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Advances in the Local and Targeted Delivery of Anti-infective Agents for Management of Osteomyelitis

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Structured Abstract

Introduction—Osteomyelitis, a common and debilitating invasive infection of bone, is a frequent complication following orthopedic surgery and causes pathologic destruction of skeletal tissues. Bone destruction during osteomyelitis results in necrotic tissue, which is poorly penetrated by antibiotics and can serve as a nidus for relapsing infection. Osteomyelitis therefore frequently necessitates surgical debridement procedures, which provide a unique opportunity for targeted delivery of antimicrobial and adjunctive therapies.

Areas Covered—Following surgical debridement, tissue voids require implanted materials to facilitate the healing process. Antibiotic-loaded, non-biodegradable implants have been the standard of care. However, a new generation of biodegradable, osteoconductive materials are being developed. Additionally, in the face of widespread antimicrobial resistance, alternative therapies to traditional antibiotic regimens are being investigated, including bone targeting compounds, antimicrobial surface modifications of orthopedic implants, and anti-virulence strategies.

Expert Commentary—Recent advances in biodegradable drug delivery scaffolds make this technology an attractive alternative to traditional techniques for orthopedic infection that require secondary operations for removal. Advances in novel treatment methods are expanding the arsenal of viable antimicrobial treatment strategies in the face of widespread drug resistance. Despite a need for large scale clinical investigations, these strategies offer hope for future treatment of this difficult invasive disease.

Keywords

antimicrobial; biomaterial; bone; orthopedic implant; osteomyelitis; PMMA; *Staphylococcus aureus*; vancomycin

1.0 Introduction

Osteomyelitis is a devastating inflammatory state of bone most commonly triggered by invasive infection and characterized by pathologic changes in bone remodeling [1]. The incidence of osteomyelitis is increasing for all patterns of disease, most notably diabetic foot osteomyelitis due to the increased incidence of diabetes [2]. Three clinical patterns of osteomyelitis are recognized: 1) osteomyelitis occurring secondary to vascular insufficiency [e.g. diabetic foot infection], 2) osteomyelitis resulting from spread from a contiguous source [e.g. accidental trauma or surgical contamination], and 3) hematogenous osteomyelitis, which is more common among pediatric patients [1–3]. Each pattern of osteomyelitis has its own characteristic array of etiologic pathogens, but across all three patterns of disease, the gram-positive *Staphylococcus aureus* is by far the most common causative agent. [2,4,5]. A secondary cause of osteomyelitis is *Staphylococcus epidermidis*, which is particularly common in cases of implant-associated infection following surgery [2,6]. Patients with osteomyelitis experience profound morbidity and mortality [2]. Osteomyelitis, particularly involving an orthopedic prosthetic, causes a significant economic burden beyond the physical hardships of invasive infection [6,7]. On average, diagnosis of *S. aureus* infection in a patient who has undergone orthopedic surgery increases costs by \$56,000, increases length of stay by 14 days, and increases mortality rate by 8% [8]. Of particular clinical importance are strains of *S. aureus* bearing antimicrobial resistance, such as methicillin-resistant *S. aureus* (MRSA), which is responsible for approximately 46% of deaths related to antimicrobial-resistant pathogens in the United States [9].

Due to the difficulty of eradicating established infection in bone, treatment guidelines generally suggest long-term antibiotic therapy with or without surgery, though specific strategies vary depending on the location and pattern of disease [10–15]. In addition to widespread antimicrobial resistance, treatment of osteomyelitis is complicated by a number of microbial and host factors such as biofilm formation, metabolic changes that promote bacterial tolerance to antibiotics, and poor antibiotic penetration into infected bone. *S. aureus* is capable of forming biofilms on both the extracellular matrix of bone and foreign bodies such as orthopedic implants [13,16]. The formation of biofilms, frequently defined as aggregates of bacteria embedded in an extracellular polymeric matrix, leads to reduced antimicrobial susceptibility and prolonged infection [13,17,18]. Furthermore, *S. aureus* small colony variants (SCVs) and persisters are known to enhance the chronicity of infection through antibiotic tolerance [19–21]. Such bacterial populations are capable of dynamic reversion to the wildtype state and can exhibit enhanced virulence when growing in bacterial communities that share nutrients [22]. These pathogen-associated treatment challenges necessitate long-term antibiotic therapy with high penetration of the infectious foci. However, treatment of osteomyelitis is also challenged by host factors, such as the formation of sequestrum, defined as avascular and necrotic bone, which limits the penetration of antimicrobial agents into the infectious focus and serves as a nidus for relapsing infection [1]. Taken together, these microbial and host factors significantly challenge effective antimicrobial therapy, and therefore surgical debridement is a common adjunctive therapy for osteomyelitis.

