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REVIEW ARTICLE

Ultrasound as a stimulus for musculoskeletal disorders

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Summary Ultrasound is an inaudible form of acoustic sound wave at 20 kHz or above that is widely used in the medical field with applications including medical imaging and therapeutic stimulation. In therapeutic ultrasound, low-intensity pulsed ultrasound (LIPUS) is the most widely used and studied form that generally uses acoustic waves at an intensity of 30 mW/cm^2, with 200 ms pulses and 1.5 MHz. In orthopaedic applications, it is used as a biophysical stimulus for musculoskeletal tissue repair to enhance tissue regeneration. LIPUS has been shown to enhance fracture healing by shortening the time to heal and reestablishment of mechanical properties through enhancing different phases of the healing process, including the inflammatory phase, callus formation, and callus remodelling phase. Reports from *in vitro* studies reveal insights in the mechanism through which acoustic stimulations activate cell surface integrins that, in turn, activate various mechanical transduction pathways including FAK (focal adhesion kinase), ERK (extracellular signal-regulated kinase), PI3K, and Akt. It is then followed by the production of cyclooxygenase 2 and prostaglandin E2 to stimulate further downstream angiogenic, osteogenic, and chondrogenic cytokines, explaining the different enhancements observed in animal and clinical studies. Furthermore, LIPUS has also been shown to have remarkable effects on mesenchymal stem cells (MSCs) in musculoskeletal injuries and tissue regeneration. The recruitment of MSCs to injury sites by LIPUS requires the SDF-1 (stromal cell derived factor-1)/CXCR-4 signalling axis. MSCs would then differentiate differently, and this is regulated by the presence of different cytokines, which determines their fates. Other musculoskeletal applications including bone—tendon junction healing, and distraction osteogenesis are also explored, and the results are promising. However, the use of LIPUS is controversial in treating osteoporosis, with negative findings in clinical settings, which may

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be attributable to the absence of an injury entry point for the acoustic signal to propagate, strong attenuation effect of cortical bone and the insufficient intensity for penetration, whereas in some animal studies it has proven effective.

Introduction

Ultrasound is an inaudible sound wave at frequencies higher than 20 kHz that is widely used in clinical imaging and sonography, and is also used in physiotherapy in medicine [1]. Therapeutic ultrasound is alternating compression and rarefaction of longitudinal sound waves with a frequency between 0.7 MHz and 3.3 MHz to maximise energy absorption at a depth of 2—5 cm of soft tissue. At intensities of 0.2—100 W/cm^2, the therapeutic benefits of ultrasound have also been explored and these benefits were generated using low-intensity pulsed ultrasound (LIPUS) [2]. LIPUS generally uses acoustic waves at intensity of 30 mW/cm^2, with 200 ms pulses and 1.5 MHz.

An ultrasound wave is generated when an electric current is applied to an array of piezoelectric crystals located on the transducer surface. Passing through the tissue, absorption of the ultrasound signal results in energy conversion to heat [3]. This thermal effect of ultrasound could cause various responses in biological tissues. Generally speaking, an increase of 1°C in temperature by ultrasound could increase metabolism; an increase of 2—3°C could decrease pain and muscle spasm; an increase of 4°C or above could increase collagen extensibility and decrease joint stiffness; meanwhile, at temperatures higher than 45°C, the thermal effect from ultrasound will be damaging. The thermal effect is extremely small using low intensity as in the LIPUS, which is advantageous in minimising the thermal effect observed in ultrasound forms at higher intensities; therefore, it is used to enhance bone and soft tissue healing [4]. Moreover, the nonthermal effects of ultrasound such as cavitation and acoustic microstreaming occur simultaneously with thermal effects. Therefore, ultrasound may be able to normalise or reestablish the effective metabolic temperatures in healing regions [1].

As LIPUS is reported to demonstrate clinical efficacy in enhancing repair in various musculoskeletal tissues, we review the related literatures on the effect of LIPUS on the most widely used musculoskeletal tissue repair, including fracture repair, the effect on stem cell recruitment and the mechanism of action of LIPUS during that process. We also look into the effect of LIPUS on other musculoskeletal injury repair such as distraction osteogenesis (DO), bone—tendon junction healing, muscle repair as well as osteoporosis.

