# Bone healing in 2016

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**Summary**

**Delayed fracture healing and nonunion occurs in up to 5-10% of all fractures, and can present a challenging clinical scenario for the treating physician. Methods for the enhancement of skeletal repair may benefit patients that are at risk of, or have experienced, delayed healing or nonunion. These methods can be categorized into either physical stimulation therapies or biological therapies. Physical stimulation therapies include electrical stimulation, low-intensity pulsed ultrasonography, or extracorporeal shock wave therapy. Biological therapies can be further classified into local or systemic therapy based on the method of delivery. Local methods include autologous bone marrow, autologous bone graft, fibroblast growth factor-2, platelet-rich plasma, platelet-derived growth factor, and bone morphogenetic proteins. Systemic therapies include parathyroid hormone and bisphosphonates. This article reviews the current applications and supporting evidence for the use of these therapies in the enhancement of fracture healing.**

*KEY WORDS: fracture; healing; nonunion; bone defects; growth factors.*

## Introduction

Of the estimated 7.9 million fractures that occur annually in the United States, approximately 5-10% have either delayed or impaired healing (1, 2). Furthermore, of these 600,000 fractures with delayed healing, nearly 100,000 progress to nonunion (3). Trauma is the most expensive medical condition after heart conditions, costing the United States $56 billion dollars every year (3, 4). Of that, $21 billion is used for the treatment of fractures. For these reasons, the efficacious and expedient treatment of fractures is of paramount importance to the patient, physician, and healthcare system as a whole. Fortunately, our understanding of fracture healing has made tremendous strides in the last two decades. To assist with the understanding and management of fractures and fracture healing, this review summarizes the most current concepts in the enhancement of fracture healing.

## Fracture healing and delayed healing

Fracture healing involves a complex interplay between several anatomical, biomechanical, and biochemical processes. Skeletal repair, unlike many other tissues, may result in complete restoration of the biochemical and mechanical properties of the injured tissue. Bone is unique in that regeneration may occur without a fibrous scar. Although skeletal tissue has a robust regenerative capacity, the healing process may fail, resulting in delayed healing, non-unions, and malunions (5). There is currently no standard criteria to define when a fracture is considered a nonunion. According to the United States Food and Drug Administration (FDA), a nonunion is defined as a fracture that has not completely healed within 9 months of injury, with serial radiographs demonstrating no progression of healing during the final three months (3). In clinical practice, there is a considerable variability for what is considered a nonunion, with definitions of nonunion ranging from 2-12 months (6). When a nonunion does occur, it presents a challenging clinical scenario for the treating physician. There are many different factors that can contribute to the formation of a nonunion, including which bone is involved, fracture site, initial degree of bone loss, time elapsed since injury, extent of soft-tissue injury, as well as a host of patient factors such as smoking, diabetes, and other systemic diseases (7). A thorough knowledge of the methods of enhancing fracture healing would therefore benefit the physician treating patients that are at risk of, or have experienced, delayed healing or nonunion.

## Methods of enhancement of bone repair

Methods to enhance fracture healing are an important way to ensure the patients rapid recovery, which includes return to work, recreation, and family life. Autologous bone grafting, the current "gold-standard" in the enhancement of fracture repair, is costly, time-consuming, and associated with morbidity including pain, injury, hematoma, and fracture (8). Therefore, the ability to heal skeletal injuries without the use of autologous iliac crest bone graft is highly desirable. Methods for the enhancement of skeletal repair can be categorized into either physical stimulation therapies or biological therapies. Biological therapies can be further classified into local or systemic therapy based on the method of delivery.

## Physical stimulation therapies

There is a large number of devices that are marketed under the category of "bone growth stimulators". These modalities are appealing because they are less invasive, and the complications associated with bone graft harvest are eliminated. The three major

categories of physical stimulation therapies used for fracture healing include electromagnetic fields, low-intensity pulsed ultrasonography, and extracorporeal shock wave therapy.

