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Infection and tissue engineering in segmental bone defects – A mini review

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Abstract

As tissue engineering becomes more of a clinical reality through the ongoing bench to bedside transition, research in this field must focus on addressing relevant clinical situations. While most *in vivo* work in the area of bone tissue engineering focuses on bone regeneration within sterile, surgically created defects, there is a growing need for investigation of bone tissue engineering approaches within contaminated or scarred wound beds, such as those that may be encountered following traumatic injury or during delayed reconstruction/regeneration. Significant work has been performed in the area of local drug delivery via biomaterial carriers, but there is little intersection in the available literature between antibiotic delivery and tissue regeneration. In this review, we examine recent advances in segmental bone defect animal models, bone tissue engineering, and drug delivery with the goal of identifying promising approaches and areas needing further investigation towards developing both a better understanding and new tissue engineering approaches for addressing infection control while simultaneously initiating bone regeneration.

Introduction

The treatment of infected segmental bone defects continues to be a troublesome problem in orthopedics. The incidence of osteomyelitis is high in the United States as approximately 112,000 orthopedic device-related infections occur annually, with 10 – 15% of these infections associated with open bone fractures [1]. On average, about 2–5% of initially inserted internal fixation devices become infected, and the average cost of combined medical and surgical treatment is estimated at US \$15,000 [2,3].

In general, microorganisms, the most common of which is *Staphylococcus aureus* [4,5], infect bone through one or more of three basic methods: (a) hematogenous spread from other sources of infection, (b) from the skin or other external contaminants through open fracture or trauma, or (c) following placement of internal fixation devices [6–8]. Once the bone is infected, pus spreads affecting both the medullary and periosteal blood supplies, thereby impairing blood flow. Decreased vascular function likely decreases the amount of oxygen available to tissues at and around the fracture site, impairing the exchange of nutrients, and potentially leads to problems during recruitment of cells to the injury site [9]. These events, alone or in combination, lead to the formation of sequestra or necrotic bone,

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which is considered the hallmark of chronic disease and may contribute to delayed healing of any segmental defect.

Following diagnosis, which is most commonly accomplished through magnetic resonance imaging or the gold standard diagnostic modality for osteomyelitis – bone biopsy, conventional treatment includes drainage, extensive debridement of all necrotic tissue, obliteration of dead spaces, adequate soft tissue coverage, and restoration of an effective blood supply. This treatment is coupled with systemic administration of antibiotics for four to six weeks [10,11]. The rationale for this duration of antibiotic therapy is based on the results of animal studies and the observation that revascularization of bone after debridement takes about four weeks [4]. Even after the introduction of antibiotics, osteomyelitis remains difficult to treat because the limited vascular supply to the tissue precludes obtaining therapeutic levels of antibiotic in the infected bone [12].

For the past few years, an alternative to systemic antibiotic therapy has been proposed, i.e., the *in situ* implantation of a local antibiotic delivery system, which initially works to obliterate bacteria in the area and eventually reduces the dead space. The most extensively studied and commercially available material for local antibiotic delivery is polymethylmethacrylate (PMMA), which is typically combined with antibiotics like gentamycin [13,14], tobramycin [15], and vancomycin [16]. The use of PMMA, however, is associated with several significant drawbacks [17,18]. During mixing and curing of PMMA, an exothermic reaction occurs, and the temperature may increase up to 100°C, which decreases the effectiveness of some antibiotics [17]. Another drawback includes the requirement for surgical removal after treatment since PMMA is non-degradable. Surgery to remove the implanted material is usually more difficult than implantation because of localized scarring and adhesion and may impart additional risk for postoperative infection depending on the condition of the patient. Additionally, a significant proportion of antibiotics may be retained within the cement (less than 10% of the entrapped drug is eventually released), potentially generating resistant bacteria on the carrier-surface during later stages [19,20].

Various biodegradable devices made from natural or synthetic polymers have also been investigated as local antibiotic delivery systems. The degradation properties of these polymers can be tailored for the sustained release of antibiotics for long periods and eliminates the need for surgical removal of the implant. Materials used for this application include natural polymers such as collagen [21,22], fibrin [23,24], and chitosan [25], as well as synthetic polymers including polyhydroxyalkanoates [26–28], poly(ricinoleic-co-sebacic-ester-anhydride) [29,30], poly(lactic acid) [31], poly(lactic-co-glycolic acid), and poly(ϵ -caprolactone) [32]. Antibiotic release from each of these polymers has proven to be effective towards reducing infection, but none of these systems has been investigated for simultaneous or subsequent bone regeneration in infected segmental bone defects.

