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Dual delivery of an antibiotic and a growth factor addresses both the microbiological and biological challenges of contaminated bone fractures

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Introduction: Open fractures are plagued by high complication rates, among which infection and nonunion are the most common, leading to higher morbidity and poor patient outcomes. Despite meticulous surgical care and employment of adjunctive therapies, infection rates remain at 20%, due to the limitations of conventional therapies.

Areas covered: Persistent bacteria often survive initial debridement and treatment with antibiotics. Thus, the bone graft subsequently implanted to promote healing can be the nidus for infection. The principles of biofilm theory and the "race to the surface" have been applied to develop dual-purpose bone grafts that are protected by a sustained release of an antibiotic, thereby preventing bacterial colonization. A simultaneous sustained release of a recombinant human growth factor allows the defect to become vascularized and heal.

Expert opinion: Current therapies fail to meet the challenges of open fractures. Tissue engineering and drug delivery approaches can address the challenges of healing large bone defects while protecting the implant from infection. When combined as an adjunctive therapy with existing clinical practices for management of open fractures, dual-purpose bone grafts that release both an antibiotic and a growth factor at biologically relevant time scales can potentially reduce infection rates and improve patient outcomes.

Keywords: antibiotic, bone, bone morphogenetic protein, dual purpose, graft, growth factor, infection, scaffold, tissue engineering

Expert Opin. Drug Deliv. (2011) 8(12):1555-1569

1. Introduction

Tissue engineering and drug delivery hold promise for treating many diseases and clinical conditions that are incurable today. Two of the most active areas have focused on preventing infection and promoting bone healing which can help in the treatment of open fractures resulting from high-energy insults to a limb. These fractures are often grossly contaminated, have large segments of bone missing, and are associated with concomitant soft tissue injury to the surrounding soft tissue envelope. Delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) to heal bone defects is a rapidly expanding area of research in bone regeneration and has been reviewed extensively [1-4]. The Food and Drug Administration (FDA) has approved the use of rhBMP-2 delivered on an absorbable collagen sponge (ACS) as INFUSE® Bone Graft (Medtronic, Memphis, TN USA) for treatment of posterior-lateral spine

10.1517/17425247.2011.628655 © 2011 Informa UK, Ltd. ISSN 1742-5247

Article highlights.

- Despite meticulous care from well-trained surgeons who employ staged management of the most severe open fractures, high infection rates (generally 20% but as high as 50% for the most severe) for open fractures persist.
- Open fractures are treated with systemic and local antibiotics to control infection. However, a small number (0.1 – 10%) of persister cells can survive antimicrobial therapy. Subsequent implantation of an avascular bone graft after removal of the antibiotic depot initiates a "race to the surface" between bacterial colonization and tissue integration.
- To protect the bone graft from infection and enable wound healing and angiogenesis to progress, a sustained release of the antibiotic exceeding the MIC is required for at least 6 weeks post implantation. While antibiotic delivery depots with sustained release of antibiotics have been reported, these materials at best provide an osteoconductive matrix for cells to infiltrate and do not address the biological requirements for healing of large osseous defects.
- Dual-purpose bone grafts that incorporate antibiotics and rhBMP-2 control infection and promote new bone formation in contaminated segmental defect models. The dual-delivery strategy could be an effective therapy for reducing the incidence of infection and nonunion in the clinic.

This box summarizes the key points contained in the article.

fusions, tibial fractures, and sinus and alveolar ridge augmentations. Considering recent reports that rhBMP-2 promotes bone healing as well as autograft [5], it has become a common standard of care for treating severe fractures.

Prevention of infection is another clinical challenge associated with the healing of open fractures. While the surgical debridement and irrigation of the wound, along with systemic and local depot delivery of antibiotics, kills most of the bacterial cells, bacteria inevitably can reside within the wound and are present when definitive grafting of the bone defect occurs. Consequently, implantation of an avascular graft that is not integrated with host tissue into a contaminated wound bed can be a contributing cause of infection [6]. In this review, we discuss the burden of nonunion and infection in open fractures and the conventional surgical management of severe open fractures. Biofilm theory and the "race to the surface" concept are used to highlight the shortcomings in current clinical practice, and tissue engineering and drug delivery strategies to address both the biological and microbiological challenges are reviewed. Several key performance criteria essential to the clinical success of novel therapies for treating contaminated fractures are discussed, such as improved bone regeneration relative to currently available bone grafts, minimizing adverse effects of the antimicrobial on bone healing and release of the antimicrobial for a sufficient period of time (e.g., 6 – 8 weeks) to allow vascularization of the implant. Dual-purpose bone grafts releasing both rhBMP-2 and an antibiotic at biologically relevant time scales have been developed to protect the graft from contamination until the wound area is vascularized and immunocompetent, thereby preventing the required vulnerary graft from triggering a foreign body effect [7-10]. As summarized in **Figure 1**, dual-purpose grafts are anticipated to ensure more predictable healing of open contaminated fractures, thereby resulting in more favorable clinical outcomes.

2. Clinical burden and management of contaminated open fractures

2.1 Significance and impact of complications on patient outcomes

Mangled extremities often result in poor outcomes. A large prospective clinical study (Lower Extremity Assessment Project; LEAP) demonstrated that severe, traumatic extremity injuries that underwent limb salvage and amputation resulted in equally poor outcomes after 2 years, at which time only about 50% of 569 patients had returned to work [11]. A multivariate analysis revealed that a significant predictor of a poor outcome was rehospitalization for a major complication, the most common of which are infection and nonunion [12]. Considering that many orthopedic trauma patients are young, complications associated with mangled extremities constitute a substantial financial burden if patients do not return to a productive life.

In the LEAP study, 37% of the patients who underwent limb salvage had nonunion or malunion of their fracture, and 32% had either a wound infection or osteomyelitis (a bone infection that is generally caused by *Staphylococcus aureus*) [12]. The relationship between infection and nonunion is further illustrated in a small clinical study on severe open tibia fractures [13], in which patients who had an infection experienced twice the rates of delayed union and amputation compared with patients without an infection. Thus, wounds that become infected do not heal.

2.2 Clinical management of contaminated open fractures

High infection rates (generally 20% but as high as 50% for the most severe [14]) for open fractures persist despite meticulous care from well-trained surgeons who employ staged management of the most severe open fractures. After thorough debridement and irrigation of the wound and administration of systemic antibiotics, the limb is stabilized with a bridging external fixator to allow the soft tissues to improve and recover (**Figure 1**). Radical debridement of all necrotic tissue and foreign material, which removes most of the bacteria along with the environment that allows bacteria to thrive, is paramount in managing severe open fractures. External fixation spans the wound and allows temporary stabilization without introducing hardware to the wound. A temporary local antibiotic depot,

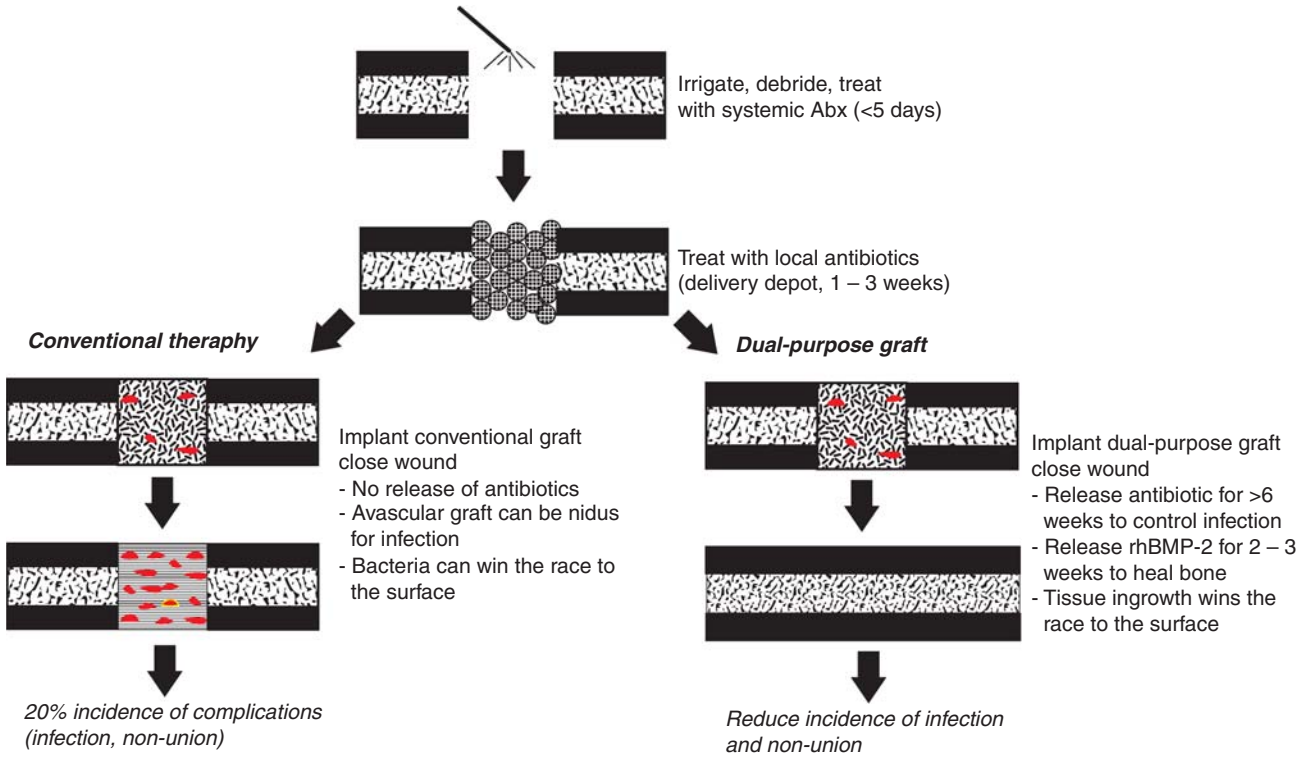

Figure 1. A schematic diagram illustrating the conventional treatment for contaminated open fractures (left) and the concept of the dual-delivery device (right). Open fractures present a biological challenge that requires a graft for bone union. In conventional therapy, there is no release of antibiotics from the bone graft. The residual bacteria (represented by the red particles) residing in the wound and the avascular graft act as a foreign body and can be the nidus for infection. In the dual-purpose graft, delivery of an antibiotic locally for more than 6 weeks controls infection until the graft is vascularized and immunocompetent. The release of a recombinant growth factor (e.g., rhBMP-2) for 2 – 3 weeks promotes bone healing. The anticipated clinical outcome is reduced incidence of infectious and nonunion complications.

