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ORTHOPEDIC MANAGEMENT OF FRACTURES (D LITTLE AND T MICLAU, SECTION EDITORS)

Orthobiologics in the Augmentation of Osteoporotic Fractures

J. Tracy Watson · Daemeon A. Nicolaou

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Abstract Many orthobiologic adjuvants are available and widely utilized for general skeletal restoration. Their use for the specific task of osteoporotic fracture augmentation is less well recognized. Common conductive materials are reviewed for their value in this patient population including the large group of allograft adjuvants categorically known as the demineralized bone matrices (DBMs). Another large group of alloplastic materials is also examined—the calcium phosphate and sulfate ceramics. Both of these materials, when used for the proper indications, demonstrate efficacy for these patients. The inductive properties of bone morphogenic proteins (BMPs) and platelet concentrates show no clear advantages for this group of patients. Systemic agents including bisphosphonates, receptor activator of nuclear factor κβ ligand (RANKL) inhibitors, and parathyroid hormone augmentation all demonstrate positive effects with this fracture cohort. Newer modalities, such as trace ion bioceramic augmentation, are also reviewed for their positive effects on osteoporotic fracture healing.

Keywords Orthobiologics · Osteoporosis · Fragility fractures · DBM · Bone void fillers · Calcium phosphate cements · Bisphosphonates · RANKL inhibitor · Parathyroid hormone · Trace element bioceramics

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J. T. Watson (✉) · D. A. Nicolaou
Orthopaedic Trauma Service, Department of Orthopaedic Surgery, Saint Louis University School of Medicine, 3635 Vista Ave., 7th Floor Desloge Towers, St. Louis, MO 63110, USA
e-mail: watsonjt@slu.edu

D. A. Nicolaou
e-mail: daemeon5@gmail.com

Introduction

Osteoporosis is defined as low bone mass and decreased microarchitecture as a result of the imbalance between bone resorption and bone formation. According to the Surgeon General's report, it results in over 1.5 million fractures with an annual cost of over $18 billion. These resultant fragility fractures can present challenges in management due to the severity of comminution, and inferior implant fixation because of the poor bone quality. In addition, osteoporotic fractures have shown an impaired ability to heal and increased healing time [1]. This article will look at some of the orthobiologic adjuvants and their use in the augmentation of osteoporotic fractures.

Allograft Bone

Demineralized bone matrix (DBM) is formed by acid extraction of the mineralized extracellular matrix of allograft bone. It contains type-1 collagen, noncollagenous proteins, and osteoinductive growth factors including the bone morphogenic proteins (BMPs) and other inductive factors found in the TGF-β group of proteins [2]. The factors that are known to be osteoinductive are the BMPs, growth differentiation factors (GDFs), and possibly TGF-β 1, 2, and 3 [3]. DBM is highly osteoconductive due to its particulate nature and presents a large surface area and three-dimensional architecture to serve as a site of cellular attachment [4, 5]. Thus, when demineralized bone matrix is implanted in an animal, all of these factors potentially work in combination to produce the observed osteogenic response. This material represents an attractive alternative for the treatment of fractures, nonunions, and fusion augmentation for the osteoporotic population where autogenous harvest would be less than a satisfying experience, with low bone yields expected and all the attendant risks associated with the second surgical site.

It is clear that donor selection, graft processing, and sterilization techniques as well as carrier admixtures have significant effects on DBM viability. With this in mind, the clinician should be aware of how these factors influence the efficacy of each particular DBM product that they may choose to use in each clinical application. These products are clearly not equal in their BMP concentrations or their inductive potential as based on efficacy assays.

While the preclinical data is impressive for DBM forming de novo bone in lesser animal models, the human clinical data is deficient with only isolated case reports and uncontrolled retrospective reviews [6]. These are only level III and IV studies that have suggested potential therapeutic effects of demineralized bone matrix [7]. Unfortunately, most clinical series combined DBM with other adjuvants and, as noted above, the singular effectiveness of DBM alone is difficult to elucidate.

There is early data suggesting that DBM putty enriched with bone marrow may be comparable to autograft for treating long bone fractures and nonunions. This option offers the distinct advantages of decreased morbidity, reduced costs, and shorter hospital stay compared to iliac crest bone graft [8]. These studies did not specifically address the osteoporotic population however. The ease of application and the theoretical advantages of DBM make it an attractive adjuvant when dealing with the osteoporotic population. There is certainly no reported "downside" to using these materials as the safety and biocompatibility have been abundantly demonstrated.

Conductive Agents

There is considerable interest in creating osteoconductive matrices using nonbiological porous structures implanted into or adjacent to the bone. The host substrate must mimic the cancellous bony architecture and have very specific surface kinetics to facilitate the migration, attachment, and proliferation of mesenchymal stem cells, which then differentiate into osteoprogenitor cells. Broad categories of these materials are available and in general are classified as calcium ceramics. These include the specific materials of calcium sulfate, calcium phosphate, synthetic tricalcium phosphate as well as beta tricalcium phosphate, and coralline hydroxyapatite.

