Low-Intensity Pulsed Ultrasound Stimulation for Bone Fractures Healing

A Review

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Low-intensity pulsed ultrasound (LIPUS) is a developing technology, which has been proven to improve fracture healing process with minimal thermal effects. This noninvasive treatment accelerates bone formation through various molecular, biological, and biomechanical interactions with tissues and cells. Although LIPUS treatment has shown beneficial effects on different bone fracture locations, only very few studies have examined its effects on deeper bones. This study provides an overview on therapeutic ultrasound for fractured bones, possible mechanisms of action, clinical evidences, current limitations, and its future prospects.

Key Words—bone healing; delayed unions; fresh fractures; low-intensity pulsed ultrasound; nonunions; ultrasound therapy

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Abbreviations

BMD, bone mineral density; COX-2, cyclooxygenase-2; FDA, Food and Drug Administration; LIPUS, low-intensity pulsed ultrasound; MSC, mesenchymal stem cells; PGE2, prostaglandin E_2; RANKL, receptor activator of nuclear factor kappa-B ligand; VEGF, vascular endothelial growth factor

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Post-fracture rehabilitation has been a major clinical burden worldwide, which results in limited mobility and weakened musculoskeletal function of an able body. It is considered as a complex orthopedic challenge, where insufficient and delayed treatment can cause numerous complications such as bone weakening, abnormal healing, and function losing.[1] The duration of the patient's recovery from the fracture is determined by certain factors such as the site of injury, strength of impact, types of bone involved, and biological processes.[2,3] A fracture or a broken bone may result in nonunion, a condition of the bone that fails to heal even after 9 months of fracture; whereas delayed unions are defined as the condition of the bone that failed to show radiographic progression between 3 and 9 months after a fracture event.[4] It is estimated that 10% of the people are facing problems related to nonunion and delayed unions in the United States alone.[5] It was estimated that nearly 4.39 million people experienced fractures due to trauma in China in 2014.[6] Even though a fractured bone can undergo self-regeneration, there are unhealed bones, with the cause remaining unknown.[7] Many orthopedic surgeons are concerned about the fracture care of the patients.[8] Traction is the most commonly used method classically in which alignment of bone is done by stretching certain parts around the broken areas. For some cases, immobilization of fractured bone is needed, by using braces, plaster casts, and splints to stabilize the broken bone. Furthermore, surgical insertion of metal plates, rods, and screws are used to stablilize and fix the bone

soon after a severe fracture. Apart from these, treatments, analgesics, and anti-inflammatory medications are commonly used as pain relief and to suppress the inflammation caused by the injury.[9–11] However, the choice of the treatment procedures for the fracture healing greatly depends on many factors, including the nature of fracture, location of the bone, type of fracture, and so on. Recent developments in stimulation technologies on bone fracture healing have increased significant interests of the researchers to find an effectual way to improve the healing and to speed up the recovery. Among them, ultrasound stimulation has been found to be a safe approach to accelerate the bone fracture healing process.[12,13] Studies reported that ultrasound stimulation enhances bone formation by accelerating the process at the inflammatory stage[14–16] and subsequent phases of fracture healing.[17,18]

Therapeutic Ultrasound for Fractured Bone

Ultrasound stimulation for the human body is safe and noninvasive. The therapeutic use of ultrasound started in the early 1930s.[19] Initially, a frequency of 800 kHz and an intensity between 4000 and 5000 mW/cm^2 were used in the treatment of neuralgia, myalgia, and other diseases. The higher ultrasound intensity caused more heating in biological tissues.[19,20] In the 1940s, ultrasound treatment was limited to treat young bones in humans and dogs.[21] It was criticized that ultrasound treatment might induce bone damages until the discovery of Barth,[22] who reported that a low dose of ultrasound did not affect the bone or surrounding tissues. Meanwhile, other early clinical studies revealed that ultrasound stimulation with higher intensity ranging from 5000 to 25000 mW/cm^2 caused complications such as necrosis, ceased bone healing and formation of fibrous tissue.[23,24] It was also reported that ultrasound had the ability to stimulate osteogenesis.[25] Maintz used ultrasound with low intensities ranging from 500 to 2000 mW/cm^2 for treatment in limbs of rabbit and noticed the formation of new periosteal bone.[26] The first successful formation of new callus in the fracture site was noticed using continuous ultrasound stimulation with an intensity of 1500 mW/cm^2.[27] In order to minimize the thermal effects on soft tissues, it was proposed to use low-intensity and pulsed ultrasound signals for stimulation, which resulted in bone growth in tibia of rabbits with an intensity of 200 mW/cm^2.[28] Later in 1983, Xavier and Duarte treated 27 nonunion cases with low-intensity pulsed ultrasound (LIPUS) and generated successful treatment results.[29] When the nonunions were treated for 20 minutes per day for 18 days, they showed a success rate of 70%. The efficacy of LIPUS in fracture repair process with 38% of acceleration rate in tibial fractures was further demonstrated by Heckman and colleagues in 1994 with a spatial average-temporal average intensity of 30 mW/cm^2.[30]