such as antibiotic-impregnated PMMA, can be placed within the wound to increase the antimicrobial level within the defect [15]. The second stage is definitive fixation and bone grafting, where the PMMA beads are removed from the wound and the graft is implanted. Typical bone grafts comprise either autograft, often harvested from the iliac crest of the patient, or INFUSE, which has been shown to achieve healing in large defects [16] and is comparable to or better than autograft without the need for a second harvesting surgery [5]. Use of intramedullary (IM) nails are the conventional fixation device used, but there is a growing concern that this hardware placement within the wound may increase infection rates, prompting investigation into alternative devices (e.g., ring fixators) [17]. A significant advantage of ring fixators is their ability to provide stabilization without placing hardware into the wound; however, the cost is significantly higher than that of the conventional IM nail.

Systemic antibiotics are given to the injured patient promptly as delay may increase the risk of infection [18]. For the most severe and contaminated fractures, broad-spectrum antibiotics are administered for up to 5 days [19] and then again at the time of wound closure. Systemic antibiotics are often administered in combination with local depot delivery via antibiotic-impregnated PMMA beads, which has the advantage of providing high local concentrations with low serum concentrations to avoid systemic toxicities [20]. Biodegradable calcium sulfate pellets impregnated with 4% tobramycin (Osteoset®, Wright Medical) have also been used to treat osteomyelitis in both the limbs and spine [21-23] and during grafting of open fractures [24].

3. Current strategies in tissue engineering and drug delivery

3.1 Current strategies in bone regeneration

Local delivery of rhBMP-2 is a rapidly expanding area of research in bone tissue engineering [1-4]. As the primary non-mineral component of bone, collagen has attracted considerable attention due to its biocompatibility and ability to bind rhBMP-2. Considerable research has focused on the use of ACS carriers for rhBMP-2 [25], in which lyophilized rhBMP-2 is reconstituted with water, added to the sponge and allowed to impregnate the carrier prior to implantation. The rapid release of rhBMP-2 from the collagen sponge [1,26]

has been suggested to recruit osteoprogenitor cells, thereby initiating the osteogenic cascade [27]. The composition of the ACS carrier and the delivery conditions of rhBMP-2 affect the binding of the protein to the sponge [28], but most of the rhBMP-2 is released in the first few days [29]. This bolus release of protein has been associated with the need to deliver supra-physiological doses of rhBMP-2, which has prompted research into alternative carriers that are efficacious at lower doses by providing a more sustained release. Other natural polymers have been investigated as carriers for rhBMP-2, which have been reviewed [30]. Due to their similarities in composition to the natural environment of bone tissue, an advantage of natural polymers is their ability to signal cells at various stages of their development and thus accelerate healing [31]. Natural polymers investigated as carriers for rhBMP-2 include hyaluronic acid [32-34], alginate [35], chitosan [36], fibrin [37], and silk fibroin [38], which have been shown to promote new bone formation in rat and rabbit bone defects.

Biodegradable synthetic polymers have also been extensively investigated as delivery systems for rhBMP-2, starting with poly(lactic acid) (PLA) [39]. However, the PLA releases acidic degradation by-products [30], prompting a search for alternatives as recently reviewed [40]. Combining PLA with poly(glycolic acid) to yield poly(lactic-*co*-glycolic acid) (PLGA) copolymer scaffolds has proven to be an effective delivery system for rhBMP-2 in several preclinical models, including repair of alveolar cleft defects in dogs [41] and augmentation of alveolar bone in rats [42]. Scaffolds fabricated from poly(propylene fumarate) incorporating rhBMP-2 have also been reported to promote new bone formation in rat cranial critical size defects [43], a goat ectopic implantation model [44] and in rat femoral segmental defects [45]. Recent studies have focused on reducing the dose of rhBMP-2 required to induce new bone formation. A hybrid nanofiber mesh/alginate delivery system [46] was compared with the ACS carrier at rhBMP-2 doses ranging from 0.1 and 1.0 µg in 8-mm rat femoral segmental bone defects [47]. At a dose of 1.0 µg, the hybrid delivery system resulted in greater connectivity by week 4 and 2.5-fold greater bone volume by week 12. Similarly, resorbable poly(ester urethane) scaffolds incorporating 2 µg (60 µg cm^{-3}) yielded a 50% increase in bone volume compared with the ACS carrier when implanted in 6-mm rat femoral segmental bone defects. In both studies, the increase in new bone formation was attributed to a more sustained release of rhBMP-2 (up to 21 days) for the PUR and hybrid delivery systems, suggesting that carriers delivering both a burst and sustained release of rhBMP-2 can induce new bone formation at doses substantially less than that recommended for the ACS carrier (200 µg cm^{-3} in rats).

In situ gelling systems have also been investigated as carriers for rhBMP-2. The injectable poloxamines Tetronic® 908, 1107 (Tetronic, BASF, Florham Park, NJ USA) and 1307, which undergo sol-gel transitions near 37°C, induce osteoblast differentiation both solely and in combination with rhBMP-2 *in vitro* [48]. In another approach, thermosensitive poly(ε-caprolactone-*co*-lactide)-poly(ethylene glycol)-poly(ε-caprolactone-*co*-lactide) block copolymers capped with pH-sensitive sulfamethazine oligomers yield pH- and thermosensitive block copolymers that can be injected as a liquid and gel *in situ*. Block copolymer solutions containing human mesenchymal stem cells (hMSCs) and rhBMP-2 injected subcutaneously into the backs of mice revealed mineralized tissue formation and high levels of alkaline phosphatase activity [49]. Nongelling injectable delivery systems for rhBMP-2 have been investigated as well. An injectable biopolymer comprising chitosan and inorganic phosphates seeded with mesenchymal stem cells and rhBMP-2 supported significantly more new bone formation than groups not treated with MSCs and rhBMP-2 [50]. Biodegradable sol-gel-derived porous silica carriers have also been investigated for proteins, in which a sustained release of the protein can be achieved by varying the processing conditions and is both diffusion and degradation controlled [51,52].

3.2 Current strategies in the prevention of infection

Contamination of hardware used to fix open fractures is a significant clinical problem, and strategies for reducing infection are currently under investigation. Ti-6Al-4V rods coated with a vancomycin-containing Ca-P film using a sol-gel technique reduced *S. aureus* number, showed minimal signs of infection and reduced bone resorption in a rat osteomyelitis model [53]. Degradation of the sol-gel films was found to be the main mechanism regulating drug release, and concentrations exceeding the minimal inhibitory concentration (MIC) of vancomycin against *S. aureus* were achieved by controlling sol-gel processing parameters [54].

Prior to bone grafting, open fractures are treated with an antibiotic depot (e.g., PMMA beads) to reduce the burden of infection. A number of approaches to extending the period of antibiotic release from local depot delivery systems have been investigated, such as degradable chitosan sponges [55], injectable particles and hydrogels, and scaffolds fabricated from degradable polymers. Silica sol-gels (xerogels) have been reported to achieve a long-term (up to 6 weeks) sustained release of vancomycin with tunable release kinetics [56,57]. Combining silica xerogels with chitosan yielded hybrid materials with increased yield strength, yield strain and work to fracture, and the mesoporous structure of the materials allowed the effectual loading of vancomycin [58]. In a recent study, an anesthetic (lidocaine) and an antibiotic (mupirocin) embedded in electrospun poly (L-lactic acid) (PLLA) nanofibrous scaffolds showed release of active drug for up to 3 days, and the presence of the two drugs with different lipophilicities in the same polymer matrix altered the release kinetics [59]. However, by encapsulating the hydrophilic antibiotic doxycycline in PLGA nanospheres followed by embedding in nanofibrous PLLA scaffolds, sustained release of antibiotic for more than 6 weeks was accomplished [60]. The composition and molecular weight of the PLGA nanospheres were modified to achieve release kinetics ranging from days to weeks.