Although surgical debridement improves osteomyelitis treatment outcomes by removing biofilm- adherent and necrotic tissue, such procedures can hamper tissue healing by creating what is known as “dead space” [15]. Dead space is a void in the normal architecture of bone that must be revitalized to allow for future healing [1,15]. Methods currently in practice for the management of dead space include the placement of cancellous allograft (i.e., spongy, decellularized cadaveric bone), grafting of musculocutaneous or osteocutaneous flaps, or placement of antibiotic-laden poly(methyl methacrylate) (PMMA) beads capable of releasing antibiotic locally [1]. Allograft is at risk of colonization by pathogens [16] and also has limited capacity for mechanical stability due to the current methods required to wash and prepare the graft prior to implantation [23]. Autografts (tissue grafts obtained from one donor site and placed at the injury site on the same individual) do not require washing and irradiation prior to use because the tissue is inherently patient-compatible. However, a major drawback of methods involving autograft placement is the required surgical morbidity of the donor site. PMMA beads offer several advantages over allograft bone and autograft flaps, including local drug delivery and dead space maintenance without the need for graft tissue. Local drug delivery was first used to control bone infection in the 1960s with closed irrigation of antibiotic containing solutions [24]. Shortly thereafter, PMMA was introduced in orthopedic medicine as a cement between the femoral prosthetic and the surrounding native bone to assist in the stability of prosthetic hip joints [25]. A decade later, PMMA was used as a substrate to carry antibiotics for prophylaxis of infection during prosthetic surgery [26]. PMMA has since been formulated into commercially available, antibiotic-containing beads for the treatment of osteomyelitis following surgical debridement [27]. Since the advent of antibiotic-containing PMMA beads in clinical practice, research of local drug delivery to bone has significantly improved. These advances include the development of biodegradable polymers and titanium alloys as well as novel pharmaceutical strategies to both prevent and treat infection of bone. In this manuscript, we review 1) recent advances in polymeric carriers for local delivery of antimicrobial compounds, 2) novel medications for the treatment of osteomyelitis, 3) methods for bone-targeted delivery of systemic treatments, and 4) improvements in antimicrobial coatings of orthopedic implants to prevent osteomyelitis.

2.0 Body

2.1 Polymeric Carriers of Antimicrobials

Antibiotic-impregnated PMMA beads are commonly used in the treatment of osteomyelitis, yet several disadvantages exist that have led to the search for improved methods of drug delivery. PMMA beads are not biodegradable, which means that for some patients, a second operation is required for removal [26]. Furthermore, PMMA is formed in a highly exothermic reaction that limits the breadth of antibiotics to those thermally stable enough for use in the PMMA matrix, excluding the use of certain agents such as the tetracyclines [28]. Antibiotic-loaded PMMA will continue to serve important roles in a limited set of cases in which removal is not necessary, such as in the anchoring of a prosthetic device. This was the initial application of antibiotic-loaded PMMA cement, and this practice is still common today [26,29]. Currently, antibiotic-laden PMMA formulations continue to be improved, with combination therapies such as vancomycin or clindamycin added to gentamicin for a

synergistic effect in preventing infection [29–31]. On the other hand, bone tissue engineering has advanced greatly in the last few decades, facilitating the development of improved biodegradable scaffolds of both natural and synthetic polymers. Tissue engineered bone scaffolds can be fabricated as highly tunable co-polymers that allow for improved control of the kinetics of biodegradation, drug release, biocompatibility, and biomechanical strength [32]. Advances in therapeutic scaffolds for bone have led to the development of improved antibiotic delivery that allows for a broader range of compatible antibiotics and no need for a secondary operation. The entirety of biodegradable bone scaffolds for local antibiotic delivery is reviewed elsewhere [33]. The following paragraphs review the recent advances in biodegradable polyurethane (PUR) scaffolds, three-dimensional (3D) printing of scaffolds, and polymeric microspheres for the treatment of osteomyelitis.

