BIOLOGICAL ADJUVANTS IN ORTHOPEDIC SURGERY (J DINES AND D GRANDE, SECTION EDITORS)

Biologic adjuvants and bone: current use in orthopedic surgery

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Abstract Normal bone healing is a complex process that eventually restores original structure and function to the site of trauma. However, clinical circumstances such as nonunion, critical-sized defects, systemic bone disease, and fusion procedures have stimulated a search for ways to enhance this normal healing process. Biologics are an important part of this search and many, including bone marrow aspirate concentrate, demineralized bone matrix, platelet-rich plasma, bone morphogenic proteins, and platelet-derived growth factor, are currently in clinical use. Many others, including mesenchymal stem cells, parathyroid hormone, and Nel-like molecule-1 (NELL-1) will likely be in use in the future depending on the results of preclinical and clinical trials.

Keywords Orthopedic surgery · Biologic · Adjuvant · Bone · Graft extender · Growth factor

Introduction

Unlike some musculoskeletal tissues, bones have the ability to regenerate after injury. Under ideal biomechanical conditions, which include proper alignment and stability of the fracture site, bone will heal and remodel into its original form [1]. However, circumstances exist in the field of orthopedic surgery in which this normal healing process is inadequate. Fracture nonunion is defined as a lack of complete healing after 6 months. Depending on the site and type of fracture, rates can vary between 4.8 and 10 % [2]. In addition, critical-sized bone defects caused by trauma or excision of neoplasms derive their very definition as those that will not heal without augmentation [3]. Surgical fusion procedures, commonly performed to join two or more vertebral bodies, involve the use of bone graft in order to remodel the targeted area into one piece of bone. One type of failure of this procedure, pseudoarthrosis, has been reported to range between 0 and 56 %, depending on many factors [4]. Finally, metabolic disorders such as osteoporosis and more rare genetic disorders like osteogenesis imperfecta disrupt normal bone physiology and can result in poor bone quality and healing [5].

Due to these conditions, many techniques and materials have been developed as a way of improving bony regeneration and healing. Of particular interest and relevance to this review is the use of biologic adjuvants to augment this process. There is no consensus for the definition of what a biologic therapy is, but it usually must be derived from a living system [6]. In other fields, this usually refers to molecules involved in signaling or generating a certain immune response. However, for the purpose of this review, cell-based therapies will be included as well. While it is tempting to include the field of gene therapy under the biologic umbrella, the authors determined that this was beyond the scope of this review.

Review criteria

Articles for this review were chosen from the authors' personal libraries and from searches of PubMed using the search terms "bone, biologic adjuvant, biologics, orthopedic surgery" alone and in various combinations. Additional references were found by reviewing the reference lists of pertinent articles and previous reviews for relevant articles. All articles

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were English language, full-text papers. Searches were performed up to December 2014.

Normal bone healing

Typical fracture healing is commonly described in three phases: inflammation, repair, and remodeling [7] (Fig. 1). These phases proceed in order, but with considerable overlap. Initial disruption of the bone macrostructure leads to extravasation and hematoma formation at the site. Then, inflammatory cytokines and growth factors, including transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and bone morphogenetic proteins (BMPs), coordinate inflammatory cell and stem cell migration [8]. In the repair phase, a fibrous callus is formed by chondrocytes and fibroblasts. This is subsequently mineralized by osteoblasts under a similar growth factor milieu. Finally, osteoclasts resorb and remodel the mineralized hard callous to approximate the original structure and function of the underlying bone. Giannoudis et al. popularized the diamond concept of bone healing, in which osteogenic cells, osteoinductive scaffolds, growth factors, and mechanical forces interact to lead to ideal bone healing [9]. This review will focus on the use of cells and growth factors to augment normal bone healing in orthopedic surgery.

Biologic adjuvants

Platelet-derived growth factor

PDGF is responsible for various cellular responses that are critical to tissue repair in the musculoskeletal system. Some examples are chemotaxis, mesenchymal stem cell (MSC) recruitment, angiogenesis, and upregulation of vascular endothelial growth factor [10–12]. Importantly, PDGF acts as an initiator of the native bone-healing cascade at the implantation site [13]. Due to these effects, it was investigated as an adjuvant treatment of complex nonunion fractures. Recombinant human PDGF (rhPDGF) was combined with a tricalcium phosphate (TCP) bone substitute scaffold prior to implantation. The rhPDGF saturated scaffold allowed for an initial burst release, followed by a slower release to mimic endogenous PDGF from platelets. Advantages of this type of treatment are limited systemic exposure, local delivery, and a continual dosage [14]. PDGF was further investigated in clinical trials involving hind foot and ankle fusion. A similar technique was used, combining rhPDGF with a TCP bone substitute. This resulting fusion was compared to autogenous bone grafts and was shown to be equally as effective in stimulating bone healing. This method spared the patient from any donor site morbidly or complications [14]. These studies have shown rhPDGF is a promising recent addition to the surgical treatments for bone repair. Further studies and trials are planned to optimize its therapeutic use in these applications as well as other orthopedic areas such as tendon-to-bone healing, intra-tendon, and articular cartilage regeneration [13].