Calcium sulfate hemihydrate has been used for many years as a self-setting biomaterial due to its good setting properties. The fairly rapid degradation rate of these materials which occurs in 3 to 4 months was once viewed as an advantage [9]. However, as these materials began to be used to support articular subchondral surfaces in cases of periarticular plateau and pilon fractures, this rapid degradation becomes a distinct disadvantage [10]. This combination of rapid degradation rate, speedy loss of compressive strength, and lack of bioactivity has currently limited its application for bone defect management especially when used in patients with deficient skeletal architecture [11].

The porosity of these materials is the primary factor in determining the ability to foster ingrowth and osteointegration. No osseous ingrowth occurs with pore sizes of 15 to 40 μm. Osteoid formation requires minimum pore sizes of 100 μm, with pore sizes of 300 to 500 μm reported to be ideal for osseous ingrowth [12]. Some authors, however, have reported that pore size may be less critical than the presence of interconnecting pores for osseous ingrowth. Interconnecting pores prevent the formation of blind alleys, which are associated with low oxygen tension; low oxygen tension prevents osteoprogenitor cells from differentiating into osteoblasts [13, 14].

The phosphate materials are highly porous interconnected materials that have abundant sites available for cellular interactions, thus the more pores, the faster these materials will osteointegrate. This is accompanied by a corresponding decrease in the compressive strength afforded. If the material is designed with minimal porosity, the rate of osteointegration will be very prolonged because of the paucity of cellular interactions. The corresponding compressive strength will also be very high. This may be an advantage in patients that have substantially weakened bone. Some of these material properties approach compressive strengths much greater than that for cortical bone, greater than 200 megapascals. As noted, their ability to provide structural support is dependent on the degree of porosity inherent in each unique material which can be highly manipulated [13, 15]. These materials have the advantage of incorporating at a slower rate than calcium sulfate materials. They increase bone formation by providing an osteoconductive matrix for host osteogenic cells to create bone under the influence of host osteoinductive factors shores.

Calcium phosphate can also be manufactured as cement, by adding an aqueous solution to dissolve the calcium, which is followed by a precipitation reaction in which the calcium phosphate crystals grow and the cement hardens. The primary advantage of cements over blocks, granules, or powders is the ability to custom-fill defects and produce increased compressive strength [15, 16]. However, cement can be extruded beyond the boundaries of the fracture, potentially damaging the surrounding tissue. This is especially problematic if these materials extrude into a joint cavity following repair of a subchondral defect such as in tibial plateau fractures. This presents a potential disadvantage of these phosphate materials, as they will not dissolve if they happen to migrate into the joint [17•]. The ability of calcium phosphate bone substitutes to act as a bone-void filler has been documented in multiple preclinical animal studies and biomechanical and human case series [18].

The use of injectable calcium phosphate cements offers the opportunity to support the reduced joint surface without open bone grafting. This is a valuable adjuvant, as less invasive fixation approaches are becoming widely accepted as is the

desire to limit the exposures for grafting and subchondral defect augmentation.

Many studies have specifically evaluated these materials as bone graft substitutes in the management of subchondral bone defects associated with tibial plateau fractures. A meta-analysis study compared calcium phosphate cement substitutes directly to these other conductive substrate materials used for plateau augmentation: hydroxyapatite granules, calcium sulfate, bioactive glass, tricalcium phosphate, demineralized bone matrix, allografts, autografts, and xenografts [19•]. Fracture healing was uneventful in over 90 % of the cases over the variable time period of the meta-analysis.

Secondary collapse of the knee joint surface ≥2 mm was highest in the biological substitutes group, 8.6 % (allograft, DBM, autograft, and xenograft); the group that experienced the highest rate of subsidence was the calcium sulfate cases, 11.1 % [20]. This is consistent with the rapid dissolution time and relative biomechanical properties of this material discussed previously in this review.

These materials have also been extensively used for the augmentation of distal radius fractures. With the widespread use of locked plating for these injuries, the efficacy of these materials for use in this situation must be questioned. A recent randomized study sought to determine whether augmentation of volar locking plate fixation with calcium phosphate bone cement had any benefit over volar locking plate fixation alone in an elderly patient population with unstable distal radial fractures [21•].

The authors concluded that augmentation of metaphyseal defects with calcium phosphate bone cement after volar locking plate fixation offered no benefit over volar locking plate fixation alone in elderly patients with an unstable distal radial fracture. This study documents the biomechanical superiority that locked plating can provide for fracture fixation in this patient population. However, prospective studies are required to determine the role that conductive substrates play in augmenting fracture fixation when combined with locked plating techniques [22].

