

# Bone

## **1) Low-intensity pulsed ultrasound treatment for postoperative delayed union or nonunion of long bone fractures.**

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**BACKGROUND:** Postoperative delayed union and nonunion is the most common complication in fracture treatment. Recent studies have shown an accelerating effect of low-intensity pulsed ultrasound (LIPUS) on fracture repair. However, the indications for delayed union and nonunion are not clear. To clarify the factors which influence the effects of LIPUS, the data from a previous prospective multicenter study on LIPUS treatment for postoperative delayed union and nonunion of long bone fractures were reanalyzed. **METHODS:** Seventy-two cases of long bone fracture, including those of the femur, tibia, humerus, radius, and ulna, were analyzed. The mean time from the most recent operation to the beginning of LIPUS treatment was 11.5 (3-68) months. The relationship between the background factors and the union rate was analyzed using a logistic regression method. In addition, long bone fractures in an upper extremity or in a lower extremity were analyzed separately. **RESULTS:** The union rate was 75% in all the cases of long bone fracture. There was a significant relationship between the union rate and the period from the most recent operation to the beginning of LIPUS treatment in all cases and in those that had long bone fracture of an upper extremity. There was also a significant relationship between the union rate and the time when a radiological improvement was first observed after the beginning of the treatment in all cases and in those with fractures in a lower extremity. When LIPUS treatment was started within 6 months of the most recent operation, 89.7% of all fractures healed. When an improvement in the radiological changes at the fracture site was observed after 4 months in those cases, then the sensitivity and specificity for union were more than 90%. **CONCLUSIONS:** LIPUS treatment should be started within 6 months of the most recent operation. Because LIPUS has been shown to be effective without causing either serious invasiveness or any undue risk to the patient, it may be considered the treatment of first choice for cases of postoperative delayed union or nonunion.

## **2) The enhancement of bone regeneration by ultrasound.**

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Millions of fractures occur every year worldwide, with nearly 6.2 million fractures reported annually in the United States alone. Even though treatment methods have improved over the last few decades, 5-10% of fractures still show delayed healing. A significant subpopulation of these delayed healings do not heal by nine months and are thus termed non-unions. Experimental studies have shown some evidence that low intensity pulsed ultrasound stimulation (LIPUS) results in enhanced bone regeneration during fracture healing and callus distraction. LIPUS treatment has led to increased callus area and accelerated return of bone strength following fracture. Histological studies suggest that LIPUS influences all major cell types involved in bone healing, including osteoblasts, osteoclasts, chondrocytes and mesenchymal stem cells. The affect of LIPUS seems to be limited to cells in soft tissue, whereas cells in calcified bone seem not to be effected. In vitro cell culture studies as well as tissue culture studies have shown some effects on cell differentiation and protein synthesis. Even though the energy used by LIPUS treatment is extremely low, the effects are evident. The most probable source of the therapeutic benefits observed with LIPUS treatment involves nonthermal mechanisms that influence cell membrane permeability and increase cellular activity. Despite clinical and experimental studies demonstrating the enhancing effect of LIPUS on bone regeneration, the biophysical mechanisms involved in the complex fracture healing process remain unclear and requires further research.

## **3) Use of low-intensity pulsed ultrasound for posttraumatic nonunions of the tibia: a review of patients treated in the Netherlands.**

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**BACKGROUND:** Low-intensity pulsed ultrasound is effective in fresh fracture healing, resulting in a 40% reduction in healing time. The aim of this study is to determine the effect of ultrasound treatment on established tibia nonunions.

**METHODS:** The study group consists of all Dutch patients of posttraumatic consecutive nonunion of the tibia, who started their ultrasound treatment between January 2000 and February 2003. In total, 71 cases have been included, which involve 56 men and 15 women. Mean age was 40 years. Low-intensity pulsed

ultrasound was the only new treatment. Strict criteria of enrollment minimized any spontaneous healing chance. According to literature, the spontaneous healing rate was between 5% and 30%. The study outcome, healed or failed, was the primary efficacy parameter. Thirty percent was chosen to represent the maximum expected spontaneous healing and was the basis for statistical evaluation. Stratification was performed for the variables at the ultrasound treatment start. RESULTS: The overall healing rate is 52 of 71 cases (73%). Ultrasound treatment shows a statistically significant higher healing rate compared with that of the spontaneous healing chance ( $p < 0.0001$ ). Stratification shows no statistical significance for any of the variables analyzed. The long-term follow-up shows high compliance rate and no refractures. CONCLUSION: Tibia nonunions have a high occurrence rate and cause significant impairment to daily functioning. This study shows that low-intensity pulsed ultrasound is effective in the treatment of established tibia nonunions and can be seen as a good, safe, and cheaper alternative to surgery.