Fracture repair

LIPUS was first reported to be able to accelerate fracture repair in 1983 [5] and was shown to accelerate biomechanical healing in the rabbit fibula osteotomy animal model [6]. In 1994, EXOGEN obtained approval from the United States Food and Drug Administration for accelerated healing of certain fresh fractures, and used this in clinical trials in tibial fracture healing [7] and distal radial fracture healing [8]. LIPUS was also shown to be an effective and safe treatment for delayed union or nonunions in clinical trials [9—12] and animal studies [13]. Recent studies showed that fracture healing in aged animal [14,15] in ovariectomy-induced osteoporotic bone was enhanced by LIPUS [16]. Four systematic review and meta-analysis journal articles found in Medline all concluded the positive effect of LIPUS on fracture repair [17—20]. These studies provided various lines of evidence that LIPUS had a positive effect on bone fracture healing in many types of fractures, especially on the processes of converting cartilaginous callus to hard callus, endochondral ossification, mineralised callus, and increasing the mechanical stability of the healing fracture [21] that spans over the various phases during the healing process. However, a recent large-scale TRUST randomised controlled trial involving 501 patients reported no positive effect of LIPUS on tibial fracture healing, in which the moderate compliance rate at 73% administered $>$ 50% of all recommended treatments might explain the differences from previous positive studies [22].

In Azuma et al's [23] study, a rat closed femoral fracture model was performed and treated at different periods of repair in order to determine the effect of LIPUS on each phase of the fracture repair process. Rats were divided into four groups based on the timing and duration of LIPUS treatment: Day 1—8 for the haematoma phase, Day 9—16 for the soft callus phase, Day 17—24 for the mineralisation phase and Day 1—24 for all phases of the fracture repair process. The results showed that even partial treatment with LIPUS during different phases of fracture healing could improve the fracture repair, but the treatment that lasted for all 24 days was the most effective. The results further confirmed the positive effect of LIPUS on fracture repair, and meanwhile indicated that LIPUS could act on various cellular reactions in all phases of fracture healing process such as inflammation, angiogenesis, chondrogenesis and endochondral ossification. Many studies showed the effect of LIPUS on different phases of fracture repair.

Inflammatory cells such as macrophages played an important role during the inflammatory phase of fracture repair. It was reported that LIPUS could accelerate macrophage phagocytosis, which removed debris and bacteria to facilitate fracture healing during the inflammation stage [24]. Ultrasound was also shown to stimulate the proliferation of fibroblasts within 24 hours after exposure [25,26].

After the inflammatory stage, angiogenesis was stimulated and activated. Vascularity was increased in an

osteotomised dog model treated by LIPUS [27]. An *in vivo* study of diabetic rat model showed that the vascular endothelial growth factor (VEGF) was significantly increased with the addition of LIPUS after 7 days of treatment [28]. Basic fibroblast growth factor, which is another cytokine regulating angiogenesis, was also found to be upregulated by LIPUS in human osteoblasts [25].

Endochondral ossification during fracture repair involves invasion of newly formed blood vessels into the cartilage tissue, degradation of cartilage tissue and bone formation. Cartilage formation was reported to be enhanced by LIPUS in rabbit osteochondral defect model [29] and rat osteoarthritis model [30]. *In vitro* studies showed that proliferation, gene expression, and matrix production were increased by LIPUS using rat and human chondrocytes [31,32]. The enhancement of blood vessel and cartilage regeneration indicated the role of LIPUS in advancing endochondral ossification. An *in vitro* study showed that LIPUS activated ossification via a direct effect on osteoblasts and ossifying cartilage [33], and the enhancement of endochondral ossification by LIPUS was clearly shown in an *in vivo* study in which prolonged endochondral bone healing in aged mice was shortened by LIPUS [14]. Another study of aged mice with femur fracture showed a similar result, in which LIPUS accelerated the healing process by reducing the time of endochondral ossification completion in aged mice [15]. At the cellular level, cells in the local environment are the key players during the enhanced endochondral ossification process, which helps to convert soft cartilaginous or fibrous callus to calcified callus. The osteogenic differentiation of osteoblast was positively mediated by LIPUS in several studies [34—36]. Leung et al [37] showed that LIPUS could stimulate the osteogenic activities of human periosteal cells. An independent study using human haematoma-derived progenitor cells showed enhanced osteogenesis after LIPUS treatment [38]. The osteogenic differentiation and expression of bone morphogenic proteins in response to LIPUS has also been enhanced in rat osteosarcoma cell line ROS 17/2.8 cell [39,40] and UMR-106 cell [41]. Finally, bone resorption during the remodelling phase of the fracture repair was also reported to be influenced by LIPUS by increasing the number of osteoclasts and the removal of cortical bone in a specific manner in time and site [42].