Thus a current, ideal goal for any such system is the elimination of bacteria together with the regeneration of bone in an infected segmental defect. This review summarizes two main aspects in this area. The first section explains the challenges associated with the creation of an experimental animal model and the second section deals with current research modalities in this area.

(a) Experimental animal model

The most commonly used animal model for studying segmental bone defects is the rat, a small animal whose use is relatively economical and which can tolerate broad spectrum antibiotic therapy well [17]. Still, the creation of an infected segmental defect in an animal model is a great challenge. Usually, the segmental defect has to be stabilized with external

or internal fixation devices, the presence of which poses many problems in infected bone. Soon after insertion of fixation devices, adhesive proteins adsorb on the surface of the implant facilitating adherence of microbial organisms followed by the secretion of glycocalyx. This allows the formation of a three-dimensional protective structure or “biofilm” that is less susceptible to antibacterial therapy [33,34] and necessitates the removal of fixation devices. However, in terms of gaining bone union and bone formation, the removal of fixation devices is counterproductive unless the fracture has attained some degree of stability [35].

Thus, the main question is how to create an infected segmental bone defect in an animal model with some degree of associated bone lysis without reducing the stability of bone fixation and without creating an untreatable biofilm due to the fixation method. Chen *et al.* [36] characterized a new model of chronic osteomyelitis to determine the bacterial inoculum and time from contamination that would reliably result in an infected defect without excessive bone lysis and loss of fixation stability. A segmental defect of critical size was surgically created in the rat femur, stabilized with a polyacetyl plate and 6 Kirschner (K) wires, and all animals were inoculated with lyophilized type I collagen wetted with 4 inocula of *S. aureus* (10^3 , 10^4 , 10^5 , 10^6) for 4 time points (1, 2, 3 or 4 weeks). A 10^4 colony forming unit (CFU) inoculum over 2 weeks was found to consistently create an infection without severe loss of fixation stability. The same inoculum and time points were repeated in two further studies, where the application of BMP-2 [37] and BMP-7 [38] were investigated. Further reports found an inoculation dose of 10^5 CFU of *S. aureus* in a rat model was not sufficient to establish infection when BMP-2 [39] and BMP-7 [40] were applied along with the bacterial inoculum to accelerate bone formation.

Other studies have been performed with the objective of preventing, rather than treating, bone infection in a segmental defect. The objective of this prophylactic approach is to prevent the proliferation of naturally occurring or exogenous, contaminant microbes to a level that might cause active infection. There are conflicting opinions regarding the timing of antibiotic administration to prevent infection. A 6 h post implantation decisive period has been identified during which prevention of bacterial adhesion is critical, because certain species of bacteria are capable of forming a biofilm over extended periods [20]. Brown *et al.* created a segmental defect stabilized with polyacetyl plates and K wires in a rat model, implanted 30 mg of type I collagen wetted with 10^5 CFU of *S. aureus* for 2, 6 or 24 h, and then treated with antibiotic impregnated PMMA beads. Two weeks after inoculation and subsequent treatment, there was a significant increase in infection between 2 and 6 h and a further increase between 6 and 24 h for groups treated with debridement alone as well as debridement plus local antibiotic delivery [41]. Recently, a similar study was performed in a rat model (with 10^5 CFU of *S. aureus* inoculated 6 h before treatment) demonstrating effective infection control via vancomycin released from polyurethane scaffolds [42]. In these prophylaxis studies, the challenge associated with fixation devices can be mitigated, provided the inoculum is not allowed to establish a biofilm on these devices; however, such methods are of limited utility for modeling the treatment of active infection.