#### **4) The effect of low-intensity pulsed ultrasound therapy on time to fracture healing: a meta-analysis.**

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BACKGROUND: The effect of low-intensity ultrasonography on fracture healing is controversial, and current management of fractures does not generally involve the use of ultrasound therapy. We describe a systematic review and meta-analysis of randomized controlled trials of low-intensity pulsed ultrasound therapy for healing of fractures. METHODS: We searched 5 electronic databases (MEDLINE, EMBASE, Cochrane Database of Randomised Clinical Trials, HealthSTAR and CINAHL) for trials of ultrasonography and fracture healing, in any language, published from 1966 to December 2000. In addition, selected journals published from 1996 to December 2000 were searched by hand for relevant articles, and attempts were made to contact content experts in the area of ultrasound therapy and fracture healing as well as primary authors of reviewed trials. Trials selected for review met the following criteria: random allocation of treatments; inclusion of skeletally mature patients of either sex with 1 or more fractures; blinding of both the patient and the assessor(s) as to fracture healing; administration of low-intensity pulsed ultrasound treatments to at least 1 of the treatment groups; and assessment of time to fracture healing, as determined radiographically by bridging of 3 or 4 cortices. Two reviewers independently applied selection criteria to blinded articles, and selected articles were scored for methodologic quality. The internal validity of each trial was assessed with the use of a 5-point scale that evaluates the quality of trial method on the basis of description and appropriateness of randomization and double-blinding, and

assessment of study withdrawals and likelihood of bias. RESULTS: We identified 138 potentially eligible studies, of which 6 met our inclusion criteria. Agreement beyond chance of quality assessments of the 6 trials was good (intraclass correlation coefficient 0.77,  $p = 0.03$ ). One trial was a repeat analysis of previously reported data, and 2 trials appeared to report on a shared group of subjects. Three trials, representing 158 fractures, were of sufficient homogeneity for pooling. The pooled results showed that time to fracture healing was significantly shorter in the groups receiving low-intensity ultrasound therapy than in the control groups. The weighted average effect size was 6.41 (95% confidence interval 1.01-11.81), which converts to a mean difference in healing time of 64 days between the treatment and control groups. INTERPRETATION: There is evidence from randomized trials that low-intensity pulsed ultrasound treatment may significantly reduce the time to fracture healing for fractures treated nonoperatively. There does not appear to be any additional benefit to ultrasound treatment following intramedullary nailing with prior reaming. Larger trials are needed to resolve this issue.

#### **5) A new direction for ultrasound therapy in sports medicine.**

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Ultrasound therapy is a widely available and frequently used electrophysical agent in sports medicine. However, systematic reviews and meta-analyses have repeatedly concluded that there is insufficient evidence to support a beneficial effect of ultrasound at dosages currently being introduced clinically. Consequently, the role of ultrasound in sports medicine is in question. This does not mean that ultrasound should be discarded as a therapeutic modality. However, it does mean that we may need to look in a new direction to explore potential benefits. A new direction for ultrasound therapy has been revealed by recent research demonstrating a beneficial effect of ultrasound on injured bone. During fresh fracture repair, ultrasound reduced healing times by between 30 and 38%. When applied to non-united fractures, it stimulated union in 86% of cases. These benefits were generated using low-intensity ( $<0.1 \text{ W/cm}^2$ ) pulsed ultrasound (LIPUS), a dose alternative to that traditionally used in sports medicine. Although currently developed for the intervention of bone injuries, LIPUS has the potential to be used on tissues and conditions more commonly encountered in sports medicine. These include injuries to ligament, tendon, muscle and cartilage. This review discusses the effect of LIPUS on bone fractures, the dosages introduced and the postulated mechanisms of action. It concludes by discussing the relevance of these latest findings to sports medicine and how this evidence of a beneficial

clinical effect may be implemented to intervene in sporting injuries to bone and other tissues. The aim of the paper is to highlight this latest direction in ultrasound therapy and stimulate new lines of research into the efficacy of ultrasound in sports medicine. In time this may lead to accelerated recovery from injury and subsequent earlier return to activity.

#### **6) Management of non-union with pulsed low-intensity ultrasound therapy-- international results.**

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In prospective, randomized, double-blind, placebo-controlled, multi-center clinical studies, pulsed, low-intensity ultrasound has been proven to be effective in decreasing the time to heal in both fresh diaphyseal (tibia) and metaphyseal (distal radius) fractures. It also decreases the likelihood of a delayed union (>150 days to heal) in tibia fractures and loss of reduction in distal radius fractures. World-wide clinical studies, using pulsed, low-intensity ultrasound for treatment of non-union in a self-paired control study design, have demonstrated a heal rate of 88% with an average treatment time of 4.5 months in non-unions and an average fracture age of 23 months. The therapy is safe and non-invasive, and is used by the patient at home for a 20-minute treatment session per day.