LIPUS promoted different processes of fracture repair from several aspects during each phase of the healing process. The molecular mechanism of action of LIPUS has also been widely studied to understand how it triggers and influences the fracture repair processes.

Prostaglandin E2 (PGE2), which stimulates osteoblast formation by increasing nuclear factor kappa B ligand mRNA expression [43] through cAMP-protein kinase A pathways, can accelerate the fracture healing. Production of PGE2 was increased by LIPUS via induction of cyclooxygenase-2 (COX-2) mRNA in mouse osteoblasts [44]. Stimulation of COX-2 production after LIPUS treatment was also demonstrated in murine bone marrow-derived cells [34], rat alveolar mononuclear cell—osteoblast coculture system [45] and human osteoblasts [46], whereas endochondral bone healing became delayed in the COX-2 knockout mice model [14]. These studies suggested that COX-2 is a key molecule stimulated by LIPUS and if COX-2 inhibitor was used, the mineralisation enhancement by LIPUS would be significantly reduced [47]. The important role of COX-2 in fracture healing was proven by the impaired fracture by the inhibition of COX-2 [48] and the reduced expression in aged mice [49]. A further study investigating the mechanism of LIPUS on COX-2 showed that the upregulation was through the integrin/focal adhesion kinase (FAK)/PI3K/Akt and extracellular signal-regulated kinase (ERK) signalling pathways [47]. The integrin and PI3K/Akt signalling pathways were activated by LIPUS in mandibular osteoblasts [50] and chondrocytes [51]. These results showed the mechanism through which the mechanical signal in the form of LIPUS was converted to biological signal to influence the cells during the fracture repair process. The integrin as the mechanoreceptors was involved in this process. LIPUS could activate the integrin pathway through focal adhesions formation, linking integrin cytoplasmic domain to the cytoskeleton and activated integrin-associated signalling pathways on the surface of cells [26]. One of the key focal adhesion proteins involved in the transduction of the LIPUS signal from a mechanical force to a biochemical signal is FAK, a potential signalling molecule regulating the integrin-mediated signalling active by LIPUS [47]. As the downstream of FAK, ERK signalling linked with the simulation of integrins also played an important role in the transmission of LIPUS [26,47,52]. Another subsequent cell surface integrin cluster promoted by LIPUS in macrophage cells and the fibroblasts was the enhancement of actin polymerisation that could activate the downstream Src tyrosin kinase, the following ERK and p38 or RhoA/Rho-associated kinase pathways to accelerate phagocytosis, which contributes to the fracture repair or fibroblast proliferation during the inflammatory phase [24,26]. Moreover, LIPUS has also been shown to be effective in enhancing both normal and osteoporotic fractures, where mechanical sensitivity is related to the differential estrogen receptor expression in rats [53]. Taking these together, the mechanical signal of LIPUS influenced the mechanosensors, integrins on the surface of cells, to activate related signal pathways such as FAK/PI3K/Akt and ERK triggering downstream biochemical signalling molecule such as COX-2 expression in osteoblast. COX-2 signalling was a key pathway of the LIPUS-induced intracellular pathway associated with initiating the production of PGE2 to regulate the expression of osteogenic genes, which could enhance the endochondral ossification of the callus.

Stem cell differentiation and recruitment

Mesenchymal stem cells (MSCs) play a key role in fracture repair [54]. During the repair process where the microenvironment is dynamically changed, effect of LIPUS can enhance osteogenesis and chondrogenesis. LIPUS was proven to upregulate the osteogenic mRNA in mouse MSC clone ST2 cells [55]. The osteogenic stimulation was also found in another pluripotent mesenchymal myoblast cell line, C2C12 [56]. A study using rat bone MSCs showed that LIPUS and BMP-2 stimulated the expression of several genes associated with osteogenesis. Core-binding factor subunit alpha-1 (Cbfa-1), a primary regulator of osteogenesis, was upregulated more quickly with LIPUS than BMP-2 [57]. A

preclinical study also confirmed that the combined treatment of MSCs and LIPUS was beneficial to fracture healing in a rat fracture model [58]. In human MSCs, similar results were observed, in which LIPUS alone could increase osteogenic differentiation and LIPUS with BMP induced alkaline phosphatase (ALP) and Cbfa-1 mRNA expression; and when hMSCs were treated with LIPUS and dexamethasone/transforming growth factor β1, chondrogenesis was enhanced [59]. A recent study also demonstrated that LIPUS could restore normal osteogenic differentiation of adipose-derived human stem cells (Ad-hMSC) from disuse by daily stimulation (20 min/d), which showed significant increases in ALP, OSX, RANKL (receptor activator of nuclear factor-kappa B ligand), and RUNX2, and decreases in OPG after LIPUS treatment on microgravity-simulated Ad-hMSC culture [60]. Therefore, the use of LIPUS could unleash various differentiation potentials of MSCs during fracture repair at different phases with different microenvironments.