Injectable antibiotic delivery systems provide advantages such as conformability to irregularly shaped bone defects as well as administration using minimally invasive techniques. Microspheres fabricated from resorbable polyesters (e.g., poly(lactic-*co*-glycolic acid) have been reported to release antibiotics such as tobramycin [61], doxycycline [62], and gentamicin [63] for sustained periods exceeding 2 – 4 weeks. Silica sol gels microspheres have also been shown to support controlled release of antibiotics such as vancomycin [64]. Elastin-like polypeptides comprising repeating penta-peptide sequences that are thermally triggered to undergo *in situ* depot formation at body temperature have also been shown to release therapeutic concentrations of vancomycin for more than 30 days [65]. In another approach, biodegradable thermosensitive poly(ethylene glycol) monomethyl ether (mPEG) and PLGA copolymers containing teicoplanin, characterized by near zero-order release of drug and *in situ* gelling, have been shown to be effective for treating osteomyelitis in rabbits [66].

However, a significant disadvantage of polymeric delivery systems is their inability to actively promote bone healing. To address the need for osteointegration as well as infection control, osteoconductive biomaterials supporting the sustained release of antibiotics have been investigated. Osteoconductive hydroxyapatite (HA) biomaterials have been investigated as a delivery system for antibiotics such as amoxicillin, clavulanic acid and erythromycin [67]. While HA granules released the entire antibiotic during the first several hours, HA microspheres incorporating nanoscale (e.g., <100 nm) features showed sustained release of antibiotic for 3 weeks. In another study, HA scaffolds incorporating ceftriaxone and sulbactam supported sustained release of the drug at concentrations exceeding the minimum inhibitory concentration against *S. aureus* for 42 days, and demonstrated a superior infection control and new bone formation compared with the parenteral administration of the drugs in a contaminated rabbit tibia model [68]. Resorbable bioactive silica–calcium phosphate nanocomposites have also been reported to deliver a sustained release of a therapeutic dose of vancomycin for up to 35 days [69]. Due to the stimulatory effects of the degradation products on osteoblast gene expression, bioactive glass scaffolds incorporating antimicrobials are promising materials for bone regeneration and treatment of infection. Vancomycin-loaded scaffolds releasing the drug over 4 days significantly reduced MRSA infection compared with the untreated control at 8 weeks when implanted into the right tibiae of rabbits infected with osteomyelitis [70]. Furthermore, the scaffolds showed ingrowth of blood vessels and new bone at 8 weeks. In another study, incorporation of bioactive glass foam containing 2 mol% silver has been reported to release silver ions at a rate that is bactericidal, but not toxic, to bone cells [71]. Another study has shown that bioactive SiO2-CaO-P2O5 sol-gel glass with gentamicin sulfate supported a local release of gentamicin as well as new bone formation and integration with host bone in a rabbit femur model [72].

Composite scaffolds comprising a degradable polymer and an osteoconductive mineralized component combine the advantages of controlled release of antibiotic due to the polymeric component and stimulation of new bone formation due to the mineralized component. Combining PLGA with a novel mesoporous silica-HA composite increased the release of gentamicin from a period of 12 h for silica-HA alone to over a month for the PLGA-silica-HA scaffolds. Furthermore, growth of mesenchymal stem cells was higher on the PLGA-silica-HA scaffolds than for PLGA alone [73]. Similarly, coating allograft bone particles with a poly(ε-caprolactone) film incorporating tobramycin has been shown to extend the release of the drug for 6 – 8 weeks [74].

4. Microbiological challenges and biological principles to be considered

4.1 Need for bone healing and vascularization

Large defects in bone do not heal without intervention and must be grafted to achieve union of the fracture. While osteoconductive scaffolds delivering an antibiotic are efficacious in healing small lesions, large open fracture defects require a more potent osteoinductive factor such as rhBMP-2. Previous studies have shown that a burst release of rhBMP-2 promotes rapid infiltration of cells into the scaffold [1], and that sustained release of rhBMP-2 for more than 3 weeks enhances new bone formation relative to a burst release alone [29,47,75]. The role of BMP-2 in stimulating vasculogenesis is also well established [76-79], and bone wound healing is dependent on vascularization [80-83]. The processes of vasculogenesis and bone repair are highly coupled, as exemplified by the stimulation of angiogenesis through osteoblast-derived vascular endothelial growth factor (VEGF) [78], so enhancement of either process is beneficial to the other. A recent study has shown that a burst release of the angiogenic factor rhVEGF and sustained release of rhBMP-2 from a poly(propylene fumarate) scaffold did not increase vasculogenesis or new bone formation relative to rhBMP-2 alone [84], which further demonstrates the angiogenic effects of rhBMP-2. Sufficient blood flow is essential not only for new bone formation, but also for preventing infection in extremity injuries [85,86]. The bacteriocidal capacities of granulocytes, as well as the regenerative capacities of macrophages, fibroblasts, and endothelial cells, are highly dependent on the local perfusion of oxygenation, metabolites and cytokines [87]. Considering that vascularization may require up to 6 weeks to occur throughout the scaffold [83], sustained release of an antibiotic at concentrations exceeding the MIC for 6 – 8 weeks is desirable to kill the persistent bacteria and allow the processes of vasculogenesis and new bone formation to proceed unimpeded by infection [61,88].

4.2 Biofilm theory and the race to the surface

Surgical debridement and copious amounts of irrigation solution remove detectable necrotic tissue and foreign

materials, thereby both reducing the bioburden as well as removing the potential culture medium. Debridement ensures that the residual tissue is more effective in combating the bacteria that inevitably remain in the wound [89]. While antibiotics are also effective in reducing the bacteria after debridement, they must be administered immediately to kill the bacteria while they are in the planktonic stage, and too low a concentration of antibiotic may induce antibiotic resistance [90]. Bacteria adhere to the surface of the wounds shortly after injury and biofilms can form and mature within 5 – 10 h [91], while the average time for debridement and irrigation of open fractures is approximately 6 h [92] or longer [93]. Thus, early administration of antibiotics is anticipated to lower infection rates, as shown by a clinical study demonstrating that patients who were given antibiotics within 3 h after debridement had a lower infection rate compared to those who received antibiotics later [18]. A preclinical study that was designed specifically to evaluate the temporal relationship between treatment and infection demonstrated that delaying treatment drastically reduces the effectiveness of antibiotics [94].

Bacteria adhering to a surface produce a glycocalyx, an organic polymeric matrix known as biofilm. The biofilm and planktonic phenotypes differ substantially, as evidenced by the observation that the antibiotic concentration required to kill bacteria in a biofilm is up to 1000 times higher than that required for the planktonic phenotype [95]. There are many plausible reasons for the increased antimicrobial resistance of biofilms. Three of the more accepted explanations are the barriers to mass transfer and perfusion, extreme microenvironmental conditions, and the reduced growth rates associated with the biofilm [96]. The perfusion barrier hypothesis suggests that there is slow mass transfer of the antimicrobial into the biofilm. There is also evidence that the hypoxic and acidic conditions cause micro niches that are antagonistic to antibiotics or antimicrobials [97]. In addition, the nutrient-scarce microenvironment results in very slow metabolic and growth rates for some of the bacteria within the biofilm. Since many antibiotics require bacterial growth to be effective [98], minimal growth of bacteria in the biofilm can render the antibiotic ineffective. Regardless of the mechanism for survival, bacteria can survive antimicrobial therapy. These so-called "persister cells" can constitute 0.1 – 10% of a biofilm [97] as shown in **Figure 2** [99]. While the number of cells surviving the antimicrobial therapy is small, these persister cells grow rapidly in the presence of nutrients released from their lysed community partners after antimicrobial exposure is removed [97]. Furthermore, biofilms are difficult to detect, as evidenced by a study reporting that *S. aureus* within biofilms recovered from patients could not be detected by swabbing and culturing on agar media [100].

The "foreign body effect" describes the increased susceptibility to and morbidity of infection that can occur in the presence of an intracorporeal foreign body because its inanimate surfaces are the environmentally eminent domain not of tissue or host defense cells, but rather of microbes [6]. The "race to the surface" concept is useful for understanding the relationship between tissue integration and bacterial colonization [6]. For most orthopedic implants (e.g., screw, plate or rod), tissue integration typically wins the race since host tissue cells arrive to the implant first and form a cohesive bond. Consequently, bacteria will be confronted by host immune cells and will be less likely to colonize and form a biofilm. Infections centered on biomaterials or bone grafts are difficult to eliminate and usually require removal of the device [6], which underscores the importance of rapid tissue integration. A preclinical study in rabbits has shown that bone defects treated with autograft required four times lower bacterial concentrations to sustain an infection compared with empty defects [101], suggesting that avascular bone grafts potentially function as a nidus of infection. A canine study further confirms these results [102], where various levels of bacteria in suspension along with different biomaterials were placed into the femoral canal to determine the amount of bacteria required to produce an infection in 50% of the femora. The presence of an implant reduced the inoculum of *S. aureus* needed to produce an infection by two to four orders of magnitude. Thus, in the context of bacterial contamination the foreign body effect can be elicited by avascular biomaterials, even those that are known to be biocompatible.

5. Shortcoming of previous approaches

5.1 Clinical care

Conventional clinical practice dictates that devitalized tissue and gross contaminants be removed from the wound bed of open fractures. Most of the bacteria are removed during debridement and irrigation, and the vast majority of the remaining bioburden is killed by systemic antibiotics. However, systemic antibiotics also present the potential for systemic toxicity, difficulty in achieving high concentrations of antimicrobial agents at the site of infection and problems with patient compliance. Antibiotic-impregnated PMMA beads may be placed to maintain space and provide high levels of local antibiotics, which has been shown to reduce infection in preclinical models [103] and in a large retrospective clinical study [104] but not in a small prospective clinical study [105]. Since the PMMA beads do not resorb and antibiotic elution decreases after 2 to 6 weeks, they require surgical removal prior to definitive grafting [106]. In contrast, calcium sulfate pellets are biodegradable and can be mixed with antibiotics and used either as or with the definitive bone graft [24]. However, calcium sulfate pellets have in some cases been associated with seromas and drainage problems [107], and they do not meet the biological challenge of regenerating bone in a large defect.