#### **7) Stimulation of fracture healing in a canine ulna full-defect model by low-intensity pulsed ultrasound.**

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Because no report has been issued on the healing effects of low-intensity pulsed ultrasound on moderate to large fracture gaps, we performed an experimental study using a canine ulna full-defect model. Ten mongrel male dogs were divided into two groups: a small defect group and large defect group. The defects were made on the middle one third of both ulnae and one side only was randomly selected for ultrasound sonication, at 1 MHz, 200 microsecond bursting sine wave in 50 mW/cm<sup>2</sup> spacial average and temporal average. Sonication was started on the day after surgery and applied for 15 minutes once a day for six days a week. In the small defect group, the means of the radiologic scores, as described by

Lane and Sandhu, were 0.6, 4.4, and 8.4 in the control side and 1.8, 6.0, and 10.4 in the treatment side one, three, and five months after the operation, respectively ( $p=0.0372$ ). In the large defect model, the corresponding means were 2.2, 3.4, and 6.0 in the control side and 3.3, 5.4, and 9.2 in the treatment side ( $p= 0.009$ ). Low-intensity pulsed ultrasound enhanced new bone formation in small and large full-defects and decreased the incidence of nonunion in the large defect model.

### **8) Enhancement of fracture healing by low intensity ultrasound.**

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Fracture healing is a highly complex regenerative process that is essentially a replay of developmental events. These events include the action of many different cell types, a myriad of proteins, and active gene expression that in the majority of cases ultimately will restore the bone's natural integrity. Several biologic and biophysical approaches have been introduced to minimize delayed healing and nonunions, some with promising results. One example of such an approach is low intensity pulsed ultrasound, a noninvasive form of mechanical energy transmitted transcutaneously as high frequency acoustical pressure waves in biologic organisms. Numerous in vivo animal studies and perspective double blind placebo controlled clinical trials have shown that low intensity ultrasound is capable of accelerating and augmenting the healing of fresh fractures. Preliminary evidence suggests efficacy in the treatment of delayed healing and nonunions as well. This article reviews the animal and clinical studies that consider the effects of ultrasound on fracture healing, and the in vivo and in vitro work that strives to identify the biologic mechanism(s) responsible for the ultrasound induced enhancement of osteogenesis and fracture healing.

### **9) Low-intensity pulsed ultrasound stimulates osteogenic differentiation in ROS 17/2.8 cells.**

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There have been no studies investigating the effects of the mechanical stimulation provided by Low-intensity pulsed ultrasound (LIPUS) treatment on periodontal

disease accompanying bone loss. LIPUS is known to accelerate mineralization and bone regeneration, but the precise cellular mechanism is unclear. Here, we investigated the effect of LIPUS on osteogenesis by examining the effect of LIPUS stimulation on cell proliferation, alkaline phosphatase (ALPase) activity, osteogenesis-related gene expression, and mineralized nodule formation in a rat osteosarcoma cell line. The cells were cultured in medium with or without the addition of LIPUS stimulation. The ultrasound signal consisted of 1.5 MHz at an intensity of 30 mW/cm<sup>2</sup> for 20 min for all cultures. LIPUS stimulation did not affect the rate of cell proliferation. ALPase activity was increased at day 7 of culture after LIPUS stimulation. Real-time PCR analysis indicated that LIPUS significantly increased the expression of mRNA for the transcription factors Runx2, Msx2, Dlx5, and Osterix and for bone sialoprotein, whereas the mRNA expression of AJ18 was significantly reduced. The mineralized nodule formation and the calcium content in mineralized nodules were markedly increased on day 14 of culture after LIPUS stimulation. Our study demonstrates that LIPUS stimulation directly affects osteogenic cells, leading to mineralized nodule formation. In view of the widespread use of LIPUS for the clinical therapy of periodontal disease, it is likely that LIPUS has an important influence on key functional activities of osteoblasts in alveolar bone.