The first commercially available LIPUS product is EXOGEN® device, which was approved by the Food and Drug Administration (FDA) for treatment of fresh fractures in 1994. Kristiansen et al reported a shortened time to achieve bone union, acceleration of radiographic stage of healing, and a significant decrease in loss of reduction was achieved with LIPUS treatment.[31] In the year 2000, FDA approved the use of LIPUS for nonunion treatment.[32] The most commonly prescribed LIPUS bone growth stimulators are Exogen 4000+™, Exogen 3000™, Exogen 2000+™, and Exogen 2000™.[33] It was reported that LIPUS with an ultrasound intensity of 30 mW/cm^2 and a dosage of 20 minutes/day could accelerate the bone maturation in distraction osteogenesis in rabbits.[34] LIPUS treatment healed 86% of cases in nonunions in an average of 22 weeks.[32] Heybeli et al used low ultrasound intensity of 11.8 mW/cm^2 and demonstrated increased bone density in rat femora as well as radiographic fracture healing.[35] A further study reported by Rutten and colleagues showed LIPUS was effective in the treatment of established tibial fracture, for which they achieved 73% of overall success rate with 40% reduction in healing time.[36] The studies conducted by Gebeaur et al and Nolte et al in the treatment of nonunion fractures have achieved 85 and 86% of success rate, respectively.[32,37] Because of the positive reports from the above studies, the usage of LIPUS on delayed unions and nonunions was supported by the UK National Institute for Health and Care Excellence (NICE) in 2010.[38] The most widely used LIPUS parameters for stimulation consisted of an ultrasound frequency of 1.5 MHz burst waveform with 200 μs on and 800 μs off, signal repetition of 1 kHz, a spatial

average-temporal average intensity (I_{SATA}) of 30 mW/cm^2, and a dosage of 20 minutes per day.[12] Up to now, most of the experiments reported have used EXOGEN® devices approved by FDA, though there are other systems being used, with the intensity of the pulsed wave commonly below 100 mW/cm^2.[39]

Role of LIPUS on Fracture Healing

LIPUS therapy has been widely accepted for enhancing endochondral bone formation.[17,34] It has also been demonstrated to increase the blood flow near the injured area[18] and reduce the healing time in the cases of scaphoid fractures, tibial, and distal radius fractures.[30,31,40] The therapeutic ultrasound used in LIPUS is harmless and does not require any subsequent surgeries,[41] and higher efficiency of the treatment can be achieved when it is performed in the initial stage of the fracture impact. In addition, LIPUS treatment can be used along with the metallic fixtures, without causing any adverse side effects to the tissues.[42] Apart from these, the clinical application of ultrasound therapy has been extended to healing of maxillofacial bones.[43–45] LIPUS has shown positive effects on patients, irrespective of age, smoking, fracture gap, and absence of fibular fracture as well as distal fracture location.[46] The acceleration of callus formation in diabetic fractures has also been reported.[47] Konno et al further reported that LIPUS increased the acceleration of callus formation in the stimulation side compared with the nonstimulation side.[16]

Clinical Evidences on Fracture Healing

Effects on Fresh Fractures

Fractures less than 1 week are considered as fresh fractures.[48] It is clinically proved that LIPUS plays an important role in the fresh fracture healing with an ultrasound intensity of 30 mW/cm^2.[49] Some of the studies that showed effective results using LIPUS treatment on fresh fractures are listed in Table 1.

These studies demonstrated that LIPUS could effectively reduce the healing time in fresh fractures. One factor that affects healing process is smoking habit.[31] LIPUS stimulation has shown acceleration of bone formation and reduction of healing time even in smoking persons.[30,31,50] The meta-analysis conducted by Lou et al also suggests that LIPUS treatment has positive effects on adult fresh fractures.[49] However, some patients were reported to have adverse effects with LIPUS treatment, such as muscle cramps, swelling in the cast, and skin irritation.[30,53] Furthermore, some studies reported that LIPUS did not reduce the healing time and functional recovery with metallic fixations.[50,52,55] A recent review has suggested that LIPUS holds Grade B recommendation in case of fresh fracture healing.[33] Furthermore, LIPUS is more suitable for nonoperative treatments because during the operative treatments there is a higher possibility of osteonecrosis.[56] The studies listed in Table 1 show that the functional recovery of the LIPUS treatment has been sparsely studied. Future research should be focused on the treatment outcomes such as pain reduction, weight-bearing ability, and time to return to work.

Effects on Nonunions

Nonunions are the fractures that have failed to heal even after 9 months of the fracture event and with the possibility of healing with or without treatment intervention.[4] Treatment of nonunions using internal or external fixations along with bone grafts is considered as the ''gold standard.'' The surgical treatments for nonunions can achieve success rates between 70 and 90%, depending on the location of the fracture and the type of treatment.[41] The success rate for nonunions when treated with LIPUS is determined by three key factors, namely age of the fracture, size of the maximum gap, and stability of the fracture site.[57] The detailed outcomes of LIPUS-treated nonunion fractures at various bone fracture locations are shown in Table 2.