While osteogenic or osteoinductive grafts induce new bone formation, they are not protected from the persistent bacteria remaining in the wound or from the nosocomial or hematological introduction of the bacteria to the wound. Autograft and

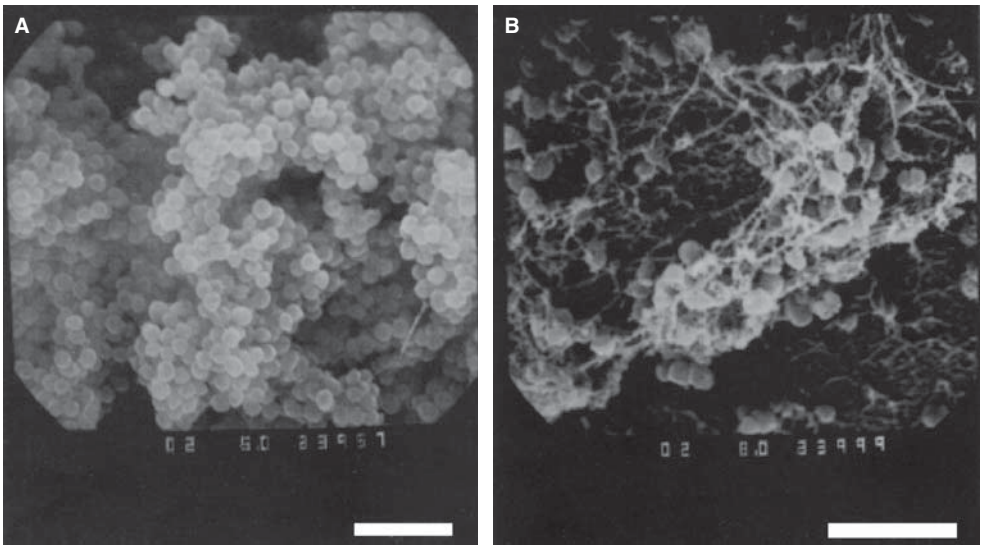

Figure 2. (A) Scanning electron micrograph of an untreated biofilm of *Staphylococcus epidermindis* formed on the surface of an equilibrium dialysis membrane and **(B)** the same biofilm after 72 h of contact with vancomycin and rifampin in concentrations that exceeded the MIC and MBC for the organism [99]. Despite marked differences in the appearance of the biofilm, viable organisms were recovered from the membrane shown in panel (B) for which MICs of either agent were unaltered. The scale bars in the lower right of each panel are approximately 5 μm. Reproduced with permission from American Society for Microbiology.

rhBMP-2 generally promote bone union, but they are not always effective, as evidenced by a study reporting that only 77% of large open fractures (average defect size of 4 cm) treated with these materials healed without requiring an intervention [5]. Furthermore, for some patients receiving an autograft, harvesting a sufficient amount of material from the iliac crest was difficult. In addition, the burst release of rhBMP-2 from the collagen sponge requires high doses of rhBMP-2 which makes its use expensive. The LEAP study also demonstrated high nonunion and malunion rates (37% combined) [12]. Thus current approaches for treating severe open fractures do not address the need to both control infection and also induce new bone formation to ensure that tissue integration predictably wins the race to the surface, which contributes to the high incidence of infection for severe open fractures (≥20% [14]).

5.2 Current tissue engineering and drug delivery strategies

While rhBMP-2 delivered on a collagen sponge enhances new bone formation, rapidly released rhBMP-2 may diffuse from the fracture site prior to achieving a critical density of newly infiltrated cells in the scaffold [1]. The requirement for supra-physiological doses of rhBMP-2 to induce a robust osteogenic effect has been attributed to the rapid release of the drug, and may result in complications such as severe inflammation and ectopic bone formation [16,108]. Complications have also been associated with the off-label use of INFUSE, which have been estimated to comprise >85% of principal procedures using rhBMP-2 [109]. INFUSE is approved by the FDA for use with the lumbar tapered fusion device to perform single-level anterior lumbar interbody fusions (ALIF: L2-S1 levels), but it is also used off-label for anterior and/or posterior cervical, thoracic, and lumbar surgery [110]. Reported complications include ectopic bone formation, paralysis, dural tears, and respiratory failure, and are particularly prevalent in the anterior cervical spine.

INFUSE is contra-indicated for infection [110]. Furthermore, infection is known to inhibit new bone formation even in the presence of rhBMP-2 [111]. Systemic delivery of antibiotics and local delivery of rhBMP-7 from a collagen carrier have been observed to enhance bone formation relative to local delivery of rhBMP-7 alone [112], but systemic delivery of antibiotics is limited by the difficulty of providing local concentrations exceeding the MIC without damaging distant organs. Therefore, infection control is only provided prior to bone grafting and definitive closure, leaving the graft unprotected against bacteria remaining in the wound bed. To protect the bone graft from infection during healing, osteoconductive scaffolds supporting the release of antibiotics above the MIC for the 6 – 8-week period required for full vascularization [83] have been investigated [68]. A recent clinical study has reported that antibiotic-loaded allograft bone dramatically reduced the incidence of infection in patients with infected orthopedic implants [113], which underscores the need to protect the

bone graft from infection during the healing process. While this approach is effective for treating small osteomyelitis lesions in trabecular bone, it does not address the biological challenges of regenerating bone in large contaminated open fractures.

6. Development of a dual-purpose graft

6.1 Need for protection from contamination while healing: dual-delivery concept

The dual-delivery concept addresses both the biological challenge of healing large bony defects and the microbiological challenge of killing residual bacteria [7-10]. Local sustained delivery of antibiotics from the bone graft for 6 – 8 weeks is anticipated to protect the graft from infection and reduce the frequency of secondary complications. Therefore, protecting the implant from bacterial colonization until the site is vascularized, and the host immune system can prevent colonization, is an important strategy for reducing infection. This hand-off from chemical (e.g., through antibiotics) to immune (e.g., through vasculogenesis) defense reduces the risk of the graft material causing infection. Biologically, the challenge of healing large defects with concomitant soft tissue injury can be daunting. Therefore, a dual-purpose graft must heal bone as well as, and ideally better than, the current standards of care.

6.2 The appropriate antimicrobial should not compromise healing

Dual-purpose bone grafts present additional challenges for bone healing due to the potential effects of the antibiotics on recruitment and differentiation of osteoprogenitor cells, as well as the degradation rate of the scaffold. Many antibiotics, such as ciprofloxacin, are cytotoxic and a hindrance to osteoblast differentiation at bacteriocidal doses [114], which underscores the importance of selecting a suitable antibiotic for the dual-purpose graft that does not hinder migration or differentiation of osteoprogenitor cells into the scaffold. Despite the relatively high toxicity of gentamicin, which has been shown to hinder osteoblast proliferation and differentiation *in vitro* at concentrations >100 μg ml^{-1} [114], when incorporated into HA scaffolds it did not stimulate or inhibit osteointegration or bone apposition [115]. These observations are in agreement with a recent study investigating dual delivery of the antibiotic teicoplanin and rhBMP-2 from a synthetic degradable block copolymer of poly(D,L-lactic acid) and polyethylene glycol (PLA-DX-PEG) [116]. Rat calvarial defects treated with PLA-DX-PEG discs loaded with rhBMP-2 with or without teicoplanin showed restoration of normal anatomy within 6 weeks, suggesting that the biological activity of rhBMP-2 was retained in the presence of antibiotics. Other studies have investigated the effects of infection on scaffold degradation and remodeling. Borate bioactive glass scaffold devices loaded with vancomycin formed HA resulting from the conversion of the glass, which served as structure to support the growth of new bones and blood vessels, in a rabbit tibia osteomyelitis model [70,117]. In another study, biodegradable poly(ester urethane) scaffolds incorporating rhBMP-2 and vancomycin showed accelerated scaffold degradation when implanted in a contaminated rat segmental defect compared with a noncontaminated defect [9]. Thus, the composition of the scaffold is anticipated to affect its degradation and remodeling in the presence of infection.