2.1.1 Polyurethane Scaffolds—PUR scaffolds are comprised of long chain polymers linked at isocyanate and alcohol terminal groups. The bonds linking the monomers in the scaffold are split *in vivo* at rates controlled by altering the monomers or co-polymers [34]. The scaffolds can be made into a foam with porosity that permits cell infiltration. Furthermore, PUR scaffolds can be loaded with pharmaceutical payloads for local drug delivery as well as osteoconductive materials such as hydroxyapatite (the inorganic mineral of bone). Recently, biodegradable PUR scaffolds have been used for the delivery of vancomycin in a post-traumatic osteomyelitis model [35]. PUR scaffolds containing vancomycin were compared to vancomycin delivered by PMMA, the current clinical standard of care. In this study, the authors initially discovered that vancomycin delivery by PUR is complicated by the rapid release of vancomycin, thereby limiting the duration of effective therapy. To overcome this challenge, the authors formed free-base vancomycin from the standard formulation to prolong release without loss of antimicrobial potency. This method enhanced therapeutic benefit compared to conventional vancomycin formulations. PUR beads impregnated with vancomycin equally reduced bacterial burden when compared to PMMA delivery. However, delivery of vancomycin with PUR is potentially superior, when considering that, unlike PMMA beads, PUR scaffolds are biodegradable and do not require a secondary operation for removal. These results complement a recent small-scale, randomized clinical trial that compared a clinically available bioabsorbable ceramic (calcium sulfate) impregnated with antibiotic to PMMA impregnated with antibiotic. The study found the methods to be equally efficacious [36]. Several other studies have shown mixed results with ceramics, which are the only bioabsorbable carriers that have been studied clinically [33,37–40]. Before translation into clinical practice, it will be necessary to conduct randomized clinical trials of both bioabsorbable and biodegradable methods.

2.1.2 Three-Dimensional Printing in Clinical Practice—Both *in vivo* animal studies and clinical experience suggest that biodegradable carriers will benefit patients suffering from osteomyelitis following surgical debridement. Rapid and custom fabrication of drug delivery scaffolds would allow appropriate management of dead space on a case-by-case basis. With specific attention to customized treatment of dead space, researchers have investigated the formulation of antibiotic-laden PUR as an injectable liquid with *in vivo* polymerization to form a patient-specific shape [41]. Additionally, another method for the fabrication of patient-specific polymer scaffolds is 3D printing of antimicrobial-impregnated

polymers. As proof of principle, 3D printed polycaprolactone (PCL), a biodegradable polymer, was recently used for the treatment of tracheobronchomalacia [42]. A custom PCL scaffold was successfully placed to maintain patency of the airway. Similarly, a proof of concept for three-dimensionally printed scaffolds for the treatment of osteomyelitis was created by blending of PCL with poly(lactic-co-glycolic acid) (PLGA) and tobramycin [43]. Tobramycin was chosen for its broad activity and thermal stability for extrusion of the polymer blend during the 3D printing process, mimicking the limitation of PMMA requiring thermally stable antibiotics. Blended hydrophobic (PCL) and hydrophilic (PLGA) polymers with tobramycin was proven to be efficacious *in vitro* and *in vivo*, using an experimental model of osteomyelitis [43]. 3D printed scaffolds therefore show promise in personalized medicine due to the ability to rapidly build custom scaffolds specific to the shape of the orthopedic defect.

2.1.3 Microspheres—Beyond pre-fabricated bulk scaffolds, polymers have also been used to form microspheres for improved drug delivery to bone. Measuring on the order of microns in diameter, microspheres are often composed of a polymer shell around a pharmaceutical product and have a variety of potential uses such as controlling a drug's half-life or increasing its solubility in an aqueous environment [44]. Since the 1990s, antibiotics have been loaded into microspheres for local treatment of experimental osteomyelitis in animal models [45]. In 2004, Ambrose et al. examined the use of PLGA microparticles for the local delivery of antibiotics [46]. Comparing PLGA-tobramycin microparticles to tobramycin-loaded PMMA with or without additional systemic therapy, the PLGA-tobramycin microparticles combined with systemic antibiotics were superior to other groups in eradicating detectable infection. Furthermore, a reactive oxygen species (ROS)-responsive microsphere was recently developed [47]. While not initially developed for osteomyelitis, the method has the potential for broad applicability. ROS-responsive microspheres are fabricated from poly(propylene sulfide) (PPS), which serves as a pharmaceutical carrier that unloads its payload in oxidative environments. The PPS microspheres are capable of improving healing in a limb ischemia model through the selective delivery of an antioxidant compound to sites of high oxidation, namely those areas with ischemia and inflammation [47]. This method has the potential to improve the localized delivery of systemically administered drugs to regions of high inflammation, such as infected bone. Microparticles can be delivered more easily than bulk scaffolds but may not be as capable of maintaining debrided dead space due to the relative fluidity of the delivered load. Nevertheless, microparticles offer potential promise for targeted delivery of systemically administered compounds while maintaining the capacity for highly tunable parameters (biodegradation kinetics, etc.) innate to polymeric scaffolds.