Bone marrow aspirate concentrate

Initially described in 1952, bone marrow aspirate concentrate (BMAC) is a natural extension of the iliac crest bone graft (ICBG) that has been used for many years. Through aspiration of the marrow, a small percentage of mesenchymal stem cells (MSCs) can be obtained. In the past, these cells would have been expanded through culture in the lab, but the more recent impetus was to condense the harvest and implantation down to one surgical procedure. Currently, the standard is aspiration from the posterior iliac crest [15, 16], as this site is potentially psychologically less traumatic [16] and is easily accessible with the needle. Compared to harvesting from the anterior pelvis, there is no difference in the total number of cells obtained, but there are more connective tissue progenitors from a posterior aspiration [15]. After spinning the cells down, between 0.001 and 0.01 % of the cells obtained were found to be MSCs, with the total number of viable cells obtained decreasing with age [17]. As a result, the growth factors that are
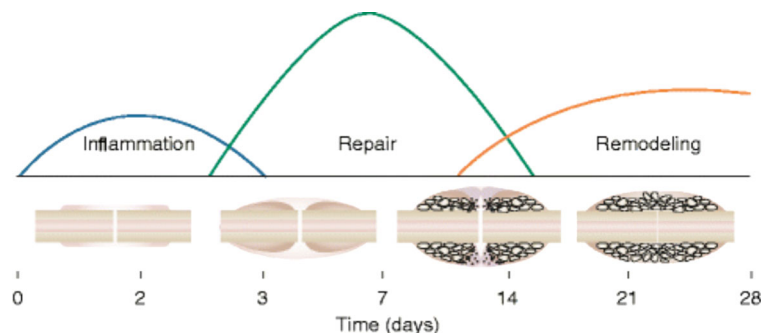

Fig. 1 Graphical representation of the three overlapping phases of bone healing. Cytokines direct inflammatory and stem cell migration to the injured area. A fibrous callous is formed during the repair phase that is ultimately remodeled in the final phase to more closely resemble the original structure. *Figure 5a* from Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. Nat Rev Rheumatol. 2012;8(3):133–43

contained in the aspirate, PDGF, TGF-β, and VEGF are likely to be more important [18].

While often studied in conjunction with cartilage repair [19] and combined with a microfracture technique, BMAC had initially been studied in tibial shaft fractures because of the historically elevated nonunion rate. More recently, BMAC has been used successfully in osteochondritis dessicans (OCD) lesions of the talus, not only alone, but to assist in the ingrowth of allograft plugs [20–22]. The same principle has since been applied to OCD lesions in knees [23], and in addition, it has been used in many cases of nonunion in order to stimulate new bone growth [24]. However, while there are multiple level IV studies applying BMAC to use in aseptic nonunions, there is no good level 1 evidence [25].

While promising, use of BMAC is not always appropriate. In a study of aseptic nonunions, the authors found that the number of progenitor cells can vary widely and has a direct impact on the volume of formation of new bone [24]. Age and sex also play a role, as there were fewer colony forming units (CFUs) found as age increases [26]. In a separate study, BMAC was used in conjunction with TCP to fill benign cavitary lesions, and there was no reported benefit [27]. Overall, BMAC is an osteoinductive product that often must be combined with an osteoconductive material to produce effective bone formation. With high variability in samples between patients not only in aspiration volume [28] and technique [26], more research needs to be done to further define the indications of use.

Platelet-rich plasma

Platelet-rich plasma (PRP) is one of the newest innovations in orthopedic healing and repair, but most of the research has been focused on soft tissue rather than bone. While there is still debate as to whether the white blood cells (WBCs) included in the PRP preparations are beneficial or harmful to the healing response [29], PRP does contain very high concentrations of PDGF, VEGF, and FGF. Because of this, it is thought that PRP may be able to assist in many situations that require tissue healing [30].