#### **10) Dose-dependent effect of low-intensity pulsed ultrasound on callus formation during rapid distraction osteogenesis.**

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Distraction osteogenesis of bone or callotaxis causes poor bone formation when the distraction rate is beyond the optimal rate. Low-intensity pulsed ultrasound was reported to enhance fracture healing, treatment of nonunion, and accelerate bone maturation and remodeling during consolidation stage of distraction osteogenesis. In this study, we evaluated the efficacy of different durations of LIPUS treatments during rapid bone lengthening. After 7-day latent period, osteotomized New Zealand white rabbit tibiae were lengthened at the rate of 2 mm per day for 1 week. Two different LIPUS treatment durations of 20 min and 40 min were selected for treatment groups. Rabbits without treatment served as the control group. Plain X-ray, peripheral quantitative computed tomography (pQCT) and histology were performed to assess bone acquisition in the distraction callus. The results showed that LIPUS increased bone mineral content and volume of the mineralized tissue of distraction callus in a dose-dependent manner. The different regions of distraction callus exhibited various spatial response to LIPUS treatment. Moreover, LIPUS enhanced dose-dependant endochondral formation. Compared with 20-min treatment, the 40-min LIPUS treatment was a more

favorable treatment duration for bone regeneration in the distraction callus. In conclusion, LIPUS was able to enhance bone regeneration under rapid distraction, and its effect was dose-dependent. Copyright (c) 2006 Orthopaedic Research Society.

### **11) Low intensity pulsed ultrasound accelerated bone remodeling during consolidation stage of distraction osteogenesis.**

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Bone regeneration in distraction osteogenesis occurs under tensile stress with axial rhythmic distraction after osteotomy. In this study, we evaluated if the low intensity pulsed ultrasound (LIPUS) was also effective on enhancement of bone remodeling during consolidation stage of distraction osteogenesis. Open osteotomy of seventeen 18-week-old female New Zealand rabbit tibiae were performed. The distraction was applied with the rate of 1 mm per day. LIPUS (30 mW/cm<sup>2</sup>, 1.5 MHz) was delivered for 20 min per day during 4-week consolidation stage (n=10). The animals without treatment served as sham group (n=7). Plain X-ray, peripheral quantitative computational tomography (pQCT), and torsional test were performed. Results showed that smaller radiolucent interzone of LIPUS treatment group was gradually occupied by calcified tissue in plain X-ray at week 2. The bone mineral density (BMD) measured on radiographs increased by 9.18% in the LIPUS group. Bone mineral content (BMC), hard callus volume, and bone strength index (BSI) measured by pQCT were 83%, 116%, and 94%, respectively, in LIPUS group that were significantly greater than those of the controls. At the 4th week, LIPUS-treated callus showed the development of neocorticalization in the proximal and distal region. The BMC, hard callus volume, and BSI of LIPUS group decreased and was not significantly different from control. This was also confirmed by the maximum torque of LIPUS-treated callus (1424.2±457.3 N . mm) obtained at week 4, which did not differ from that of the sham group (1968.8±895.1 N . mm). In conclusion, the effective period of LIPUS treatment was at the initial stage of consolidation, with accelerated bone formation and remodeling. (c) 2005 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res.

## **12) Low-intensity pulsed ultrasound for bone healing: an overview.**

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Low-intensity ultrasound is a biophysical form of intervention in the fracture-repair process, which through several mechanisms accelerates healing of fresh fractures and enhances callus formation in delayed unions and nonunions. The goal of this review is to present the current knowledge obtained from basic science and animal studies, as well as existing evidence from clinical trials and case series with the different applications of ultrasound in the management of fractures, delayed unions, nonunions and distraction osteogenesis. Low-intensity pulsed ultrasound is currently applied transcutaneously, although recent experimental studies have proven the efficacy of a trans-osseous application for both enhancement and monitoring of the bone healing process with modern smart implant technologies.

## **13) Pulsed low-intensity ultrasound: a new salvage procedure for delayed unions and nonunions after leg lengthening in children.**

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From 1998 to 2001, 112 lengthening procedures with or without deformity correction were performed in 108 children by external fixation with the Ilizarov method. Of these cases, 16.9% did not lead to a solid bone consolidation. Two children were operated the second time, mainly because of the parent's decision. Seventeen delayed unions or nonunions in 13 children were treated with low-intensity pulsed ultrasound. All 17 cases healed within 3 to 12 months without any risk of surgical intervention.

#### **14) Low-intensity pulsed ultrasound: effects on nonunions.**

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To study the efficacy of low-intensity pulsed ultrasound (US), or LIPUS, of 85 treated nonunion cases with a minimum fracture age of 8 months, 67 cases met the study criteria. These were: no surgical intervention during 4 months before US treatment and radiographically ceased healing for 3 months before US. In a self-paired control study, the mean fracture age of the 67 patients was 39 +/- 6.2 months. After a daily 20-min US treatment at home for an average of 168 days, 85% (57 of 67) of the nonunion cases were clinically and radiographically healed. The study did not include any cases that were malaligned, grossly instable, actively infected or that had extensive bone loss. The results demonstrate that the specific US can effect heal rates similar to those achieved by surgical means, without the associated risks and complications, and to those achieved by electrical bone growth stimulation or by extracorporeal shock-wave therapy.