***Electrical stimulation***

Electrical stimulation is based on the premise that mechanical stress applied to bone results in the generation of electric potentials (9). Compression results in electronegative potentials, which result in bone formation. Tension results in electropositive potentials, which act to resorb bone (9, 10). In theory, the generation of an electrical field at the fracture site should therefore have the ability to enhance bone formation. While several basic science studies have demonstrated the benefit of electrical stimulation, clinical studies have had mixed results (11-14). In a 2008 meta-analysis of 11 randomized controlled trials, the Authors found that of 4 trials including 106 delayed or ununited fractures there was an overall non-significant pooled relative risk of 1.76 (95% confidence interval, 0.8 to 3.8; p=0.15) in favor of electromagnetic stimulation (15). The Authors concluded that although they found no significant effect of electromagnetic stimulation on delayed unions or nonunited long bone fractures, that the heterogeneity of studies creates uncertainty as to this conclusion (15). Future high-quality studies are needed to determine the true benefit of electromagnetic stimulation.

***Low-Intensity pulsed ultrasonography***

The second major category of physical stimulation therapies is low-intensity pulsed ultrasonography (LIPUS), which is believed to work by creating sound waves that generate micromechanical stress at the fracture site. These micromechanical stresses then stimulate various cellular and molecular changes to promote healing (16, 17). LIPUS has been primarily studied in patients with long bone fractures, patients undergoing osteotomies, and smokers with fractures (18, 19). In a well-designed trial, LIPUS has been shown to be a safe treatment for acute fractures of long bones and nonunions (20). The Authors did note, however, that the treatment is very time consuming (average treatment is 20 minutes per day for 5 months) (20). A recent meta-analysis of 13 randomized controlled trials found that the evidence in favor of LIPUS being effective in the promotion of bone healing is limited and the data are conflicting (21).

Interestingly, a 2014 network meta-analysis was performed to indirectly compare electrical stimulation with LIPUS for fracture healing (22). In patients with an acute fracture, the study demonstrated a suggested but not significant benefit of LIPUS at 6 months after fracture (risk ratio [RR] 1.17, 95% confidence interval [CI] 0.97-1.41) (22). In patients with a nonunion or delayed fracture healing, electrical stimulation also had a non-significant but suggested benefit at 3 months (RR 2.05, 95% CI 0.99-4.24) compared to the standard of care alone. There was very low-quality evidence to suggest a potential benefit of LIPUS over electrical stimulation in improving union rates at 6 months in acute fractures (RR 0.76, 95% CI 0.58-1.01), but the Authors conclude that direct comparative trials are required to support the validity of their findings (22).

***Extracorporeal shock wave therapy***

Extracorporeal shock wave therapy has been employed in the treatment of union and nonunion (23). An applicator produces shock waves, which are single high-amplitude sound waves generated by electrohydraulic, electromagnetic, or piezoelectric methods that propagate through tissue. In animal models, these shockwaves have biological effects by forming free radicals and oxygen radicals, which lead to the production of a number of different growth factors (24, 25). Zelle et al. performed a systematic review of the effect of extracorporeal shock wave therapy on the treatment of delayed unions or malunions; 10 studies including 924 patients with delayed union had an overall union rate of 76% (confidence interval 73-79%) (26). The Authors concluded that extracorporeal shock wave therapy may stimulate the healing process in delayed unions or nonunions (26). Considering that all studies included for analysis provided only level 4 evidence, future studies will be needed to confirm the benefit of this treatment modality.

**Local strategies for the repair and regeneration of bone**

There is a variety of local strategies that have been used for the repair and regeneration of bone. These strategies are further classified by the mechanism through which they promote bone regeneration, and are considered either osteogenic, osteoconductive, osteoinductive or tissue repair factors. Osteogenic materials are those that contain viable cells (either osteoprogenitor or osteogenic precursor) capable of bone formation, and include either autologous bone or autologous bone marrow. Osteoconductive materials serve as scaffolding for new bone formation, and include materials such as calcium sulfate, calcium phosphate, allograft, xenograft, and ceramics. Osteoinductive materials are those that can induce into differentiating into bone cells, and include bone morphogenetic proteins (BMPs) and Wnt proteins. Tissue repair factors play an important role in wound regeneration and healing in a variety of tissues, and are not specific to bone healing or fracture. The two most important tissue repair factors that have been used in the context of fracture healing are fibroblast growth factor and platelet-derived growth factor.