2.1.4 Polymeric Scaffolds Combined with Microspheres—Finally, a combined polymeric approach for delivery of antibiotics is the use of a biodegradable scaffold that has been imbedded with antibiotic-containing microparticles. A recent study used biodegradable PUR scaffolds containing osteoconductive nano-hydroxyapatite and mesoporous microspheres with levofloxacin [48]. The authors demonstrated profoundly improved bone healing through histological and microcomputed tomography relative to a debridement-only control. The biodegradable scaffold performed as well as a PMMA-levofloxacin comparator

for the metrics of bone healing. A major drawback of the study was the lack of direct evaluation of bacterial burden at any time point, though the authors noted that gross osteomyelitis (“sequestrum, bone swelling and abscess formation”) was only present in the debridement-only control. Despite this drawback, the study did demonstrate a highly adaptable method of drug delivery to bone. By combining a bulk scaffold with polymeric microparticles, the authors created a system in which (1) the osteoconductive scaffold maintained the dead space in bone and promoted osteogenic healing and (2) the microparticle delivered the antibiotic with tunable release kinetics independent of scaffold degradation. In conclusion, advances in polymeric scaffolds and microparticles enhance the breadth and customizability of drug delivery vehicles for the treatment of osteomyelitis.

2.2 Novel Therapeutics for Osteomyelitis

2.2.1 Development of New Antibiotics—Although improved methods for antibiotic delivery could significantly improve outcomes for osteomyelitis, widespread antimicrobial resistance necessitates the concomitant development of new therapeutics for treatment of invasive infections such as osteomyelitis [9,49]. Though the development of novel antibiotics has waned over the decades following 1980, many new antibiotics have been developed and are reviewed elsewhere [50]. Furthermore, *S. aureus* vaccine development remains both elusive and highly desired as *S. aureus* has become the most common cause of invasive bacterial infection [51]. Research into *S. aureus* vaccine development is reviewed elsewhere and is beyond the scope of this review [51,52]. This section will highlight recently developed antibiotics with proven activity against methicillin-resistant staphylococcal species. These antibiotics include dalbavancin, oritavancin, tedizolid, ceftaroline, and Debio-1450, which have all been recently investigated or are currently being investigated in clinical trials related to bone infection [50,52]. Dalbavancin and oritavancin are each lipoglycopeptide derivatives of vancomycin with increased potency against MRSA and available in intravenous (IV) formulations [50]. Unlike vancomycin, however, both dalbavancin and oritavancin have pharmacokinetics to support infrequent dosing, such as once weekly [50]. In 2017, two phase II clinical trials were in progress assessing the safety and efficacy of dalbavancin in the treatment of osteomyelitis versus the standard of care [53,54]. The new oxazolidinone compound, tedizolid, has been developed as an IV formulation that is also being tested for treatment of osteomyelitis in clinical trials [50,55]. A novel fifth-generation cephalosporin, ceftaroline, with increased activity against MRSA and other gram-positive pathogens has been developed [50]. In addition to IV formulations, intramuscular formulations are in development for potential single administration therapy of certain infections [56]. In 2017, a clinical trial was recruiting patients for investigation of ceftaroline for treatment of hematogenously acquired *S. aureus* osteomyelitis in children [57]. Another new antibiotic, Debio-1450, targets the enzyme FabI in the fatty acid synthesis pathway of *S. aureus* [52]. Debio-1450 has activity against MRSA and is currently being tested in clinical trials to assess its ability to penetrate bone in healthy patients [52,58]. Though antibiotic development has waned in the preceding decades, these data indicate that important new drugs are currently under investigation for management of osteomyelitis.

2.2.2 Repurposing of Older Antibiotics—In addition to the development of new therapeutics, an alternative approach is the re-evaluation and repurposing of older antibiotics

that have fallen out of favor in the current antimicrobial armamentarium [59]. In line with this approach, the drug fusidic acid, which inhibits microbial protein synthesis, has recently shown promise for use against multi-drug resistant pathogens [60]. Fusidic acid has been used clinically since 1962 and functions in a bacteriostatic manner against *S. aureus* [59]. The drug is available in an IV formulation as well as orally due to its high bioavailability [59,61]. In 2017, a clinical trial was recruiting participants to evaluate the effectiveness of twice daily, oral fusidic acid in suppressing chronic osteomyelitis [62]. Other antibiotics being considered for repurposing include pristinamycin, fosfomycin, and minocycline [59,63].