A recent meta- analysis was undertaken to evaluate the concept of hip fracture augmentation using these materials to augment fixation in these fractures that commonly occur in an osteoporotic population. Because there were only a few, randomized, controlled studies, there is currently poor evidence for the use of any orthobiologic bone cement in the treatment of fractures of the hip and should not be undertaken [23].

Inductive Agents

Platelet-Rich Plasma

The clinical use of platelet-rich plasma (PRP) has been reported for a wide variety of clinical applications, most predominately for the problematic wound, maxillofacial applications, and spine. Collectively, these studies provide variable support for the clinical use of PRP. The use of this autogenous material as an adjuvant to augment the healing potential of osteoporotic fracture patients makes it an attractive alternative to the true BMP materials currently available. However, many reports are anecdotal, and few level I studies with control group comparison are available to definitively determine the role of PRP. There are no studies evaluating its effectiveness in this specific population.

Currently, there is no level I evidence to indicate using PRP alone or in combination with other materials has a substantial effect on bone healing. The available evidence (level III and IV) indicates that PRP may have a positive effect as an adjunct to local bone graft and has been suggested for use to increase the rate of bone deposition and quality of bone regeneration in fusion and nonunion situations; specifically to augment ankle fusions in a diabetic population. Overall, there is clearly a lack of scientific evidence to support the routine use of PRP in combination with bone grafts during augmentation procedures [24].

Inductive Substrates

The bone morphogenic proteins (BMPs) belong to the TGF-β superfamily of growth and differentiation factors. Unlike DBM, which is a mixture of BMPs and immunogenic noninductive proteins, the pure form of BMP is nonimmunogenic and nonspecies specific. The BMPs are true "osteoinducers." As they are released, they feed back onto circulating undifferentiated perivascular mesenchymal cells (stem cells), changing them directly into osteoprogenitor cells.

There are only two currently approved indications for use of these adjuvants in a fracture population. In a large prospective, randomized, controlled, partially blinded, multicenter study, Friedlaender et al. assessed the efficacy of the OP-1 Device (Olympus Corporation, Tokyo, Japan) (3.5 mg of rhBMP-7 in a bovine bone-derived type-1 collagen-particle delivery vehicle), in comparison with that of autografting in the treatment of 122 patients with a total of 124 tibial nonunions. All of the nonunions were at least 9 months old and had shown no progress toward healing for the 3 months prior to the patient's enrollment in the study. OP-1 statistically proved to be a safe and effective alternative to bone graft in the treatment of tibial nonunions [25]. Limited approval of OP-1 by the FDA for use in the treatment of tibial nonunions and other long bone nonunions was designated as a Humanitarian Use Device (HUD) for this particular indication [26].

A prospective randomized study evaluated rhBMP-2 for the treatment of open tibial shaft fractures was carried out. The BMP-2 Evaluation in Surgery for Tibial Trauma (BESTT) Study Group reported the results of a large multinational, prospective, randomized, controlled study of the effects of

INFUSE (rhBMP-2 on an absorbable type-1 collagen sponge; Medtronic Sofamor Danek, Memphis, TN) in the treatment of open tibial fractures [27•]. At the time of definitive wound closure, the patients were randomized to one of three groups: standard closure, standard closure and the addition of 6 mg of rhBMP-2 to the fracture site, or standard closure and the addition of 12 mg of rhBMP-2 to the fracture site.

The group treated with the higher dose of rhBMP-2 (1.5 mg/kg) had fewer secondary interventions. Interestingly, although not used as primary outcome measures, an accelerated time to union, improved wound healing, and a reduced infection rate were also found in the patients treated with the high dose of rhBMP-2. The FDA subsequently granted approval for the treatment of acute, open fractures of the tibial shaft.

Following the approval of these two BMPs for specific traumatic conditions (acute open tibial shaft fractures and recalcitrant nonunions), very limited data has been published using these devices with their strict on-label indications. Many investigators have sought to combine these materials with other biologic adjuvants. The ability to combine multiple inductive, conductive, and/or osteogenic factors continues with the BMPs as well. This has been done for a variety of clinical issues regarding the clinical handling and application of these materials, as well as to attempt to augment the healing potential of these specific BMPs for their trauma application.

Currently, their use in this specific population is restricted to the on-label indications and may be an attractive alternative for those patients that have insufficient bone stock for skeletal augmentation.

Systemic Agents

Bisphosphonates

Bisphosphonates are the most commonly prescribed agents for the treatment of osteoporosis. Their antiresorptive properties are due to their inhibition of osteoclast-mediated bone resorption. The result is an increase in bone mineral density and a decrease in fracture risk [28]. This function could adversely affect bone healing, primarily the remodeling phase. It has also been shown that bisphosphonates are preferentially deposited at acute fracture sites in a rat model and this could further impact bone repair [29].