***Autologous bone marrow***

Bone marrow aspirated from the iliac crest contains progenitor cells that have both osteogenic and angiogenic properties (27). These cells are self-regenerating, and are able to produce factors such as BMP and vascular endothelial growth factor (VEGF) for successful bone healing (27). The effect of bone marrow aspirate can be further enhanced by centrifugation to further concentrate the number of cells, or by combining bone marrow with grafts to provide structural support (28). Hernigou et al. used autologous bone marrow to treat 60 patients with aseptic nonunions, and achieved fracture union in 53 of 60 patients (28). The Authors demonstrated that there was a correlation between the number of progenitor cells and the rate of healing, as defined by the volume of mineralized callus at 4 months (28). While the use of bone marrow cells to enhance fracture healing is effective and relatively cheap, further work is required on both the harvesting technique and cell preparation to improve this method.

***Autologous bone graft***

For larger defects in bone, many surgeons prefer to use autologous bone graft harvested from the iliac crest instead of autologous bone marrow. Historically, this technique has been associated with donor site morbidity (e.g. bleeding and hematoma) and pain at the harvest site (29). Recent reports suggest that the actual rate of persistent pain at the harvest site 1 year post-operatively was <3%, much lower than previous reports (30). In addition to donor site morbidity, other disadvantages of autologous bone grafting include the limited volume of graft available, and the lack of structural capability of the graft. Despite these drawbacks, there are several advantages of autologous bone graft. It is relatively low cost, and there is no risk of disease transmission or immunological rejection. In addition, autologous bone graft has osteoinductive, osteoconductive, and osteogenic properties.

***Fibroblast growth factor-2 (FGF-2)***

Fibroblast growth factors (FGFs) are a family of polypeptides known to play crucial roles in the mitogenesis of mesenchymal stem cells (31). Mutations in genes of FGF or their receptors lead to severe skeletal abnormalities and therefore the FGF signal has been suggested to play a crucial role in osteogenesis (32). Of the FGF family members, FGF-2 has the highest expression in early stages of bone formation and is most abundantly accumulated in bone matrix (33, 34). Using a rat model of a femur fracture, Rundle et al. found that there was abundant expression of FGF receptors throughout the fracture callus, emphasizing the role of this family of proteins in this process (35). In a randomized controlled trial of 70 patients with transverse or short oblique fractures of the tibial shaft, patients were randomized to receive either placebo, low dose (0.8mg), or high dose (2.4mg) of recombinant FGF-2 hydrogel (rhFGF-2) injected into the fracture site (36). The percentage of patients with radiographic bone union was higher in the rhFGF-2 treated groups compared with the placebo group (p= 0.03 and 0.009 in low- and high-dosage group, respectively) (36). There was no difference in adverse events between the two groups. Although early studies are promising, further studies are needed.

***(Platelet-Rich Plasma) PRP***

Platelets play an important role in the native fracture hematoma, in which they aggregate and subsequently degranulate to release a number of growth factors including PDGF, transforming growth factor-β, VEGF, and FGF. These growth factors have a substantial influence on wound and fracture healing. As such, there has been a lot of interest in harnessing the power of platelets and their derivatives to promote fracture healing. The use of platelet-rich plasma has a number of advantages, including its autologous nature, ease of application, and relatively low cost. There is *in vitro* evidence that PRP enhances osteoprogenitor cell proliferation, increases extracellular matrix formation, and promotes angiogenesis (37). The clinical support for PRP is less convincing. In a 2012 systematic review, Sheth et al. evaluated the evidence to support the use of autologous PRP in decreasing pain and improving healing in a variety of orthopaedic bone and soft tissue injuries (38). The Authors included 23 RCT's and 10 prospective cohort studies that met all inclusion criteria. Although there was a lack of consistency across outcome measures, the Authors concluded that PRP provided no significant benefit over control therapy for the majority of outcome measures included (38). To date, the clinical evidence for PRP use in fracture healing largely anecdotal and somewhat lacking.