2.2.3 Anti-virulence Strategies—Besides combating multi-drug resistance with a broader range of antibiotics, alternatives to traditional antibiotic therapy are being investigated for osteomyelitis. Anti-virulence medications offer an attractive adjunct to traditional therapy by preventing the activity or production of toxic virulence factors without impacting microbe viability. The anti-virulence approach is theorized 1) to allow for more specific targeting of the pathogen to minimize effects on the microbiota, 2) to limit the emergence of resistant strains by limiting selective pressure on the microbe, and 3) to facilitate immune clearance of the infecting microorganism [64,65]. In the targeting of osteomyelitis caused by staphylococci, anti-virulence drugs have been identified [66] or recently developed [67]. The quorum-sensing system of *S. aureus* is encoded by the accessory gene regulator (*agr*) locus and allows *S. aureus* to sense its relative density to regulate its growth and virulence [68]. Several compounds have been shown to target the *agr* quorum-sensing system of *S. aureus*, which has been viewed with particular importance due to the broad impact on staphylococcal virulence pathways [65–67]. A recent study found that the FDA-approved nonsteroidal anti-inflammatory drug diflunisal inhibits the *agr* quorum-sensing system and downstream toxin production through inhibition of AgrA's binding to the promoter P3 (possibly interrupting the phosphorylation of AgrC) to induce the transcription of various virulence factors [66]. Local delivery of diflunisal during experimental osteomyelitis was highly efficacious in limiting bone destruction in an experimental model of osteomyelitis, despite having no significant effect on bacterial burdens at the site of infection [69]. *In vitro*, diflunisal decreased the production of osteolytic toxins known as phenol-soluble modulins, providing a mechanistic basis for the decrease in cortical bone destruction *in vivo* [69]. Future studies will determine whether such an approach improves traditional antimicrobial therapy by limiting bone destruction and potentially improving antimicrobial penetration into infected tissues. Moreover, given the mechanistic link between quorum sensing and biofilm formation, it will be critically important to ensure that anti-virulence strategies targeting quorum sensing do not enhance biofilm-associated infections [68].

2.2.4 Biofilm Targeting Agents—*S. aureus* forms biofilms on bone surfaces during osteomyelitis, which limits antibiotic penetrance and prolongs infection [13,16,17,70,71]. In addition to surgical methods that remove necrotic bone fragments that often have attached biofilms, biofilm dispersal agents are being investigated as adjunctive therapies for invasive infection [70,71]. Biofilm targeting agents can be divided into those that limit initial attachment and maturation steps, and those that lead to degradation or dispersal of

established biofilms. Initial attachment can be prevented in orthopedic devices by altering the surface chemistry of the prosthetic (see Section 2.4: Anti-infective Orthopedic Implants). However, small molecules have also been developed for this purpose. One such class of molecules are the aryl rhodanines, which have been shown to inhibit biofilm formation by staphylococci without impacting planktonic bacteria or causing mammalian cell cytotoxicity [72]. A greater challenge that is more applicable to treatment of established infection is dispersal of mature biofilms. Several molecules have been investigated for this purpose including fatty acids, amino acids, and proteolytic enzymes. A more thorough review of biofilm dispersal agents may be found elsewhere [70]. The fatty acid messenger, *cis*-2-decenoic acid, has been shown *in vitro* to inhibit biofilm formation of *S. aureus* as well as to disperse established biofilms [73,74]. D-amino acid mixtures limit biofilm formation and promote biofilm dispersal *in vitro*, possibly by increasing oxidative stress within the biofilm [75–77]. *In vivo* studies of D-amino acids delivered via PUR scaffolds demonstrated that D-amino acids may decrease biofilm formation during osteomyelitis [75]. However, research on biofilm dispersal by D-amino acids has led to mixed results, which necessitates further research to determine the validity and clinical potential of this approach [78]. A final mechanism for biofilm dispersal is the use of proteolytic enzymes, such as the agent dispersin B. Dispersin B, a β -*N*-acetylglucosaminidase produced by the gram-negative bacterium *Actinobacillus actinomycetemcomitans*, proteolytically dissolves *S. epidermidis* biofilms and has been shown to act synergistically with concomitant administration of an antibiotic (e.g. the second-generation cephalosporin, cefamandole) *in vitro* [79]. Biofilm dispersal agents are largely underexplored in 3D *in vitro* or through *in vivo* analyses. Future studies must broaden investigations into these areas to validate the promise of this novel anti-infective technique. Furthermore, as noted above, any agents that modulate quorum sensing as a mechanism to disperse biofilms will need to be carefully evaluated to ensure that quorum-responsive virulence pathways are not activated in kind.

2.2.5 Heavy Metals—In addition to development of novel anti-infective techniques and the repurposing of older antibiotics, the reintroduction of intrinsically antimicrobial elements such as silver and copper is an area of active investigation. The antimicrobial properties of select metals have been exploited since well before antibiotic therapy was introduced [80]. Silver has long been used as an antimicrobial compound and, though its relevance has waned since the dawn of antibiotics, it continues to be important for topical treatment and control of multidrug-resistant pathogens [81]. Recently, a biodegradable scaffold composed of nano-hydroxyapatite/polyamide with titanium dioxide and silver was fabricated and studied *in vitro* and *in vivo* in an experimental osteomyelitis model [82]. The titanium dioxide and silver exhibited strong antimicrobial properties while promoting osteogenic proliferation and demonstrating general biocompatibility. Animals treated with debridement and the silver-containing scaffold showed decreased colony forming unit (CFU) burden, decreased systemic markers of inflammation, and increased bone formation [82]. The authors therefore demonstrated the potential benefits of local delivery of a highly antimicrobial compound with limited potential for systemic toxicity. Emerging anti-virulence strategies and the incorporation of forgotten antimicrobial compounds into local delivery platforms will serve increasingly important roles in controlling infection of multi-drug resistant pathogens moving forward.