Since the majority of open fractures do heal, it is also essential that the infection control strategy does not increase nonunion rates. Clinically, antibiotics are selected for their spectrum or ease of administration to the patient. The effects on eukaryotic cells traditionally have not factored in the selection, but must be considered when developing a dual-purpose graft. A recent *in vitro* study evaluating the effect of eight concentrations (0 – 5000 μg/ml) of 21 antibiotics on the viability and activity of osteoblasts reported that vancomycin, a tricyclic glycopeptide antibiotic that is efficacious for treating infections caused by gram-positive bacteria such as *S. aureus* [32,33], had the least detrimental effects on osteoblast function (**Figure 3**) [114]. All other antibiotics reduced the alkaline phosphatase (ALP) activity at doses that were 10 – 50 times lower than that of vancomycin [114], which is consistent with previous studies showing that vancomycin has less adverse effects on osteoblasts than other commonly used antibiotics *in vitro* [118,119]. Although data from cell culture studies do not always predict the *in vivo* response (such as interactions between antibiotics and rhBMP-2 that affect osteoblasts), vancomycin has also been shown not to impede bone growth in fractures *in vivo* [120]. These observations are in agreement with our unpublished data investigating the effects of vancomycin released from a dual-purpose implant incorporating both vancomycin and rhBMP-2 on new bone formation in a 6-mm critical-sized segmental defect model. While the vancomycin dose (8160 μg cm^{-3} scaffold) exceeded the concentration (5000 μg cm^{-3} scaffold) above which vancomycin adversely affects osteoblast ALP activity *in vitro* [114], the presence of vancomycin did not hinder new bone formation induced by rhBMP-2, which is attributed to the sustained release of the drug from the scaffold. This observation underscores the need for a sustained release of vancomycin to both provide infection control for up to 6 – 8 weeks as well as minimize the cytotoxic effects of the antibiotic on osteoblasts. Modulating the hydrophilicity of the antibiotic has also been investigated to control its release from polymeric scaffolds. For example, converting hydrophilic vancomycin hydrochloride to the more hydrophobic free base form of the drug extended its release from a polymeric scaffold for more than 8 weeks above the MBC and MIC [121].

6.3 Improving healing and reducing infection in a contaminated bone defect

Several approaches to the development of a dual-purpose graft have been evaluated in critical-size (6 mm) segmental defects contaminated with *S. aureus* in rat femora. A dual-purpose graft supporting sustained release of

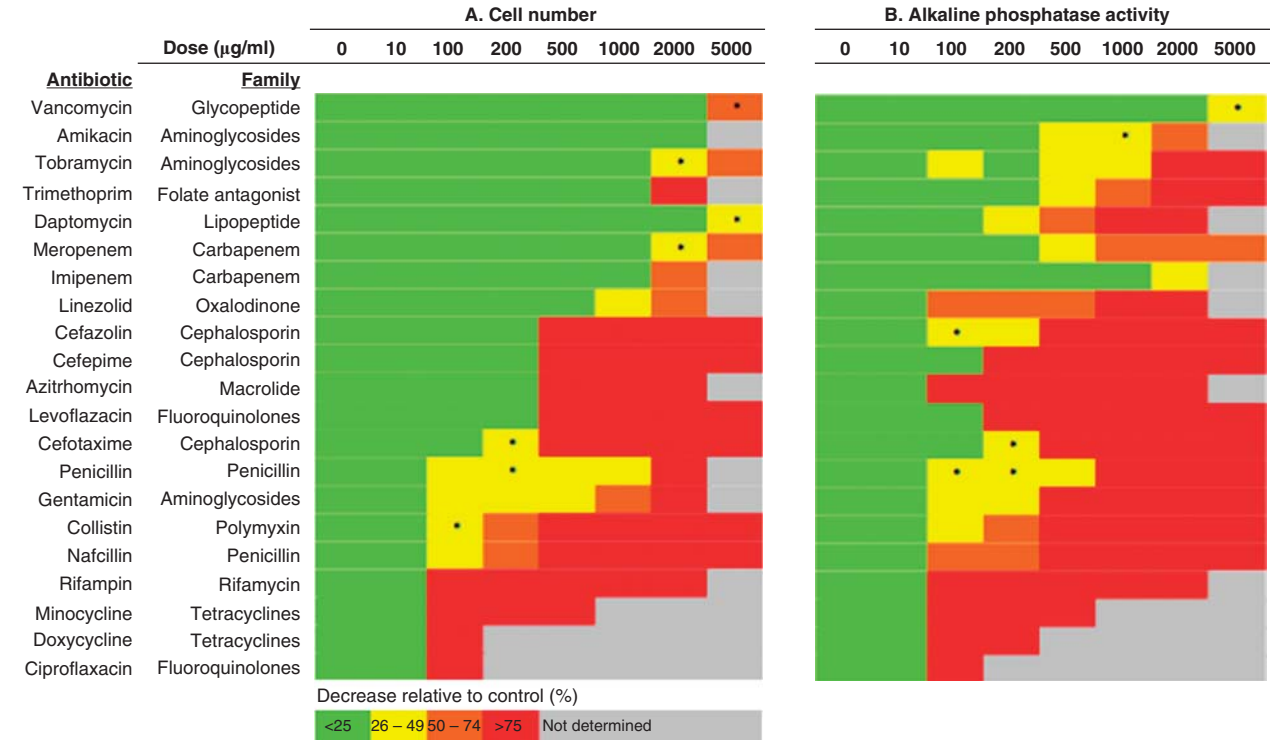

Figure 3. Effects of treatment with different antibiotics on osteoblast cell number and ALP activity [114]. The mean % decreases in osteoblast cell number (A) and ALP activity (**B**) are classified as <25%, 26 – 50, 51 – 74, and >75% of control after incubation with 0, 10, 100, 200, 500, 1000, 2000 and 5000 μg ml^{-1} of each antibiotic for 10 and 14 days (n = 5 – 6 per dose after data are pooled). Not determined: ALP activity and/or cell number was untestable for some of the antibiotics, presumably because of precipitation and incompatibility with the test assays used. Decreases in osteoblast cell number and ALP activity >25% were significant, $p < 0.05$, with exceptions indicated by (*) where the value at that dose was not different from control.

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vancomycin (8160 μg cm^{-3}) scaffold above the MIC for 8 weeks as well as release of rhBMP-2 (60 or 600 μg cm^{-3} scaffold) for more than 3 weeks controlled infection and promoted three times more new bone formation compared with the ACS carrier with rhBMP-2 and without vancomycin [9]. In another study, the use of silver nanoparticles (2 wt%) and rhBMP-2 (30 μg ml^{-1}) released from PLGA scaffolds both healed the defect and prevented infection [8]. The control group (grafts containing rhBMP-2 without silver) did not heal and contained bacteria 12 weeks after implantation. Another group has reported using a polypropylene fumarate (PPF) cylinder as the scaffold [7]. Four side portals were loaded with dicalcium phosphate dehydrate that contained rhBMP-2 (10 μg). The implant was protected from infection by applying a paste to the outside surface of the scaffold that consisted of gentamicin (10 or 20 mg) mixed with an absorbable gelatin sponge. The gentamicin paste improved healing and reduced infection in 5-mm rat segmental defects contaminated with a mixed microbial population (*S. aureus* and *Escherichia coli*). Another approach is the use of a chitosan-calcium phosphate composite scaffold [10].

The carrier as well as the antibiotic and rhBMP-2 release kinetics are anticipated to have a significant effect on the healing of contaminated segmental bone defects. Previous studies have shown that both polymeric carriers (PUR [9] and PLGA [8]) as well as polymer/ceramic composites (PPF/TCP) [7] are useful materials for dual-purpose grafts. However, differences in antibiotics and preclinical models between the few studies reported to date confound the effects of the carrier on healing. Partial depletion of rhBMP-2 by bacteria has been suggested to slow down the healing of contaminated fractures, and may necessitate using higher doses or more efficient delivery systems (e.g., microspheres) capable of achieving sustained release of rhBMP-2 for a longer period of time [8]. It is anticipated that future studies on dual-purpose bone grafts will identify key material properties, doses of biologics, and release kinetics that optimize both infection control and bone healing.

7. Expert opinion

Contaminated open fractures are generally treated by a two-stage procedure comprising an initial treatment with local delivery of antibiotics followed by a second bone grafting step. However, current treatment strategies do not promote predictable healing, as evidenced by a recent clinical study reporting that one-third of patients with severe lower extremity trauma experienced complications such as infection and nonunion resulting in rehospitalization [12]. Preclinical and clinical studies have demonstrated that avascular bone grafts can function as a nidus for infection when implanted in a contaminated wound bed [101,102], and that local delivery of an antibiotic from the graft can dramatically reduce the incidence of infection [113]. These observations underscore the need to protect the bone graft from infection during the healing process. Significant advances in materials science and engineering have contributed to the development of local delivery depots with sustained release of antibiotics for >6 weeks. However, these materials at best merely provide an osteoconductive matrix for cells to infiltrate and do not address the biological requirements for healing of large osseous defects.

Infection rates of severe open fractures have not decreased substantially since antibiotics were introduced into clinical practice, and microvascular surgery became available to provide soft tissue coverage. We suggest that the lack of suitable bone graft materials has contributed to the inability to reduce the incidence of infection. The dual-purpose graft approach addresses, for the first time, both the microbiological and biological challenges. Besides potentially reducing complications in severe open fractures, dual-purpose grafts may be used for treating recalcitrant infections such as osteomyelitis or smaller bony injuries in patients with co-morbidities that increase their risk for nonunion. This strategy can be modified to heal other tissues, such as skin equivalents for severe burns and diabetic ulcers, which also have a challenging healing environment and can become infected. The ultimate goal is to improve outcomes by addressing all of the challenges of the injury.