In addition to improved animal models, other advances, such as the development of luminescent bacterial strains that allow for easy *in vivo* imaging and quantification may greatly impact research in this area [43].

b) Bone growth in an infected segmental defect

Bone regeneration in a critical size segmental defect is a formidable clinical challenge in orthopedics, particularly if the defect or surrounding bone is infected. As previously alluded to, this is due to impaired vascularization of the infected tissues, which delays bone healing [9]. Therefore, recent reports have studied local delivery of growth factors alone or in

combination with systemic antibiotic therapy with the aim of improving vascularization at the infected site and allowing or promoting bone regeneration. Many studies have shown the importance of bone morphogenetic proteins (BMPs), multifunctional cytokines and members of the transforming growth factor (TGF)- β super family, in the regulation of skeletal growth and development. During fracture repair, BMPs are produced by mesenchymal stem cells (MSCs), osteoblasts, and chondroblasts, and trigger a cascade of events, such as MSC proliferation and differentiation, angiogenesis, and synthesis of extracellular matrix [44]. The most commonly utilized BMPs for fracture repair are BMP-2 and BMP-7 (also referred to as osteogenic protein-1 or OP-1) [45]. BMP-2 acts on global cellular mobilization and is also present during the later stages of osteogenesis and chondrogenesis, whereas BMP-7 acts on bone differentiation [46,47]. In addition, several studies have shown that BMPs can stimulate the expression of VEGF and its receptor to facilitate angiogenesis during fracture repair [48,49].

Chen *et al.* studied whether BMP-2 [37] and BMP-7 [38,39] are capable of inducing bone formation in the presence of bacterial contamination. A 6 mm segmental defect was created in the femur of rats, stabilized with plates and wires, and the defect was either left untreated or subjected to various combinations of growth factor, lyophilized bovine type I collagen carrier, and inocula of *S. aureus*. Systemic ceftriaxone therapy was also administered to some of the groups. The results showed that there was very little, if any, bone formation in the untreated defects and in the contaminated defects with or without collagen carrier. However, bone formation was greater in a dose dependent fashion in the contaminated defects with both BMP-2 and BMP-7. The systemic antibiotic administration also had a positive effect on both bone regeneration and the mechanical strength of the treated bone when compared to the contralateral intact bone, indicating the importance of addressing both bone regeneration and infection clearance or control.

In another study, which further demonstrates the need for addressing existing infection along with initiating growth factor mediated bone regeneration [40], rhBMP-2 (200 μ g) was delivered to a segmental rat femoral defect, and expression of key genes in bone formation (collagen types I and II, osteocalcin, and BMP type II receptor mRNA expression) was measured in the presence of acute infection. The results showed that all four genes were upregulated in infected defects in the presence of rhBMP-2; however, infected defects not treated with BMP-2 expressed little collagen I and II and osteocalcin mRNAs.

Few studies have been published in this area, and even fewer reports have demonstrated the local delivery of both antibiotics and growth factors towards both regenerating bone and treating or preventing infection [50–52]. This may be due to the challenges associated with the dual delivery of antibiotics and growth factors during the same time period from the same carrier. Since two events must occur in concert with one another (elimination of bacteria and enhancement of bone regeneration), the local concentration and release kinetics of both antibiotic and growth factor will be a major issue in such cases.

In all these studies, collagen was used as the carrier for the delivery of BMPs, although collagen lacks the mechanical stability usually considered to be necessary for facilitating bone growth through a scaffold. In the future, mechanically stable materials or combinations of materials capable of releasing growth factors for a sufficient period of time should be explored in these applications. In such a system, the release of antibiotic must be prolonged as well to match the degradation rate of the material, since the long-term presence of an alloplastic material in an infected wound would be a prime nidus for infection. As with any application of BMPs, the material expense and potential side effects will also be important considerations that must be taken into account when developing a treatment strategy.

Conclusion

In conclusion, significant yet disparate progress has been made in the areas of bone tissue engineering and local drug delivery for treating infection. Although significant overlap exists in the technology used to regenerate bone tissue and deliver local antibiotics, there are a limited number of studies in the literature that attempt to address bone regeneration in the infected wound. Animal models must be developed allowing for the study of bone regeneration in the presence of a clinically relevant infection model. Such a model would greatly expand our basic understanding of the physiologic processes central to bone healing or the lack thereof in the presence of infection, while also facilitating evaluation of emerging strategies designed to engineer bone in the infected wound environment. While promising work has recently expanded our understanding of the problems inherent to this process, significant hurdles remain that must be addressed before any of the described strategies become a clinical reality.

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