Currently, more than 40 commercial PRP concentrations systems are available, and there is high variability in the concentrations of growth factors present, even within a given technique [31–33]. Initially thought to assist in bone healing, the results have not been promising in spinal fusion or high tibial osteotomy trials [34, 35]. However, it has been shown in a small study that the speed at which distraction osteogenesis occurs is increased with the addition of PRP, but that has not been consistent with the outcome of larger trials. PRP has been shown to decrease pain after patellar tendon autograft ACL reconstruction and rotator cuff repair, but in neither of those cases has it been shown to significantly change the overall clinical outcome [36, 37]. Currently, the American Academy of Orthopedic Surgeons (AAOS) clinical practice guidelines say that the organization is "unable to recommend for or against" the use of PRP [38] in general, but when examined solely in the setting of bone healing, PRP is not an effective adjuvant or substitute for bone graft [39]. There are simply too many confounding factors such as preparation, type of tissue being stimulated, and type of repair for the authors to recommend the use of PRP specifically to augment bone healing, though future research might show benefits.

Demineralized bone matrix

The biologic adjuvant demineralized bone matrix (DBM) is created by demineralizing ground cadaver bone and it contains BMPs, collagen, and other growth factors that are then combined with a carrier. Much like PRP, each process of DBM creation from the harvesting to the final product is unique from company to company, with no standardization across the industry [40]. Overall, most of the research has been done in spinal fusions, utilizing it as a bone graft extender to be combined with existing bone. It has not been shown to produce efficient or durable fusion when used alone. More recently, the use has been expanding, with good results seen in ankle fusions when compared with ICBG [41] and some success with femoral nonunions or segmental defects.

The best use seems to be that it provides not only osteoinductive proteins but also physical structure that can help position any allograft used in conjunction [42]. This has been seen with the treatment of unicameral bone cysts (UBCs) where the residual void is filled with graft. Often, ICBG alone is not an option because of the limit on how much can be harvested, but when combined with DBM, a small amount can fill the entire defect. In humeral shaft nonunions, it has been successfully used to produce solid fusion of the fragments [43, 44] but compared to ICBG, there was no difference found in clinical function or healing time. Overall, DBM has been shown to produce solid fusions in multiple bony applications and allows the surgeon to avoid harvesting ICBG in some situations, which reduces donor site morbidity. However, it is worth noting that in one spine model, almost half of the products tested had a 0 % fusion rate, speaking to the vast differences in preparation and delivery that each of the unique companines provide [45].

Bone morphogenic proteins

Over the last few years, the use of BMPs has increased dramatically as they are developed and marketed. BMPs are a collection of many different proteins in the body, but only BMP-2 and BMP-7 were approved for human use. However, production of BMP-7 was stopped in May 2014 and is currently unavailable. BMP-2 is only FDA-approved for certain indications, one being anterior lumbar interbody fusion (ALIF) with a titanium or polyetheretherketone (PEEK) cage.

Multiple studies have shown that the addition of BMP-2 produces good fusion rates in the lumbar spine [46, 47], and surgeons around the country have started using it off-label for multiple indications. The other major indication where BMP has shown benefit is in the treatment of open tibial shaft fractures. These can be notoriously difficult to heal and have a very high rate of nonunion secondary to multiple factors including the energy of the trauma and possible infection. However, in the BMP-2 Evaluation in Surgery for Tibial Trauma (BESTT) trial [48], the authors found accelerated fracture healing and more importantly, a decreased rate of secondary intervention such as need for reoperation when BMP was included in the bone graft at the site. This has led to the FDA approving BMP-2 for the treatment of open tibial fractures after intramedullary nailing.

The biggest concern of using BMP is safety. The initial trials were controversial, as some thought they were too closely associated with industry and that complications were underreported. As a result, the study and use of BMP has a stigma attached to it [49]. Indeed, complications can be serious, including seroma formation, severe soft tissue swelling, radiculitis, and retrograde ejaculation. However, it has been shown to be a potent former of new bone in multiple animal models [50], and further research should be done to determine accurate dosing and delivery systems to minimize the side effect profile and expand its safe use. It is currently unknown what percentage of surgeons use BMP, and what types of surgery they are using it for, though as stated, the highest percentage is likely to be in spinal fusion.

Future directions

Clinical trials

The authors searched ClinicalTrials.gov to determine recent trends in the use of biologics for bone in orthopedic surgery.

The terms "fracture repair," "bone fusion," "critical defect bone," and "orthopedic surgery and bone and biologic" were searched and the resulting trials reviewed. Trials that did not involve biologic treatment, did not involve orthopedic surgery, or had statuses that were unknown or terminated were excluded. Fifty-nine trials met the inclusion criteria and the number of trials involving biologics is shown in Fig. 2. In addition to giving a general overview of the state of investigation of biologics in orthopedic surgery, the authors noted two interesting trends that deserve discussion.