***Platelet-derived growth factor (PDGF)***

Platelet-derived growth factors play an important role in the process of fracture healing, and attract neutrophils, macrophages, progenitor cells, VEGF and interleukin-6 (IL-6) to the fracture site in order to regulate angiogenesis and promote bone healing (39). While there are three subtypes of PDGF, PDGF-BB is considered the universal PDGF due to the fact that it can signal through both α and β receptors and has therefore been developed for therapeutic use (39). PDGF is typically delivered to the fracture site in one of two forms: a platelet gel consisting of platelet-rich plasma mixed with thrombin, or recombinant human PDGF-BB with a beta-tricalcium phosphate scaffold. In a recent prospective RCT of 434 patients undergoing either hindfoot or ankle arthrodesis, the Authors compared the use of PDGF-BB with a beta-tricalcium phosphate scaffold to autograft in promoting fusion (40). The Authors found that compared to autograft, the use of PDGF-BB with a beta-tricalcium phosphate scaffold resulted in comparable fusion rates, less pain, and fewer side effects (40). Largely as a result of this trial, the US Food and Drug Administration granted Premarket Approval for the use of PDGF-BB with a beta-tricalcium phosphate scaffold (Augment® Bone Graft, Wright Medical) for ankle and hindfoot fusions (41).

***BMPs***

Bone morphogenetic proteins (BMP) are members of the TGF-β superfamily, and have diverse roles in development, repair, and regeneration. Of the 15 different BMPs found in humans, BMP-2 and BMP-7 have been most studied in the context of bone healing (42). BMPs mediate their effect by binding to osteoprogenitor cells, thereby increasing the transcription of osteoinductive genes such as RUNX2 to enhance osteoblast differentiation (43). While BMP-2 has received premarket approval for several clinical uses, BMP-7 may only be used under humanitarian device exemption approval. Their testing and regulation by the FDA has been different, further explained below.

In a randomized controlled trial of 450 patients undergoing irrigation and debridement and intramedullary nailing of open tibial shaft fractures, the Authors studied the efficacy of recombinant human BMP-2 in fracture healing (44). Patients were randomized to either standard of care or standard of care plus either 0.75mg/kg or 1.50mg/kg BMP-2 embedded in a collagen sponge. At 12 months, there was a significantly decreased rate of secondary interventions, increased rate of healing, fewer fractures of the nail, fewer infections, and faster wound healing in the BMP-2 group compared to controls (44). The FDA granted approval for the use of recombinant human BMP-2 for the treatment of open tibial shaft fractures. This product was also approved for fusion of the lumbar spine in skeletally mature patients with degenerative disc disease (DDD) at one level from L2-S1. Since the approval of BMP-2 for use in open tibial shaft fractures, several studies of other fracture types have been conducted with less favorable results. In a double-blind randomized controlled trial of patients with closed tibial shaft fractures, the Authors compared intramedullary nailing with intramedullary nailing in addition to treatment with recombinant human BMP-2 (45). The study was terminated at 6 months after an *interim* analysis of the first 180 patients revealed no difference in the time to fracture union (45). A related study of patients with open tibial shaft fractures treated with either intramedullary nail or intramedullary nail plus BMP-2 revealed no difference between treatment groups (46). Recombinant human BMP-2 is still available under various regulatory conditions, but there is a need for the development of safer and more effective therapies.

Recombinant BMP-7 was initially studied in the treatment of 124 patients with a tibial nonunion, which had to be present for a minimum of 9 months with no improvement in healing in the 3 months prior to study enrollment. All patients were treated with a statically locked intramedullary nail, and then randomized to either recombinant human BMP-7 in a type I collagen carrier, or autologous bone graft (47). At 9 months after treatment, 85% of the autologous bone graft treated patients and 81% of the BMP-7 patients were healed (p=0.524) as determined by lack of pain at the fracture site (47). Likewise, 85% of the autologous bone graft patients and 75% of the BMP-7 patients were healed by radiographic assessment, respectively (47). While the Authors concluded that BMP-7 is safe and effective for the treatment of tibial nonunion, the FDA did not provide premarket approval for the use of BMP-7 as there was no improvement compared to autologous bone grafting. Instead, a humanitarian device exemption was issued, allowing a limited distribution to 4,000 patients at institutions were an IRB is present to monitor the use of this device. Of note, BMP-7 is also approved as an alternative to autograft in compromised patients requiring revision posterolateral lumbar spinal fusion for whom autologous bone and bone marrow harvest are not feasible or are not expected to promote fusion.