#### **15) Transforming growth factor-beta1 mediates the effects of low-intensity pulsed ultrasound in chondrocytes.**

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Low-intensity pulsed ultrasound (LIPUS) has been shown to accelerate fracture healing, but the precise mechanism is still unknown. We used aggregate chondrocyte culture system to analyze LIPUS-induced effects on chondrocytes. First, Northern analyses revealed that LIPUS maintained higher expression levels of type II collagen and aggrecan mRNA and delayed the appearance of type X collagen mRNA expression. We also showed that DNA content was increased and that alkaline phosphatase activity was maintained low by daily treatment. Moreover, LIPUS significantly promoted transforming growth factor (TGF)-beta1 mRNA expression and the protein production at 2 h and 12 h after the treatment, respectively. Furthermore, recombinant TGF-beta1 protein mimicked the LIPUS effect and anti-TGF-beta1 neutralizing antibody reversed all these changes induced by the LIPUS treatment. These results indicate that LIPUS promotes the proliferation and retains the differentiation state of chondrocytes in the aggregate culture and that TGF-beta1 plays an important role in mediating the LIPUS effects in chondrocytes.

## **16) Pulsed low-intensity ultrasound for fracture healing.**

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A large body of evidence confirms the stimulatory effect of pulsed low-intensity ultrasound on fresh fractures, nonunions, and other osseous defects such as osteotomies. This article presents the findings of some of these studies and briefly discusses our current understanding of its molecular mechanisms.

## **17) Low-intensity ultrasound stimulates proteoglycan synthesis in rat chondrocytes by increasing aggrecan gene expression.**

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We evaluated the effect of low intensity-pulsed ultrasound stimulation on rat chondrocytes in vitro using two different 1.0-MHz ultrasound signals with spatial and temporal average intensities of 50 or 120 mW/cm<sup>2</sup>. The pulses had a duration of 200 microseconds and were repeated every millisecond, with corresponding average peak-pressure amplitudes of 230 or 360 kPa, respectively. Cells were stimulated one, three, or five times for 10 minutes each day starting the third day after plating. One group of cells was exposed to sham ultrasound as a control. The cultures were evaluated for cell proliferation (by [<sup>3</sup>H]thymidine incorporation and DNA measurement), steady-state mRNA levels of alpha1(I) and alpha1(II) procollagens and aggrecan (by Northern blotting), and proteoglycan synthesis (by [<sup>35</sup>S]sulfate incorporation). The results revealed that ultrasound causes increases in the level of aggrecan mRNA ( $p < 0.05$ ) and in proteoglycan synthesis ( $p < 0.03$ ) after three and five treatments. Expression of mRNA for alpha1(II) procollagen increased over time, but ultrasound had no stimulatory effect. Expression of mRNA for alpha1(I) procollagen was initially low and remained unchanged with time. Although cell proliferation increased with time in both groups, there was no statistically significant difference between the cultures treated with ultrasound and the controls ( $p = 0.1$ ). The in vitro results support our previous in vivo findings that low-intensity ultrasound stimulates aggrecan mRNA expression and proteoglycan synthesis by chondrocytes, which may explain the role of ultrasound in advancing endochondral ossification, increasing the mechanical strength of fractures, and facilitating fracture repair.

### **18) How does pulsed low-intensity ultrasound enhance fracture healing?**

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Pulsed low-energy ultrasound, a non-invasive therapeutic treatment modality, may improve callus formation and enhance fracture healing by initiating enhanced angiogenesis.

### **19) Power Doppler assessment of vascular changes during fracture treatment with low-intensity ultrasound.**

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**OBJECTIVE:** To study the use of power Doppler sonography for assessing changes in vascularity during treatment of fracture sites with low-intensity ultrasound. **METHODS:** In 6 dogs (3 pairs), subcutaneous dissection of the midshaft of the ulna allowed symmetrical osteotomies to be made with a reciprocation saw. Three dogs were treated and 3 were used as controls. The fracture site was subjected to 1.5-MHz low-intensity ultrasound (30 mW/cm<sup>2</sup>) for 20 minutes daily from a therapeutic ultrasonic device. Gray scale sonography was performed to evaluate the fracture site. Power Doppler sonography was used to assess flow patterns at the fracture site and surrounding soft tissue. A sonographic contrast agent was administered intravenously. The dose was 0.2 mL/kg. **RESULTS:** Power Doppler sonography showed an increase in flow around the fracture site in the treated dogs compared with that in control dogs. Color pixel values on day 7 were 3-fold higher in treated legs than control legs and on day 11 were 33% higher in treated legs than control legs. Enhancement after contrast agent administration was consistently higher in treated legs than control legs. **CONCLUSIONS:** Power Doppler sonography showed increased vascularity around the fracture sites in treated dogs with and without contrast agent administration.