2.3 Systemically-Administered Tissue-Targeted Therapy

An alternative to local administration of a drug is systemic administration of a tissue-targeted compound. Bisphosphonates have been used for over the last half-century in the care of human bone diseases, most notably osteoporosis, due to their ability to rapidly bind hydroxyapatite in bone [83]. Bisphosphonate conjugation to other drugs has been theorized and explored as a potential method of targeted delivery of therapeutics in diseases afflicting human bone [84]. Successful delivery of a bisphosphonate-conjugated therapeutic to bone was performed as early as the 1980s with delivery of chemotherapeutics to osteosarcomas in a rat model [85]. Local or systemic delivery of unconjugated bisphosphonates also acts synergistically with local delivery of vancomycin in preventing loss of bone mineral density during osteomyelitis [86]. Importantly, this study showed that vancomycin was necessary for maintenance of bone mineral density suggesting that the increased bone mineral density was not solely a function of the known antiresorptive function of bisphosphonates. Therefore, beyond the role of bisphosphonates in carrying drugs to bone, bisphosphonates may help improve bone healing during infection. Bisphosphonates conjugated to select glycopeptide antibiotics, vancomycin and oritavancin, have been developed and have high binding to bone *in vitro* [87]. Despite conjugation, the compounds continue to have efficacy *in vitro* against microbes, presumably upon dissociation of the conjugated compounds [87]. Several studies have gone on to explore the potential of bisphosphonate-conjugated antimicrobials *in vivo*. Fluoroquinolones linked to bisphosphonates have continued efficacy *in vitro* and are successful in the prophylaxis of osteomyelitis when administered prior to infection. Importantly, unlike the unconjugated parent drug that was cleared from animals, the bisphosphonate-conjugate showed prolonged efficacy [88] and improved potency [89] despite administration well before inoculation of bacteria. While the bisphosphonates have not been studied in the context of established infection *in vivo*, these compounds show promise as prophylactic agents of *S. aureus* in elective orthopedic procedures based on *in vivo* experiments [88]. Because of bone's unique wealth of hydroxyapatite, drugs that target hydroxyapatite could significantly enhance the local concentration of therapeutics at the site of infection in bone.

2.4 Anti-infective Orthopedic Implants

Prophylaxis of infection during orthopedic procedures is particularly important due to the risk of orthopedic-implant associated infection and its related physical and economic hardships [7,14]. Efforts have been made to improve the prophylaxis of infection, largely through systemic administration of a cephalosporin or a comparable antibiotic before and during operations [90], but innovations are still necessary to lower the risk of infection. One promising approach is to create orthopedic implants with intrinsic antimicrobial properties. More thorough reviews have analyzed the breadth of innovations related to creating innately antimicrobial and biocompatible orthopedic prostheses [91,92], but the key developments will be highlighted below.

2.4.1 Doping Titanium Surfaces with Heavy Metals—Many orthopedic devices are made of titanium alloys due to the favorable strength-to-weight ratio, biocompatibility, and corrosion resistance [93]. Titanium dioxide (TiO₂), which frequently coats titanium-based orthopedic devices due to titanium's high reactivity with oxygen, is known to have some

antimicrobial properties [94]. Despite the intrinsic antimicrobial features of titanium alloys, infection still occurs and necessitates additional antimicrobial coatings to reduce bacterial colonization and infection [95]. Surface modification of titanium alloys to inhibit microbial growth include: adding heavy metals (e.g. silver, copper), linking antibiotics, and nanopatterning of the surface. As discussed above, metals such as copper have significant antimicrobial activity, and therefore have been considered for incorporation into titanium alloys. Norambuena et al. supplemented (“doped”) the alloy of a titanium surface with copper to form titanium-copper oxide (TiCuO) at various concentrations of copper [96]. *In vitro* studies showed that high doses of copper (80%) were most effective in reducing biofilm and planktonic growth of *S. epidermidis* [96], a coagulase-negative species of staphylococcus that is an important clinical cause of implant-associated osteomyelitis [2,6]. Although microbial growth was reduced, no significant changes were observed in the viability of osteoblast cells, clarifying the potential clinical utility [96]. Further studies have demonstrated similar effects with a second heavy metal, silver, as discussed above [82]. Through *in vivo* studies, an inoculation of *S. epidermidis* was successfully treated prophylactically with implantation of a titanium implant coated in silver-containing phosphonate [97]. The doping of titanium with heavy metals therefore improves the antimicrobial quality of titanium alloys.