In order to accomplish predictable bone healing and infection control, the dual-purpose bone graft must be designed such that the process of tissue integration will win the "race to the surface" [6]. Upon implantation of the bone graft in the contaminated defect, it is at risk of being colonized by bacteria present in the wound bed. Infection can result from gram-negative or gram-positive bacteria and is often polymicrobial [13], which underscores the need to select antibiotics that are effective against a broad spectrum of bacteria at supra-MIC levels. Vancomycin is effective against gram-positive bacteria, particularly the most common infecting organisms (*S. aureus*), and has some coverage against gram-negative bacteria. To protect the bone graft from infection and enable wound healing and angiogenesis to progress, a sustained release of the antibiotic exceeding the MIC is required for at least 6 weeks post-implantation [121]. In addition, a burst followed by a sustained release of rhBMP-2 for more than 3 weeks has been reported to enhance new bone formation relative to a burst release or a sustained release alone [1,30]. Thus the release kinetics for both the antibiotic and rhBMP-2 must be tuned to targets determined by the biological requirements for wound healing. Currently available therapies, such as PMMA beads for local delivery of antibiotics or the ACS carrier for rhBMP-2, do not provide sustained release of the drug for a sufficiently long time.

Recent studies have shown that a dual-purpose bone graft supporting sustained release of rhBMP-2 and vancomycin [9], gentamicin [7], or nanosilver [8] controls infection and promotes new bone formation in a critical-sized infected rat segmental defect model. In the vancomycin and nanosilver studies, the defect was contaminated with a strain of *S. aureus* prior to implantation of the bone graft. The gentamicin study utilized a mixed microbial population (*S. aureus and E. coli*), which is more relevant to the polymicrobial infections encountered clinically. In the gentamicin and nanosilver studies, the dual-purpose graft was implanted to treat the infection immediately after inoculation of the wound [7,8]. A more rigorous test for the efficacy of dual-purpose grafts can be accomplished using preclinical models with an established infection. Although delaying the placement of the dual-purpose implant for several hours after necessitates another surgical procedure, it ensures that the bacteria are able to adhere to the surface of the wound and begin forming biofilms [94], which is considered to be more representative of the clinical scenario. Immediate treatment with the dual-purpose graft after inoculation offers the advantage of rapid screening, but likely does not provide as stringent a model since the burst release of antibiotics is anticipated to kill the vulnerable planktonic bacteria. While the initial studies in rats provide compelling evidence demonstrating the feasibility of the concept, dual-purpose grafts must be validated in more challenging preclinical models in larger animals prior to evaluation in a clinical study.

Dual-purpose bone grafts are a promising adjunctive therapy for open fractures. Although the results from initial preclinical studies are compelling, the exact mechanisms by which bacteria and osteoblasts interact with the surface in the presence of antibiotics and growth factors are not known. Novel imaging techniques capable of co-localizing bacterial colonization and a new bone formation on the surface of the graft may prove useful for elucidating the relevant molecular mechanisms [122]. The development of injectable dual-purpose grafts potentially offers the advantage of administration using minimally invasive surgical techniques [123]. The dual-delivery approach is

not intended to replace existing treatments or surgical tenets. Thus by building on the strengths of existing practices, the strategy of protecting the graft from contamination has the potential to reduce complications and associated morbidity of open fractures.

Acknowledgments

The authors thank B Li, KV Brown, T Guda, AE Hafeman and Baek-Hee Lee for their contributions to developing the dual-purpose graft.

Declaration of interest

The development of the dual-purpose graft was funded by the Orthopedic Extremity Trauma Research Program (DoD Grant #W81XWH-07-1-0211). S Guelcher is a consultant for Medtronic, which has licensed aspects of the technology from Vanderbilt. J Wenke has nothing to declare. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Seeherman H, Wozney JM. Delivery of bone morphogenetic proteins for orthopedic tissue regeneration. Cytokine Growth Factor Rev 2005;16:329-45
2. Haidar ZS, Hamdy RC, Tabrizian M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part B: delivery systems for BMPs in orthopaedic and craniofacial tissue engineering. Biotechnol Lett 2009;31:1825-35
3. Haidar ZS, Hamdy RC, Tabrizian M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part A: current challenges in BMP delivery. Biotechnol Lett 2009;31:1817-24
4. Li RH, Wozney JM. Delivering on the promise of bone morphogenetic proteins. Trends Biotechnol 2001;19:255-65
•• **An excellent review of rhBMP-2 delivery systems and clinical applications.**
5. Jones AL, Bucholz RW, Bosse MJ, et al. Recombinant human BMP-2 and allograft compared with autogenous bone graft for reconstruction of diaphyseal tibial fractures with cortical defects. A randomized, controlled trial. J Bone Joint Surg Am 2006;88:1431-41
6. Gristina AG, Naylor P, Myrvik Q. Infections from biomaterials and implants: a race for the surface. Med Prog Technol 1988;14:205-24
•• **This paper establishes the key concept of the race to the surface and infection in biomedical implants.**
7. Stewart RL, Cox JT, Volgas D, et al. The use of a biodegradable, load-bearing scaffold as a carrier for antibiotics in an infected open fracture model. J Orthop Trauma 2010;24:587-91
•• **This paper reports that poly(propylene fumarate) dual-purpose grafts incorporating gentamicin and rhBMP-2 were effective at decreasing radiographically defined osteomyelitis in an acutely infected open fracture.**
8. Zheng Z, Yin W, Zara JN, et al. The use of BMP-2 coupled - Nanosilver-PLGA composite grafts to induce bone repair in grossly infected segmental defects. Biomaterials 2010;31:9293-300
•• **This paper reports that PLGA dual-purpose grafts incorporating nanosilver and rhBMP-2 promote more new bone formation and provide improved infection control in an acutely infected segmental defect model.**
9. Guelcher SA, Brown KV, Li B, et al. Dual-purpose bone grafts improve healing and reduce infection. J Orthop Trauma 2011;25:477-82
• **This paper reports that biodegradable polyurethane dual-purpose bone grafts incorporating vancomycin and rhBMP-2 promote more new bone formation and provide improved infection control relative to the collagen sponge in a contaminated segmental defect model.**
10. Reves BT, Bumgardner JD, Cole JA, et al. Lyophilization to improve drug delivery for chitosan-calcium phosphate bone scaffold construct: a preliminary investigation. J Biomed Mater Res B Appl Biomater 2009;90:1-10
11. Bosse MJ, MacKenzie EJ, Kellam JF, et al. An analysis of outcomes of reconstruction or amputation after leg-threatening injuries. N Engl J Med 2002;347:1924-31
• **This paper highlights the complications of infection and non-union associated with open fractures.**
12. Harris AM, Althausen PL, Kellam J, et al. Complications following limb-threatening lower extremity trauma. J Orthop Trauma 2009;23:1-6
13. Johnson EN, Burns TC, Hayda RA, et al. Infectious complications of open type III tibial fractures among combat casualties. Clin Infect Dis 2007;45:409-15
14. Okike K, Bhattacharyya T. Trends in the management of open fractures. A critical analysis. J Bone Joint Surg Am 2006;88:2739-48
15. McLaren AC. Alternative materials to acrylic bone cement for delivery of depot antibiotics in orthopaedic infections. Clin Orthop Relat Res 2004;427:101-6
16. Swiontkowski MF, Aro HT, Donell S, et al. Recombinant human bone morphogenetic protein-2 in open tibial fractures. A subgroup analysis of data combined from two prospective randomized studies. J Bone Joint Surg Am 2006;88:1258-65
17. Keeling JJ, Gwinn DE, Tintle SM, et al. Short-term outcomes of severe open wartime tibial fractures treated with ring external fixation. J Bone Joint Surg Am 2008;90:2643-51
18. Patzakis MJ, Wilkins J. Factors influencing infection rate in open fracture wounds. Clin Orthop Relat Res 1989 Jun;(243):36-40.
19. Murray CK, Obremskey WT, Hsu JR, et al. Prevention of Combat-related Infections Guidelines Panel. Prevention

of infections associated with combat-related extremity injuries. J Trauma 2011;71:S235-57

20. Kanellakopoulou K, Giamaraellos-Bourboulis E. Carrier systems for the local delivery of antibiotics in bone infections. Drugs 2000;59:1223-32
21. Beardmore AA, Brooks DE, Wenke JC, Thomas DB. Effectiveness of local antibiotic delivery with an osteoinductive and osteoconductive bone-graft substitute. J Bone Joint Surg Am 2005;87:107-12
22. Gitelis S, Brebach GT. The treatment of chronic osteomyelitis with a biodegradable antibiotic-impregnated implant. J Orthop Surg (Hong Kong) 2002;10:53-60
23. McKee MD, Wild LM, Schemitsch EH, Waddell JP. The use of an antibiotic-impregnated, osteoconductive, bioabsorbable bone substitute in the treatment of infected long bone defects: early results of a prospective trial. J Orthop Trauma 2002;16:622-7
24. Helgeson MD, Potter BK, Tucker CJ, et al. Antibiotic-impregnated calcium sulfate use in combat-related open fractures. Orthopedics 2009;32:323
25. Kirker-Head C. Potential applications and delivery strategies for bone morphogenetic proteins. Adv Drug Deliv Rev 2000;43:65-92
26. Uludag H, Gao T, Porter T, et al. Delivery systems for BMPS: factors contributing to the protein retention at an application site. J Bone Joint Surg 2001;83-A:S128-35
27. Cho TJ, Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. J Bone Miner Res 2002;17:513-20
28. Geiger M, Li RH, Friess W. Collagen sponges for bone regeneration with rhBMP-2. Adv Drug Deliv Rev 2003;55:1613-29
29. Brown KV, Li B, Guda T, et al. Improving bone formation in a rat femur segmental defect by controlling bone morphogenetic protein-2 release. Tissue Eng Part A 2011;17:1735-46

•• **This paper reports a 50% increase in new bone formation when rhBMP-2 is delivered from a resorbable polyurethane scaffold compared to the collagen sponge.**

30. Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery). J Tissue Eng Regen Med 2008;2:81-96
31. Mano JF, Reis RL. Osteochondral defects: present situation and tissue engineering approaches. J Tissue Eng Regen Med 2007;1:261-73
32. Aebli N, Stich H, Schawalder P, et al. Effects of bone morphogenetic protein-2 and hyaluronic acid on the osseointegration of hydroxyapatite-coated implants: an experimental study in sheep. J Biomed Mater Res A 2005;73:295-302
33. Eckardt H, Christensen KS, Lind M, et al. Recombinant human bone morphogenetic protein 2 enhances bone healing in an experimental model of fractures at risk of non-union. Injury 2005;36:489-94
34. Arosarena OA, Collins WL. Bone regeneration in the rat mandible with bone morphogenetic protein-2: a comparison of two carriers. Otolaryngol Head Neck Surg 2005;132:592-7
35. Saito A, Suzuki Y, Kitamura M, et al. Repair of 20-mm long rabbit radial bone defects using BMP-derived peptide combined with an alpha-tricalcium phosphate scaffold. J Biomed Mater Res A 2006;77:700-6
36. Park DJ, Choi BH, Zhu SJ, et al. Injectable bone using chitosan-alginate gel/mesenchymal stem cells/BMP-2 composites. J Craniomaxillofac Surg 2005;33:50-4
37. Jung RE, Schmoekel HG, Zwahlen R, et al. Platelet-rich plasma and fibrin as delivery systems for recombinant human bone morphogenetic protein-2. Clin Oral Implants Res 2005;16:676-82
38. Kirker-Head C, Karageorgiou V, Hofmann S, et al. BMP-silk composite matrices heal critically sized femoral defects. Bone 2007;41:247-55
39. Miyamoto S, Takaoka K, Okada T, et al. Evaluation of polylactic acid homopolymers as carriers for bone morphogenetic protein. Clin Orthop Relat Res 1992;278:274-85
40. Saito N, Takaoka K. New synthetic biodegradable polymers as BMP carriers for bone tissue engineering. Biomaterials 2003;24:2287-93
41. Mayer MH, Hollinger JO, Ron E, Wozney J. Repair of alveolar clefts in dogs with recombinant bone morphogenetic protein and poly(alpha-hydroxy acid). Plast Reconstr Surg 1996;98:247-59
42. Shimazu C, Hara T, Kinuta Y, et al. Enhanced vertical alveolar bone augmentation by recombinant human bone morphogenetic protein-2 with a carrier in rats. J Oral Rehabil 2006;33:609-18
43. Young S, Patel ZS, Kretlow JD, et al. Dose effect of dual delivery of vascular endothelial growth factor and bone morphogenetic protein-2 on bone regeneration in a rat critical-size defect model. Tissue Eng Part A 2009;15:2347-62
44. Kempen DH, Kruyt MC, Lu L, et al. Effect of autologous bone marrow stromal cell seeding and bone morphogenetic protein-2 delivery on ectopic bone formation in a microsphere/poly(propylene fumarate) composite. Tissue Eng Part A 2009;15:587-94
45. Chu TM, Warden SJ, Turner CH, Stewart RL. Segmental bone regeneration using a load-bearing biodegradable carrier of bone morphogenetic protein-2. Biomaterials 2007;28:459-67
46. Kolambkar YM, Dupont KM, Boerckel JD, et al. An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. Biomaterials 2011;32:65-74
47. Boerckel JD, Kolambkar YM, Dupont KM, et al. Effects of protein dose and delivery system on BMP-mediated bone regeneration. Biomaterials 2011;32:5241-51
48. Rey-Rico A, Silva M, Couceiro J, et al. Osteogenic efficiency of in situ gelling poloxamine systems with and without bone morphogenetic protein-2. Eur Cell Mater 2011;21:317-40
49. Kim HK, Shim WS, Kim SE, et al. Injectable in situ-forming pH/thermo-sensitive hydrogel for bone tissue engineering. Tissue Eng Part A 2009;15:923-33

50. Stephan SJ, Tholpady SS, Gross B, et al. Injectable tissue-engineered bone repair of a rat calvarial defect. Laryngoscope 2010;120:895-901

51. Santos EM, Radin S, Ducheyne P. Sol-gel derived carrier for the controlled release of proteins. Biomaterials 1999;20:1695-700

52. Viitala R, Jokinen M, Rosenholm JB. Mechanistic studies on release of large and small molecules from biodegradable SiO2. Int J Pharm 2007;336:382-90

53. Adams CS, Antoci V Jr, Harrison G, et al. Controlled release of vancomycin from thin sol-gel films on implant surfaces successfully controls osteomyelitis. J Orthop Res 2009;27:701-9

54. Radin S, Ducheyne P. Controlled release of vancomycin from thin sol-gel films on titanium alloy fracture plate material. Biomaterials 2007;28:1721-9

55. Noel SP, Courtney HS, Bumgardner JD, Haggard WO. Chitosan sponges to locally deliver amikacin and vancomycin: a pilot in vitro evaluation. Clin Orthop Relat Res 2010;468:2074-80

56. Aughenbaugh W, Radin S, Ducheyne P. Silica sol-gel for the controlled release of antibiotics. II. The effect of synthesis parameters on the in vitro release kinetics of vanomycin. J Biomed Mater Res 2001;57:321-6

57. Radin S, Ducheyne P, Kamplain T, Tan BH. Silica sol-gel for the controlled release of antibiotics. I. Synthesis, characterization, and in vitro release. J Biomed Mater Res 2001;57:313-20

58. Lee EJ, Jun SH, Kim HE, et al. Silica xerogel-chitosan nano-hybrids for use as drug eluting bone replacement. J Mater Sci Mater Med 2010;21:207-14

59. Thakur RA, Florek CA, Kohn J, Michniak BB. Electrospun nanofibrous polymeric scaffold with targeted drug release profiles for potential application as wound dressing. Int J Pharm 2008;364:87-93

60. Feng K, Sun H, Bradley MA, et al. Novel antibacterial nanofibrous PLLA scaffolds. J Control Release 2010;146:363-9

61. Ambrose CG, Gogola GR, Clyburn TA, et al. Antibiotic microspheres: preliminary testing for potential treatment of osteomyelitis. Clin Orthop Relat Res 2003;415:279-85

62. Aishwarya S, Mahalakshmi S, Sehgal PK. Collagen-coated polycaprolactone microparticles as a controlled drug delivery system. J Microencapsul 2008;25:298-306

63. Virto MR, Elorza B, Torrado S, et al. Improvement of gentamicin poly(D,L-lactic-co-glycolic acid) microspheres for treatment of osteomyelitis induced by orthopedic procedures. Biomaterials 2007;28:877-85

64. Radin S, Chen T, Ducheyne P. The controlled release of drugs from emulsified, sol gel processed silica microspheres. Biomaterials 2009;30:850-8

65. Adams SB Jr, Shamji MF, Nettles DL, et al. Sustained release of antibiotics from injectable and thermally responsive polypeptide depots. J Biomed Mater Res B Appl Biomater 2009;90:67-74

66. Peng KT, Chen CF, Chu IM, et al. Treatment of osteomyelitis with teicoplanin-encapsulated biodegradable thermosensitive hydrogel nanoparticles. Biomaterials 2010;31:5227-36

67. Ferraz MP, Mateus AY, Sousa JC, Monteiro FJ. Nanohydroxyapatite microspheres as delivery system for antibiotics: release kinetics, antimicrobial activity, and interaction with osteoblasts. J Biomed Mater Res A 2007;81:994-1004

68. Kundu B, Soundrapandian C, Nandi SK, et al. Development of new localized drug delivery system based on ceftriaxone-sulbactam composite drug impregnated porous hydroxyapatite: a systematic approach for in vitro and in vivo animal trial. Pharm Res 2010;27:1659-76

69. El-Ghannam A, Jahed K, Govindaswami M. Resorbable bioactive ceramic for treatment of bone infection. J Biomed Mater Res A 2010;94:308-16

70. Liu X, Xie Z, Zhang C, et al. Bioactive borate glass scaffolds: in vitro and in vivo evaluation for use as a drug delivery system in the treatment of bone infection. J Mater Sci Mater Med 2010;21:575-82

71. Shi X, Wang Y, Ren L, et al. Novel mesoporous silica-based antibiotic releasing scaffold for bone repair. Acta Biomater 2009;5:1697-707

72. Sevy JO, Slawson MH, Grainger DW, Brooks AE. Assay method for polymer-controlled antibiotic release from allograft bone to target orthopaedic infections - biomed 2010. Biomed Sci Instrum 2010;46:136-41

73. Jones JR, Ehrenfried LM, Saravanapavan P, Hench LL. Controlling ion release from bioactive glass foam scaffolds with antibacterial properties. J Mater Sci Mater Med 2006;17:989-96

74. Meseguer-Olmo L, Ros-Nicolas M, Vicente-Ortega V, et al. A bioactive sol-gel glass implant for in vivo gentamicin release. Experimental model in Rabbit. J Orthop Res 2006;24:454-60

75. Jeon O, Song S, Kang S-W, et al. Enhancement of ectopic bone formation by bone morphogenetic protein-2 released from a heparin-conjugated poly(L-lactic-co-glycolic acid) scaffold. Biomaterials 2007;28:2763-71