Nolte et al reported that LIPUS treatment for established nonunions had no side effects.[58] Ultrasound helps to achieve the bone union similar to surgical means without causing any complications to patients, which is particularly crucial to the elderly patients with low healing ability.[37] It has been discovered that the efficiency of bone union using LIPUS treatment was closely related to the time duration between the most recent surgery and ultrasound

Table 1. Details of Studies About the Effects of LIPUS on Fresh Fractures

Study	Location of Fracture	No of Patients (LIPUS Group)	No of Patients (Control Group)	Start of Treatment After Fracture (Days)	Treatment Time per Day (Minutes)	Treatment Period (Days)	Maximum Healing Period (Days)	Surgery or Not	Patient Follow-up Rate	Healing Time (Days)	Major Findings
Heckman 1994[30]	Tibial shaft	33	34	4 ± 0.3	20	140	180	No	88%	Active group: 96 ± 4.5 Control group: 154 ± 13.7	Significant decrease in healing time, weight bearing not affecting efficacy of the results, smoking patients healed in 115 ± 11.2 days, (LIPUS accelerates bone formation even in smokers), 38% clinical and radiographic acceleration
Kristiansen 1997[31]	Radius	30	31	3 ± 0.4	20	70	128	No	75%	Active group: 61 ± 3 control group: 98 ± 5	Significant decrease in healing time, weight bearing excluded, smoking persons healed in 48 ± 5 days, 34–39% acceleration
Emami 1998[50]	Tibial shaft	15	17	4	20	75	361	Yes	100%	Active group: 155 ± 22 Control group: 125 ± 11	Healing time not reduced with intramedullary rods fixed on fractures, healing time reduced in smokers up to 40–50%
Mayr 2000[40]	Scaphoid	15	15	2 ± 3.5	20	42	43.2 ± 10.9	No	100%	Active group: 43.2 ± 10.9 Control group: 62 ± 19.2	Decrease in healing time, 31% of acceleration of bone formation, 70% of cases healed
Leung 2004[51]	Tibia	16	14	N/A	20	90	84 ± 14	Yes	100%	Active group: 66 ± 15 Control group: 110 ± 21	Disappearance of tenderness of fracture noticed at 6.1 ± 2.1 weeks, full weight bearing at 15 weeks
Handolin 2005[52]	Lateral malleolus	11	11	14	20	42	63	Yes	100%	Active group: 84 Control group: 84	Bone healing was assessed using multidetector CT, no significant effect on lateral malleolar fracture healing
Lubbert 2008[53]	Clavicle shaft	52	49	5	20	28	27	No	85%	Active group: 26.77 Control group: 27.09	Ability to return to work in 17 days, healing time not reduced
Gan 2014[54]	Metatarsals, fibula, tibia	10	13	N/A	20	28	84	No	100%	N/A	No significant changes in the MRI grading and bone marrow edema size measured.
Busse 2016[55]	Tibial shaft	250	251	N/A	20	365	N/A	Yes	73%	N/A	9% ability to weight bearing, reduction in healing time, fails in improving functional recovery with intramedullary nails

LIPUS, low-intensity pulsed ultrasound; N/A, not available or not reported.

Table 2. Details of Studies About the Effects of LIPUS on Nonunions

Study	Fracture Location	No. of Patients	Start of Treatment After Surgery (Days)	Treatment Time per Day (Minutes)	Treatment Period (Days)	Max Healing Period (Days)	Patient Follow-up Rate	Overall Success Rate	Major Findings
Nolte 2002[58]	Femur, tibia, radius, scaphoid	29	< 90	20	119	154	93%	91%	Healing noticed in smokers, ability to bear weight without pain
Gebauer 2005[37]	Femur, tibia, radius, humerus	66	120	20	168	180	94%	85%	Healing noticed in few smokers; higher success rate seen in ≤1 year of fracture time
Rutten 2007[36]	Tibia	71	90	20	160	179	98%	73%	Healing rate in smokers 63%, nonsmokers 84%, better healing achieved if the treatment started within 3 months
Jingushi 2007[59]	Humerus, radius, femur, tibia, ulna	72	180	20	219	219	N/A	89.7%	Higher efficiency noticed if the treatment started within 6 months after surgery
Zura 2015[60]	Multiple bones	767	365	20	179.5	179.5 ± 127.9	21%	86.2%	Data from patient registry, higher success rate noticed in fractures >1 year
Elvey 2020[61]	Hand and wrist bones	26	84	20	104	365	100%	62%	2 nonunion cases had second surgery after 12 months of LIPUS treatment. No significant difference found in bone union rate of LIPUS vs surgery

LIPUS, low-intensity pulsed ultrasound; N/A, not available or not reported.

treatment and it should be less than 3 months.[36] Furthermore, it is showed to be more effective in the treatment of postoperative nonunions when LIPUS therapy was started within 6 months after the surgery.[59] It was also reported that LIPUS was an effective approach for acute fractures like long bones, especially nonunions.[62] It appears that better bony union would be achieved if the treatment is started at the right time after the fracture events. In addition to timing, a recent report on established nonunions demonstrated that LIPUS treatment on bone healing also depended on factors such as fracture type and treatment approach to the injury.[63] Meanwhile, it was reported that LIPUS failed to promote the healing of nonunion fractures in the case of the fracture gap size greater than 1 cm, where the average healing time was 5.3 months, with only 20 out of 60 patients reported to have bone formation.[64] The systematic review and meta-analysis conducted by R. Leighton et al supported the use of LIPUS treatment for nonunions as the average success rate was greater than 80%.[65] In summary, LIPUS treatment appears to be a suitable substitute for the surgical therapy in nonunions for its lower cost and fewer complications. However, there was no report that LIPUS on nonunion cases show any improvement on weight-bearing ability, pain reduction, and time reduction in radiographic healing. Further research in this area needs to be conducted.

Effects on Delayed Unions

Delayed union can be referred to as the fractures that failed to show radiographic progression between 3 and 9 months.[66] Similar to nonunions, the treatment is mostly preferred to be started within 6 months after the most recent operation to achieve better results.[59] LIPUS effects on delayed unions are summarized in Table 3.