Another remarkable reparative ability is the homing or recruitment of MSCs to the injury site. Recruitment of circulating progenitor cells to the fracture site is a normal biological stage of the fracture process [61]. The success or level of MSCs recruited to the fracture site was also an important factor to determine the success of fracture healing. LIPUS can induce the homing of circulating osteogenic progenitors to the fracture site for possible contribution to new bone formation [62]. A study showed that the migration of exogenously administered labelled-MSCs was promoted by LIPUS to the fracture site in a rat closed fracture model [63] through the pathway of stromal cell derived factor-1 (SDF-1) and its receptor, CXCR4, as they were proven to play a key role in the recruitment of circulating cells to the fracture site for repair [64]. The study also showed that the SDF-1 expression of rat MSCs was directly increased by LIPUS *in vitro* [63]. In another independent study using a parabiotic mice fracture model cojoining a green fluorescent protein mouse and a syngeneic wild-type mouse, more SDF-1 and CXCR4 were immunohistochemically identified at the fracture site in the LIPUS treatment group 2 weeks after fracture [62]. These findings demonstrated that LIPUS enhanced stem cell recruitment to the fracture site through the SDF-1/CXCR4 pathway.

Therefore, the efficacy of LIPUS on musculoskeletal tissue repair with respect to stem cell is generally attributed to its ability to enhance the recruitment of stem cells to the injury site, and the differentiation potential being exploited at various stages to direct them into either the osteogenic or chondrogenic lineage of cells, eventually contributing to earlier consolidation at the injured site.

Distraction osteogenesis

The effect on fracture repair is the most and best investigated application of LIPUS in musculoskeletal tissue repair; meanwhile, the LIPUS was also applicable and utilised in DO. A study on the rat DO model revealed that healing of ultrasound-treated bones preceded that of the sham group by approximately 1 week, with significantly higher bone volume fraction and trabecular bone pattern factor, yet no difference was observed in bone mineral density (BMD) [65]. Another study on rabbit DO model demonstrated that ultrasound could accelerate bone maturation with significantly higher callus area, BMD and mechanical properties at a normal distraction rate (0.5 mm/12 hours), and bone maturation could also be achieved well in the ultrasound group at a fast distraction rate (1.5 mm/12 hours) [66]. Shimazaki et al [66] further investigated the different timing of LIPUS treatment in DO rabbit model, which indicated that the LIPUS effect was mediated through endochondral pathways, and it is most effective to treat at the lengthening phase. Another research group showed that LIPUS could shorten the distraction period under a normal distraction rate (1 mm/d for 1 week) by accelerating bone formation at the initial stage of consolidation with significantly higher BMD (+9.18%), hard callus volume (+116%), and bone strength index (+94%) [67], and was able to enhance bone regeneration under rapid distraction (2 mm/d for 1 week) with dose-dependent effect (20 minutes vs. 40 minutes LIPUS treatment) in the rabbit DO model [68]. By contrast, two reports showed negative findings in rabbit DO models: Uglow et al [69] showed no difference between ultrasound and control groups with respect to bone mineral content, cross-sectional area and strength; Tis et al [70] also reported no positive effect on the mechanical properties or density of bone regenerate despite the significantly larger callus in the ultrasound group.

Clinically, LIPUS was reported to accelerate bone maturation in DO, and reduce the overall time to removal of the fixation device in clinical trials [71—73]: El-Mowafi et al [71] indicated that the mean healing index in the ultrasound group was 30 (27—36) d/cm, as compared with the control group with 48 (42—75) d/cm in a 20-patient trial [71]; Salem et al [72] reported 27% faster callus maturation and 33% more radiographic callus density in the LIPUS group in a 21-patient trial; Dudda et al [73] showed that the fixation gestation period was decreased for 43.6 days in the ultrasound group with no negative effects in a 36-patient trial. A meta-analysis report [20,74] concluded that LIPUS was an effective treatment to reduce the overall treatment time for DO, yet the lines of evidence were weak; thus, large-scale clinical studies are needed to confirm its efficacy.