Persons that sustain an osteoporotic fracture are more likely to have additional fragility fractures. The HORIZON (Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly Recurrent Fracture) Trial demonstrated that use of zoledronic acid reduced the risk of subsequent low-energy fractures [30]. The benefits of bisphosphonates versus concerns over potential impaired fracture healing have called into question timing or continuation of therapy in the setting of a fracture. Most studies have shown an increase in callus formation or no effect at all on healing [29, 31]. An increase in mechanical strength of the callus was found when compared to controls [29, 32]. This increased callus mineralization has also been seen in a randomized study of osteoporotic distal radius fractures treated with clodronate [33].

The complications of extended bisphosphonate therapy including osteonecrosis of the jaw as well as atypical subtrochanteric femur fractures have been well documented [34–37]. Even in the setting of atypical femur fractures, healing is generally reliable, but sometimes delayed [38]. Most recommendations today are for drug holidays after about 2 to 5 years of use in low-risk patients [34].

A recent meta-analysis demonstrated no clinically detectable delay in indirect fracture healing compared to control groups and no difference in early versus late administration of bisphosphonates [39•]. It has even been shown that a twofold increase in screw fixation extraction torque was achieved in osteoporotic hip fractures treated with bisphosphonates [40]. It is the recommendation of Xue et al. that bisphosphonate therapy should be initiated following fracture fixation [39•].

Denosumab

Denosumab is a human monoclonal antibody that binds to the receptor activator of nuclear factor κβ ligand (RANKL) and interferes with the RANK and RANKL interaction. This results in decreased bone resorption by inhibiting osteoclast formation, function, and survival [41]. It has been approved for the treatment of postmenopausal osteoporosis in women with a high risk of fracture in both Europe and the USA.

The FREEDOM (Fracture Reduction Evaluation of Denosumab in Osteoporosis every 6 Months) study evaluated the effects on bone mineral density (BMD) and fracture risk in postmenopausal women with osteoporosis at a dose of 60 mg every 6 months for 3 years. The primary end point of the study was the incidence of new vertebral fractures with secondary end points being the time to first nonvertebral and hip fractures [42]. All patients (denosumab and control groups) also received calcium and vitamin D supplementation. The incidence of new vertebral fractures was reduced in the denosumab group, 2.3 versus 7.2 % [42]. Nonvertebral fractures as well as hip fractures were also reduced with incidences of 6.5 versus 8.0 % and 0.7 versus 1.2 %, respectively [42]. The increases in BMD in the denosumab group over placebo were 9.2 % in the lumbar spine and 6.0 % at the hip [42].

A preplanned subgroup analysis of the FREEDOM trial looked at the effect of denosumab on fracture healing. A total of 667 patients (303 in the denosumab group and 364 in the placebo group) had 851 nonvertebral fractures (386 in the denosumab group and 465 in the placebo group) with 199 fractures treated surgically (79 and 120, respectively) [43•]. There was no delayed healing or nonunion in the denosumab and one nonunion seen in the placebo group. This supported

data from animal studies that showed RANK inhibition was not associated with delayed union or changes in the mechanical integrity of healing fractures [44, 45]. In addition, total complication rates associated with the fracture or its management were also decreased in the denosumab group (1.7 %) versus the control placebo group (5.5 %) [43•]. The conclusion from the group is that denosumab may be administered at the time of, or soon after, a nonvertebral fracture and does not result in an increased risk of complications or delayed healing [43•].

Parathyroid Hormone

Parathyroid hormone (PTH) is a major controller of bone turnover through the regulation of calcium, phosphate, and vitamin D. The first 34 of the 84 amino acids of this molecule represent the active site. Teriparatide is a recombinant human PTH (1–34) from the N terminus and is the first anabolic agent approved for the treatment of osteoporosis. Constant physiologic doses of PTH enhance osteoclast activity through increasing RANKL and decreasing osteoprotegerin with the final result being increased bone resorption. On the other hand, pulsatile PTH in the form of daily subcutaneous injections increases differentiation of osteoblast precursors and diminishes osteoblast apoptosis resulting in increased bone formation [46]. Supraphysiologic doses of recombinant PTH have shown enhanced fracture healing in animal studies, but limited studies in humans [47–49].

There have only been two randomized prospective control trials looking at the potential improved fracture healing related to PTH administration. The first study by Aspenberg et al. compared daily injections of teriparatide 40 μg versus teriparatide 20 μg versus a placebo for 8 weeks in 102 postmenopausal women with a distal radius fracture treated conservatively [50]. The time to healing was determined by radiographic evidence of cortical bridging in three out of four cortices. The median healing time was 8.8, 7.4, and 9.1 weeks for teriparatide 40 and 20 μg and placebo, respectively [50]. There was only a statistical difference in healing between the teriparatide 20 μg group and the placebo group (p=0.006), and the effect was not dose dependent in the 40 μg group [50]. The second more recent randomized control trial by Peichl et al. studied the effect of the full molecule of PTH (1–84) on postmenopausal women with pelvic rami fractures [51]. The mean time to cortical bridging seen on CT scan was 7.8 weeks for the PTH (1–84) 100 μg versus 12.6 weeks for the placebo group [51]. In addition, at 8 weeks, the PTH (1–84) group were found to have significantly less pain than the control group on the visual analog scale, 3.2 versus 6.5 (p<0.001). The PTH (1–84) group also had significantly improved functional outcome as assessed by the Timed "Up and Go" test (p<0.001) at 12 weeks.