### Systemic biological factors

The systemic administration of therapies to enhance fracture healing has several potential benefits over local therapies, such as obviation of the need for surgery in certain situations or the enhancement of healing at multiple fracture sites. The observation that patients with closed head or spinal cord injuries oftentimes have enhanced skeletal healing suggests that either a neurological mechanism or a circulating factor could be used to enhance fracture repair. In the future, a pill or injection might help to enhance fracture healing.

#### *Parathyroid hormone (PTH)*

PTH is a naturally occurring hormone and a key regulator of mineral metabolism. The intermittent injection of the active 1-34 PTH metabolite is an FDA-approved treatment for osteoporosis, which has been shown to both increase bone mass and reduce the risk of fracture (48). PTH has also been shown to enhance fracture healing in both animal studies and clinical trials (48-52). Although the mechanism is not fully understood, this is thought to occur by PTH binding to osteoprogenitor cells, which then interacts with PTHrP and Indian Hedgehog to mediate chondrocyte development and differentiation (49). There is also evidence to suggest that PTH interacts with the Wnt-signaling pathway to enhance intramembranous bone healing (53). In a study of post-menopausal women with pelvic fractures, patients treated with a once daily injection of 100μg PTH (1-84) beginning 2 days after admission to the hospital had a significantly faster time to fracture healing (7.8 weeks) compared to controls (12.6 weeks, $P<0.001$) (52). The Authors concluded that PTH (1-84) may accelerate fracture healing in postmenopausal women with pelvic fractures. In a RCT of post-menopausal women undergoing closed reduction and immobilization of distal radius fractures, the patients were randomly assigned to 8 weeks of daily injections of placebo, or of 20μg or 40μg PTH (1-34) within 10 days of sustaining the fracture (51). The median time from fracture to radiographic healing was 9.1, 7.4, and 8.8 weeks, respectively ($p=0.015$). The time to healing was significantly shorter in the 20μg PTH (1-34) group as compared to the placebo group ($p=0.006$). The Authors concluded that fracture repair may be accelerated by 20μg PTH (1-34).

#### *Bisphosphonates*

Bisphosphonates inhibit osteoclastic bone resorption, and are the most widely used class of compounds for the treatment of diseases characterized by enhanced osteoclastic activity, such as osteoporosis and Paget disease (54). Because the remodeling phases of fracture healing involve bone resorption, and bisphosphonates effectively reduce bone resorption, there has been interest in the possible effect of bisphosphonates on the enhancement of fracture healing. There are two different categories of bisphosphonates, which have different structures and different mechanisms of action. Nitrogen-containing bisphosphonates inhibit components of the mevalonate pathway, which results in impaired membrane localization of small guanosine triphosphatases (GTPases). These small GTPases are an important signaling molecule for osteoclast cell morphology and cell survival (55). Non-nitrogen-containing bisphosphonates preferentially bind to the mineral phase of bone, are accumulated in osteoclasts during osteoclastic bone resorption, and thereby induce apoptosis (55). Although there is a number of animal studies to support the use of bisphosphonates in fracture healing, there are few studies in humans dedicated to this question. In a case report of a 9-year-old girl that developed a tibial non-union 7 months after a motor vehicle accident, Kakar noted that administration of two doses of 0.025 mg/kg zolodronic acid intravenously six weeks apart was followed by a rapid increase in bridging callus at the tibial fracture site (56). This case suggests that there was sufficient anabolic activity to heal the fracture, but that bisphosphonates were required to downregulate the catabolism in order for healing to occur. Future studies are needed on this topic.

### Conclusion

Fractures are the most common traumatic large-organ injuries in humans. Successful healing depends on a complex biological process that results in fracture union. Nonunion is a devastating clinical complication of fractures that presents a complex clinical challenge to the treating physician. There has been a number of advances in both physical and biological therapies aimed at promoting fracture healing at both a local and systemic level. Physicians must continue to optimize conditions for the harvest, selection, expansion, and formulation of osteogenic stem cell preparations. The targeted delivery of these cells and other local osteoinductive substances is critical to their success. In addition to local therapies, the development of systemic therapies has a number of potential advantages. Future studies on these therapies must have appropriate settings and meaningful clinical endpoints to advance our ability to enhance fracture healing.

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