## **20) Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells.**

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The effect of low intensity pulsed ultrasound on human periosteal cells was investigated. Normal human periosteum was obtained to culture the periosteal cells. After characterization, cultures of periosteal cells at Days 2 and 4 were treated with ultrasound for 5, 10, and 20 minutes respectively. Assessments were done to assess total number of viable cells, cell proliferation, alkaline phosphatase activity, osteocalcin secretion, vascular endothelial growth factor expression, and calcium nodule formation. With the cells not treated with ultrasound as the control, the results showed that ultrasound did not affect the total number of viable cells. It stimulated cell proliferation at the early phase of cell culture. The activity of alkaline phosphatase was increased significantly in the culture at Day 4. A similar effect was seen with osteocalcin secretion and the responses were dose-dependent. The vascular endothelial growth factor secretion increased in Day 2 and Day 4 cultures with the dose-dependent effect. Formation of calcium nodules was significantly higher with ultrasound treatment. We think that low intensity pulsed ultrasound stimulated periosteal cell proliferation and differentiation toward osteogenic lineage. The dose-dependent effect on osteogenic activities may modify the existing treatment regimen. Ultrasound treatment should be started from the beginning of fracture healing.

## **21) Complex tibial fracture outcomes following treatment with low-intensity pulsed ultrasound.**

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A clinical study was conducted to investigate the effect of low-intensity pulsed ultrasound (US) stimulation (LIPUS) on the healing of complex tibial fractures. Thirty complex tibial fractures were randomly assigned to the treatment with LIPUS (n = 16) or by a dummy machine (sham-exposed: n = 14). The fractures were immobilized by either internal or external fixations according to the clinical indications. LIPUS was given 20 min/day for 90 days. Fracture healing was monitored by clinical, radiological, densitometric and biochemical assessments. The LIPUS-treated group showed statistically significantly better healing, as

demonstrated by all assessments. Complications were minimal in the LIPUS group. There were two cases of delayed union, with one in each group. There were two cases of infection in the control group. The delayed-union cases were subsequently treated by LIPUS and the infection cases were treated with standard protocol. Fracture healing in these patients was again treated by LIPUS.

**22) Low-intensity pulsed ultrasound and nonsteroidal anti-inflammatory drugs have opposing effects during stress fracture repair.**

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Low-intensity pulsed ultrasound (LIPUS) and nonsteroidal anti-inflammatory drugs (NSAIDs) were used to treat stress fracture. Bilateral stress fractures were induced in the ulnas of 48 adult rats. Animals were divided into two groups (NSAID and VEH), and treated 5 days per week with celecoxib (5 mg/kg) mixed in a vehicle solution of polyethylene glycol and saline (NSAID) or vehicle alone (VEH). One-to-three hours following drug administration, all animals were treated with unilateral active-LIPUS and contralateral inactive-LIPUS. Equal numbers of ulnas from each drug group were histologically evaluated at 2, 4, and 8 weeks following induction of stress fracture. Neither LIPUS nor NSAID influenced bone resorption, but each had significant and opposite effects on intracortical bone formation rate. These effects indicate that LIPUS may be used to facilitate stress fracture repair whereas NSAID may delay tissue level repair of stress fractures. There was no interaction between LIPUS and NSAID, indicating that the beneficial LIPUS effect was not mediated by the cyclooxygenase-2 pathway. LIPUS accelerated stress fracture healing, whereas the NSAID delayed repair. When used in combination, the beneficial LIPUS effect was not impaired by the detrimental NSAID effect. Copyright 2007 Orthopaedic Research Society. Published by Wiley Periodicals, Inc.

### **23) Effects of timing of low-intensity pulsed ultrasound on distraction osteogenesis.**

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We investigated the effects of low-intensity pulsed ultrasound (LIPUS; 30 mW/cm<sup>2</sup> spatial and temporal average) on the timing of LIPUS treatment in distraction osteogenesis. Lengthening of the right tibia was performed in 75 male Japanese white rabbits using unilateral fixators (waiting period, 7 days; distraction rate, 1.5 mm/day; distraction period, 7 days). Rabbits were divided into four groups according to the timing of the LIPUS treatment. Control group had no stimulation. Waiting group was treated with a daily 20-min session of LIPUS during a 7-day latency period. Lengthening group was treated during the lengthening period. Maturation group was treated for the first 7 days after completion of distraction. We evaluated the distraction site by radiography and histology every week for 4 weeks. Bone mineral density (BMD) and mechanical strength were tested and microfocus X-ray computed tomography was performed on specimens 2 weeks after completion of distraction. The lengthening group had greater BMD and mechanical strength than the other groups, bone regeneration was enhanced more in the maturation group than in the control or waiting groups. Histologically, endochondral bone formation in the lengthening and the maturation groups occurred earlier than in the control or waiting groups. These results suggest the LIPUS effect is mediated via endochondral pathways. We concluded that LIPUS stimulates bone formation in distraction osteogenesis and is most effective during the lengthening phase.