LIPUS has been reported to improve bone mineral density (BMD), thus can be an adjuvant therapy after surgical intervention.[67] One study has reported that LIPUS could achieve 74.3% bone union without any surgical procedures.[57] It is also demonstrated that a higher healing rate could be achieved if the treatment was started immediately after the injury in delayed union fractures. LIPUS has received C grade recommendation in delayed unions.[33]

Effects on Distraction Osteogenesis

Distraction osteogenesis is performed to lengthen the bones by using an external fixator.[71] This method is usually carried out to treat the deformities of long bones greater than 3–4 cm. The treatment is complicated, which involves presurgical, surgical, and post-surgical stages. Some clinical studies of LIPUS treatment on distraction osteogenesis are listed in Table 4.

LIPUS has shown significant improvement in BMD of tibial bones and promotes bone maturation by reducing the treatment time in distraction osteogenesis.[72,73] Furthermore, it was also reported that LIPUS helps to accelerate callus maturation by 27% and reduced the usage time of external fixator in distraction osteogenesis.[75] However, the chain smokers treated with LIPUS after distraction osteogenesis in tibia did not attain a higher success rate.[71]

Besides the human studies, Shimazaki et al investigated 70 Japanese male rabbits by performing callus distraction on right tibia.[34] Ultrasound treatment was administered 20 minutes every day to 35 rabbits whose normal distraction rate was 0.5 mm/12 hours, and the remaining were in the control group. Both the groups were subjected to mechanical testing, BMD measurement, and radiography examination, and the results showed a higher rate of callus maturation in the LIPUS group. Tis et al reported that LIPUS increased callus size with reduced fibrous tissues while the BMD remains unchanged.[77] A meta-analysis conducted in 2016[78] concluded that LIPUS therapy was more effective in the earlier stages of distraction osteogenesis, and further efficacy of LIPUS should be confirmed with more clinical trials. The effects of LIPUS on distraction osteogenesis have been classified as Grade B recommendation.[33] Hence, LIPUS works well on distraction osteogenesis by lessening the consolidation period. The major drawback of this method is its being time consuming, as the consolidation phase in distraction osteogenesis takes up to 6 months, which leads to a prolonged treatment period, and the treatment period depends on the size of the bone defect.[78]

Effects of LIPUS on Mechanical Properties of Bone

LIPUS has an influence over the mechanical ability of bone by increasing bone density.[35] Earlier studies conducted by Azuma et al demonstrated the

Table 3. Details of Studies About the Effects of LIPUS on Delayed Unions

Study	Fracture Location	No. of Patients	Start of Treatment After Fracture (Day)	Treatment Time per Day (Minutes)	Treatment Period (Days)	Maximum Healing Period (Days)	Patient Follow-up Rate	Overall Success Rate	Major Findings
Schofer 2010[67]	Tibial shaft	51	≥120	20	112	112	90%	N/A	Increased BMD, reduced bone gap area
Rutten 2012[68]	Fibula	20	>180	20	150	365	100%	65%	LIPUS decreased healing time by 29%
Watanabe 2013[57]	Long bones	101	90	20	Until healed	180	100%	74.3%	Some bones unhealed due to instability, gap size not >9 mm
Farkash 2015[69]	Scaphoid	29	>90	20	67	67	N/A	76%	Immobilization to be a major part of the treatment
Teoh 2018[70]	Metatarsal	30	101	20	75	88 ± 5.9	100%	90%	Three patients found with nonunion even after LIPUS treatment

BMD, bone mineral density; LIPUS, low-intensity pulsed ultrasound; N/A, not available or not reported.

relationship between duration and mechanical properties of rat femur.[17] The rat group was divided into three phases and treated with LIPUS for up to 25 days. The acceleration of fracture healing was noticed with no adverse effects irrespective of treatment duration. The mechanical testing demonstrated that the stiffness and torque of bone was increased in all the phases of treatment. They suggested that longer treatment could be more effective. A recent study with rat tibial metaphyseal bone showed that the stiffness of the bone increased after the LIPUS therapy for about 2 weeks.[13] In another study with a group of mice treated with LIPUS intensity of 30 mW/cm^2 on the femur for 4 weeks, the femoral elastic modulus and ultimate strength were enhanced by 42 and 39%, respectively.[79] The research conducted by Lu et al on bone–tendon junction healing demonstrated that LIPUS results in higher ultimate strength, failure load, and energy at failure.[80] It has been suggested that LIPUS has influences over certain factors, including collagen cross-linking, collagen alignment, and porosity that determine the mechanical properties of the bone.[79] LIPUS treatment on rabbit mid-tibia that had undergone osteotomy with fixator produced significant improvement in the callus mineral density at the end of 8th week, but no significant effect on the flexural strength of the fractured bone was observed.[81] Overall, it has been demonstrated clearly that LIPUS stimulation helps in improving the mechanical properties of bone during fracture healing process.