2.4.2 Antibiotic Surface Coatings—A second method to enhance the antimicrobial nature of titanium surfaces is the conjugation of antibiotics directly to the metal. Antoci et al. demonstrated that a titanium surface with covalently linked vancomycin is resistant to biofilm formation by *S. epidermidis in vitro* [98]. The authors showed that the antimicrobial property of the material was likely a result of vancomycin rather than titanium by testing and finding that the gram-negative bacterium, *Escherichia coli*, was not susceptible to the vancomycin-linked titanium surface. With an *in vivo* model of implant-associated osteomyelitis, a titanium implant covalently linked to vancomycin at its surface effectively prevented biofilm formation and improved bone healing [99]. A similar concept has been developed and put into clinical practice. Poly(lactic acid) (PLA) polymer was imbedded with gentamicin and used to coat titanium nails for fixation of tibial fractures. In two published clinical studies, the PLA-gentamicin-coated titanium was associated with no cases of osteomyelitis [100,101]. A non-randomized clinical trial enrolling 100 patients evaluated the use of PLA-gentamicin-coated titanium and found 5 deep surgical site infections but no cases of osteomyelitis [102]. An alternative vancomycin delivery method is also under development. Using a phosphatidylcholine wax loaded with vancomycin or amikacin, researchers developed an “antibiotic crayon” that can be effectively drawn on the surface of any prosthetic device to reduce risk of colonization and infection [103]. Though still in development, this tool would allow for rapid prophylactic preparation of any unique surface prior to implantation. Taken together, these data indicate that surface modification of orthopedic prostheses with antibiotics reduces infection risk.

2.4.3 Nanopatterned Surfaces—A final method of titanium surface modification is the direct alteration of the surface at the nanoscopic level. In a recent study, oxidative nanopatterning was used to modify the surface topology of titanium [104]. The authors showed through *in vitro* studies that the nanopatterned titanium was resistant to adhesion of

S. aureus and *E. coli*, and the aggregation of *Candida albicans* yeast. The authors had previously demonstrated that the nanopatterned surface was biocompatible and permitted the binding and proliferation of osteoblast lineage cells [105]. Though experimental, nanopatterned surfaces offer an exciting new space for tuning implant properties to produce a biocompatible surface for human cell lineages while preventing microbial colonization.

3.0 Conclusion

Osteomyelitis continues to be a highly morbid infection with substantial risk of relapse. Therapeutic strategies must deliver highly active compounds to poorly vascularized sites of infection. This avascular tissue limits the efficacy of systemic therapy and often requires surgical debridement and local delivery of antimicrobial compounds. Although surgical debridement procedures increase the morbidity of osteomyelitis, they offer a unique opportunity for targeted therapeutics. While PMMA carriers of antibiotics continue to be used in clinical practice, basic research has made great progress in designing biodegradable scaffolds for local delivery of antibiotics with highly tunable release and degradation kinetics, osteoconduction, and biomechanical support. Targeted drug therapies using bisphosphonate conjugates offer future potential but have yet to be proven clinically. Multiple methods of titanium implant coating have shown promise in preventing microbial colonization. Further research is needed to determine 1) the optimal co-polymer and drug combination to support bone healing while inhibiting microbial growth during acute and chronic osteomyelitis, 2) improved methods and confirmation of the effectiveness of bisphosphonate-conjugated antimicrobial compounds, and 3) the ideal surface coating of titanium for osteogenic growth while preventing short-term and long-term infection of the prosthesis. Additionally, as clinical trials progress, it will be vitally important to test the safety of these methods. Namely, the toxicities and potential hypersensitivity reactions to both local antibiotic elution and biodegradable polymer scaffolds must be assessed to ensure clinical safety of these delivery strategies.

4.0 Expert Commentary

Invasive staphylococcal infections such as osteomyelitis are increasingly common. Difficulties in treating osteomyelitis necessitate further investigation as current methods of therapy are often inadequate in bringing about an expedient recovery. Multidisciplinary collaborations are vital to expand anti-infective techniques and pharmaceuticals. Expansion of treatment strategies relies on microbiological investigation of novel treatment targets, pharmaceutical development of novel agents to safely inhibit such targets, engineering research to improve methods of delivery that limit systemic side effects and potentiate drug efficacy, and medical accommodation of clinical trials research to validate and translate new technologies.

Microbiologists must continue to investigate novel targets. Areas of considerable interest include methods of antimicrobial resistance, anti-virulence strategies, and processes of biofilm formation and dispersal. Study of skeletal cell biology will be a necessary adjunct to development of new drug targets, fueling both the development of immunomodulatory compounds, as well as testing the host-compatibility of locally delivered compounds.