Bone—tendon junction healing

The repair of the patella—patellar tendon complex can influence the functional outcomes of the knee joint. In one study, it was shown that in the rabbit partial patellectomy and surgical reconstruction between patella and patellar tendon animal model, VEGF expression and chondrogenesis was significantly enhanced at Week 4 after the treatment using LIPUS [75]; the bone formation increased by 96% at Week 8 and 57% at Week 16, whereas the failure load and ultimate strength of the bone—tendon junction increased by 35% at Week 16 after the treatment with LIPUS [76,77]. These results indicated that LIPUS showed very high efficacy for enhancing not just the morphological, but also the functional, outcomes of bone—tendon junction repair. The same research group also conducted a study to investigate the combined effect of LIPUS and functional electrical

stimulation (FES) on bone—tendon junction healing in a rabbit model, which showed more new bone formation and higher tensile properties in the combined group over the LIPUS or FES alone groups [78].

More recent studies have reported that the effect of LIPUS was better if it started on Day 7 postoperatively than immediately or on Day 14 [79], whereas if it started at 14 days postoperatively (after inflammation stage) it could accelerate bone formation and improve healing quality [80]. These findings indicated that the initiation time of LIPUS should also be considered during application of LIPUS in bone—tendon junction healing. However, to date, there has been no clinical trial conducted to report the efficacy of LIPUS on bone—tendon junction healing in patients.

Osteoporosis

Although osteoporotic fracture healing can be enhanced by LIPUS, there have been only a few reported positive effects on osteoporotic animals. Wu's group [36] reported that LIPUS could prevent bone loss in ovariectomised rats, with increased wet weight of femur and increased trabecular spongiosa; Carvalho and Cliquet [81] demonstrated more bone formation and less microarchitectural deterioration in osteopenic rats treated with LIPUS; another study revealing different ultrasound intensities on osteopenic rats reported that 100 mW/cm^2 LIPUS could increase bone volume fraction (BV/TV) (+33%), trabecular number (Tb.N), trabecular thickness (Tb.Th), structure model index (SMI), apparent level elastic modulus (+42%) and trabecular mechanical strength in the treatment group over ovariectomised controls, whereas 30 mW/cm^2 LIPUS could increase Tb.Th only [82].

By contrast, many studies reported negative or zero effect on enhancing BMD in osteoporotic patients [37,41,83]. In a clinical trial in which patients suffering from osteoporosis had their distal radius treated daily with LIPUS for 3 months, and evaluated 3 months after discontinuing the treatment, the results showed that the rate change of trabecular bone mineral density and integral bone mineral density was not significantly different between the site treated with LIPUS and the contralateral control at either 3 months or 6 months after treatment, suggesting that LIPUS could not change the anabolic effect on bone mass. Another preclinical study showed that ovariectomy induced significant bone loss in the rodent hind limb, but LIPUS could not interfere with the decline in BMD [84]. The discrepancy in these results on intact osteoporotic bone might be attributable to the strong attenuation effect of intact cortical bone, insufficient energy of ultrasound and the lack of an entry point for the LIPUS signals in intact bones [21]. These clinical and preclinical results indicate that the application of LIPUS on osteoporosis remains disputable, and more research is warranted to confirm its efficacy on osteoporosis, especially the effect of different ultrasound intensities.

Conclusion

LIPUS, the most applied form of therapeutic ultrasound, is used widely in musculoskeletal tissue repair especially in fracture healing. The positive effect of LIPUS on fracture healing was confirmed by many clinical and preclinical studies including normal and osteoporotic fractures. In this paper, the mechanism of action of LIPUS converting a mechanical signalling to biological signal and how it affects the downstream pathways and products in a multiscale cascade were reviewed, in which COX-2 signalling pathway was enhanced at the molecular and cellular levels thereby affecting the differentiation and recruitment of MSCs during the repair process. Besides the application in fracture repair, LIPUS was also beneficial to other musculoskeletal injuries such as bone—tendon junction healing and DO; however, more research is necessary to look into their mechanisms as the current related lines of evidence are far fewer than those of fracture healing. However, the effect of LIPUS on intact osteoporotic bone is debatable, with discrepancy between clinical and preclinical results, which might be attributable to the strong attenuation effect of intact cortical bone, inadequate ultrasound energy for penetration, and lack of entry point for ultrasound signals. In conclusion, LIPUS would be a very useful biophysical stimulus for different musculoskeletal disorders.

Conflicts of interest

The authors have no conflicts of interest to declare.

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