76. Moser M, Patterson C. Bone morphogenetic proteins and vascular differentiation: BMPing up vasculogenesis. Thromb Haemost 2005;94:713-18

77. Langenfeld EM, Langenfeld J. Bone morphogenetic protein-2 stimulates angiogenesis in developing tumors. Mol Cancer Res 2004;2:141-9

78. Deckers MM, van Bezooijen RL, van der Horst G, et al. Bone morphogenetic proteins stimulate angiogenesis through osteoblast-derived vascular endothelial growth factor A. Endocrinology 2002;143:1545-53

79. Ripamonti U, van den Heever B, Sampath TK, et al. Complete regeneration of bone in the baboon by recombinant human osteogenic protein-1 (hOP-1, bone morphogenetic protein-7). Growth Factors 1996;13:273

80. Gung YW, Cheng CK, Su CY. A stereomorphologic study of bone matrix apposition in HA-implanted cavities observed with SEM, being prepared by a microvascular cast and freeze-fracture method. Med Eng Phys 2003;25:565-71

81. Lienau J, Schmidt-Bleek K, Peters A, et al. Differential regulation of blood vessel formation between standard and delayed bone healing. J Orthop Res 2009;27:1133-40

82. Li R, Stewart DJ, von Schroeder HP, et al. Effect of cell-based VEGF gene

therapy on healing of a segmental bone defect. J Orthop Res 2009;27:8-14

83. Geiger F, Bertram H, Berger I, et al. Vascular endothelial growth factor gene-activated matrix (VEGF165-GAM) enhances osteogenesis and angiogenesis in large segmental bone defects. J Bone Miner Res 2005;20:2028-35

84. Kempen DH, Lu L, Heijink A, et al. Effect of local sequential VEGF and BMP-2 delivery on ectopic and orthotopic bone regeneration. Biomaterials 2009;30:2816-25

85. Cierny G III, Byrd HS, Jones RE. Primary versus delayed soft tissue coverage for severe open tibial fractures. A comparison of results. Clin Orthop Relat Res 1983 Sep;(178):54-63

86. Fischer MD, Gustilo RB, Varecka TF. The timing of flap coverage, bone-grafting, and intramedullary nailing in patients who have a fracture of the tibial shaft with extensive soft-tissue injury. J Bone Joint Surg Am 1991;73:1316-22

87. Hunt TK. The physiology of wound healing. Ann Emerg Med 1988;17:1265-73

88. Zilberman M, Elsner JJ. Antibiotic-eluting medical devices for various applications. J Control Release 2008;130:202-15

89. Bowyer G. Debridement of extremity war wounds. J Am Acad Orthop Surg 2006;14:S52-6

90. Thomes B, Murray P, Bouchier-Hayes D. Development of resistant strains of Staphylococcus epidermidis on gentamicin-loaded bone cement in vivo. J Bone Joint Surg Br 2002;84:758-60

91. Harrison-Balestra C, Cazzaniga AL, Davis SC, Mertz PM. A wound-isolated Pseudomonas aeruginosa grows a biofilm in vitro within 10 hours and is visualized by light microscopy. Dermatol Surg 2003;29:631-5

92. Anglen JO. Comparison of soap and antibiotic solutions for irrigation of lower-limb open fracture wounds. A prospective, randomized study. J Bone Joint Surg Am 2005;87:1415-22

93. Pollak AN, Jones AL, Castillo RC, et al. The relationship between time to surgical debridement and incidence of infection after open high-energy lower extremity trauma. J Bone Joint Surg Am 2010;92:7-15

94. Brown KV, Walker JA, Cortez DS, et al. Earlier debridement and antibiotic administration decrease infection. J Surg Orthop Adv 2010;19:18-22

95. Nickel JC, Ruseska I, Wright JB, Costerton JW. Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother 1985;27:619-24

96. Dunne WM Jr. Bacterial adhesion: seen any good biofilms lately? Clin Microbiol Rev 2002;15:155-66

•• **This paper reports that treatment with antibiotics does not completely kill the bacteria and that a some cells survive on the surface of the implant.**

97. Percival SL, Hill KE, Malic S, et al. Antimicrobial tolerance and the significance of persister cells in recalcitrant chronic wound biofilms. Wound Repair Regen 2011;19:1-9

•• **This paper shows that persister cells survive antibiotic treatment and can colonize the implant after the antibiotics are no longer present.**

98. Tuomanen E, Cozens R, Tosch W, et al. The rate of killing of Escherichia coli by beta-lactam antibiotics is strictly proportional to the rate of bacterial growth. J Gen Microbiol 1986;132:1297-304

99. Dunne WM Jr, Mason EO Jr, Kaplan SL. Diffusion of rifampin and vancomycin through a Staphylococcus epidermidis biofilm. Antimicrob Agents Chemother 1993;37:2522-6

100. Veeh RH, Shirtliff ME, Petik JR, et al. Detection of Staphylococcus aureus biofilm on tampons and menses components. J Infect Dis 2003;188:519-30

101. Cordero J, Munuera L, Folgueira MD. Influence of bacterial strains on bone infection. J Orthop Res 1996;14:663-7

102. Petty W, Spanier S, Shuster JJ, Silverthorne C. The influence of skeletal implants on incidence of infection. Experiments in a canine model. J Bone Joint Surg Am 1985;67:1236-44

103. Wenke JC, Owens BD, Svoboda SJ, Brooks DE. Effectiveness of commercially-available antibiotic-impregnated implants. J Bone Joint Surg Br 2006;88:1102-4

104. Osterman P, Seligson D, Henry S. Local antibiotic therapy for severe open fractures: a review of 1085 consecutive cases. J Bone Joint Surg Br 1995;77:93-7

105. Moehring HD, Gravel C, Chapman MW, Olson SA. Comparison of antibiotic beads and intravenous antibiotics in open fractures. Clin Orthop Relat Res 2000 Mar;(372):254-61

106. Ostermann PA, Seligson D, Henry SL. Local antibiotic therapy for severe open fractures. A review of 1085 consecutive cases. J Bone Joint Surg Br 1995;77:93-7

107. McLaren AC. Alternative materials to acrylic bone cement for delivery of depot antibiotics in orthopaedic infections. Clin Orthop Relat Res 2004 Oct;(427):101-16

108. Govender S, Csimma C, Genant HK, et al. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J Bone Joint Surg Am 2002;84-A:2123-34

109. Ong KL, Villarraga ML, Lau E, et al. Off-label use of bone morphogenetic proteins in the United States using administrative data. Spine 2010;35:1794-800

110. Epstein NE. Pros, cons, and costs of INFUSE in spinal surgery. Surg Neurol Int 2011;2:10

111. Chen XQ, Kidder LS, Lew WD. Osteogenic protein-1 induced bone formation in an infected segmental defect in the rat femur. J Orthop Res 2002;20:142-50

112. Chen XQ, Tsukayama DT, Kidder LS, et al. Characterization of a chronic infection in an internally-stabilized segmental defect in the rat femur. J Orthop Res 2005;23:816-23

113. Winkler H, Kaudela K, Stoiber A, Menschik F. Bone grafts impregnated with antibiotics as a tool for treating infected implants in orthopedic surgery - one stage revision results. Cell Tissue Bank 2006;7:319-23

114. Rathbone CR, Cross JD, Brown KV, et al. Effect of various concentrations of antibiotics on osteogenic cell viability

and activity. J Orthop Res 2011;29:1070-4

•• **This paper reports that vancomycin has a significantly less adverse effect on osteoblast proliferation and differentiation compared to 20 other antibiotics.**

115. Rogers-Foy J, Powers D, Brosnan D, et al. Hydroxyapatite composites designed for antibiotic drug delivery and bone reconstruction: a caprine model. J Invest Surg 1999;12:263-75

116. Suzuki A, Terai H, Toyoda H, et al. A biodegradable delivery system for antibiotics and recombinant human bone morphogenetic protein-2: a potential treatment for infected bone defects. J Orthop Res 2006;24(3):327-32

117. Zhang X, Jia W, Gu Y, et al. Teicoplanin-loaded borate bioactive glass implants for treating chronic bone infection in a rabbit tibia osteomyelitis model. Biomaterials 2010;31:5865-74

118. Edin ML, Miclau T, Lester GE, et al. Effect of cefazolin and vancomycin on osteoblasts in vitro. Clin Orthop Relat Res 1996 Dec;(333):245-51

119. Antoci V, Adams CS, Hickok NJ, et al. Antibiotics for local delivery systems cause skeletal cell toxicity in vitro. Clin Orthop Relat Res 2007;462:200-6

120. Haleem AA, Rouse MS, Lewallen DG, et al. Gentamicin and vancomycin do not impair experimental fracture healing. Clin Orthop Relat Res 2004 Oct;(427):22-4

121. 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;145:221-30

•• **This paper reports that vancomycin free base released from polymeric scaffolds for more than 8 weeks provides comparable infection control to PMMA beads in a contaminated segmental defect model.**

122. Swanson PA II, Kumar A, Samarin S, et al. Enteric commensal bacteria potentiate epithelial restitution via reactive oxygen species-mediated inactivation of focal adhesion kinase phosphatases. Proc Natl Acad Sci USA 2011;108:8803-8

123. Dumas JE, Zienkiewicz K, Tanner SA, et al. Synthesis and characterization of an injectable allograft bone/polymer composite bone void filler with tunable mechanical properties. Tissue Eng Part A 2010;16:2505-18

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