Engineering techniques may play a unique role in the management of osteomyelitis due to the frequent necessity of surgical intervention. Surgery provides ready access to the invasive infectious focus. While biodegradable synthetic scaffolds appear to be a natural progression from PMMA beads for the local delivery of antibiotics, several studies must be performed before translating these technologies to clinical practice. Prior to clinical implementation, these materials and methods must be properly assessed to determine clinical safety, with a particular focus on local toxicity and cellular integration. It will be important to continue to innovatively form composite materials of fibrous polymers and brittle ceramics to better match the structure and mechanics of bone. These materials must not sacrifice the ability to deliver important pharmaceutical agents in order to gain mechanical, osteoconductive advantages.

Surface modification of orthopedic implants will continue to serve as an important method of antimicrobial prophylaxis by allowing for enhanced healing while inhibiting microbial growth. As recognized in other fields, prevention is the best treatment method. Preventative methods will become extremely valuable and ubiquitous due to the high morbidity and economic burden of implant-associated infections. It is also critical to quickly expand clinical trial data to expedite translation and lessen the burden of this common complication of orthopedic operations.

5.0 Five Year View

It is expected that biodegradable or bioabsorbable methods will become routine in lieu of nonbiodegradable methods except in cases in which secondary operation is necessary, or where rigid materials are desired to enhance the stability of implanted prosthetics. Successful implementation of new local delivery vehicles for the treatment of osteomyelitis will depend upon clinical trials over the next five years to measure efficacy and safety. There is a barrier to translation of these technologies among biomedical engineers in academic institutions that must be overcome with increased collaboration between physicians and engineers. Furthermore, we anticipate that as the prevalence of infections triggered by antimicrobial resistant pathogens increases, there will be an urgent need to develop therapies that overcome both intrinsic and extrinsic factors contributing to treatment failure. Potential strategies to address this urgent need include continued chemical modifications of available antibiotic classes, repurposing of drugs for antimicrobial and anti-virulence effects, development of small molecules that re-sensitize pathogens to antibiotics, local delivery of highly concentrated antibiotic payloads to overcome intermediate levels of resistance, and identification of new bacterial targets amenable to drug discovery. Finally, there has been an explosion of evidence supporting the beneficial role of the human microbiota in fundamental physiologic processes. We therefore anticipate that pathogen-selective and local therapies will receive increasing interest in both the basic science and translational research realms. Patients suffering from osteomyelitis are at high risk for potentially pathologic changes in the microbiota given that they often receive prolonged systemic treatment with broad-spectrum antimicrobials.

With advances in medical care, patients with multiple comorbidities are living longer, and therefore undergoing more surgical procedures, particularly the placement of arthroplastic

implants. Accordingly, we expect an increasing emphasis on strategies to prevent surgical site infections. In terms of musculoskeletal surgeries, it is feasible that all orthopedic implants will carry surface modifications that will inhibit antimicrobial colonization and subsequent infection. Antibiotic-containing coatings will likely be the first modality to see clinical translation as PLA-gentamicin-coated titanium nails are already in use in orthopedic procedures. Future studies will be necessary to translate alternative methods such as nanopatterning to clinical practice. In concert with local antibiotic delivery, implant surface modifications effectively address infection prevention in an increasingly complex medical population, where prevention may be the most important cure.

6.0 Key Issues

6.1 Design requirements of ideal local antibiotic delivery

- Prolonged retention of antibiotic for continued antimicrobial delivery
- Biodegradation of the carrier that inversely matches the kinetics of bone healing
- Biocompatibility that accelerates bone healing
- Custom-shaped scaffolds for personalized medicine
- Resistance to biofilm formation on the polymer surface
- Activity against multidrug resistant pathogens by incorporating anti-virulence pharmaceuticals and/or intrinsically antimicrobial compounds

6.2 Design requirements of ideal prophylactic orthopedic implants

- Biocompatibility that accelerates integration of the prosthetic into the bony matrix
- Efficacy in the prevention of infection in the short-term and long-term

6.3 Miscellaneous

- Novel therapeutics will include tissue-targeted drugs and drugs that target virulence rather than microbial viability.
- Older antibiotics continue to be investigated for potential use in modern medical practice.
- Many of these methods are still experimental and will require clinical trials before they will be accessible for treatment of human disease.

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Abbreviations

agr accessory gene regulator

CFU	colony forming unit
IV	intravenous
MRSA	methicillin-resistant <i>S. aureus</i>
PCL	polycaprolactone
PLA	Poly(lactic acid)
PLGA	poly(lactic-co-glycolic acid)
PMMA	poly(methyl methacrylate)
PPS	poly(propylene sulfide)
PUR	polyurethane
ROS	reactive oxygen species
SCV	small colony variant
3D	three-dimensional

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