### **24) In vitro effects of low-intensity ultrasound stimulation on the bone cells.**

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Mechanical perturbations serve as extracellular signals to a variety of cells, including bone cells. Low-intensity pulsed ultrasound produces significant multifunctional effects that are directly relevant to bone formation and resorption. Ultrasound stimulation has been shown to accelerate bone-defect healing and trabecular bone regeneration. In this study, we use an in vitro bone cell culture model to investigate the effect of low-intensity pulsed ultrasound. The rat alveolar mononuclear cell-calvaria osteoblast coculture system was used in this study. Before treatment, the bone cells were cultured for 3 days to facilitate their

attachment and differentiation. Then, ultrasound exposure (frequency = 1 MHz, intensity = 0.068 W/cm<sup>2</sup>) or sham exposure for 20 min per day was applied until the end of the experiment. Half of the culture media were obtained on the 4th, 5th, 6th, 7th, 8th, 9th, and 10th days for the analysis of cytokines and biochemical parameters. At the end of the experiment, cells were fixed and stained for identification and quantification of the osteoblast and osteoclast cells. After low-intensity pulse ultrasound stimulation, the osteoblast cell counts were significantly increased, whereas the osteoclast cell counts were significantly decreased. The total alkaline phosphatase amount in the culture medium was increased after 7 days of ultrasound stimulation, and tumor necrosis factor- $\alpha$  in ultrasound-stimulated bone cells was significantly increased after the 7th day of culture and reached 474.77% of the control medium on the 10th day of culture. The results of this study suggest that low-intensity ultrasound treatment may have a stimulatory effect on bone-healing processes. Copyright 2001 John Wiley & Sons, Inc. *J Biomed Mater Res* 57: 449-456, 2001

**25) Low intensity pulsed ultrasound exposure increases prostaglandin E2 production via the induction of cyclooxygenase-2 mRNA in mouse osteoblasts.**

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Both prostaglandin E2 (PGE2) and low intensity pulsed ultrasound (U/S) exposure have been reported to accelerate fracture repair. We hypothesized that these two pathways are interactive. To verify this hypothesis, we examined the regulation of PGE2 production by U/S exposure (sine wave of 1.5MHz repeating at 1kHz, 30mW/cm<sup>2</sup>, 20 minutes) in mouse osteoblastic cell line, MC3T3-E1. The production of PGE2 in osteoblasts was augmented by U/S, which was threefold at 60 min. in comparison with unexposed samples. Then we evaluated the expression of cyclooxygenase-2 (COX-2) mRNA, which is a critical enzyme for PGE2 production. U/S rapidly up-regulated the expression of COX-2 mRNA in a time dependent manner. In addition, PGE2 production by U/S was drastically suppressed by a selective inhibitor of COX-2. These results provide strong evidence that PGE2 production in osteoblasts is dependent upon the induction of COX-2 mRNA expression by U/S and offer a mechanistic insight into how U/S accelerates fracture repair. Copyright 1999 Academic Press.

## **26) Effects of low-intensity pulsed ultrasound on the differentiation of C2C12 cells.**

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Low-intensity pulsed ultrasound (LIPUS) is known to accelerate bone regeneration, but the precise cellular mechanism is still unclear. The purpose of this study was to determine the effect of LIPUS on the differentiation of pluripotent mesenchymal cell line C2C12. The cells were cultured in differentiation medium with or without the addition of LIPUS stimulation. The ultrasound signal consisted of 1.5 MHz at an intensity of 70 mW/cm<sup>2</sup> for 20 min for all cultures. To verify the cell lineage after LIPUS stimulation, mRNA expression of cellular phenotype-specific markers characterizing osteoblasts (Runx2, Msx2, Dlx5, AJ18), chondroblasts (Sox9), myoblasts (MyoD), and adipocytes (C/EBP, PPARgamma) was studied using real-time polymerase chain reaction analysis. The protein expression of Runx2 and activated phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (p38 MAPK) were performed using Western blotting. The mRNA expression of Runx2, Msx2, Dlx5, AJ18, and Sox9 was increased markedly by the LIPUS stimulation, whereas the expression of MyoD, C/EBP, and PPARgamma was drastically decreased. In the Western blot analysis, LIPUS stimulation increased Runx2 protein expression and phosphorylation of ERK1/2 and p38 MAPK. Our study demonstrated that LIPUS stimulation converts the differentiation pathway of C2C12 cells into the osteoblast and/or chondroblast lineage via activated phosphorylation of ERK1/2 and p38 MAPK.