A recent review of the PTH literature by Zhang et al. included 16 studies, 2 of which were the abovementioned randomized controlled trials and the remainder being case reports with the limited availability of prospective trials, the authors had to combine randomized trials using teriparatide as well as PTH. Additionally, anecdotal evidence in the form of case reports was also included in this preliminary review. Their final conclusion was that teriparatide is a viable treatment option which can address the underlying osteoporosis, but may also enhance fracture healing without additional adverse events [52•].

There has been a concern for the development of osteosarcoma as a result of treatment with teriparatide based on preclinical trials with rats [53, 54]. This causality has not been demonstrated in humans. A 7-year surveillance study performed in the USA looked at the relationship between teriparatide treatment and osteosarcoma. There were 1448 new cases of osteosarcoma in the registry, and of the 549 patients that were interviewed, none had a history of treatment with teriparatide [55]. This was further corroborated by a retrospective cohort study looking at patients in the Danish registry after 7 years of recombinant PTH both (1–34) and (1–84) being available. There were over 4100 patients treated with both recombinant forms versus nearly 41,000 patients as age- and gender-matched controls. None of the patients in the treatment group were diagnosed with osteosarcoma, and there was no significantly increased cancer risk with recombinant PTH treatment [56].

Bioceramics

Early bioceramics, named inert ceramics such as alumina and zirconia, were utilized for their low reactivity. At that time, the only expected responses to foreign material were inflammation and rejection [57]. Newer generations of bioceramics aim to induce specific tissue responses based on the composition of their surfaces. There has been a paradigm switch from osteointegration to osteoregeneration through the binding of bioceramics to various elements. This is defined as functionalization. In osteoporotic models, the osteoblastic differentiation of bone marrow stromal cells (BMSCs) has been shown to be significantly reduced [58]. The use of functionalization could help to stimulate the osteoblastic differentiation and potentially inhibit osteoclastogenesis in setting of osteoporosis.

As a trace element, strontium (Sr) has been shown to positively affect bone metabolism, by stimulating bone formation and inhibiting bone resorption [59, 60]. The proposed mechanism is related to not only an increase in osteoblast-related gene expression as well as phosphatase activity of mesenchymal stem cells but also the inhibition of osteoclast differentiation through inhibition of RANKL [61, 62]. Strontium ions have also been shown to stimulate the expression of osteoprotegerin (OPG), which inhibits the differentiation and

activity of osteoclasts by preventing the binding of RANK with its ligand, RANKL [63].

Silicate (Si) ions released from calcium silicate (CS) have shown promising effects on osteogeneration. An in vivo study showed CS promoted early bone formation when compared to traditional calcium phosphate [64, 65]. The Si ions released could provide an environment that would encourage osteogenic differentiation of BMSCs as well as promote angiogenesis through human umbilical vein endothelial cell (HUVEC) proliferation [64].

The hypothesis of Lin et al. was that the combination of Sr and Si within bioceramic scaffolds could have potential synergistic effects on osteoporotic bone regeneration [66••]. In their study, they used the ovariectomized (OVX) rat model which has been approved by the FDA to evaluate and treat postmenopausal osteoporosis [67]. Macroporous Sr-substituted calcium silicate (SrCS) ceramic scaffolds were constructed. Their results demonstrated the proposed synergistic effect from the bioactive Sr and Si ions from SrCS directly causing BMSCs-OVX differentiating toward osteoblasts along with stimulation of endothelial cells causing angiogenesis. Both CS and SrCS stimulated OPG production and downregulated RANKL resulting in inhibition of osteoclastogenesis, but SrCS showed a longer and greater inhibitory effect. The gene and protein expression in the BMSCs-OVX from this synergistic upregulation were similar to those of BMSCs from healthy tissue. Their conclusion was that these macroporous SrCS ceramic scaffolds have osteoinductive activity to promote early bone formation as well as cause angiogenesis and are a "promising candidate for the regeneration of osteoporotic bone defects."

Conclusions

This is an exciting time in the development of orthobiologic therapies for enhancement of bone healing for traumatic conditions in osteoporotic patients. Areas of application for new technologies include the acceleration of fracture healing, treatment of nonunions, enhancement of fusion mass, and the treatment of massive segmental bone loss. The ideal bone-graft augmentation material is biocompatible, bioresorbable, osteoconductive, osteoinductive, structurally similar to bone, easy to use, and cost-effective and at minimal risk for material-based complications.