Possible Mechanisms of LIPUS on Bone Healing

After a bone fracture occurs, it undergoes three phases of healing including the inflammatory, reparative, and remodeling phases.[82] During the inflammatory phase, blood vessels supplying the periosteum and bone are injured, and a mass of blood clot is formed near the fracture site, which is called as hematoma. The area of fracture becomes swollen and painful. Following the inflammatory phase, new blood vessel formation occurs, which is called as angiogenesis. At the reparative phase, proliferation and osteoblastic differentiation of mesenchymal stem cells (MSCs) take place to form soft callus that

Table 4. Details of Studies About the Effects of LIPUS on Distraction Osteogenesis

Study	Fracture Location	No. of Patients	Treatment Time per Day (Minutes)	Treatment Period (Days)	Distraction Rate (mm/Day)	Surgery or Not	Follow-up (Year)	Major Findings
Tsumaki 2004[72]	Tibia	21	20	30	1	Yes	N/A	Increased mineralization of callus with active group: 0.20 ± 0.12 g/cm^2, and control group: 0.13 ± 0.10 g/cm^2
Schortinghuis 2005[73]	Mandible	8	20	30	6.6 ± 1.1	Yes	2.6	Mineralized tissue formed in distraction gap: 1.9 ± 1.7 mm^2
El-Mowafi 2005[71]	Tibia	20	20	30	1	Yes	1	Chain smoker does not show improvement in success rate
Dudda 2011[74]	Tibia	36	20	165.1 ± 95.7	N/A	Yes	0.7	Reduced healing time, callus maturation at distraction gap: 2 cm, fixator gestation period: active group: 218.6 days, control group: 262.2 days
Salem 2014[75]	Tibia	21	20	33	1	Yes	N/A	Bone density: 0.49 ± 0.14 g/cm^2, increased by 33%
Simpson 2016[76]	Tibia	62	20	133	0.75	Yes	N/A	No significant difference in time to maturation of bone or length of the distraction: active group: 101.9 days, control group: 102.7 days

N/A, not available or not reported.

eventually matures to hard callus. The last phase is the bone remodeling, where the excess callus is removed by osteoclasts to achieve the actual structure of bone (Figure 1).

Extensive research studies have been conducted to demonstrate the biological effects of LIPUS on all the phases of fracture healing. It has been demonstrated by Azuma and colleagues that LIPUS has advantageous effects on all the phases of fracture healing in closed femoral fracture of rats.[17] During the inflammation and callus formation stages, ultrasound boosts the deposition of certain proteins such as collagen and aggrecan.[83] Harrison et al studied the molecular mechanism and found that LIPUS generated "nano motion" at the fracture site.[84] When the ultrasound waves are transmitted to the bone through the tissues, the cells around the fracture site convert the biomechanical stimulation into biochemical response through integrins: the molecular mediators that are highly important in sensing the mechanical signals (Figure 1). A cadaveric experiment demonstrated that bone responded to mechanical stimulation and displacement, which is called as "micro motion."[85] Another possible biological effect of ultrasound stimulation is that it creates mechanical stress in tissues, which further promotes osteogenesis, protein synthesis, calcium uptake, and DNA synthesis in various types of cell.[86] In summary, previous studies revealed that the mechanism of LIPUS should be understood from different types of effects happened at various phases of fracture healing.

Inflammatory Response at the Early Phase

The effect of LIPUS on the inflammatory phase was investigated using mouse osteoblastic cell line by exposing it to ultrasound with an intensity of 30 mW/cm^2 for 20 minutes.[87] The results were compared with control samples, and it was found that ultrasound has augmented the production of prostaglandin E_2 (PGE2). A greater upregulation of cyclooxygenase-2 (COX-2) was also observed, which is purely responsible for the production of PGE2 by initiating the inflammatory response. These prostaglandins are produced by the osteoclastic cells, which have a large amount in bones.[88] This reaction is vital for the inflammatory phase of healing. During the inflammatory phase, certain mediators such as TGF-b, PGE2, platelet-derived growth factor

(PDGF), basic fibroblast growth factor (bFGF), and interleukin-1 (IL-1) are released from platelets.[87] Although a few publications have shown that the expression of COX-2 has increased more after the application of LIPUS,[89,90] reduced expression of COX-2 in older mice was also noticed during ultrasound stimulation.[91] In addition, COX-2 and prostaglandins are the key processes involved in mineralization and remodeling phases of bone healing.[62] The findings of Tang et al demonstrated that when cells cultured for 24 hours were subjected to ultrasound stimulation, the expression of integrins and COX-2 in osteoblasts was increased.[89] Some in vitro studies have shown that PGE2 increased the synthesis of collagen in the cultured bones and further stimulated the cell proliferation of osteoblastic cells.[92–94] These results have shown that PGE2 is essential in the bone repair process, and it has been further reported that the production of PGE2 by ultrasound stimulation depends on exposure time.[87] Furthermore, COX-2 upregulation was noticed after 15 minutes of LIPUS exposure and the expression reached to peak at 60 minutes and diminished after 3 hours.[87] Hence, the results of these studies lead to a conclusion that LIPUS enhances the osteoblastic activity and COX-2 generation with inhibition of osteoclastic activity at the fracture site during the early inflammatory stage of the healing process. It is found that LIPUS induces an anti-inflammatory response at the fracture site (Figure 1). A recent study conducted by Yang et al demonstrated LIPUS could activate anti-inflammatory response by upregulating the anti-inflammatory gene expression.[14] They also found that LIPUS could improve immunosuppressive cells and enhance exosome biogenesis along with docking. Moreover, it was shown that lower ultrasound intensity also promoted anti-inflammatory effects.[95] In addition, few studies have reported that LIPUS inhibited the inflammatory factors.[96,97] According to the published results, we have a good understanding that LIPUS enhances inflammation; however, more research efforts are needed to understand the exact mechanism behind the anti-inflammatory response of LIPUS.