## **27) Ultrasound-biophysics mechanisms.**

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Ultrasonic biophysics is the study of mechanisms responsible for how ultrasound and biological materials interact. Ultrasound-induced bioeffect or risk studies focus on issues related to the effects of ultrasound on biological materials. On the other hand, when biological materials affect the ultrasonic wave, this can be viewed as the basis for diagnostic ultrasound. Thus, an understanding of the interaction of ultrasound with tissue provides the scientific basis for image production and risk assessment. Relative to the bioeffect or risk studies, that is,

the biophysical mechanisms by which ultrasound affects biological materials, ultrasound-induced bioeffects are generally separated into thermal and non-thermal mechanisms. Ultrasonic dosimetry is concerned with the quantitative determination of ultrasonic energy interaction with biological materials. Whenever ultrasonic energy is propagated into an attenuating material such as tissue, the amplitude of the wave decreases with distance. This attenuation is due to either absorption or scattering. Absorption is a mechanism that represents that portion of ultrasonic wave that is converted into heat, and scattering can be thought of as that portion of the wave, which changes direction. Because the medium can absorb energy to produce heat, a temperature rise may occur as long as the rate of heat production is greater than the rate of heat removal. Current interest with thermally mediated ultrasound-induced bioeffects has focused on the thermal isoeffect concept. The non-thermal mechanism that has received the most attention is acoustically generated cavitation wherein ultrasonic energy by cavitation bubbles is concentrated. Acoustic cavitation, in a broad sense, refers to ultrasonically induced bubble activity occurring in a biological material that contains pre-existing gaseous inclusions. Cavitation-related mechanisms include radiation force, microstreaming, shock waves, free radicals, microjets and strain. It is more challenging to deduce the causes of mechanical effects in tissues that do not contain gas bodies. These ultrasonic biophysics mechanisms will be discussed in the context of diagnostic ultrasound exposure risk concerns.

### **28) Stimulation of bone cell differentiation by low-intensity ultrasound--a histomorphometric in vitro study.**

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Several investigations have established a stimulatory effect of low-intensity ultrasound treatment on osteogenesis and fracture healing. The objective of this study was to examine whether the stimulatory effect of low-intensity ultrasound results in increased bone cell activity and/or proliferation. Twenty-four paired triplets of metatarsal bone rudiments of twelve 17-days-old fetal mice were dissected and divided into two groups. One group of bone rudiments was treated with pulsating low-intensity ultrasound (30 mW/cm<sup>2</sup>; 1.5 MHz) for 20 min/day for a period of 3 or 6 days. The other group served as controls. After culture, the metatarsal bone rudiments were prepared for computer aided light microscopy. The following histomorphometric parameters were determined: length, width and volume of the calcified cartilage and of the bone collar, and cell number. GLM analysis demonstrated that bone collar volume and calcified cartilage percentage

were significantly higher in the ultrasound-stimulated rudiments compared to untreated controls. Further, the calcified cartilage volume bordering the hypertrophic zone was significantly higher than in the center of the bone rudiment. Ultrasound treatment did not change the number of the cells. These results suggest that the stimulatory effect of low-intensity ultrasound on endochondral ossification is likely due to stimulation of bone cell differentiation and calcified matrix production, but not to changed cell proliferation.

### **29) Pulsed low intensity ultrasound enhances mineralisation in preosteoblast cells.**

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Pulsed low intensity ultrasound has been shown to be highly efficacious in the treatment of nonunion fractures and in the acceleration of fresh fracture healing. MC3T3-E1 subclone 14 cells were cultured for up to 25 days either with or without a daily treatment with low intensity pulsed ultrasound. It was determined that, on day 10 there was a dramatic increase in alkaline phosphatase and MMP-13 mRNA levels detected in ultrasound-treated cultures compared with untreated controls. The activity of alkaline phosphatase was significantly increased at days 6, 8 and 10. On day 10, the amount of mineralisation within cultures, assessed using alizarin red staining, was significantly increased in ultrasound-treated cultures compared with untreated controls. These results suggest that one of the mechanisms that low intensity pulsed ultrasound has on fracture repair is to enhance the process of endochondral ossification where the soft callus is converted to mineralised hard callus.