Alternatively systemic treatments offer distinct advantages without the surgical risks. Systemic agents including bisphosphonates, denosumab, or PTH analogs can be started immediately after fracture fixation with no evidence of impaired bone healing and potentially increased callus formation and fixation strength of implants. Ensuring that patients are started on some form of osteoporosis therapy should be included in the postoperative care plan and managed in conjunction with primary medical team. Newer adjunctives on the horizon such as bioceramics could help further to fill osteoporotic defects to strengthen implant fixation as well as aid in healing. Future comprehensive strategies for therapeutic application will combine concepts of tissue engineering with a simple delivery mechanism and biologic scaffolding,

Compliance with Ethics Guidelines

Conflict of Interest JT Watson has received a speaker honorarium from Medtronic and served on an advisory panel for Bioventus.

DA Nicolaou declares no conflicts of interest.

Human and Animal Rights and Informed Consent All studies by JT Watson involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Giannoudis P et al. Fracture healing in osteoporotic fractures: is it really different? A basic science perspective. Injury. 2007;38 Suppl 1:S90–9.
2. Friedlaender GE. Immune responses to osteochondral allografts. Current knowledge and future directions. Clin Orthop Relat Res. 1983;174:58–68.
3. Ramoshebi LN et al. Tissue engineering: TGF-beta superfamily members and delivery systems in bone regeneration. Expert Rev Mol Med. 2002;4(20):1–11.
4. Kirk JF et al. Osteoconductivity and osteoinductivity of NanoFUSE((R)) DBM. Cell Tissue Bank. 2013;14(1):33–44.
5. Moore ST et al. Osteoconductivity and osteoinductivity of Puros(R) DBM putty. J Biomater Appl. 2011;26(2):151–71.
6. Han B, Yang Z, Nimni M. Effects of moisture and temperature on the osteoinductivity of demineralized bone matrix. J Orthop Res. 2005;23(4):855–61.
7. Hierholzer C et al. Plate fixation of ununited humeral shaft fractures: effect of type of bone graft on healing. J Bone Joint Surg Am. 2006;88(7):1442–7.
8. Wilkins RM, Chimenti BT, Rifkin RM. Percutaneous treatment of long bone nonunions: the use of autologous bone marrow and allograft bone matrix. Orthopedics. 2003;26(5 Suppl):s549–54.
9. Peters CL et al. Biological effects of calcium sulfate as a bone graft substitute in ovine metaphyseal defects. J Biomed Mater Res A. 2006;76(3):456–62.
10. Yu B et al. Treatment of tibial plateau fractures with high strength injectable calcium sulphate. Int Orthop. 2009;33(4):1127–33.
11. Beuerlein MJ, McKee MD. Calcium sulfates: what is the evidence? J Orthop Trauma. 2010;24 Suppl 1:S46–51.
12. Kuhne JH et al. Bone formation in coralline hydroxyapatite. Effects of pore size studied in rabbits. Acta Orthop Scand. 1994;65(3):246–52.

