrogels eliminate bacteria in infected fractures. A, Schematic diagram of mouse femur infection model. Quantification of *Staphylococcus aureus* UAMS-1 recovered from the (B) tissue surrounding the femur, (C) femur bone, and (D) stabilization needle 7 d postfracture. Dashed line indicates detection limit. * $P < .05$, ** $P < .01$. E, Histological sections of femurs 7 d postfracture stained for H&E, Saf-O/FG, and Gram. Black arrows indicate gram-positive bacteria. Kruskal–Wallis test with Dunn’s multiple comparisons test. Ox., oxacillin; Sol., soluble. Mean $\pm$ SD $n = 4-8$, compilation of four independent experiments. Reproduced from Johnson, C. T. et al. Hydrogel delivery of lysostaphin eliminates orthopedic implant infection by *S. aureus* and supports fracture healing. *Proc. Natl. Acad. Sci.* (2018). <https://doi.org/10.1073/pnas.1801013115>. (CC BY-NC-ND 4.0)

Table 1:**Delivery Vehicles and Therapeutics Used to Combat Osteomyelitis**

Delivery Vehicles	Therapeutic	Citations
Hydrogels	Antibiotics	21, 103–107, 110–114
	Bacteriophages	32, 34, 42, 47, 49, 108
	Cell Healing and Other Antimicrobials	49, 73, 76, 96, 100–102, 109
Cements	Antibiotics and Other Antimicrobials	60, 63, 67, 115–125
Micro/Nanoparticles	Antibiotics	104, 107, 112, 114, 126–130, 134, 135, 137
	Bacteriophages	46, 47, 131, 132, 138
	Other Antimicrobials	96, 127, 133, 136
Coatings/Films	Antibiotics	99, 113, 137, 139, 141, 142, 144–146, 148
	Bacteriophages	35
	Other Antimicrobials	98, 133, 136, 140, 143, 147
Scaffolding	Antibiotics	149, 150, 152, 153
	Other Antimicrobials	91, 151
Sponges	Antibiotics	29, 118, 154–160