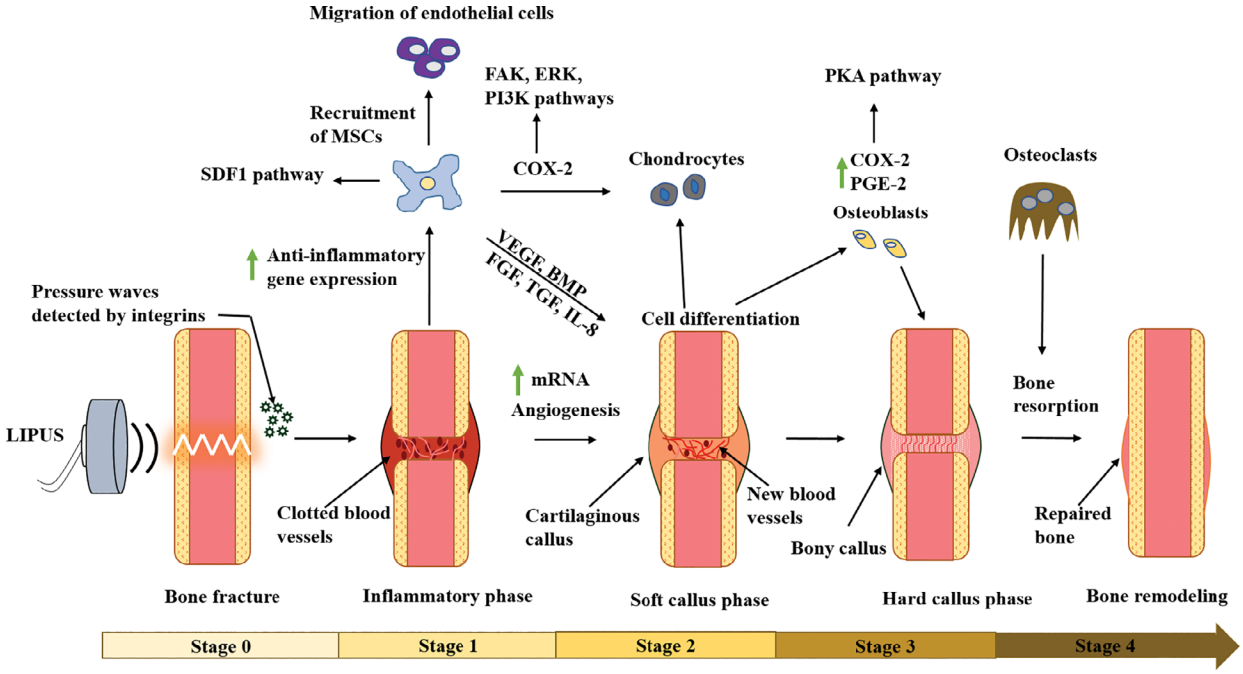
Angiogenesis at the Fracture Site

When bone is broken, the damage of blood vessels occurs in the surrounding tissues and a group of clotted blood is formed near the fracture site, called as hematoma. During this period, the blood flow is affected and some cells die near the fracture site. In this phase, LIPUS helps to increase the formation of new blood vessels in a process, called as angiogenesis. It has been demonstrated that LIPUS promoted the expression of mRNA that is responsible for angiogenesis.[98] Angiogenesis process consists of proliferation and migration of endothelial cells (Figure 1). Metalloproteinase (MMP) is an extracellular matrix that degrades to perform angiogenesis.[99] Many growth factors are involved in the angiogenesis process such as fibroblast growth factor (FGF), bone morphogenic protein (BMP), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and interleukins (IL-8).[100] IL-8 has been reported as an important factor to promote angiogenesis.[101] However, the major growth factor responsible for angiogenesis is VEGF.[102] VEGF is produced by osteoblasts and periosteal cells. This growth factor plays a crucial role in differentiation of osteoblasts and activation of osteoclasts.[103] A few studies have also reported the importance of LIPUS in angiogenesis process.[104–106] Vavva et al demonstrated that angiogenesis process depends on the ultrasound frequency by their mathematical model.[107] From all these studies, it is understood that LIPUS enhances angiogenesis process. In addition to LIPUS treatment, high-frequency vibration treatment has also been used to increase the blood flow as well as angiogenesis process at fracture site monitored by 3D power Doppler ultrasonography in rat models.[108,109] In summary, previous studies have revealed that LIPUS enhances angiogenesis process by increasing the expression of angiogenic genes and that VEGF promotes new blood vessel formation at the fracture site.

Callus Formation

Callus is the hard tissue formed around the areas of the broken bone. One of the studies has noticed that MSCs are involved in callus formation.[110] The MSCs are attracted and migrate to the fracture site by certain growth factors in the inflammatory phase (Figure 1). These MSCs are differentiated into osteoblasts and chondroblasts by the activation of osteochondral progenitor cells influenced by hormones, with proteins such as PTH and BMP, respectively. Osteoblasts are the cells that produce

Figure 1. Summary of possible mechanism of LIPUS on bone fracture healing. When bone fracture occurs, it initially undergoes inflammatory phase (Stage 1). It is followed by reparative phase namely soft callus and hard callus formation phase (Stages 2 and 3). Finally, the bone maturation occurs in remodeling phase (Stage 4).