Mesenchymal stem cells

The first trend is the heavy investigation of mesenchymal stem cells. MSCs are found in many tissues in the human body, including blood, adipose tissue, skin, trabecular bone, liver, and lungs [51]. Briefly described above, stem cell migration and differentiation is thought to be an integral part of normal bone healing [52]. In contrast to the autologous MSCs contained in perioperatively harvested BMAC, many of the current MSC trials involve the use of isolated and cultured *allogenic* cells. Allogenic MSCs have been shown to avoid immune detection by lacking major histocompatibility complex type II (MHC II) and costimulatory molecules as well as modulating the surrounding inflammatory environment [53]. A study of rabbit fractures has shown that allogenic MSCs performed just as well as autologous MSCs in repairing bone, with no increase in immunogenicity [54]. Although the view of the future clinical utility of these cells is not uniformly optimistic [55], the successful outcomes of these trials have the potential to significantly change the field of orthopedic surgery.

Parathyroid hormone

Another interesting trend identified in the trial search is the use of teraperatide, which is recombinant parathyroid hormone
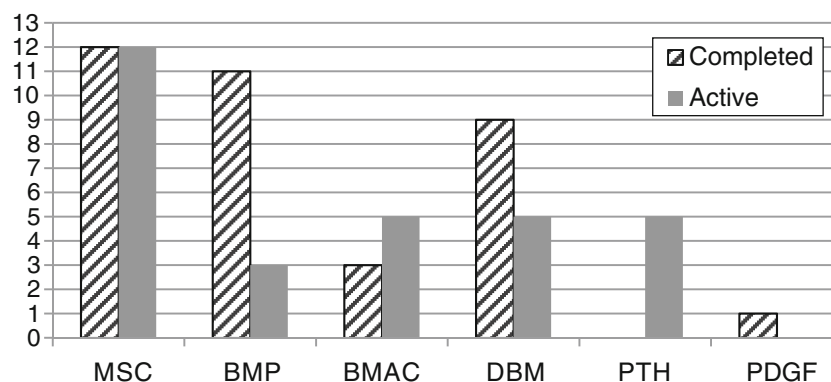

Fig. 2 ClinicalTrials.gov was searched for relevant trials using biologic adjuvants in orthopedic surgery for bone. The number of trials investigating each category of biologic is displayed above. *MSC*

mesenchymal stem cell, *BMP* bone morphogenetic protein, *BMAC* bone marrow aspirate concentrate, *DBM* demineralized bone matrix, *PTH* parathyroid hormone, *PDGF* platelet-derived growth factor

(PTH). Physiologically, PTH acts on osteoblasts and osteoclasts to resorb bone, increasing serum calcium. However, when given in a pulsatile fashion during treatment of osteoporosis, it increases bone mineral density (BMD) and improves bone architechture [56]. This likely occurs due to preferential osteoblast differentiation over osteoclast bone resorption [57]. Limited clinical studies of off-label use have shown positive effects on bone repair [58]. Therefore, the results of these clinical trials involving bone repair and fusion have the potential to add another treatment option in these difficult cases.

Nel-like molecule-1

Protein kinase C-binding protein Nel-like molecule-1 (NELL-1) is a growth factor originally identified in patients with craniosyntosis [59]. Since its discovery, animal studies involving overexpression as well as deficiency have confirmed its importance in bone formation [60–63]. Investigators have attempted to utilize the osteoinductive effects of NELL-1 to enhance bone repair in experimental animal models. Rat and mouse explant studies demonstrated its ability to induce and accelerate bone growth [64, 65]. NELL-1 has also been investigated in many in vivo models, including a rat critical-sized femoral defect model and rat and sheep spine models, showing positive effects on bone growth and repair [66–68]. The exact actions of NELL-1 in the signaling cascade are still being defined, but it is thought to act through Runx2 [62, 69, 70] and interact with BMPs and the sonic hedgehog (SHH) signaling pathway [71–74]. This protein has demonstrated enormous potential in preclinical studies and further progress towards a clinical use is anticipated.

Coating implants with biologics

In addition to fracture repair and fusion procedures, biologic materials are being investigated as a coating material for orthopedic implants. Implants are an important part of orthopedic surgery but are susceptible to several drawbacks that accompany placing a foreign material in the body such as nonintegration, inflammation and immune response, and infection. The rationale for coating implants is to reduce these risks. In both oral and orthopedic implants, animal models show that ceramic coatings improve osteointegration. Biologic coatings such as extracellular matrix peptides, collagen, BMPs, and other growth factors have been investigated but are still in the early phases of pre-clinical study [75, 76]. Continued research study is needed to develop biologic implant coatings for routine human use.

Conclusion

The field of orthopedic surgery currently uses several biologic adjuvants in the treatment of a vast array of bone pathologies. Research is actively seeking ways to refine and optimize currently used treatments while simultaneously investigating promising new molecules. These advances combined with breakthroughs in other fields such as materials science, tissue engineering, and surgical technique ensure the continued dynamic evolution and progress of the field as a whole.

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