13. De Long Jr WG et al. Bone grafts and bone graft substitutes in orthopaedic trauma surgery. A critical analysis. J Bone Joint Surg Am. 2007;89(3):649–58.
14. Nakahara H, Goldberg VM, Caplan AI. Culture-expanded periosteal-derived cells exhibit osteochondrogenic potential in porous calcium phosphate ceramics in vivo. Clin Orthop Relat Res. 1992;276:291–8.
15. Watson JT. Overview of biologics. J Orthop Trauma. 2005;19(10 Suppl):S14–6.
16. Watson JT. The use of an injectable bone graft substitute in tibial metaphyseal fractures. Orthopedics. 2004;27(1 Suppl): s103–7.
17.• Mauffrey C et al. Incidence and pattern of technical complications in balloon-guided osteoplasty for depressed tibial plateau fractures: a pilot study in 20 consecutive patients. Patient Saf Surg. 2013;7(1): 8. *This study looked at the complications and steep learning curved involved with the use of inflation bone tamps to reduce depressed tibial plateau fractures*.
18. Szpalski M, Gunzburg R. Applications of calcium phosphate-based cancellous bone void fillers in trauma surgery. Orthopedics. 2002;25(5 Suppl):s601–9.
19.• Goff T, Kanakaris NK, Giannoudis PV. Use of bone graft substitutes in the management of tibial plateau fractures. Injury. 2013;44 Suppl 1:S86–94. *Review of 19 studies comparing various bone graft substitutes which showed greater tolerance of early weight bearing and improved early functional outcomes with injectable calcium phosphate*.
20. Bajammal SS et al. The use of calcium phosphate bone cement in fracture treatment. A meta-analysis of randomized trials. J Bone Joint Surg Am. 2008;90(6):1186–96.
21.• Kim JK, Koh YD, Kook SH. Effect of calcium phosphate bone cement augmentation on volar plate fixation of unstable distal radial fractures in the elderly. J Bone Joint Surg Am. 2011;93(7):609–14. *Augmentation of metaphyseal defects with calcium phosphate bone cement after volar locking plate fixation offered no benefit over volar locking plate fixation alone in elderly patients with an unstable distal radial fracture*.
22. Suhm N, Gisep A. Injectable bone cement augmentation for the treatment of distal radius fractures: a review. J Orthop Trauma. 2008;22(8 Suppl):S121–5.
23. Lindner T et al. Fractures of the hip and osteoporosis: the role of bone substitutes. J Bone Joint Surg (Br). 2009;91(3):294–303.
24. Sanchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. Int J Oral Maxillofac Implants. 2003;18(1):93–103.
25. Friedlaender GE et al. Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. J Bone Joint Surg AmSuppl. 2001;83(A Suppl 1(Pt 2)):S151–8.
26. Blokhuis TJ, Calori GM, Schmidmaier G. Autograft versus BMPs for the treatment of non-unions: what is the evidence? Injury. 2013;44 Suppl 1:S40–2.
27.• Lad SP et al. Cancer after spinal fusion: the role of bone morphogenetic protein. Neurosurgery. 2013;73(3):440–9. *This study showed increased incidence of benign tumors especially of the nervous system in lumbar fusion patients exposed to BMP*.
28. Morris CD, Einhorn TA. Bisphosphonates in orthopaedic surgery. J Bone Joint Surg Am. 2005;87(7):1609–18.
29. Amanat N et al. Optimal timing of a single dose of zoledronic acid to increase strength in rat fracture repair. J Bone Miner Res. 2007;22(6):867–76.
30. Lyles KW et al. Zoledronic acid and clinical fractures and mortality after hip fracture. N Engl J Med. 2007;357(18):1799–809.
31. Li J et al. Concentration of bisphosphonate (incadronate) in callus area and its effects on fracture healing in rats. J Bone Miner Res. 2000;15(10):2042–51.
32. Amanat N et al. A single systemic dose of pamidronate improves bone mineral content and accelerates restoration of strength in a rat model of fracture repair. J Orthop Res. 2005;23(5):1029–34.
33. Adolphson P et al. Clodronate increases mineralization of callus after Colles' fracture: a randomized, double-blind, placebo-controlled, prospective trial in 32 patients. Acta Orthop Scand. 2000;71(2):195–200.
34. Schmidt GA et al. Risks and benefits of long-term bisphosphonate therapy. Am J Health Syst Pharm. 2010;67(12):994–1001.
35. Watts NB. Long-term risks of bisphosphonate therapy. Arq Bras Endocrinol Metabol. 2014;58(5):523–9.
36. Beninati F, Pruneti R, Ficarra G. Bisphosphonate-related osteonecrosis of the jaws (Bronj). Med Oral Patol Oral Cir Bucal. 2013;18(5):e752–8.
37. Janovska Z. Bisphosphonate-related osteonecrosis of the jaws. A severe side effect of bisphosphonate therapy. Acta Med (Hradec Kralove). 2012;55(3):111–5.
38. Egol KA et al. Healing delayed but generally reliable after bisphosphonate-associated complete femur fractures treated with IM nails. Clin Orthop Relat Res. 2014;472(9):2728–34.
39.• Xue D et al. Do bisphosphonates affect bone healing? A meta-analysis of randomized controlled trials. J Orthop Surg Res. 2014;9: 45. *This recent meta-analysis looked at eight RCTs and found no delay in fracture healing with bisphosphonate therapy and recommended infusion after fracture fixation and lumbar fusion*.
40. Moroni A et al. Alendronate improves screw fixation in osteoporotic bone. J Bone Joint Surg Am. 2007;89(1):96–101.
41. Moen MD, Keam SJ. Denosumab: a review of its use in the treatment of postmenopausal osteoporosis. Drugs Aging. 2011;28(1):63–82.
42. Cummings SR et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009;361(8):756–65.
43.• Adami S et al. Denosumab treatment in postmenopausal women with osteoporosis does not interfere with fracture-healing: results from the FREEDOM trial. J Bone Joint Surg Am. 2012;94(23): 2113–9. *The three year results of this placebo-controlled trial showed no evidence of impaired healing in 303 patients treated with 60mg Denosumab showed no delay in fracture healing or increased complications even when administered immediately after surgery.*
44. Flick LM et al. Effects of receptor activator of NFkappaB (RANK) signaling blockade on fracture healing. J Orthop Res. 2003;21(4): 676–84.
45. Ulrich-Vinther M, Andreassen TT. Osteoprotegerin treatment impairs remodeling and apparent material properties of callus tissue without influencing structural fracture strength. Calcif Tissue Int. 2005;76(4):280–6.
46. Wu CC et al. Enhanced healing of sacral and pubic insufficiency fractures by teriparatide. J Rheumatol. 2012;39(6): 1306–7.
47. Alkhiary YM et al. Enhancement of experimental fracture-healing by systemic administration of recombinant human parathyroid hormone (PTH 1-34). J Bone Joint Surg Am. 2005;87(4):731–41.
48. Nozaka K et al. Intermittent administration of human parathyroid hormone enhances bone formation and union at the site of cancellous bone osteotomy in normal and ovariectomized rats. Bone. 2008;42(1):90–7.
49. Rowshan HH et al. Effect of intermittent systemic administration of recombinant parathyroid hormone (1-34) on mandibular fracture healing in rats. J Oral Maxillofac Surg. 2010;68(2):260–7.
50. Aspenberg P et al. Teriparatide for acceleration of fracture repair in humans: a prospective, randomized, double-blind study of 102 postmenopausal women with distal radial fractures. J Bone Miner Res. 2010;25(2):404–14.