substance for bone formation in hard callus phase,[111] and chondroblasts are the cells that produce chondrocytes in growing cartilage matrix in the soft callus phase.[112] In addition, PGE2 increases the maturation of osteoblasts by decreasing the osteoclastic activity.[113] Osteoclasts are the bone cells that resorb the bone tissue to inhibit the bone healing.[111] PTH increases the osteoblastic cells in the bone marrow.[114] An in vitro study reported the augmentation process of callus formation during ultrasound exposure.[115] The authors utilized the cultured chondrocytes to be exposed with LIPUS and found the upregulation of specific gene expression, namely aggrecan which occurs in the earlier stage of fracture healing process. A recent study has investigated the effects of callus formation using ultrasound on rat femur.[16] It was observed that callus formation was initiated during the inflammatory phase with more callus formed at the ultrasound stimulated side compared with the nonstimulated side by radiographic analysis of the same bone. This study concludes that the inflammatory phase is an important early phase for the initiation of callus formation. Effective bone union can be achieved by starting the ultrasound stimulation at the initial stage of fracture. Cheung et al demonstrated LIPUS effects on osteoporotic fractures, and their study proved that LIPUS promotes fracture healing by activating callus formation.[104] In summary, LIPUS has the ability to enhance the callus formation even in the presence of distraction osteogenesis.[116]

Bone Remodeling

Callus remodeling is the resorption process induced by osteoclasts toward the end of the entire healing process (Figure 1). In this phase, woven bone is also replaced with the lamellar bone. The osteoclasts resorb the woven bone and the fracture callus is transformed into original bone shape. An in vitro study has demonstrated that LIPUS enhanced the osteoclastic resorptive activity.[117] They found that if the treatment time was longer, the resorption rate would be higher. Azuma et al studied the effects of LIPUS on the right femur of rat, which was treated with ultrasound stimulation for 20 minutes daily, and their

results showed that LIPUS supported early remodeling of trabecular bone at day 17, which was earlier than the control group.[17] A study conducted on ovariectomized rat model demonstrated that LIPUS increased the rate of endochondral ossification between 4 and 8 weeks.[104] Furthermore, bone remodeling was influenced by osteoclastic and osteoblastic processes.[118] Other studies have also shown positive results on bone remodeling through LIPUS treatment.[41,119,120] Hence, it has been proven that LIPUS accelerates the formation of osteoclastic cells and promotes resorption process in bone remodeling phase.

Role of Cellular Signaling Pathways

Ultrasound stimulation enhances the cellular signaling pathways during the healing process. Integrins help to convert the mechanical stimuli into chemical stimuli.[121] It has been reported that the mechanical stimuli can be transmitted through interaction with certain protein signaling molecules.[122] This activity is promoted by a key protein named focal adhesion kinase (FAK) by phosphorylation.[83,89] These focal adhesions promote translocation of intracellular proteins inside the cell where integrins are present. Some evidence showed that cAMP-protein kinase A (PKA) pathways were involved in osteoblastic activity. The enhancement of receptor activator of nuclear factor kappa-B ligand (RANKL) mRNA expression accelerated by this pathway is done by PGE2.[84] LIPUS could increase the expression of RANKL, which led to the activation of osteoclast and chondroclast for resorption.[123,124] Furthermore, enhancement of osteoblastic differentiation was observed when a longer time of ultrasound stimulation was performed.[123] The signaling pathways involved in the bone formation through increasing the expression of COX-2 include the activities of FAK, the extracellular signal-regulated kinase (ERK), and the phosphatidylinositol 3-kinase (PI3K),[89] which in turn promotes the cellular proliferation and differentiation (Figure 1). A study has been reported on whether mechanical stimuli are related to primary cilia through hedgehog signaling.[125] Primary cilia are the organelle in mechanical sensing cells that detect and process the molecular and mechanical signals. In this study, the authors observed that LIPUS enhanced the callus formation at the left femur of mice when treated with a frequency of 3 MHz and daily exposure of 20 minutes for 5 weeks. The findings revealed an increased osteoblastic differentiation and mineralization through hedgehog signaling pathway. It has been proved that LIPUS stimulates the osteogenic process through primary cilia of osteoblasts cells. This process plays an important role in bone remodeling. The mechanism of LIPUS on osteoclast was analyzed in an in vitro study with an average intensity of 30 mW/cm^2, and it is revealed that LIPUS reduced the RANKL-induced osteoclast gene expression via ERK, c-Fos, and NFATc1 signaling pathways.[118] Another in vitro study demonstrated that LIPUS stimulation enhanced the stromal cell-derived factor-1 signaling pathway, which increased the migration of MSCs to the fracture site. In addition, Wnt signaling pathways supporting the process of cell migration, renewal of stem cells, and cell polarity could also be altered by LIPUS.[126] These pathways help in the callus formation phase in fracture healing process. Therefore, LIPUS enhances the osteogenic process by increasing the osteoblastic activity and promotes the cellular differentiation.

Current Limitations and Future Directions of Ultrasound Treatment for Fracture Healing

There are a large number of clinical trials showing positive effects of LIPUS, but still with certain limitations. A few studies reported that ultrasound does not reduce the pain caused during fracture healing process.[127,128] LIPUS helps to accelerate the quality of fracture union without affecting the functional recovery of the patients, such as ability to return to work[53,127] and weight-bearing capacity.[51,129] Furthermore, it was reported that LIPUS treatment failed to reduce the incidence rate of recurrent fracture among nonunion and delayed union fracture cases.[49] Moreover, the effects of LIPUS were not noticed when they were applied over internally fixed tibial fractures.[50] This implies that ultrasound stimulation may not have a positive effect on all musculoskeletal problems. Due to the availability of commercial LIPUS devices, most of reported studies have used similar set of stimulating parameters, no matter for human, animal or even cells, hence the study of optimized LIPUS parameter sets and their effects needs to be

further carried out to enhance the outcome of healing therapy. This is particularly important when LIPUS is used for different types and locations of bone fracture or nonunion. A better understanding is needed about how the changes of ultrasound frequency, intensity, signal waveform, treatment dosage, and duration, and so on would affect the outcome of LIPUS. Although most of the studies have argued on the efficiency of LIPUS, a comparison of LIPUS therapy with other available treatments is needed to investigate whether LIPUS itself is a more effective therapy or it must be used as a supportive therapy to existing fracture treatments. A meta-analysis by Ebrahim and team in 2014 compared LIPUS with electrical stimulation for fracture healing. They reported that the benefit of LIPUS is not significant in case of fresh fractures.[130] This is because invasive treatments can provide continuous stimulation when implanted near the fracture site. Due to the heterogeneity of the studies from the literature, it is still difficult to conclude the efficiency of the LIPUS treatment. The studies differ in the protocol, stimulation parameter, treatment time, and nature of the trauma. Hence, this requires a greater number of randomized controlled trials to be conducted in humans to examine the efficiency of the LIPUS therapy. In addition, further research is still needed to investigate the mechanism underlying behind the fracture healing process with the application of LIPUS. Previous studies have demonstrated that ultrasound stimulation appears to have the effects in all the healing phases of the bone fracture; however, it is still not clear stimulation at which phase gives the best benefit for the healing.

The review of Watanabe et al demonstrated that LIPUS possesses weak evidence to treat deep bones.[46] The healing rates achieved in the past studies on deeper bones such as femur and humerus bones are 65 and 75%, respectively.[66,131] This may be due to various factors such as increased ultrasound attenuation, difficulties in targeting ultrasound beam, and insufficient ultrasound penetration for deeper fracture treatment using LIPUS. On the other hand, Fung et al conducted a very inspiring experiment on rat femoral fracture using LIPUS stimulation with an intensity (I_{SATA}) of 30 mW/cm^2 (measured at the rat skin surface) but arranging the ultrasound transducer at three different distances, 0, 6, and 13 cm.[132] Their results showed that LIPUS had effects for all the three distances, indicating that ultrasound can achieve treatment effects even for bone fractures located as deep as 13 cm from the transducer, given that high enough intensity of ultrasound is delivered at the fracture location. Meanwhile, this study also showed that the tissue depth affected the types of effects, with the 13-cm group achieved significantly larger changes in BMD and stiffness at week 4, while the other two groups having significantly higher woven bone percentage. They found that patterns of intensity distribution along the cross-section of the ultrasound beam at different distances apart from the transducer were different, and suggested that the beam intensity pattern caused the observed depth-dependent effect. If this effect can be further verified, the intensity distribution of ultrasound beam at the location of the fracture would become another stimulation parameter that we should control and investigate in future studies, particularly when deep bone fracture is a target.

Athough there are limited reports about successful LIPUS treatment for deep bone fracture healing, the impacts of some deep bone fractures, such as hip fractures, are huge. Among elderly, hip fractures are the most common life-threatening injury, which usually requires surgical procedures. It is estimated that hip fractures may have significantly increased across worldwide from 1.66 million now to 6.26 million by 2050.[133] The common causes for hip fractures are sudden fall, trauma, and obesity, as well as diseases like osteoporosis that reduce bone density.[134] Hip fracture healing using LIPUS therapy could be suggested mostly to the patients with chronic diseases such as cancer and diabetes, smoking persons, vascular insufficiency, or to obese patients who have a high risk of infection, and elderly persons for whom the wound healing is a very slow process. Because the aging population continuously grows all over the world, the importance of accelerating hip fracture healing becomes more obvious. Therefore, the authors of this review strongly suggest more research and development efforts should be conducted to focus on the LIPUS treatment for deep bone fracture healing, such as hip fracture, given that earlier studies have shown that LIPUS effects can be achieved for deep fracture if the intensity at the fracture location reaching 30 mW/cm^2.[132] The research questions will include what frequency of ultrasound is most suitable to deliver ultrasound into deep tissues, how larger attenuation to ultrasound caused by a thicker layer of tissues can be

overcome, how to make sure the ultrasound beam is properly targeted at the fracture location, how to achieve the optimized set of ultrasound stimulation parameters, what intensity distribution pattern should be used to optimize the treatment, how to fix LIPUS devices on various body surfaces, and how to harness the latest development in the field and ultrasonics and electronics for LIPUS device development.[132]

Conclusions

Overall, LIPUS has shown positive results on bone fracture healing through the molecular, biological, and biomechanical changes around the fracture site. It has been proven to accelerate the bone formation in fresh fractures, delayed unions, nonunions, and distraction osteogenesis. LIPUS treatment is recommended as a safe therapy when compared to existing fracture treatments, and can be used as an adjunctive therapy to accelerate the bone healing process for fresh fractures, delayed fractures, and nonunions. However, ultrasound treatment on fractures located in deeper tissues has not been widely investigated due to some factors, including high attenuation of ultrasound beam, insufficient penetration power, and difficulty in targeting ultrasound energy at the fracture location. Given the importance of enhancing deep bone fracture healing, such as hip fracture, additional research works are suggested in the area of LIPUS for deep bone fracture, by overcoming these challenges and with the consideration of optimizing ultrasound stimulation parameters and more understanding about its mechanism.

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