51. Peichl P et al. Parathyroid hormone 1-84 accelerates fracture-healing in pubic bones of elderly osteoporotic women. J Bone Joint Surg Am. 2011;93(17):1583–7.
52.• Zhang D et al. The role of recombinant PTH in human fracture healing: a systematic review. J Orthop Trauma. 2014;28(1):57–62. *Literature review of 16 publications, the majority of which are case reports, which found no evidence of impaired fracture healing in the setting of denosumab treatment and even anecdotal evidence of enhanced fracture healing.*
53. Jolette J et al. Defining a noncarcinogenic dose of recombinant human parathyroid hormone 1-84 in a 2-year study in Fischer 344 rats. Toxicol Pathol. 2006;34(7):929–40.
54. Vahle JL et al. Skeletal changes in rats given daily subcutaneous injections of recombinant human parathyroid hormone (1-34) for 2 years and relevance to human safety. Toxicol Pathol. 2002;30(3): 312–21.
55. Andrews EB et al. The US postmarketing surveillance study of adult osteosarcoma and teriparatide: study design and findings from the first 7 years. J Bone Miner Res. 2012;27(12):2429–37.
56. Bang UC, Hyldstrup L, Jensen JE. The impact of recombinant parathyroid hormone on malignancies and mortality: 7 years of experience based on nationwide Danish registers. Osteoporos Int. 2014;25(2):639–44.
57. Arcos D, Izquierdo-Barba I, Vallet-Regi M. Promising trends of bioceramics in the biomaterials field. J Mater Sci Mater Med. 2009;20(2):447–55.
58. Saidak Z et al. Strontium ranelate rebalances bone marrow adipogenesis and osteoblastogenesis in senescent osteopenic mice through NFATc/Maf and Wnt signaling. Aging Cell. 2012;11(3):467–74.
59. Peng S et al. The cross-talk between osteoclasts and osteoblasts in response to strontium treatment: involvement of osteoprotegerin. Bone. 2011;49(6):1290–8.
60. Yang F et al. Strontium enhances osteogenic differentiation of mesenchymal stem cells and in vivo bone formation by activating Wnt/catenin signaling. Stem Cells. 2011;29(6): 981–91.
61. Peng S et al. Strontium promotes osteogenic differentiation of mesenchymal stem cells through the Ras/MAPK signaling pathway. Cell Phys Biochem. 2009;23:165–74.
62. Marie PJ, Felsenberg D, Brandi ML. How strontium ranelate, via opposite effects on bone resorption and formation, prevents osteoporosis. Osteoporos Int. 2011;22(6):1659–67.
63. Saidak Z, Marie PJ. Strontium signaling: molecular mechanisms and therapeutic implications in osteoporosis. Pharmacol Ther. 2012;136(2):216–26.
64. Wang C et al. Osteogenesis and angiogenesis induced by porous beta-CaSiO(3)/PDLGA composite scaffold via activation of AMPK/ERK1/2 and PI3K/Akt pathways. Biomaterials. 2013;34(1):64–77.
65. Xu S et al. Reconstruction of calvarial defect of rabbits using porous calcium silicate bioactive ceramics. Biomaterials. 2008;29(17): 2588–96.
66.•• Lin K et al. Enhanced osteoporotic bone regeneration by strontium-substituted calcium silicate bioactive ceramics. Biomaterials. 2013;34:10028–42. *These experiments demonstrated that strontium substituted calcium silicate ceramic scaffolds dramatically enhanced bone regeneration and angiogenesis in critical sized bone defects in ovarectomized mice. This paper also gave good background information on the function of strontium and silicate ions on bone metabolism and angiogenesis, respectively.*
67. Food and Drug Administration, in Guidelines for preclinical and clinical evaluation of agents used in the prevention or treatment of postmenopausal osteoporosis. 1994: Rockville, USA: FDA.